

An Overview of University Post-graduate Research (2014-2019)

DUVASU



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Vice Chancellor

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FOREWORD

The prime mandate of DUVASU is to impart high quality teaching, research and extension in the area of Veterinary and Animal Sciences, Fisheries and Livestock Products Technology, thereby, contributing its share in building the economy of nation. The College of Veterinary Sciences and Animal Husbandry under DUVASU is amongst the premier Veterinary Institutions in the country. It is the first Veterinary College of Post independent India which was started with a B.V.Sc. & A.H. programme, initially and later Post-graduation with M.V.Sc. and Ph.D. Degree Programmes in various disciplines of Veterinary and Animal Sciences.

Post-graduate research usually focuses on the end user needs and difficulties faced at the field levels. Also, Post-graduate research reflects the academic and scientific standard of any institution. DUVASU is known to have many state of art laboratories in the various disciplines which facilitate world class research. These high class research findings are compiled in the form of thesis. Although these are available in the university library, yet, sometimes the dissemination of such vital informations suffers drastically due to non availability in original forms or in diskettes. Hence, it was felt that compilation of these into "Thesis Abstracts" is a ready at hand service to the researchers who want to trace the developmental status with concepts and design to final interpretation and outcome of various researches in various disciplines at various times.

Keeping this in mind, University had published "An Overview of University Post-graduate Research (2001-2013)" in October 2014 which served its purpose very well and was one the major guidance book for researchers. However, it has been over 7 years since its first issue, and within this period a lot of work has been done in the University. So, when the proposal to bring its second issue, covering various thesis submitted between 2014-2019, was put in front of me, I readily concurred to it. Not only this compilation will bring the glimpses of research done during this period at DUVASU, it will also avoid duplication of research work being conducted in various laboratories of the country. It will save the valuable resources and time that can actually be utilized for conducting newer research and thus, paves the way for national prosperity.

The editorial committee has done a commendable job in bringing out this second issue. I congratulate them for an excellent job. I hope that this publication will serve as a useful reference tool to the researchers engaged in various disciplines of Animal and Veterinary sciences and other allied disciplines.



(G. K. Singh)
Vice Chancellor

PREFACE

Quality research is the outcome of years of hard work. It is not only the hard work of a single person but is, more often than none, a combined effort of group of researchers from various disciplines that have tried hard to find solution to a single burning issue. These findings are often published in various journals. The “Thesis” that is submitted for the award of Masters or Doctoral Degree comprises of whole hearted hard effort of a research scholar under the able guidance of his supervisor and other members of advisory committee. It is a quality compilation that reveals the quality of mind of associated researchers.

“An Overview of University Post-graduate Research (2001-2013)” published previously by the University in the year 2014 and it was a huge hit among the researchers. It has been over 7 years and a great amount of work has been done during this time that again needed to be compiled on a single platform. With this background, the present publication has been compiled and it comprise of abstracts of M.V.Sc. and Ph.D. Degree awarded during 2014-2019. Any reader may have the glimpse of type and trend of research which have been conducted during this period. Since the past research forms the background of future research, researchers may consult the outcome of previous researches and accordingly plan their future line of research. The present volume will serve as a reference material for the researchers and will help them in choosing problems, methodology and the guidelines for smooth conduction of future research. It will also save duplication of research and will save precious time and resources at various universities.

The editors feel grateful to the Hon'ble Vice Chancellor for supporting us in brining out this publication.

Our colleagues from various departments of the University are also acknowledged for their cooperation in collection and compilation of information. Efforts of Mr. Y. N. Prasad, Assistant Librarian is also acknowledged for his help in compilation of abstracts. The Co-ordinator, Communication Center is acknowledged in bringing out this priceless publication in short span of time.

-Editors

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DEPARTMENT OF ANIMAL GENETICS AND BREEDING

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2	Molecular characterization and polymorphic studies of Secreted phosphoprotein 1 (SPP1) gene in Indian cattle	Mona Sharma	Dr. S.P. Singh	2017	2
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1	Genetic polymorphism of prolactin and leptin gene and their association with production and reproduction traits in Sahiwal cattle in India	Sumit Kumar	Dr. Deepak Sharma	2015	3

M.V.Sc. Degree Programme

1. Genetic polymorphism of prolactin receptor (PRLR) and growth hormone receptor (GHR) gene in Sahiwal and Hariana cattle

Gaurav Parihar and Deepak Sharma

Prolactin and Growth hormone both are the polypeptide hormones of the pituitary gland. Prolactin regulates mammary growth and lactogenesis. It also regulates reproductive and immunological functions, fluid balance, cellular growth and differentiation, while Growth hormone is the main regulator of postnatal growth and metabolism in mammals, stimulating anabolic process such as cell proliferation, skeletal growth and protein synthesis. Thus it has important role in reproduction, lactation and growth. The biological actions of GH/PRL mediates by receptors i.e. Prolactin receptor (PRLR) and Growth hormone receptor (GHR). In the present study, identification of PRLR and GHR gene polymorphism and its association with milk production traits was undertaken in 103 Sahiwal and Hariana cattle maintained at ILFC, DUVASU, Mathura by using PCR-RFLP technique. The amplified fragments of the PRL-I, PRL-II and GHR-I genes revealed 168bp, 582bp and 302 bp respectively, the amplified products were digested with *SmlI*, *DraIII* and *NsiI* restriction endonuclease enzymes respectively. The *SmlI*/PCR-RFLP assay of PRLR gene revealed three types of genotypes; one of them was of 168 bp (GG genotype); 123 and 45 bp (TT genotype); third of 168, 123 and 45 bp (GT genotype) with frequencies 27.18%, 6.6% and 67.90 respectively. Allelic frequency of G and T allele were 0.607 and 0.393. After χ^2 analysis the screened population of Sahiwal and Hariana cattle was found in Hardy- Weinberg Equilibrium. The *DraIII*/PCR-RFLP assay of PRLR gene revealed three types of genotypes, 582 bp (AA genotype); 399 & 183 bp (GG genotype) and 582, 399 & 183 bp bands (AG genotype), with frequencies of 17%, 16% and 67% respectively. The allelic frequencies of allele A and G were 0.505 and 0.495. In restriction digestion of GHR 302 bp product with *NsiI* revealed only one type of genotype. This revealed no polymorphism i.e. monomorphic in nature. In Association studies of PRLR/*SmlI* gene, GG genotype was associated with the higher milk yield 300 days in first lactation and total lactation yield in third lactation. There was no significant difference was observed for the production traits among all the genotypes of *DraIII*/PCR-RFLP in all the lactations.

□□□

2. Molecular characterization and polymorphic studies of secreted phosphoprotein 1 (SPP1) gene in Indian cattle

Mona Sharma and S. P. Singh

Secreted phosphoprotein 1 (*SPP1*) is a highly negative phosphorylated glycoprotein that is a prominent component of the mineralized extracellular matrices of bones and teeth and is found in plasma and milk. *SPP1* gene also has potent roles in growth, production and reproduction of the animals. It plays important role in initiation and maintenance of pregnancy, as well as in the development of the fetus. In the present study, CDS sequence of *SPP1* gene has been cloned and characterized in Hariana breed of Indian cattle and also investigated the DNA polymorphism of *SPP1* gene in Indian cattle breeds viz., Sahiwal and Hariana. Multiple sequence analysis using DNASTAR revealed that Hariana *SPP1* sequence was 99.5-99.9% and 98.9-99.6% similar to that of Indian as well as exotic cattle breeds at nucleotide and amino acid level, respectively. The *SPP1* CDS of Hariana cattle breed contained one unique nucleotide changes, C to T transition at nucleotide position 11 which

resulted into amino acid substitution of alanine (A) to valine (V). Phylogenetic analysis revealed that, Haryana was clustered together with Vechur and other exotic cattle breeds in bovine clad. In the present study, polymorphism study was undertaken in 147 Sahiwal (n = 72) and Haryana (n = 75) cattle maintained at ILFC, DUVASU, Mathura using PCR-RFLP assay. The amplified fragments of the *SPP1* intron IV (C>T) and exon 7 (G>T) regions revealed 204 bp and 290 bp, respectively. The amplified products were digested with *BsrI* and *HpyCH4IV* restriction enzymes, respectively. The *BsrI*/PCR-RFLP assay of *SPP1* gene revealed only TT genotype (290 bp; 100%) with T allele (1.0). The *HpyCH4IV*/PCR-RFLP assay of *SPP1* gene also revealed only TT genotypes (204 bp; 100%) with T allele (1.0). Therefore, association analysis could not preformed with milk production traits.

□□□

Ph.D. Degree Programme

1. Genetic polymorphism of prolactin and leptin gene and their association with production and reproduction traits in Sahiwal cattle in India

Sumit Kumar and Deepak Sharma

Prolactin (PRL) is one of the polypeptide hormones of the pituitary gland, regulates mammary growth, lactogenesis, reproductive & immunological functions, fluid balance and cellular growth & differentiation, while Leptin (LEP), the fat derived protein hormone regulates the feed intake, energy balance, fertility, immune functions, important role in lactogenesis and its receptors have been found to be expressed in mammary epithelial cells. In the present study, identification of PRL and LEP gene polymorphism and its association with milk production and reproduction traits was undertaken in 154 Sahiwal cattle maintained at NDRI, Karnal and ILFC, DUVASU, Mathura by using PCR-RFLP technique. The amplified fragments of the PRL-I, PRL-II, LEP-I and LEP-II genes revealed 156, 857, 454 and 1820 bp respectively, the amplified products were digested with *RsaI*, *HaeIII*, *HphI* and *Sau3AI* restriction endonuclease enzymes respectively. The *RsaI*/PCR-RFLP assay of PRL gene revealed three types of genotypes; 156 bp fragment (BB genotype); 82 & 74 bp fragments (AA genotype) and 156, 82 & 77 bp fragments (AB genotype) with frequencies 22.58%, 60.48% and 16.94% respectively. Allelic frequency of A & B alleles were 0.472 and 0.528, respectively. DNA sequencing revealed A→G substitution at *RsaI* recognition site GTAC. After χ^2 analysis the selected population of Sahiwal cattle was found in Hardy- Weinberg Equilibrium. In restriction digestion of PRL 857 bp product with *HaeIII* revealed only one type of genotype. This revealed no polymorphism *i.e.* monomorphic in nature, Further the presence of the restriction site for *HaeIII* in PCR products confirmed by sequencing. The obtained sequence of PRL/ *HaeIII* after aligning was revealed deletion of G from *HaeIII* recognition site GGCC. The *HphI*/PCR-RFLP assay of LEP gene revealed two types of genotypes; 309 and 145 bp fragments (BB genotype); 454, 309 and 145 bp fragments (AB genotype) with frequencies 70.97% and 29.03% respectively, whereas the AA genotype was not found in these samples (0.00%). The frequency of A and B alleles was 0.15 and 0.85, respectively. DNA sequencing revealed C→T substitution at *HphI* recognition site GGTGA(N)8. After χ^2 analysis the selected population of Sahiwal cattle was not found in Hardy- Weinberg Equilibrium. The *Sau3AI*/PCR-RFLP assay of LEP gene was revealed three types of genotypes; 730, 690 and 400 bp fragments (AA genotype); 730, 690, 400, 310 and 90 bp fragments (AB genotype); 730, 690, 310 and 90 bp fragments (BB genotype) with frequencies 40.32%, 58.87% and 0.81% respectively. The frequency of A and B alleles was 0.698 and 0.302, respectively. DNA sequencing revealed C→T substitution at *Sau3AI* recognition site GATC. After χ^2 analysis the selected population of Sahiwal cattle was not found in Hardy- Weinberg Equilibrium. In Association studies of PRL/*RsaI* gene, AB genotype was associated with the shorter Age at first calving, while BB genotype was associated with the smaller Dry period, Calving interval and Days reach to peak yield but larger Total milk yield, milk yield at 300 days and Peak yield. In Association studies of AB and BB genotypes of *HphI*/PCR-RFLP assay of LEP gene showed significant differences for Age at first calving, Dry period, Peak yield, Calving interval and Days reach to peak yield, while BB genotype was associated with the shorter Age at first calving, Dry period, Calving interval and Days reach to peak yield but large peak yield. In Association studies of AB Genotype of LEP gene by *Sau3AI*/PCR-RFLP assay was associated with the shorter Age at first calving, Dry period and Calving interval, while AA genotype was associated with the larger 300 days milk yield. □□□

DEPARTMENT OF ANIMAL NUTRITION

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14	Effect of inorganic and nano zinc supplementation on performance and immune response in growing heifers	Sharish Kumar	Dr. Vinod Kumar	2019	18
15	Effect of different organic acids and their combinations on silage quality, feed intake and growth performance of indigenous heifers	Vivekanand	Dr. Muneendra Kumar	2019	18
Ph.D. Degree Programme					
1	Effect of formaldehyde treated mustard oil cake on nutrient utilization and growing performance in heifers	Mahima	Dr. S. K. Tomar	2014	20

M.V.Sc. Degree Programme

1. Effect of feeding spent wash on growth performance and nutrient utilization in growing cattle

Pradeep Kumar Kesharwani and Vinod Kumar

Spent wash (SW) is the waste product of sugarcane or brewers distillery industry and can be used as supplement or replacement with livestock feed. The present study was planned to evaluate the effect of spent wash on growth performance and nutrient utilization in growing cattle. The study was conducted in two phases i.e. *in vitro* and *in vivo* study. In *in vitro* study, the experiment was designed to evaluate the effect of different levels (0, 5, 10, 15 and 20%) of SW containing concentrate and roughages diet in 40: 60 ratio. Microbial protein synthesis (mg) was higher in spent wash treated group than control. NH₃-N, TVFA and partitioning factor values of all groups were similar (P>0.05). IVDMD and IVOMD were reported to be higher in for 10, 15, 20% spent wash treated group. TGP, TGDM (ml/g) and TGDDM (ml/g DDM) of various groups did not show any significant difference. On the basis of *in vitro* findings, it was concluded that 10 and 20% spent wash treated group had showed best results and hence selected for further 150 days growth trial (*in vivo*) in Haryana heifers. In phase II (*in vivo* study), an experiment was conducted on 18 Haryana heifers randomly distributed on body weight basis into three groups of six animals each. The heifers in three groups were fed different dietary treatments, Control: (basal diet); 10% SW (basal diet with 10% spent wash containing concentrate) and 20% SW (basal diet with 20% spent wash containing concentrate). All animals were fed test diet for a period of 150 days. Fortnightly DMI, body weight changes and monthly blood plasma samples were analyzed for blood biochemical and minerals. Average body weights of heifers in kg or metabolic body weight (kgW^{0.75}) periodically were similar (P>0.05). Fortnightly weight and ADG were similar (P<0.05) in control and spent wash groups. No significant effect on DMI in kg/d and on percent body weight basis was observed. The FCR and FCE were found similar control and spent wash treated groups. DM, DCP and TDN intake were similar in all groups. OM, EE and NDF digestibility also improved in spent wash groups. Blood hematological parameter like RBCs and WBCs count non significantly differed (P>0.05) in all groups and PCV and Hb concentration similar in both control and spent wash groups. Plasma creatinine, glucose, cholesterol (mg/dl), albumin, globulin, ALT and ALP (IU/L) activity in experimental heifers were statistically similar (P>0.05) among three groups. The total protein, AST and urea values were significantly higher (P<0.05) in spent wash treated group. The plasma Ca, P and Mg levels were not affected by spent wash treatment groups. Immunoglobulin concentration has no significant effect in all groups but numerically better in spent wash treated groups. FRAP assay and NEFA concentration value was not different in all groups. The results revealed that feeding of 10% spent wash containing concentrate in basal diet improved growth performance and utilization of nutrient without any adverse effect in growing heifers.

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2. Effect of feeding different levels of *Azolla pinnata* on growth performance and carcass characteristics of broiler chicken.

Deepesh Bharat Mishra and Debashis Roy

Present study was conducted for 8 weeks to see the effect of replacing commercial broiler feed with different levels of *Azolla* meal on growth performance, carcass characteristics,

slaughter traits, blood biochemical attributes, immunocompetence, nutrient utilization and sustainability of rearing Chabro birds. One hundred and sixty day old Chabro broiler chicks were divided into four treatment groups each with four replicates of ten birds. The control birds (T₁) was offered basal diets without *Azolla* supplementation while in T₂, T₃ and T₄ groups basal diet was replaced with *Azolla* meal at 5%, 7.5%, and 10% levels, respectively. The composition of *Azolla* meal contain 25.64% crude protein, % ether extract, 17.29 % crude fibre, 21.67 % total ash and 32.25% nitrogen free extract on the air dry basis. Live weight and weight gain were significantly improved (P<0.05) at 5% and 7.5% replacement group. Feed conversion ratio (FCR) and protein efficiency improved significantly (P<0.05) in the diet with 5% and 7.5% *Azolla* meal in comparison to control. Overall FCR were observed as 2.54, 2.49 and 2.50 in control, 5% and 7.5% replacement group. The percent dressed yield, eviscerated yield and ready to cook yield in Chabro were not influenced by dietary supplementation of dried *Azolla*. There were no significant differences among treatment for gizzard, heart, spleen, proventriculus, large intestine and caeca relative percentage. Supplementation of 5% *Azolla* meal significantly increased breast relative percentage compared to control. Blood sample were collected at the last week of trial randomly from one bird of each replicate. The study showed that haematological profile of the Chabro bird was not affected by any treatment except heterophil and lymphocyte which was found higher in T₂ and T₃ groups and eosinophil was found higher in T₃ group than control. Blood biochemical parameter viz. glucose, creatinine, cholesterol, total protein, albumin, uric acid and tri glycerides were found similar to control and within the normal values for broiler chicken. Liver enzymes and macro mineral content in blood were found similar in all the treatment groups and within normal physiological range. Though AST was found higher in 10% replacement group than control, the value was within normal range for broiler chicken. Though anti- body titre was found similar in all the experimental groups in present study, Cell mediate immune response (response to PHA-P) was found higher in 5%, 7.5% and 10% replacement groups than control (P<0.05). Retention of crude protein & Ca was found significantly higher in *Azolla* replacement groups than control. The economics of broiler production revealed the total feed cost per kg live bird was significantly lower in all *Azolla* replacement groups than control (52.74, 50.32, 49.51 and 49.40 Rs. in control, 5%, 7.5% and 10% replacement groups, respectively). It was concluded that dietary inclusion of dried *Azolla* upto 7.5% level improved performance without having any deleterious effect on palatability and health profile.

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3. Effect of feeding biomethanated spent wash on growth and nutrient utilization in growing cattle

Sanjay Singh and Muneendra Kumar

The aim of present study was to evaluate the effect of biomethanated distilleries spent wash (BDS) supplementation on rumen fermentation parameters in vitro, growth performance, nutrient utilization, haematological and blood biochemical and economic of feeding in calves. Entire study was conducted in two phase, phase I as in vitro study and phase II as in vivo study. In phase I study, effect of replacement of 0, 5, 10, 15 and 20% of substrate with BDS was studied on IVTDMD, IVTOMD, pH, in vitro gas production, PF, NH₃-N, Total N, MBP and EMBP. Results revealed that there was no significant effect (P>0.05) of incorporation of different levels BDS on IVTDMD, pH, IVTGP, PF, Ph, total VFA production and efficiency of microbial biomass production was not affected by treatment. However, treatment with different levels of BDS had significant effect (P>0.05) on IVTOMD and molar proportion of acetate and propionate. Incubation of substrate having 10, 15 and 20% BDS had significantly

($P>0.05$) lower $\text{NH}_3\text{-N}$ concentration and the decreased was reported maximum in group treated with 10% BDS. In contrast to $\text{NH}_3\text{-N}$ concentration, amount of total N was reported highest in 10 % BDS treated groups. On the basis of findings of in vitro study, 10 and 20% levels of BDS were used for feeding of experimental calves of phase II study. Eighteen growing calves were selected from the herd of cattle maintained at Instructional Livestock Farm Complex, DUVASU, Mathura. Selected calves were randomly allocated into three groups ($n=6$) on body weight basis and duration of experiment was 150 days. Feeding regimen was same in all the groups except that the energy ingredients of concentrate mixture of treatment group were replaced with 10 and 20% BDS. Effect of supplementation of 10 and 20% BDS were recorded on physiological variables, daily DMI, fortnightly body weight change and feed utilization efficiency. Blood samples were collected at monthly intervals and analysed for haematological and blood biochemical attributes. At the end of study, a digestion trial of 6 days was conducted to study the effect of treatment on digestibility of nutrients. Replacement of concentrate mixture with 10 and 20% BDS did not had any effect on physiological variables, DMI, FCR and FCE while ADG was significantly ($P>0.05$) higher in 10% BDS supplement calves. Dietary supplementation of BDS did not exert ($P>0.05$) any effect on RBCs and WBCs counts, Hb concentration and PCV. The effect of supplementation of different levels of BDS had non significant effect on liver function test which was evidenced from similar AST, ALT and ALP activity in control, 10 and 20% BDS supplemented calves. The significant decreased ($P>0.05$) plasma urea and increased plasma total protein and plasma albumin concentration were observed in treatment group. Feeding concentrate mixture replaced by 10 and 20% BDS did not affects plasma concentration of cholesterol, triglycerides, creatinine, Ca, FRAP and NEFA. However, plasma glucose, P and total immunoglobulin showed positive correlation with dietary supplementation of BDS. The feeding of concentrate mixture replaced by 10 and 20 % BDS was economic as compare to animal fed on basal diet having compounded concentrate mixture without BDS. Furthermore, ADG was also higher in BDS supplemented calves. The results of present findings indicated that 10% of energy ingredients of concentrate mixture can be replaced with BDS in growing calves without any adverse effect.

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4. Growth performance, nutrient utilization and blood biochemical parameters in heifers supplemented with different sources of chromium

Anchal Keshari and Debashis Roy

Present study was conducted to see the effect of supplementation of Cr picolinate, Cr polynicotinate and Cr yeast in rumen fermentation parameters in vitro, growth performance, nutrient utilization, blood biochemical parameters and endocrine parameters of indigenous cattle heifers. These three sources of organic Cr were tested at five doses (0.5, 1.0, 1.5, 2.0 and 2.5 ppm) to determine their effects on in vitro rumen fermentation taking the diet at concentrate and roughages in the ratio of 50:50. Total gas production, true organic matter and dry matter degradability, ammonia nitrogen concentration, partitioning factor and microbial biomass production were remained similar in all the treatment groups at all dose levels though 1 ppm dose level from all the sources showed better ($P>0.05$) result. On the basis of these observations and available literature 1 ppm dose level of chromium from all the three sources were selected for in vivo experiment. Twenty four Haryana heifers of 1 to 2 years of age were randomly allocated into 4 groups (C, T1, T2 and T3) having 6 animals in each group, on body weight basis. Animals in control group fed with basal diet i.e wheat straw, maize fodder and compounded concentrate mixture. Animals in T1, T2 and T3 group were fed basal

ration with 1 ppm organic Cr from Cr picolinate, Cr polynicotinate and Cr yeast on DM basis, respectively. Body weight, body condition score and dry matter intake were recorded fortnightly. In present study temperature humidity index varied from 86.08 to 90.76. Respiration rate (RR), pulse rate (PR) and rectal temperature (RT) remained similar in all the experimental animals and they were found in normal physiological range. Average body weight, metabolic body weight, body weight gain and body condition score remained were not impacted by Cr supplementation. Feed efficiency and overall DM intake also remained similar in all the experimental groups. Nutrient digestibility and digestible nutrient intake were not impacted by supplementation of organic chromium sources to Haryana heifers. Dry matter intake (kg/100 kg BW) and TDN intake (g/kg W0.75) improved in chromium yeast supplemented group during digestion trial. Absorption (%) of chromium and zinc was increased in supplemented groups than control. Absorption (%) of copper, iron and manganese was not impacted by supplementation of organic chromium sources. Haematological parameters like blood haemoglobin concentration and haematocrit values were not impacted by Cr supplementation. Plasma glucose concentration of treatment groups was lower in Cr polynicotinate supplemented group than control group in last two months of growth trial. Plasma HDL-cholesterol level was found to increase in T1 and T3 groups than control. Total plasma protein, albumin and blood urea nitrogen concentration were also not impacted by Cr supplementation without any adverse effect on liver function test. Overall plasma FRAP value was found significantly higher in T2 and T3 groups than control depicting more antioxidant activity. Cr supplementation did not affect adversely plasma mineral concentration. Overall plasma cortisol concentration of treatment groups was found similar with control group. Plasma insulin concentration was lower in Cr polynicotinate (T2) supplemented group than control whereas other two treatment groups showed similar concentration to control. In conclusion, chromium supplementation at 1 ppm dose level proved beneficial in improving DMI and TDN intake in chromium yeast supplemented group and in improving the potency of insulin in chromium polynicotinate supplemented group without affecting overall growth performance and nutrient utilization in Haryana heifers.

□□□

5. Effect on neem oil on growth performance of heifers fed urea based diet

Takshi Rehalia and Vinod Kumar

Present study was conducted to see the effect of supplementation of neem oil treated urea on rumen fermentation parameters in vitro, growth performance, nutrient utilization, blood biochemical parameters of indigenous cattle heifers. The effect of different levels (5, 10 and 20% w/w) of neem oil treatment of urea, uncoated urea and commercially available neem coated urea were included @1% (DM basis) in TMR by replacing mustard cake to determine their effects on in vitro rumen fermentation taking the diet at concentrate and roughages in the ratio of 50:50. Total gas production, true organic matter and dry matter degradability, ammonia nitrogen concentration, partitioning factor and microbial biomass production remained similar in all the treatment groups at all dose levels. On the basis of these observations and 20% (w/w) dose level of neem oil treated urea was selected for in vivo experiment. Twenty four Haryana heifers of 1.5 to 2 years of age were randomly allocated into 4 groups (C, T1, T2 and T3) having 6 animals in each group on body weight basis. Animals in C, T1, T2 and T3 groups were fed basal ration (concentrate: roughage 50:50 without urea), basal ration with uncoated urea 2%, basal ration with neem coated urea 2% (commercial) and basal ration with neem oil coated urea 2% (20% w/w) on DM basis, respectively. Body weight,

body condition score and dry matter intake were recorded fortnightly. Average body weight, metabolic body weight, body weight gain and body condition score were not impacted by neem oil treated urea supplementation. Feed conversion ratio and overall DM intake also remained similar in all the experimental groups. Nutrient digestibility and digestible nutrient intake were not impacted by supplementation of neem oil treated urea to Haryana heifers. Dry matter intake (kg/100 kg BW) and TDN intake (g/kg W0.75) were similar in all the experimental groups during digestion trial. Total plasma protein, albumin, globulin, blood urea nitrogen and creatinine concentration were also not impacted by neem oil treated urea supplementation without any adverse effect on liver function test. Neem oil treated urea supplementation did not affect adversely plasma calcium and phosphorus concentration. It may be concluded that commercially available neem coated urea or 20% neem oil treated urea can be included at 1% level in total mixed ration of dairy animals without any adverse effect on animal performance.

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6. Growth performance, nutrient utilization and blood biochemicals of heifers supplemented with organic acids

Santosh Kumar Yadav and Debashis Roy

Present study was conducted to see the effect of supplementing different organic acids on in vitro rumen fermentation parameters, growth performance, nutrient utilization and blood biochemical parameters of Haryana heifers. Malic acid, fumaric acid, formic acid and aspartic acid were tested at five doses (1.0, 2.5, 5.0, 7.5, 10.0 Mm/L) to determine their effects on in vitro rumen fermentation. Organic matter and dry matter degradability were found significantly higher ($P < 0.05$) at 5.0, 7.5 and 10 mM/L dose level of fumaric acid and malic acid. Formic and aspartic acid did not show any significant difference in any of the in vitro parameters. 5 Mm/L dose levels of fumaric and malic acid were found optimum and selected for feeding in animal experimentation. Eighteen Haryana heifers of 1 to 2 years of age were taken from ILFC and randomly allocated into 3 groups (control, T1 and T2) having 6 animals in each group, on body weight basis. The nutrient requirements of heifers were met by feeding basal diet comprising of concentrate mixture, wheat straw and oat fodder as per standard requirements. Animals in T1 and T2 groups were fed basal ration with 0.5% malic and fumaric acid supplementation on DM basis. The doses were calculated on the basis of in vitro result. The average initial body weight of control, T1 and T2 were 116.80 kg. The average BW and metabolic BW of all the fortnights were similar ($P > 0.05$) between groups. The overall daily gain (g/d) and fortnightly BW were found similar ($P > 0.05$) in all the experimental groups. Dry matter intake (kg/d and kg/100kgBW) did not affected by different organic acid treatment. Feed efficiency and body condition of animals remained unaltered by supplementation of organic acids. The NDF digestibilities of control, T1 and T2 groups were reported as 54.54, 62.18 and 62.34% respectively. The NDF digestibility coefficients were significantly higher in both the treatment groups than control (P Digestibilities of other nutrients remained similar in all the treatment groups. The overall plasma concentration glucose, cholesterol, total protein and albumin were found similar in both the treatment groups with control. Creatinine concentration was found lower in malic acid supplemented group than control (P).

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7. Residual feed intake as a feed efficiency selection tool and its relationship with ingestive behavior, nutrient utilization and specific blood metabolites in dairy calves

Anil Kumar Singh and Muneendra Kumar

This study aimed to evaluate differences in feed utilization efficiency between low and high residual feed intake (RFI) Sahiwal calves by comparing growth performance, feed intake, nutrients digestibility, ingestive behavior, blood metabolites and cost of feeding. Eighteen female growing Sahiwal calves were fed ad libitum on a total mixed ration (TMR, concentrate: green oat fodder: wheat straw=50:20:30) for a period of 90 days (30 days adjustment/preliminary period and 60 days measurement period). Calves were monitored daily for dry matter intake (DMI), fortnightly for body weight (BW) gain and feed efficiency measures. Blood samples were collected at start, mid and end of experimental period and analyzed for biomarkers of protein metabolism, biomarkers of lipid and energy metabolism, enzymatic and hormonal activity and plasma concentration of calcium (Ca) and phosphorus (P). To compare the differences in nutrient digestibility in low and high RFI calves, a digestion trial for a collection period of 7 day was conducted at the end of experimental period. RFI was calculated as the difference between the actual and the expected feed intake. Expected DMI of individual calf was calculated by using linear regression models involving DMI, average daily gain (ADG) and mid test metabolic (W0.75) BW and calves were allocated into low (negative) and high (positive) RFI groups. In the present study, RFI varied from -0.53 to 0.40 kg DM/day with a mean RFI of -0.27 to 0.17 kg/day in low and high RFI Sahiwal calves, respectively. Calves had a mean DMI of 3.01 and 4.06 kg/day and an ADG of 549 and 570 g/day in low and high RFI groups, respectively showing that low RFI calves consumed 35% less feed while gaining at similar rate as high RFI calves. RFI showed positive correlation with daily DMI ($r=0.756$) and ADG ($r=0.191$) whereas, negative correlation were observed between nutrient digestibility. Low RFI calves required less (P globulin, plasma urea nitrogen (PUN), creatinine, insulin like growth factor-1 (IGF-1), growth hormone (GH) and lower albumin and triglycerides were detected. No significant differences in plasma levels of total protein, glucose, cholesterol, non-esterified fatty, (NEFA), beta-hydroxy butyrate (BHBA), enzymatic activity, Ca and P among low and high RFI calves were observed. RFI showed positive correlation with plasma albumin ($r=0.808$), cholesterol ($r=0.419$), triglycerides ($r=0.477$) and ALT ($r=0.324$) negative correlation with total protein ($r=-0.377$), globulin ($r=-0.693$), PUN ($r=-0.509$), creatinine ($r=-0.570$), glucose ($r=-0.015$), NEFA ($r=-0.268$), BHBA ($r=-0.161$), AST ($r=-0.041$), ALP ($r=-0.119$), Ca ($r=-0.115$), P ($r=-0.162$), IGF-1 ($r=-0.222$) and GH ($r=-0.766$). Low RFI calves consumed less feed compared to high RFI calves; therefore, selection of calves for low RFI is always beneficial. In conclusion, low RFI calves are more efficient in feed utilization and the variability in blood metabolites might be due to differences in feed intake and body metabolism.

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8. Investigation of the effects of dietary nickel supplementation on the performance of growing heifers

Anuj Singh and Muneendra Kumar

This study was conducted to evaluate the effect of different levels of nickel (Ni) supplementation on feed intake, nutrient utilization, growth performance, nutrients metabolism, enzymatic activity, antioxidant status and immune response in growing Haryana

heifers. Eighteen growing Haryana heifers were randomly allocated into three groups having six heifers in each groups and fed for 90 days. Feeding regimen was similar in all the groups except that treatment groups were supplemented with 0.0, 1.5 and 3.0 mg of Ni/kg dry matter (DM; ppm) in three respective groups. Group fed on basal diet supplemented with 0.0 mg Ni/kg DM served as a control. Nutrients requirement of experimental heifers were met by feeding concentrate mixture, berseem/oat fodder and wheat straw. Experimental heifers were monitored daily for DM intake (DMI) and fortnightly for body weight change. At the end of the study, a digestion trial of 7 days was conducted to study the effect of Ni supplementation on nutrients utilization. Blood samples were collected at 0, 30, 60 and 90 days post-Ni supplementation and analyzed for haematological attributes, biomarker of energy and lipid metabolism, biomarker of protein metabolism, biomarker of liver and kidney function, biomarker of antioxidant status and oxidative stress, biomarkers of immune response, urease activity and plasma mineral levels. Adding 3.0 ppm Ni to the diet of growing heifers increased (P<0.05) with Ca, P and Fe which is evidenced from similar absorption and plasma levels of these minerals in all three groups. No significant difference (P>0.05) exert any effect on biomarkers of energy and lipid mobilization i.e. plasma glucose, cholesterol, triglycerides and non esterified fatty acids (NEFA) concentration. Plasma total protein, albumin, globulin and urea nitrogen (PUN) used as protein metabolism biomarkers, found significant higher (P<0.05) of Ni supplementation upto 3.0 ppm level were observed on plasma lipid peroxide (LPO) and activity of superoxide dismutase (SOD) and catalase (CAT) whereas, total antioxidant status (TAS) was found lowest in 3.0 ppm Ni group but ranges within normal physiological limit. Adding Ni to the diet of growing heifers did not exerts any effect on plasma total immunoglobulin and immunoglobulin G (IgG) concentrations. Urease activity measured as plasma pH unit change was significantly higher (P<0.05) in Ni supplemented heifers. Finally, it concluded that dietary supplementation of Ni improved feed intake and growth performance by increasing urease activity and protein metabolism in growing Haryana heifers.

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9. Effect of vanadium supplementation on performance of indigenous heifers

Praveen Kumar Gupta and Shalini Vaswani

The present study was designed to investigate the effect of different levels of Vanadium (V) supplementation on growth performance, nutrient utilization, blood biochemical, antioxidant, immune, and hormonal parameters in indigenous Haryana heifers. For the growth experiment, 18 indigenous Haryana heifers of 1-2 years of age were selected from the herd maintained at ILFC. The heifers were divided into 3 groups (Control, T1 and T2) having 6 animals each on body weight basis. Control group was fed basal diet, whereas T1 and T2 groups were fed basal diet (as that of control) along with 2.5 and 5.0 ppm V as sodium metavanadate, respectively. Nutrient requirements of the animals were fulfilled as per NRC (2001) feeding standards. The experimental feeding continued for 90 days. During the trial DMI, body weight and BCS of the animals was taken fortnightly. At the end of experiment, a digestion trial of 7 days was conducted to assess the effect of vanadium supplementation on nutrient utilization. Blood samples were collected at 0, 30, 60 and 90 day, respectively. The results revealed no significant (P>0.05) difference in the average body weight and metabolic body weights (kg W^{0.75}) between groups. However, the overall fortnightly body weight gain and ADG was found to be significantly higher in T2 group. Similarly, the overall DMI (kg/d, kg/100 kg BW) were also found to be significantly higher in T2 group. No significant change

in FCR, FCE and BCS were reported in the experimental groups. The supplementation of V had shown no significant ($P>0.05$) difference on nutrient intake and digestibility in treatment groups. Digestible nutrient intake was found similar in all the experimental groups. DMI (kg/100 kg BW), TDN intake (g/kg W0.75), CP and DCP intake (g/kg W0.75) remained similar in treatment groups. The absorption (%) of V and Fe did not change significantly on V supplementation. The haematological parameters reported in present study viz Hb, PCV, TLC and DLC showed no significant ($P>0.05$) difference in treatment groups. The plasma glucose, cholesterol, triglycerides, total plasma protein, albumin, blood urea nitrogen, creatinine and NEFA concentration did not change significantly ($P>0.05$) in V supplemented groups. Plasma levels of enzymes like ALT, AST, ALKP remain unaltered, indicating no deleterious affect of V supplementation upto 5.0 ppm. The plasma V concentration increased significantly ($P<0.05$) on V supplementation. However, the plasma T4 was found significantly higher ($P<0.05$) in treatment groups. Plasma IGF-1 and IgG was found significantly higher in T1 and T2 group supplemented with 2.5 and 5.0 ppm V respectively. In conclusion, V supplementation upto 5 ppm improved DMI and growth of heifers without any adverse affect on nutrient digestibility. It also showed its beneficial role as an antioxidant and immunomodulator in growing indigenous Harijana heifers.

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10. Effect of supplementing Ashwagandha (*Withania somnifera*) root powder on growth performance, nutrient utilization and blood biochemicals of indigenous dairy heifers

Sandeep Bana and Debashis Roy

Present study was conducted to see the effect of supplementation of Ashwagandha root powder on growth performance, nutrient utilization, blood biochemical parameters and endocrine parameters of indigenous cattle heifers. The dried root powder of Ashwagandha were used at 2 different doses (2.5% and 5%) to determine their effects on overall growth performance taking basal diet with concentrate and roughages in the ratio of 50:50. Eighteen Harijana heifers of 1 to 2 years of age were randomly allocated into 3 groups (C, T1 and T2) having 6 animals in each group, on body weight basis. Animals in control (C) group fed with basal diet i.e wheat straw, jowar fodder and compounded concentrate mixture. Animals in T1 and T2 groups were fed basal ration supplemented with 2.5% and 5% Ashwagandha root powder on DM basis, respectively. Body weight, body condition score and dry matter intake were recorded fortnightly. Average body weight and metabolic body weight were not impacted by Ashwagandha supplementation but fortnightly body weight gain and average daily gain improved significantly. Feed efficiency remained similar in all the experimental groups. The overall DMI (kg/d) was found to be increased in T2 group than control. NDF digestibility was improved in supplemented animals. Digestibility of other nutrients and digestible nutrient intake were not impacted by supplementation of Ashwagandha to Harijana heifers. TDN intake (g/kg W0.75) improved in Ashwagandha supplemented group during digestion trial. Digestible CP intake remained similar. Haematological parameters like blood haemoglobin concentration and packed cell values were not impacted by Ashwagandha supplementation. Plasma glucose concentration of treatment groups was similar in Ashwagandha supplemented group in comparison with control group in growth trial. Plasma cholesterol level was found similar in all experimental groups over the period. Total plasma protein, albumin, creatinine and blood urea nitrogen concentration were also not impacted by Ashwagandha supplementation. Plasma NEFA concentration was decreased in both treatment groups than control in last 2 months but overall there was no significant change in

NEFA concentration. Plasma ALT and AST concentration remained similar though ALKP concentration was higher in T2 treatment group than the control without any adverse effect on liver function test. Plasma calcium and phosphorus concentrations were found similar in all experimental groups during whole trial period. Overall plasma FRAP value was found significantly higher in T1 and T2 groups than control depicting more antioxidant activity. Plasma Tri-iodothyronine (T3) and thyroxine (T4) concentration of treatment groups was found similar with control group. Plasma Insulin like growth factor-1 (IGF-1) concentration was significantly increased in both the treatment groups than control showed improved IGF-1 and overall growth. In conclusion, Ashwagandha supplementation at 5% dose level proved beneficial in improving overall growth performance, dry matter and TDN intake in supplemented group without adversely affecting the blood biochemicals in Harijana heifers.

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11. Effect of varying dietary neutral detergent fibre levels on growth performance of heat stressed heifers

Vivek Kumar Yadav and Vinod Kumar

Present study was conducted to see the effect of supplementation different levels of dietary neutral detergent fibre in vivo, growth performance, nutrient utilization, blood biochemical parameters and endocrine parameters of indigenous cattle heifers. These different levels of dietary neutral detergent fibrous diet were tested at four doses (45.46 %, 49.77 %, 54.70 %, and 59.02 %) having concentrate and roughages in the ratio of 50:50. Twenty Harijana heifers of 1 to 2 years of age were randomly allocated into 4 groups (T1, T2, T3 and T4) having 5 animals in each group, on body weight basis. All groups of Animals were fed with basal diet having different levels of dietary neutral detergent fibrous diet maintained by changes in composition of concentrate mixture. Body weight, body condition score and dry matter intake were recorded fortnightly. In present study temperature humidity index varied from 76.96 to 80.78 Respiration rate (RR), pulse rate (PR) and rectal temperature (RT) remained similar in all the experimental animals and they were found in normal physiological range. Average body weight, metabolic body weight, and body condition score remained were not impacted by different levels of dietary neutral detergent fibrous diet. Feed conversion ratio and overall DM intake also remained similar in all the experimental groups. Nutrient digestibility and digestible nutrient intake were not impacted by supplementation of different levels of dietary neutral detergent fibrous diet to Harijana heifers. Dry matter intake (kg/100 kg BW) and TDN intake (g/kg W0.75) improved in lower percentage of fibrous diet regarding higher, to supplemented group during digestion trial. Haematological parameters like blood haemoglobin concentration and pack cell volume values were not impacted by different levels of dietary neutral detergent fibrous diet. Overall plasma glucose concentration of treatment groups was lower in high dietary neutral detergent fibrous diet supplemented group and higher in low dietary neutral detergent fibrous diet supplemented group of growth trial. Plasma HDL-cholesterol level was found similar in all treatment groups. Total plasma protein, was increased with increasing concentration of NDF levels in diet but it was within the physiological range. Albumin concentration was not impacted by supplemented different levels of dietary neutral detergent fibrous diet. Blood urea nitrogen concentration was increasing with increasing NDF levels in diet. Overall plasma FRAP and SOD concentrations were vary significantly with in physiological limits. Plasma FRAP concentration was found higher in T1 and T4 groups than T2 and T3 depicting more antioxidant activity. Different levels of dietary neutral detergent fibrous diet did not affect adversely plasma mineral concentration. Plasma Insulin like growth factor (IGF-1) (ng/ml) concentration was

significantly higher in all treatment groups at 30 days and 90 days, but overall concentration of all treatment groups were similar. Overall plasma T3 and T4 hormone concentration in plasma of all treatment groups were found similar. In conclusion, lower NDF level (45.46%) showed higher nutrient intake, body weight gain, in supplemented different dietary neutral detergent fibrous diet. So dietary NDF level of below 50 is beneficial in summer stressed Haryana heifers as at higher NDF levels, stress parameters (SOD & FRAP) were adversely affected and growth was also lower.

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12. Effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on the performance of Barbari bucks

Anuj Dubey and Shalini Vaswani

The present study was designed to evaluate the effect of replacing protein source in concentrate mixture with dried *Moringa oleifera* leaf powder on growth performance, nutrient utilization, seminal attributes, haematological parameters, blood biochemicals, antioxidant and hormonal parameters of Barbari bucks. For this study, 18 Barbari bucks were selected from the herd maintained at Department of Physiology, DUVASU, Mathura (U.P.). The bucks were divided into 3 groups (Control, T10 and T20) having six animals each on body weight basis. Control group was fed basal diet consisting of concentrate mixture, green berseem and arhar straw where as T10 and T20 groups were fed basal diet (as that of control) along with replacement of soybean meal in concentrate mixture with dried *Moringa oleifera* leaf powder at level 10 and 20% respectively. Nutrient requirement of bucks were fulfilled as per NRC (2007) recommendation of feeding standard. The experimental feeding was intended for 90 days. At the end of experiment, a digestion trial of seven days was conducted to appraise the effect of *Moringa oleifera* supplementation on nutrient utilization. Blood sample were collected at 0, 30, 60 and 90 days interval, respectively. Semen was collected twice a week using artificial vagina from each buck for three weeks, total of six ejaculate from each bucks were collected after 90 days of post feeding of experimental diets. Overall 108 ejaculates were collected. The result revealed no significant ($P>0.05$) difference in the average body weight (kg) and metabolic body weight (kg W 0.75) between groups. Fortnightly body weight gain (kg) and ADG (g) was found to be significantly higher and comparable in Control and T10 group. Similarly the overall DMI (kg/d, 100 kg BW) was also higher and comparable in Control and T10 group. No significant change in FCR was reported in experimental groups. The supplementation of *Moringa oleifera* had shown no significant ($P>0.05$) difference on nutrient intake and digestibility in treatment groups. Digestible nutrient intake was found similar in the entire experimental groups. CP intake (kg/day), DCP intake (kg/day) and TDN intake (kg/day) remained similar in all experimental groups. DMI (kg/100 kg BW), TDN intake (g/kg W0.75) CP and DCP intake (g/kg W0.75) remained similar in all experiment groups during digestion trial. Seminal attributes viz. volume of ejaculated semen (ml), semen colour, seminal pH, and spermatozoa concentration (millions/ml) were found similar in all experimental groups. Whereas, mass motility, percent progressive motility, percent live spermatozoa count, HOST reactive spermatozoa percentage and intact acrosomal percentage was found significantly higher ($P< 0.05$) in T10 group and significantly lower ($P 0.05$) in experimental bucks. Plasma level of enzymes like ALT, AST remain unchanged in Control and treatments group showing no deleterious effect of *Moringa oleifera* supplementation on hepatic metabolism in experimental animal. Overall super oxide dismutase activity (SOD) activity increases significantly ($P<0.05$) in T10 *Moringa oleifera* treated group. However, no

significant difference in testosterone concentration was found between control and treatment groups. Hence the present results suggested that the soybean meal in concentrate mixture can be effectively replaced with dried *Moringa oleifera* leaf powder at 10 % level without any deleterious effect on blood parameters and hepatic metabolism, with stimulatory effects on their antioxidants status, anti stress and improved seminal attributes of Barbari bucks. □□□

13. Effect of green fodder replacement with corn silage on residual metabolizable feed consumption (RMFC) in growing cattle

Ashwani Kumar Verma and Muneendra Kumar

This study was conducted to determine the effect of green fodder replacement with corn silage on feed intake, growth performance, feed utilization efficiency and blood metabolites in growing Haryana cattle. This study was conducted in two phases, Phase 1: Silage preparation and evaluation and phase 2: Feeding trial. In Phase 1 study, corn silage was prepared in bunker silo and after ensiling for 60 days, silage was evaluated for nutrients content and physical and chemical characteristics. High pH value, lactic acid content, buffering capacity (BC), total volatile fatty acids (TVAs) and low ammonia-nitrogen (NH₃-N) content denoted that prepared corn silage was well preserved and of very good quality. In phase 2 study, 18 growing Haryana heifers were randomly allocated into three groups (n=6) on body weight and age basis. Experimental heifers either received a basal total mixed ration (TMR) devoid of corn silage (S0%) or were fed on TMR of which 50 (S50%) and 100% (S100%) berseem fodder were replaced with corn silage. Experimental heifers were monitored daily for DMI and fortnightly for body weight change, feed efficiency measures and physiological variables. At the end of the study, a digestion trial for a period of 6 days was conducted to study the effect of replacement of green fodder with corn silage on nutrient utilization. Blood samples were collected on the days 0, 30, 60 and 90 post treatments and analyzed for haematological attributes, biomarkers of protein metabolism, biomarkers of energy and lipid metabolism, liver and kidney function test, biomarker of antioxidant status and immune response and plasma mineral levels. Replacement of green fodder with corn silage had significant (P<0.05) effect on feed intake. As the level of inclusion of silage increased, dry matter intake (DMI) also increased while average daily gain (ADG) was similar among all groups. RMFC measured as difference between metabolizable energy (ME) intake and ME required showed significant (P<0.05) effect and showed linear increase with silage levels. Residual metabolizable feed consumption (RMFC) denoted that heifers in group S50% and S100% consumed 0.97 and 1.61 kg more DM/day than S0% group while gaining at the similar rate. Residual intake and body weight gain (RIG) showed significantly (P<0.05) higher value in S0% group followed by S50% and S100% groups. Other feed efficiency measures did not show any effect of treatment. The apparent digestibility of crude protein (CP) was higher in S0% group while digestibility of the other nutrients was similar among three groups. Haematological attributes, biomarkers of energy and lipid metabolism, biomarkers of liver and kidney function, antioxidant and immune response and plasma mineral levels showed non significant effect of green fodder replacement with corn silage. However, plasma concentrations of total protein and albumin were higher in S0% group compared to S50% and S100% groups which could be due to higher protein content and digestibility. Cost of feeding increased with the increased level of corn silage inclusion. In conclusion, replacement of green fodder with corn silage increased feed intake, RMFC and cost of feeding without altering growth performance in growing Haryana cattle. However, corn silage can be used as alternate forage during scarcity period without adverse impact on performance of growing cattle. □□

14. Effect of inorganic and nano zinc supplementation on performance and immune response in growing heifers

Sharish Kumar and Vinod Kumar

Present study was conducted to see the effect of inorganic and nano zinc supplementation on growth performance, nutrient utilization, blood biochemical and immune response in Haryana heifers. In present study, control group was not supplemented with any extra amount of zinc other than present in the basal diet, T1 group was supplemented with inorganic zinc @50 mg/kg of DM offered, while T2 and T3 group were supplemented with nano ZnO @25 and 50 mg/kg of DM offered. Basal diet offered to experimental groups containing 50% concentrate, 25% green berseem and 25% wheat straw. DM was offered to all experimental group at about 4% of the body weight of animals. All groups of animals were fed with basal diet having same levels of nutrients. Body weight and dry matter intake were recorded fortnightly. DMI (kg/day), DMI (kg/100kgBW), TDN intake (g/kg W0.75) and DCP intake (g/kg W0.75) remained similar in all experimental groups. Nutrient digestibility and digestible nutrient intake were not impacted by supplementation of different levels of inorganic and nano zinc supplementation to all treatment groups. Average fortnight body weight gains, ADG, metabolic body weight gain were similar in all groups. FCR and FCE were not significantly different between treatment and control group. Zinc bioavailability was high in nano zinc supplemented T2 and T3 group in comparison to inorganic supplemented group and control group. Haematological parameters like blood haemoglobin concentration and pack cell volume values were not impacted in different treatment groups. Overall plasma glucose, triacylglycerol, cholesterol, plasma total protein, plasma albumin, BUN, ALP, ALT, AST, bilirubin and creatinine were found similar in all treatment and control group. Plasma globulin is significantly different between the group at 90 day of trial and globulin concentration was found higher in T1, T2 and T3 treatment group than the control group. Plasma Ca and P in present study were similar in all the experimental groups. Plasma zinc concentration was high in nano zinc supplemented T2 and T3 group in comparison to inorganic zinc supplemented T1 and control group. Plasma copper concentration was low in all treatment groups in comparison to control group. Plasma SOD concentration was found higher in nano zinc supplemented T2 and T3 group than control group and inorganic zinc supplemented T1 group, at 30, 60, and 90 days. FRAP concentration increased within all groups over the time, and FRAP concentration were higher in all treatment groups than the control group. Plasma total immunoglobulin concentration was found higher in all treatment groups in comparison to control group. It may be concluded that nano Zn supplementation @25 and 50 ppm have better absorption, antioxidant and immunogenic effects thus may replace inorganic Zn source at lower level, @25 PPM.

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15. Effect of different organic acids and their combinations on silage quality, feed intake and growth performance of indigenous heifers

Vivekanand and Muneendra Kumar

This study was conducted to determine the effect of different organic acids and their combinations on silage quality, feed intake, growth performance and blood metabolites of growing Sahiwal cattle. It was conducted into two phases, Phase I: Silage preparation and their quality evaluation and phase II: Feeding trial. In Phase I study, sorghum silage was either

ensiled without organic acid or with 0.5% formic acid, 0.5% propionic acid or 0.25% combination of formic and propionic acid in 50 kg polythene bags. After ensiling for 60 days, silage was evaluated for nutrients content and physical and chemical characteristics. Low pH value, lactic acid content, buffering capacity (BC), total volatile fatty acids (TVFA) and low butyric acid and ammonia-nitrogen (NH₃-N) content denoted that prepared sorghum silage in different groups were well preserved and was of very good quality. However, sorghum fodder ensiled with 0.5% propionic acid showed better aerobic stability compared to other groups. In Phase II study: 24 growing Sahiwal heifers were randomly allocated into four groups (n=6) on the basis of body weight and age. Experimental heifers either fed on basal TMR (compounded concentrate: silage devoid of preservative: wheat straw in the proportion of 40: 40: 20 on DM basis) (SC) or basal TMR which contained silage preserved with 0.5% formic acid (S0.5%FA), basal TMR which contains silage preserved with 0.5% propionic acid (S0.5%PA) and basal TMR which contains silage preserved with combination of 0.25% formic acid and 0.25% propionic (S0.25%FA+0.25%PA). Experimental heifers were monitored daily for DMI and fortnightly for body weight change and feed efficiency. A digestion trial with a period of 6 days was conducted at the mid of the experiment to study the effect of treatments on the nutrients utilization. Blood samples were collected on the days 0, 30, 60 and 90 post treatments and analyzed for haematological attributes, biomarkers of protein, energy and lipid metabolism, liver and kidney function test and plasma mineral levels. Feeding of silage treated with formic and propionic acids did not exert any effect on feed intake and apparent nutrients digestibility. However, average daily gain (ADG) and feed efficiency was better (P<0.05) in S0.5%FA group compared to other groups. Red blood cells (RBCs) count, granulocyte count, lymphocytes count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean cell haemoglobin (MCH) showed non significant effect while white blood cells (WBCs) and platelet (PLT) count were significantly lower (P<0.05) in 0.5% formic acid group and haemoglobin (Hb) concentration and packed cell volume (PVC) or haematocrit (HIT) values were lower in 0.5% propionic acid group. Mean plasma total protein, albumin and globulin values showed non significant effect of silage preservatives in growing heifers. PUN showed significant effect (P<0.05) of treatment and was lowest in heifers of S0.5%FA groups compared to SC, S0.5%PA and S0.25%FA+0.25%PA groups. Plasma glucose and cholesterol concentration were significantly (P<0.05) higher in 0.5% propionic acid added groups while plasma triglyceride level was significantly (P<0.05) higher in combination group. No significant differences in the ALT and AST were observed among four different groups however; mean plasma ALP concentration was higher in 0.5% propionic acid group. No significant difference in the mean plasma Ca and P levels were observed among different groups. In conclusion, ensiling sorghum fodder with formic and propionic acids improved silage quality while aerobic stability of silage was better in group ensiled with 0.5% propionic acid. Feeding 0.5% formic acid added silage improved growth performance of growing Sahiwal cattle.

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Ph.D. Degree Programme

1. Effect of formaldehyde treated mustard oil cake on nutrient utilization and growing performance in heifers

Mahima and S.K. Tomar

The present study was conducted in two phase i.e. *in vitro* and *in vivo* study. In *in vitro* study, the experiment was designed to evaluate the effect of different levels (0, 1, 1.5 and 2% of CP) of formaldehyde treated mustard cake containing concentrate and roughages diet in 40: 60 ratio. Mustard cake used for the preparation of concentrate was untreated, treated with formaldehyde (1, 1.5 and 2% of CP) and/or mixed with mineral mixture. NH_3N was lower in formaldehyde treated group than control. Microbial protein synthesis (mg), pH and partitioning factor values of all groups were similar ($P>0.05$). TVFA level in 2% formaldehyde treated mustard cake group was lower ($P<0.05$) as compared to other groups. IVDMD and IVOMD were reported to be low for 1 % formaldehyde treated group. TGP, TGDM (ml/g) and TGDDM (ml/g DDM) of various groups did not show any significant difference. On the basis of *in vitro* findings, it was concluded that 1.5% formaldehyde per 100g of CP had showed best results and hence selected for further 120 days growth trial (*in vivo*) in Haryana heifers. In phase II (*in vivo* study), an experiment was conducted on 24 Haryana heifers randomly distributed on body weight basis into four groups of six animals each. The heifers in four groups were fed different dietary treatments, Q: negative control diet (diet containing 80% crude protein of NRC); C₂: positive control diet (diet containing 100% CP of NRC); T₁: diet containing formaldehyde treated mustard cake (diet containing 80% CP of NRC) and T₂: diet containing formaldehyde treated mineral fortified mustard cake (diet containing 80% crude protein of NRC). Concentrate mixture was composed of barley, wheat bran, mustard and mineral mixture in 55, 10, 33 and 2 parts, respectively. All animals were fed test diet for a period of 120 days. Weekly DMI, fortnightly body weight changes and BCS were recorded and blood serum samples were analyzed for blood biochemical and major and trace minerals. Average body weights of heifers in kg or metabolic body weight ($\text{kgW}^{0.75}$) periodically were similar ($P>0.05$). Fortnightly weight and ADG were higher ($P<0.05$) in positive control and formaldehyde treated groups. No significant effect on DMI in kg/d and on percent body weight basis was observed. The FCR was found lower in positive control and formaldehyde treated groups. BCS, DM, DCP and TDN intake were similar in all groups. OM digestibility was higher for group C_i, EE digestibility was higher for positive control, and formaldehyde treated groups. NDF digestibility also improved in formaldehyde treated groups. P absorption showed significant higher value ($P<0.01$) for positive control group. Ca, Mg, Fe, Cu, Zn, Mn and Se absorption were statistically similar in all groups. Serum creatinine (mg/dl), cholesterol (mg/dl), globulin, BUN (mg/ dl), ALT and AST (IU/L) activity in experimental heifers were statistically similar ($P>0.05$) among all four groups. The total protein values were higher in formaldehyde treated group at 90 and 120 days. The serum albumin values were higher ($P<0.05$) in both formaldehyde treated groups at 90 and 120 days. The serum Ca, Mg, Cu and Se levels were not affected by formaldehyde treatment of mustard cake. Serum P level was lower ($P<0.05$) for both treatment groups (T₁ and T₂) than control groups (C_j and C₂) on 120th day. Significantly higher ($P<0.05$) level of Fe, Zn and Mn concentration was observed in group C₂ than other groups. The results revealed that formaldehyde treatment (1.5%) of mustard cake protect the protein from rumen degradation and improve the weight gain, feed efficiency and nutrient utilization. Mineral absorption and serum mineral levels were not altered due to formaldehyde treatment of mustard cake.

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DEPARTMENT OF LIVESTOCK PRODUCTION AND MANAGEMENT

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M.V.Sc. Degree Programme

1. Study of environmental sound on reproductive performance of Haryana bulls

Archana Yadav and Yajuvendra Singh

The present investigation was performed to observe the effect of two different beats (100 and 150) of musical sound signals (with intensity below 85 dB) on reproductive (seminal, behavioral and endocrine attributes) performance of Haryana bulls maintained at semen biology lab of DDD Farm within the premises of Instructional Livestock Farm Complex (ILFC). The present experiment was carried out for an aggregate time period of three months (within autumn season) and was accomplished in three phases/conditions. In order to avoid individual effect of bulls as well as to overcome the limitations of limited numbers of bulls at the experimental farm, the same four Haryana bulls were used as experimental animals in all the three phases / conditions of the present experiment. During the first (control) phase of experiment the bulls weren't exposed to any additional source of sound except the normal environmental sound of semen collection site. In second and third phase, bulls were exposed to a musical instrumental sound signal of 100 and 150 BPM with intensity below 85 Db. All the three phases lasted for a period of one month (four weeks) one after other in continuation, but, the third phase was started after a gap of two weeks from the second in order to cancel out the persistent effect of sound exposure to bulls during second phase of investigation. The effect of exposure of sound signals on volume, concentration, mass and progressive motility, head, middle piece, tail and overall morphological abnormality of sperm and HOST (%) was highly significant ($P < 0.01$). But, no significant ($P > 0.05$) effect could be observed for seminal pH and percentage of live sperms. Exposure of sound signals also reflected highly significant ($P < 0.01$) effect on erection time and score, protrusion time and score, reaction time to first mount, intensity of thrust, time of first ejaculation, dismounting time and libido score, but, the effect on temperament score of bulls was observed to be non significant ($P > 0.05$). In present investigation a significant effect of individuality of bulls on semen volume, sperm concentration, head abnormality, percentage of live sperms ($P < 0.05$); and tail, overall morphological abnormality and HOST (%) was observed. Similarly, the effect of individuality of bulls was also observed to be significant on temperament score, erection time and score, protrusion time, reaction time to first mount, time of first ejaculation, dismounting time, libido score (PO.OI); and on intensity of thrust and protrusion score ($P < 0.05$). The days of observation in present investigation did not reflect any significant ($P > 0.05$) effect on any of the seminal or behavioral attribute, except on erection time and score. No significant ($P > 0.05$) effect of exposure of sound signals, individuality of bulls as well as days of observation on mean plasma concentration of both hormones (cortisol and testosterone) could be observed in present investigation. The exposure of an instrumental musical sound signal of 150 BPM with intensity below 85 dB improvised almost all the seminal and behavioral attributes of experimental Haryana bulls in present investigation. The mean values of seminal (volume, pH, concentration, mass and progressive motility, overall, head, middle piece and tail abnormality, percentage of live sperms and HOST) and behavioral (temperament score, erection time and score, protrusion time and score, reaction time to first mount, intensity of thrust, time of first ejaculation, dismounting time and libido score) attributes of Haryana bulls exposed to a sound signal of 150 BPM were 6.58 ± 0.29 ml, 6.65 ± 0.04 , 1018.72 ± 49.92 millions/ml, 3.98 ± 0.07 , $72.97 \pm 0.80\%$, $5.13 \pm 0.40\%$, $1.93 \pm 0.21\%$, $0.63 \pm 0.15\%$, $2.56 \pm 0.28\%$, $81.95 \pm 0.77\%$, 79.22 ± 0.69 and 3.06 ± 0.11 , 0.48 ± 0.15 min, 3.28 ± 0.09 , 0.79 ± 0.22 min, 3.44 ± 0.11 , 1.27 ± 0.27 min, 1.66 ± 0.08 , 1.55 ± 0.27 min, 1.64 ± 0.27 min and 8.09 ± 0.13 , respectively. Thus, from the present

investigation it could be concluded that the trend of non significant increase in plasma concentration of testosterone and non significant decrease in plasma concentration of cortisol as a consequence of this exposure indicated that exposure of a sound signal of 150 BPM with intensity below 85 dB caused a favorable change in plasma concentration of sex and stress hormones which in turn brought positive changes in seminal and behavioral attributes.

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2. Study of environmental musical sound on performances and activities of transient Haryana cows

Garima Shukla and Yajuvendra Singh

The present study was carried out to observe the effect of exposure of classical music on behavioral, physiological, hematological, blood bio chemical attributes; production, reproduction performance and endocrine profile of transient Haryana cows. Ten transient Haryana cows maintained at DDD Farm of ILFC at DUVASU, Mathura, were quasi randomly distributed into two groups. Cows of one group were exposed to a classical musical sound (7580 dB and below 120 beats per minute) 25 days before the expected date of calving. The exposure was made for an hour per day into two splits half in morning (6 AM) and half in evening (6PM). The effect of exposure was observed to be significant on rumination time ($P<0.05$), lying time ($P<0.05$). The overall mean rumination time of transient Haryana cows exposed to classical music in present investigation was observed to be 553.78 ± 9.01 sec. Mean values for rumination time, lying time, latency to first reaction, looking at experimenter for exposed experimental cows on different days of observation reflected an increasing trend from day 21 pre to day 90 post partum whereas, mean values for duration in reaching to exposure site, time spent in standing in front of the door reflected a decreasing trend during same duration. The overall mean respiration and pulse rate observed for transient Haryana cows exposed to classical music in present investigation was 20.76 ± 0.16 and 73.4 ± 1.30 per minute, which were significantly ($P<0.01$) higher than the corresponding values for cows of unexposed group. The exposure of classical music significantly affected the overall mean WBC count ($P<0.01$); and Hb and PCV estimates ($P<0.05$) of experimental cows and the mean values for them were $12.96\pm 0.16 \times 10^3 / \text{mm}^3$, 13.38 ± 0.14 mg/dl and 41.49 ± 0.48 %, respectively. Though, the WBC count on different days of observation for cows of exposed group remained apparently constant but it declined significantly for cows of unexposed group from day of calving to day 90 post partum. There was observed an apparent increase in fortnightly milk yield of cows exposed to classical music but the effect was not found to be significant ($P>0.05$). Out of various components of milk only overall mean percentage of fat could be observed to be significantly ($P<0.01$) affected by exposure of classical music and its mean value for cows of exposed group was observed to be 3.94 ± 0.04 %. At the same time the exposure of classical music also reflected a significant effect on milk flow rate (17.59 ± 0.94 ml/sec; $P<0.01$) and milk let down time (53.25 ± 1.83 sec) in Haryana cows. In reproductive traits only duration of occurrence of first post partum heat was significantly ($P<0.05$) affected by exposure (70.60 ± 7.53 days). The growth rate of calves produced from exposed cows (0.22 ± 0.01 gm/day) at fortnightly interval was observed to significantly ($P<0.01$) higher than growth rate of calves produced from unexposed cows (0.15 ± 0.01 gm/day). The exposure of classical music reflected a significant effect only on plasma creatinine, total proteins, albumin, globulin and NEFA ($P<0.01$), and glucose, phosphorus, FRAP and immunoglobulin ($P<0.05$) and plasma cortisol concentration ($P<0.01$) while the effect on plasma estrogen level could not be observed to be significant. Thus, from present investigation it could be concluded that exposure of classical

music improved the behavioral, biochemical, production and reproduction performance up to a certain extent.

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3. Study of occurrence of differential patterns of genital prolapse in indigenous cows

Kaushalendra Singh and Yajuvendra Singh

The present investigation was performed to investigate the differential patterns of occurrence of pre partum vaginal prolapse in Sahiwal and Hariana cows maintained at DDD Farm of ILFC at DUVASU, Mathura. During the experimental period of five months a total of five Sahiwal and no Hariana cow were found to be suffering from pre partum vaginal prolapse. The blood biochemical, mineral, endocrine profile, physiological and hematological attributes of prolapsed Sahiwal cows were compared with those of healthy Sahiwal and healthy Hariana cows. Moreover, changes in these attributes of prolapsed Sahiwal cows during the different stages of recovery i.e. 0, 12, 24, 36, 48 and 60 hrs after the onset of clinical signs were also observed. In affected Sahiwal cows the mean values for blood glucose (103.73 ± 6.01 mg/dl), SGOT (90.34 ± 2.31 IU/L), NEFA (127.35 ± 5.41 mEq/L) ($P < 0.05$) were observed to increase significantly while a significant ($P < 0.01$) decrease was observed for plasma ALP (87.14 ± 9.56 IU/L) concentration as compared to the corresponding values for healthy Sahiwal and Hariana cows. The mean plasma concentration of blood glucose total cholesterol ($P < 0.05$) and SGOT ($P < 0.01$) decreased significantly during the different stages of recovery in prolapsed Sahiwal cows. In present study a significant ($P < 0.05$) decrease in plasma concentration of calcium and phosphorus (6.10 ± 0.64 , 3.90 ± 0.37 mg/dl, respectively) while an increase in plasma concentration of sodium (154.40 ± 2.36 mEq/L) was observed in affected Sahiwal cows as compared to healthy Sahiwal and healthy Hariana cows. However, a significant ($P = 0.07$) decrease in plasma concentration of sodium was only observed in affected Sahiwal cows during different stages of recovery. The mean plasma concentration of relaxin (1295.19 ± 101.67 pg/ml) in affected Sahiwal cows of present study was only found to be significantly ($P < 0.01$) higher in comparison to healthy Sahiwal and healthy Hariana cows. But a significant ($P < 0.05$) decrease in plasma concentration of both estrogen and relaxin hormone of affected Sahiwal cows was observed in present study. Out of various hematological and physiological attributes only mean value for total leukocyte count (8750 ± 246.85 per mm^3) was observed to increase significantly ($P < 0.05$) in affected Sahiwal cows, remaining attributes were found to be unaffected. A significant ($P < 0.05$) decrease in mean total leukocyte count of affected Sahiwal cows was also observed during the due course of recovery. Therefore, the above changes in blood biochemical attributes, mineral and endocrine profiles may be considered as the indicator of occurrence of prolapse in Sahiwal cows. Moreover as the values of many of these attributes in prolapsed Sahiwal cows were almost closer to healthy Sahiwal cows as compared to healthy Hariana cows therefore, Sahiwal cows are more prone to prolapse.

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4. Study of urine, saliva and vaginal mucus discharge as bio-indicator of estrus in dairy animals

Krishan Kant Dhakar and Yajuvendra Singh

The present study was carried out to find out the most efficient bio indicator of estrus out of urine, saliva and vaginal mucus discharge. Simultaneously, discrimination of pre

standing, standing and end of standing heat conditions of estrus Sahiwal cows was also attempted by observing various physiological, haematological, blood biochemical, endocrine profile, pH and fern patterns in vaginal mucus discharges. The experiments were conducted on eight healthy, cyclic Sahiwal cows maintained at DDD farm of ILFC at DUVASU, Mathura. Significantly ($P < 0.01$), higher mean values for some of the behavioral responses of teasure bulls viz. Flehmen score (5.63 ± 0.18), frequency of Flehmen response (3.50 ± 0.18) and lower mean values for time elapsed in penile erection (1.00 ± 0.15 mins.), protrusion (1.30 ± 0.13 mins.) and mounting (1.61 ± 0.18 mins.) in response to urine samples as compared to saliva and vaginal mucus discharge reflected urine as the most efficient indicator of estrus in Sahiwal cows. Out of various physiological and hematological parameters studied a significant difference for three different stages of estrus in Sahiwal cows could be observed only for rectal temperature, PCV ($P < 0.01$) and hemoglobin ($P < 0.05$). During standing heat stage of Sahiwal cows, the mean values for rectal temperature ($39.00 \pm 0.10^\circ\text{C}$), hemoglobin (12.45 ± 0.20 mg/dl) and PCV (44.77 ± 0.77 %) were found to be significantly higher than during pre standing and end of standing heat stages. Interestingly, no significant difference for any of the bio chemical attribute in Sahiwal cows could be observed during three different stages of estrus but few of the parameters viz. total protein, alkaline phosphatase, total immunoglobulin, albumin and calcium, reflected non significantly higher values during standing stage as compared to other stages of estrus. This finding indicated that none of the bio chemical attribute is helpful in discrimination of the above said three stages of the estrus in Sahiwal cows. In response to standing heat condition of Sahiwal cows the mean plasma concentration of testosterone (15.62 ± 3.30 ng/ml) in teasure bulls was observed to be significantly ($P < 0.05$) higher as compared to pre standing and end of standing heat stages. Similarly, mean plasma concentration of estrogen and pH of vaginal mucus discharge during standing heat stage (21.75 ± 0.97 ng/ml and 7.71 ± 0.04) of Sahiwal cows were significantly ($P < 0.01$) higher as compared to pre standing and end of standing heat stages. Vaginal mucus discharge in Sahiwal cows reflected a typical fern pattern during standing heat, atypical fern pattern during pre standing heat and no pattern during end of standing heat stages of estrus. Thus, from present study it was concluded that urine may be used as the most efficient indicator of estrus in Sahiwal cows. Moreover, none of the biochemical, physiological and hematological attributes except, rectal temperature, blood hemoglobin, PCV up to some extent may help in discrimination of pre standing, standing and end of standing heat stages of Sahiwal cows. But, pH and fern pattern of vaginal mucus discharge and plasma concentration of testosterone in bulls and estrogen in cows may more effectively discriminate, the above said stages of estrus in Sahiwal cows.

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5. Impact of supplementation of lukewarm water during winter season on performances of dairy cows

Dikshant Kanwar and Yajuvendra Singh

The present study was carried out to find out the effect of supplementation of lukewarm water at 25°C during winter season to Sahiwal cows on their physiological, general, production, haematological, biochemical attributes and endocrine profile. For this purpose the present study was carried out with twelve healthy, lactating Sahiwal cows maintained at DDD farm within the premises of LFC of DUVASU, Mathura. These experimental cows were quasi randomly divided into two groups consisting six animals in each. All the cows were kept in conventional (tail-to-tail) system of housing for whole the day. Cows of one group i.e. control were offered drinking water stored in water trough located in a corner of the shed at

ambient temperature, whereas, the cows of supplemented group were offered lukewarm water at 25°C which was prepared by mixing of partial amount of warm water in cold water. Cows of both the groups were offered weighed amount of ad lib water thrice a day (early morning, afternoon and late evening) in graduated buckets individually. They were also provided weighed amount of ad lib feed. Various physiological, general, production, haematological, biochemical attributes and endocrine profiles of these Sahiwal cows of control and supplemented group were compared to observe the impact of supplementation of lukewarm water. Significantly, high values for mean pulse rate ($P<0.05$), morning water intake ($P<0.01$), total plasma protein ($P<0.05$) in Sahiwal cows of supplemented group (79.32 ± 1.25 per minute, 4.81 ± 0.24 liter/day and 7.53 ± 0.01 g/dL) was observed than those for control group (74.78 ± 1.25 per minute, 3.49 ± 0.24 liter/day and 7.49 ± 0.01 g/dL) and low values were observed for RBC count ($P<0.01$), BUN concentration ($P<0.05$), plasma T4 ($P=0.08$) and TSH ($P=0.07$) concentration in Sahiwal cows of supplemented group ($6.40\pm 0.11\times 10^6/\text{mm}^3$, 15.28 ± 0.31 mg/dL, 13.96 ± 0.06 µg/dl, 0.238 ± 0.005 µIU/ml) was observed than those for control group ($7.06\pm 0.11\times 10^6/\text{mm}^3$, 16.15 ± 0.31 mg/dL, 14.12 ± 0.06 µg/dl, 0.251 ± 0.005 µIU/ml). A significant difference for water intake ($P<0.01$), total solid % ($P<0.01$), SNF % ($P<0.01$), blood glucose ($P<0.01$), cholesterol ($P<0.01$) and total plasma protein concentration ($P<0.05$) in experimental Sahiwal cows among 0, 1st, 2nd, 3rd, 4th and 5th fortnights were observed. But, no significant ($P>0.05$) difference for any of the physiological, general, production, haematological, biochemical attributes and endocrine profiles between cows of supplemented and control groups on 0, 1st, 2nd, 3rd, 4th and 5th fortnights were observed. The results of the present study implied that supplementation of lukewarm water at 25 °C during winter reduced the loss of metabolic energy in the maintenance of body temperature and made it available for various productive activities. In Thus, supplementation of lukewarm water to Sahiwal cows during winter was beneficial.

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6. Effect of floor type on performance of Sahiwal heifers

Akansha Gurung and Rajneesh Sirohi

The present study was carried out to observe the effect of different flooring types on the physiological, behavioural, haematological, and blood bio-chemical attributes; hygiene and hock health scoring and endocrinal profile of Sahiwal heifers. Twenty four healthy Sahiwal heifers maintained at DDD Farm of ILFC of DUVASU, Mathura were quasi randomly distributed into four groups on the basis of body weight and age. Heifers of first group were reared on concrete flooring (T1) which served as control group, the heifers of second group were reared on Sand flooring (T2), the heifers of third group were reared on Cowdung bed flooring (T3) and the heifers of fourth group were reared on Rubber mat installed flooring (T4). The animals were exposed to their respective floorings round the clock, for which they were kept in tethered conditions (except for the period of measuring weight at every fortnight and blood collection (at monthly intervals)). Behavioural recording was done at 30th, 60th and 90th days stage using video recording devices, and scoring for health and hock health was done at fortnightly basis. The T4 group heifers showed significantly higher ($P<0.05$) values of the pooled mean of DMI, (5.04 ± 0.22 Kg/d) than the T1 group, (3.61 ± 0.22 Kg/d). Further the T3 group heifers showed comparatively higher values of the pooled mean of DMI compared to T2 and T1 group (4.34 ± 0.22 Kg/d vs. 4.19 ± 0.22 Kg/d; 3.61 ± 0.22 Kg/d). The T4 group heifers showed significantly higher ($P<0.05$) values of the pooled mean value of ADG compared to T1 group flooring (596.29 ± 28.57 vs. 420.37 ± 28.57 g/d). Further the T3 and T2 group heifers showed comparatively higher values of the pooled mean value of ADG than T1

group (512.96 ± 28.57 g/d; 492.59 ± 28.57 g/d vs. 420.37 ± 28.57 g/d). The T1 and T2 group heifers showed significantly higher ($P < 0.05$) values of the pooled mean value of ST¹ (Standing time from the period of 7:00am - 7:00 pm) compared to T3 and T4 group heifers (353.56 ± 9.93 min and 323.78 ± 9.93 min vs. 252.11 ± 9.93 min and 267.33 ± 9.93 min). The T3 and T4 group heifers showed significantly higher ($P < 0.05$) values of the pooled mean value of LT¹ (Lying time from the period of 7:00 am - 7:00pm) compared to T1 and T2 group heifers (467.89 ± 9.93 min and 452.67 ± 9.93 min vs. 366.44 ± 9.93 min and 396.22 ± 9.93 min). Similar pattern was observed on the values of the pooled mean value of ST² and LT² (Standing time and lying time in the period of 24 hrs) between the treatment groups. There was a significant effect ($P < 0.05$) observed on the values of frequency of head movements while standing and lying down, and duration of postures and leg positions while lying down in different control and treatment groups. However there were no significant effect ($P > 0.05$) seen in the haematological and biochemical attributes (except albumin) between the treatment groups. The T1 (Control) group heifers showed significantly higher ($P < 0.05$) values of the pooled mean value of total plasma albumin concentration than T2 treatment group heifers. The T3 and T4 group heifers showed comparatively higher values of the pooled mean value of total plasma albumin concentration than T2 group heifers. There was a significant effect ($P < 0.05$) observed on the mean and pooled mean values of hygiene score of heifers in different groups, which indicated that hygiene level of T3 and T4 group was much better compared to T1 and T2 groups. Similarly there was a significant effect ($P < 0.05$) observed on the pooled mean value of hock score which indicated the hock health condition in the manner (T3>T4>T1>T2). The T1 (Control) group heifers showed significantly higher ($P < 0.05$) values of the pooled mean value of Cortisol (ng/ml) than other treatment groups whereas there were no significant ($P > 0.05$) changes observed in the values of TSH (μ IU/ml) between the treatment groups.

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Ph.D. Degree Programme

1. Effect of light programme and stocking density on performance of turkey (*Meleagris gallopavo*)

Rajneesh Sirohi and P. K. Shukla

A study was conducted to find out the optimum lighting programme and SD during different seasons for the turkeys as a meat bird. In the first experiment day old turkey poults were procured from Central Avian Research Institute, Izatnagar, Bareilly, and distributed randomly on the basis of uniform body weight in to the three treatments, T-1: The birds were subjected to conventional lighting programme (16L: 8D), T-2: The birds were subjected to continuous lighting programme (24L: 0D), T-3: The birds were subjected to intermittent lighting programme (16L:3D: 2L:3D). It was found that the average weekly body weights were numerically higher for the birds reared in continuous light. The FCR during the phase of 1-8 week and the overall FCR was significantly better ($p<0.05$) in continuous lighting programme than conventional and intermittent lighting programmes. No significant difference was observed among the treatments for the biochemical and haematological attributes. Total immunoglobulins and mercaptoethanol sensitive (IgM) antibody titer (\log_2) values in response to SRBC were found to be significantly higher ($p<0.05$) in continuous lighting programme than intermittent lighting programme. Further, mercaptoethanol resistant (IgG) antibody titer, cell mediated immune response and the spleen weight as % of live weight did not differ significantly among the treatment groups. Percent dressing yield of the birds reared in continuous and intermittent lighting programme was found to be significantly higher ($p<0.05$) than that of the birds reared in conventional lighting programmes. No significant difference was found among the treatment groups for other carcass traits and individual cut-up parts and development of gastrointestinal tract. The birds reared under continuous lighting programme displayed significantly lesser behaviors than the birds reared under other lighting programmes and were less active. Average femur ($p<0.01$) and tibia ($p<0.05$) weights were significantly higher in continuous lighting programme than conventional and intermittent lighting programmes. There were no significant differences in radiographic density of femur and tibia, walking ability and the dimensions of the eye ball, tonic immobility duration among three lighting programmes.

The continuous light was selected as best lighting program on the basis of the results of first experiment. The second experiment was performed to find out the optimum SD of turkeys during winter. Day old turkey poults were distributed randomly on uniform body weight basis in the treatment groups- T-1: The birds were subjected to standard SD (2.5 ft² per bird), T-2: The birds were subjected to high SD (1.25 ft² per bird), T-3: The birds were subjected to low SD (5 ft² per bird). It was found that at 12 weeks of age, average body weight was significantly higher ($p<0.01$) in high SD than the other two SD treatments. No significant differences were found in FCR. Cholesterol level in low SD was significantly higher ($p<0.05$) than standard SD. No significant difference was observed between treatments for the other biochemical and haematological attributes as well as for the umoral and cell mediated immune response and various carcass traits. Average spleen weight and average percent liver weight were found to be significantly higher in standard SD than other two SD treatments. Average percent gizzard weight was significantly higher ($p<0.05$) in low SD than high SD. Average proventriculus weight and average small intestine weight were found to be significantly higher ($p<0.05$) in standard and low SD treatments than high SD treatment. Average small intestine length and average large intestine length were significantly more

($p < 0.01$) in low SD than other two SD treatments. Average cecal length was found to be significantly more ($p < 0.01$) in standard and low SD treatments than high SD treatment. The birds reared under high SD treatment displayed significantly lesser behaviors than the birds reared under low and standard SD treatments. These birds were displaying significantly higher standing behaviour than the other two treatments. Average femur weight and average tibia weight were found to be significantly higher ($p < 0.05$) in high SD group than the other two treatments. Average femur medulla thickness was found to be significantly higher ($p < 0.05$) in standard SD treatment than low SD treatment. Average tibia cortex thickness was significantly higher ($p < 0.01$) in standard and high SD treatments than the low SD treatment. Further, no significant difference was found in bone parameters and radiographic density of femur and tibia, gait score and tonic immobility among the three SD treatments.

The third experiment was conducted on the lines of 2nd experiment in summer. It was found that average weekly body weight during the experiment, FCR were not significantly affected among the three SD treatments. No significant differences were found between the three SD treatments for the serum biochemical parameters, haematological attributes and humoral immune response. Cell mediated immune response was found to be significantly higher ($p < 0.01$) in high and low SD treatments than standard SD treatment. No significant difference was found in different SD treatment in summer season for the spleen weight as percent of live weight, carcass traits and individual cut-up parts. Average small intestine length was found to be significantly higher ($p < 0.05$) in standard and low SD treatments than high SD treatment. Average caecal length was found to be significantly higher ($p < 0.05$) in low SD treatment than high SD treatment. However, there was no significant difference in the other digestive organs of turkeys reared in different SD. The birds reared under high SD treatment displayed significantly lesser behaviors than the birds reared under low and standard SD treatments. These birds were displaying significantly higher standing behaviour than the other two treatments. Average femur length was found to be significantly higher ($p < 0.01$) in standard and high SD treatments than the low SD treatment. Radiographic density of Tibia medulla was found to be significantly higher ($p < 0.01$) in low SD treatment than the other two SD treatments. The gait score at 8 weeks was found to be significantly different ($p < 0.05$) for three SD treatments being highest in standard SD followed by high SD and least for low SD treatment. Tonic immobility duration exhibited by turkey poults during summer did not differ significantly among the treatments. Therefore the intensive turkey farming with continuous light and high SD (1.25 ft² per bird) / (0.116 m² per bird) / (8.625 birds per m²) / (0.8 birds per ft²) can be taken as commercial enterprise without compromising the welfare of birds.

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2. Growth performance and carcass characteristics of coloured chicken fed on diets containing *Azolla* (*Azolla pinnata*) and supplemental chromium

Sandeep Singh Kashyap and P. K. Shukla

A study was conducted to find out the optimum inclusion level of *Azolla pinnata* (powder or raw form) and supplemental level of chromium picolinate and the effect of their interaction on production performance, immune response and carcass characteristics of coloured chicken. Three experiments were conducted on straight run Chabro chicks from day old to 8 weeks of age. In each experiment 120 day old chicks were distributed randomly on the basis of uniform body weight in to the four treatments groups. The first experiment was conducted to assess the performance of coloured chicken on the raw and/ or the powder form

of *Azolla pinnata*. Treatment diets were T1-basal diet, T2-Basal diet+5% Azolla meal, T3- Basal diet (+Raw azolla after 4 weeks) and T4- Basal diet+5% Azolla meal (Basal diet+ Raw azolla after 4 weeks). Average weekly body weight of the birds fed with T2 was found significantly or apparently better. The FCR during starter phase and 0-8 weeks (overall) was apparently better in the treatment group T2. Significantly higher ($P<0.01$) TLC, Heterophil, eosinophil, lymphocyte and monocyte count in T2 was recorded. No significant effect of feeding azolla on plasma protein, albumin, creatinine, uric acid, cholesterol, SGPT and ALP. The mean values observed in triglycerides and SGOT for T2, T3 and T4 were significantly higher than ($P<0.01$) the T1. Response to 1% SRBC (\log_2 titre) and cell mediated immune response to PHA-P was apparently better in T2. No significant differences in slaughter traits were recorded, only significant increase ($P<0.05$) was observed in the per cent gizzard weight in azolla fed groups. Calcium content in breast meat was apparently or significantly higher ($P<0.05$) in T2 and T4 groups. The second experiment was performed to find out the optimum supplemental level of chromium picolinate supplementation from 0, 400, 800, 1600 ppb. No significant differences on the average weekly body weight and body weight gain of coloured chicken were recorded during the course of the experiment. There was no significant difference in the average weekly feed consumption of the chicks in the duration of the experiment from first to the sixth and in eighth week. Significantly better ($P<0.05$) FCR was found at the end of third week in T1, T3 and T4 groups. Further, no significant differences were recorded in the FCR in starter and finisher phases and in overall FCR. Lymphocyte count in T2, T3 and T4 groups was significantly higher ($P<0.01$) and Monocyte count in T3 was significantly higher ($P<0.01$) than the other groups. However, the T2 and T4 groups were having apparently higher monocyte count than the control. The H:L ratio revealed a non significant decreasing trend with increase in CrPic supplementation level. Values for triglyceride and cholesterol in control group were significantly higher ($P<0.01$) than the CrPic supplemented groups. Response to 1% SRBC (\log_2 titre) and cell mediated immune response to PHA-P (foot web index) were apparently better in T3 group. No significant differences were recorded in slaughter traits, cut-up-parts and gastrointestinal tract development traits except in large intestine weight among the treatment groups, lowest weight for large intestine was found in T4. Crude protein content in breast meat were significantly higher ($P<0.01$) than the control group and ether extract content recorded apparently lower levels in CrPic supplemented groups. Whereas, in thigh meat significantly higher ($P<0.01$) crude protein and phosphorus content for CrPic supplemented groups but with significantly lower ($P<0.0$) ether extract levels were recorded. The third experiment was performed to assess the effects of the interaction of the best inclusion level of azolla in experiment no. 1 and the best performing supplemental level of chromium picolinate in experiment no. 2. Thus the treatment diets were T1- basal diet, T2- 5% of basal diet replaced with azolla meal, T3- basal die + 800 ppb CrPic and T4-basal die +5% azolla meal + 800 ppb CrPic. It was observed that the weekly body weight and body weight gain of the coloured chicken in T4 was found to be higher than the control group in nearly all the weeks whether significantly or apparently. T4 recorded significantly highest weekly feed consumption up to third week. Thereafter this trend was also observed in most of the weeks. No significant difference in the weekly, starter and finisher phases and overall FCR was recorded. At the end of fourth week the group T4 recorded significantly highest nitrogen balance. No significant differences were found in calcium and phosphorus retention among the treatment groups. Significantly higher ($P<0.05$) Hb was recorded in T2 group followed by T4, significant differences ($P<0.01$) were recorded in heterophil, eosinophil, lymphocyte and monocyte counts. The lowest H:L ratio in T3 followed by T4 group was recorded. Serum triglyceride was found highest in T2, followed by T1 and thereafter T4 group. Values of IgG were found to be significantly higher ($P<0.05$) in T4 group. CrPic supplemented groups

gained significantly higher ($P<0.01$) higher foot web index than the control group. The total antibody titre and cell-mediated Immunity was found highest in T4 whereas IgM value in T4 was lying in-between the responses of T2 and T3 groups. No significant differences in slaughter traits, cut-up-parts and development of digestive organs at eight weeks of age among the treatment groups were recorded. Significant ($P<0.01$) alleviation in the crude protein content of breast and thigh meat in both the chromium supplemented groups was noted. The average mean values recorded for crude fat content of breast were reduced in all the chromium provided groups and this reduction was significant ($P<0.01$) in thigh meat.

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3. Modulation of growth and immunity by dietary supplementation of sea buckthorn leaf meal in coloured breeder and their post hatch chicks in different seasons

Deep Narayan Singh and P.K. Shukla

The present study was conducted at the poultry farm of U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura. The basic aim of this research was to assess the modulation of growth and immunity of coloured chabro breeder birds and their post hatch chicks by dietary supplementation of sea buckthorn leaf meal in summer and winter seasons. Total four experiments were conducted to achieve this goal. The first and fourth experiments were conducted to assess the production performance and immune status of chabro breeder birds by dietary supplementation of sea buckthorn leaf meal during summer and winter seasons, respectively. Ninety coloured breeder (Chabro) hens and eighteen viable cocks in 1:5 sex ratio were randomly distributed into three treatment groups: Control (Basal), standard breeder diet (BIS, 2007); basal+0.5% and basal+1.0%SBTLM. Throughout the experimental period the birds were offered fixed weighed quantity (110g/day) feed (adequate in all nutrients) as per BIS (2007) and water *ad lib*. The dry matter, crude protein, ether extract, crude fiber, total ash, calcium and phosphorus content of dried sea buckthorn leaf meal (SBTLM) were recorded as 90.46%, 12.33%, 7.13%, 16.86, 8.19%, 1.49% and 1.14%, respectively in summer season and almost similar composition were observed during winter season. Basal diet+1.0%SBTLM supplemented group had significantly higher ($P<0.05$) Hen House Egg Production (HHEP) as compared to control group during phases of 4-8 weeks, 8-12 weeks and 0- 12 weeks (Overall) of experimentation in summer season. The weekly FCR on egg weight basis and egg dozen basis were significantly better ($P<0.05$) in basal diet+1.0%SBTLM supplemented group during 4-8 weeks, 8-12 weeks and 0-12 weeks (overall) as compared to control group during summer season while Phase wise and overall HHEP, FCR (egg weight basis) and FCR (egg dozen basis) were significantly better ($P<0.05$) in both SBTLM supplemented groups during winter season. Response to 1% SRBC (log₂ titre) and cell mediated immune response to PHA-P (foot web index) were apparently better in both SBTLM supplemented groups as compared to control group during both summer and winter seasons. The shape index and shell thickness were significantly higher ($P<0.05$), whereas the Haugh unit were comparatively better in both SBTLM supplemented groups as compared to control during both summer and winter season. Percent fertility and percent hatchability were comparatively better in both SBTLM supplemented group as compared to the control group in both the seasons. Cost of feed consumed per kg egg production (Rs.) was significantly lower ($P<0.05$) in basal diet+1.0%SBTLM supplemented group as compared to control group during phases of 4-8 weeks, 8-12 weeks and overall (0-12 weeks) experimental feeding. The second and fourth experiment was performed for assessment of growth performance and immune status of post

hatch coloured chicks by dietary supplementation of sea buckthorn leaf meal during summer and winter seasons. Two hundred seventy chicks were obtained from fertile eggs of Experiment No.1 and 2, respectively. Ninety chicks from each breeder groups were further subdivided into three groups: Control (Basal), Broiler starter till 4 weeks, broiler finisher till 8 weeks of age, BIS 2007; basal+0.5% and basal+1.0% supplementation of SBTLM supplementation. Dietary supplementation of SBTLM in breeder diet as well as post hatch diet on resulted in significantly higher ($P<0.05$) body weight and body weight gain in post hatch chicks in 0.5%SBTLM supplemented group as compared to control group both during summer and winter season. Similarly, supplementation of 0.5% SBTLM in breeder and post hatch diet resulted in a synergistic effect pertaining to body weight of coloured breeder in both the season. The overall FCR (0-8 weeks) was significantly better ($P<0.05$) in 0.5%SBTLM supplemented groups in breeder diet, post hatch diets and breeder \times post hatch diet among the various dietary treatment groups. In post hatch dietary groups, the haemoglobin concentration, packed cell volume (PCV) and total leucocyte count were significantly higher ($P<0.01$) in both SBTLM supplemented groups during both summer and winter season. The value of cholesterol, triglycerides were significantly lower ($P<0.05$) in both SBTLM supplemented groups as compared to control group where as the HDL was significantly higher ($P<0.05$) in BP+0.5%SBTLM supplemented groups in post hatch diet during both summer and winter seasons. No significant differences were recorded in slaughter traits, cut-up parts and development of digestive organs except the percent dressing, eviscerated weight, heart weight and wing weight were significantly higher ($P<0.05$) in BP+0.5%SBTLM supplemented group as compared to control group during summer season, while processing shrinkage, eviscerated weight, heart weight, wing weight were significantly higher in BP+0.5%SBTLM group during winter season. Histological study showed that supplementation of SBTLM in various post hatch dietary groups, (BB+0.5%SBTLM) +(BP+0.5%SBTLM) had some immune-stimulatory potential resulting in proliferation of lymphoid tissues in various lymphoid organs during summer and winter season. The fold changes in expression of growth related genes (IGF1 and IGF2) were better in (BB+0.5% SBTLM) +(BP+0.5% SBTLM) group whereas as expression of immunity related genes (IL2) were better in (BB+0.5%SBTLM) +(BP+1.0% SBTLM) as compared to BB+BP during both summer and winter seasons. The overall (0-8 week) feeding cost per kg body weight gain in breeder diet as well as in post hatch diet were significantly lower ($P<0.05$) in 0.5%SBTLM supplemented group as compare to control group during experimentation in both summer and winter seasons.

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DEPARTMENT OF LIVESTOCK PRODUCTS TECHNOLOGY

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M.V.Sc. Degree Programme

1. Effect of natural antioxidants on lipid oxidation of pork papad

Abhishek Singh Sengar and Vikas Pathak

The present study was envisaged to explore the effect of different natural antioxidants on the shelf life of pork papad. Formulation and processing technology for preparation of papads were optimized. Different treatments of papad were developed by using three flour viz. green gram, rice and black gram flour replaced by pork meat at the levels of 50, 60 and 70 percent. The pH and volume expansion ratio decreased significantly ($P < 0.05$) with increased levels of pork meat, while protein, fat and ash content increased significantly ($P < 0.05$). The formulation containing each flour and 60 percentage pork meat were compared among each other to select the best variant. The treatment containing rice flour and 60% pork meat (R_3) had minimum pH (5.72 ± 0.02) and fat content (16.25 ± 0.53) and maximum volume expansion ratio (54.02 ± 0.82). Texture profile analysis also showed significantly higher scores in R_3 . The variant (R_3) was also awarded highest score (6.62 ± 0.15) for overall acceptability.

The selected treatment (R_3) was incorporated with different natural antioxidants viz. lemon grass, jimbu and mint @ 0.25, 0.50 and 0.75 percent. pH decreased significantly ($P < 0.05$) with increased levels of all antioxidants and the minimum pH was observed in treatment containing 0.75 percent lemon grass. Results of sensory evaluation revealed significant increase in flavor scores with increased levels of antioxidants in formulation; however, treatments with 0.5 percent antioxidants were awarded highest overall acceptability scores. The best selected variants L_2 , J_2 and M_2 containing 0.5 percent lemon grass, jimbu and mint respectively were selected for storage studies (30 days) with R_3 as control. pH, TBA and FFA value increased significantly ($P < 0.05$) in all variants during storage while cholesterol value decreased significantly. TBA and FFA values were found highest in control (R_3) and lowest in L_2 . Total plate count, yeast & mold count and lipolytic count showed same trends i.e. highest in R_3 and lowest in L_2 throughout the storage. Results of sensory evaluation studies during storage also demonstrated decrease in sensory scores during advancement of storage period and minimum scores for all the attributes were observed for R_3 during each day of sampling. L_2 (treatment containing 0.5% lemon grass) was rated highest for appearance & colour, flavor, texture and overall acceptability during the entire study period.

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2. Development and quality assessment of fiber fortified enrobed chicken kabab

Santwana Upadhyay and Vikas Pathak

The study titled, "Development and quality assessment of fibre fortified enrobed chicken kabab" was carried out with the prime objective of enriching the fiber content of kabab simultaneously preserving its quality characteristics with edible coating. Three fibre rich vegetables viz. carrot, winter melon and jack fruit were added in the formulation of kabab replacing lean meat @ 10, 15 and 20 per cent. The moisture content increased while fat and protein content decreased with increased levels of vegetables. The incorporation of carrot and jack fruit resulted in increase in pH of kabab, however reverse trend was observed in case of winter melon. Cooking yield also decreased, significantly ($P < 0.05$) with the incorporation of winter melon and non significantly with increased levels of carrot. However the addition of jack fruit showed a significant ($P < 0.05$) increase in cooking yield. Texture profile analysis

demonstrated significant ($P<0.05$) decrease in firmness and toughness of kabab with increased levels of carrot and winter melon while contrary findings were observed with jack fruit. One treatment from each group; formulations with 20 per cent carrot (C3), 20 per cent winter melon (B3) and 10 per cent jack fruit (J1) were selected on the basis of results of sensory evaluation and compared among each other. pH (6.28 ± 0.02) and cooking yield (81.93 ± 0.20) was maximum in J1 which was significantly higher ($P<0.05$) than other variants. Similar trends were recorded for firmness and toughness. No significant difference was observed in protein and fat content. However, C3 was rated best on the basis of overall acceptability scores by sensory panelists. The selected variant was enrobed/coated with 0.5, 1.0 and 2.0 per cent aqueous solutions of guar gum, sodium alginate and carrageenan. No significant effect of coating was observed on physico-chemical properties. The results of texture profile analysis illustrated significant reduction in firmness and toughness of kabab coated with 1.0 per cent solution of guar gum. Three best variants i.e. kabab coated with 0.5 per cent guar gum (G1), 0.5 per cent sodium alginate (S1) and 2.0 per cent carrageenan (R3) were selected on the basis results of sensory evaluation and were evaluated for physico-chemical, textural and microbiological properties during storage at refrigeration (temp). The estimated mean TBA and FFA values were found significantly ($P<0.05$) higher in control throughout storage. The highest moisture content was observed in R3. No significant effect of storage was observed for mean protein, fat and ash content. The firmness and toughness decreased significantly ($P<0.05$) on 7th day. The average TPC counts increased significantly during storage and significant higher counts were observed in C3 on day 7 as compared to other treatments. The sensory attributes scores also decreased with advancement of storage. The chicken kabab incorporated with 20 per cent carrot and further enrobed by 2 per cent carrageenan was most acceptable up to 7 days stored at refrigeration temperature.

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3. Evaluation of storage stability of extended chicken nuggets incorporated with natural antimicrobials

Saraswati Ojha and Vikas Pathak

The present study was envisaged to develop chicken nuggets by the incorporation of various extenders viz. common bean, cowpea, jackfruit and natural antimicrobials viz. amla leaf powder, drumstick leaf powder, curry leaf powder to improve storage stability simultaneously cutting off the cost of formulation. Four different treatments of nuggets i.e. C, CB1, CB2 and CB3 containing 0, 10, 15, 20 per cent common bean were evaluated for various physicochemical properties and sensory attributes. The mean values for pH, cooking yield, moisture content and ash of extended chicken nuggets increased while the protein and fat content were found to be decreased significantly ($P<0.05$) with increased lean replacement. The scores of all the sensory attributes were observed to be highest in CB1 (10%) among all treatments. In the next experiment, four different treatments of nuggets i.e. C, CPI, CP2 and CP3 containing 0, 10, 15, 20 per cent cow pea were formulated. Evaluation of physico-chemical parameters revealed similar trend as obtained in extension of nuggets with common bean. The scores for all the sensory attributes decreased ($P<0.05$) with increased levels of cow pea, however, CPI was found to be comparable with control. The nuggets were also extended with jackfruit by formulating four treatments i.e. C, J1, J2 and J3 containing 0, 10, 15, 20 per cent jackfruit. Results obtained on evaluation of physico-chemical properties were in similar fashion as recorded with common bean and cow pea. The overall mean scores for all sensory attributes except overall acceptability of J1 were comparable to control. Further the selected variant from each extender were compared among each other for physico-chemical properties

and sensory attributes. The physicochemical parameters viz. pH, cooking yield and moisture were highest for J1 (10%). Sensory parameters of all treatments were comparable to each other except juiciness and overall acceptability. The juiciness and overall acceptability of J1 were higher and comparable to control. Three different natural antimicrobials viz. amla leaf powder, drumstick leaf powder and curry leaf powder at three different levels viz. 0.25%, 0.5% and 0.75% were added in the selected treatment of chicken nuggets i.e. J1. There was significant ($P < 0.05$) decrease in pH of both raw emulsion and chicken nuggets with amla leaf powder incorporation. Addition of amla leaf did not have significant effect on the physicochemical parameters of chicken nuggets except ash which increased significantly ($P < 0.05$). All the sensory attributes decreased non significantly with increased levels of amla leaf except appearance and color, texture and overall acceptability which decreased significantly ($P > 0.05$) for A3. A1 had comparable sensory scores with control. Incorporation of drumstick leaf powder resulted in significantly ($P < 0.05$) higher cooking yield, protein and ash content in D3 as compared to control. The sensory scores for different parameters decreased with increase in levels of drumstick leaf powder, however, the scores for all the sensory attributes except overall acceptability of D1 were comparable with J1. There was no significant ($P > 0.05$) decrease in pH of both raw emulsion and chicken nuggets with curry leaf powder incorporation. Similar trend was recorded for other physico-chemical parameters except moisture and ash content which was found to be significantly ($P < 0.05$) different among variants. Cyl had comparable sensory scores with control. The finally selected variants i.e. A1, D1 and Cyl along with J1 (control) were further evaluated for storage stability and quality characteristics for a storage period of 9 days at every 3 day interval. The mean pH, TBA and FFA values of control and treatments increased significantly ($P < 0.05$) during whole storage period. The highest and the lowest pH values were recorded for J1 (control) and A1 throughout storage period. The minimum TBA and FFA values were recorded for A1 on all storage days. Overall mean TPC and pschrophillic count were significantly ($P < 0.05$) higher in J1. Overall mean yeast and mould count reported to be highest of J1 followed by CY1>D1>A1. The Coliform and Salmonella were not detected during entire storage. The scores for all the sensory attributes except saltiness decreased significantly ($P < 0.05$) in all variants with progression of storage. On day 0, J1 was rated best for sensory parameters, however, with succession of storage period, the scores of J1 were found to be lower than other treatments on day 9. The mean scores for overall acceptability were found to be highest for A1. It can be concluded that chicken nuggets prepared with incorporation with 10% jackfruit and 0.25% amla leaf powder had enhanced nutritional value and was acceptable upto 9th day of storage under refrigeration.

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4. Development and quality evaluation of low fat fiber fortified milk slices

Neetu Pandit and Vikas Pathak

The study on “Development and quality evaluation of low fat fiber fortified milk slices” was envisaged to standardize the processing technology of milk slices, a milk based snack food. Milk with 6% fat was used to prepare coagulum and the final time-temperature combination for cooking was optimized as 121°C (Steam cooking) for 35 minutes followed by setting of product under refrigeration for 15 minutes, based on several preliminary trials. Three variants were prepared using milk with different fat levels viz. F1 - milk with 4.5% fat, F2 - milk with 3% fat and F3 - milk with 1.5% fat. The cooking yield increased while cooking losses decreased significantly ($P < 0.05$) with reduction in fat levels of milk. Significant ($P < 0.05$)

increase in moisture, protein and ash contents of milk slices were observed whereas fat content of milk slices was significantly ($P<0.05$) lowered. Sensory studies revealed that there was significant ($P<0.05$) increase for color and appearance scores and non significant increase for saltiness scores, whereas, flavor, texture and overall acceptability scores were decreased non significantly in milk slices prepared with three different milk fat levels. Further reduction in fat content of milk slices (F3) were attempted by use of fat replacers i.e. carrageenan with three different levels viz. C1 (0.1%), C2 (0.2%) and C3 (0.3%) and sodium alginate at three different levels viz. T1 (0.1%), T2 (0.2%) and T3 (0.3%) replacing 30, 40 and 50% of vegetable oil. The cooking yield increased non-significantly with increased levels of both carrageenan and sodium alginate. The moisture, protein and fat content of milk slices increased while fat percentage decreased significantly ($P<0.05$) with increased levels of fat replacers in the formulation. Milk slices incorporated with 0.2% sodium alginate (T2) were selected for fiber fortification based on sensory scores. Three different fiber sources i.e. sorghum (S1, S2, S3), oat (O1, O2, O3) and ragi (R1, R2, R3) at three different levels viz. 5, 10 and 15% were also tried to improve the functionality of product. Mean cooking yields were increased significantly ($P<0.05$) whereas cooking loss values were decreased significantly ($P<0.05$) with increased levels of flour in the milk slices. The estimated moisture, ash and fiber content of milk slices increased significantly ($P<0.05$) while protein and fat values decreased significantly ($P<0.05$) with increased percentage of all flours. The mean color and appearance, flavor and overall acceptability scores were found to be decreased significantly ($P<0.05$), whereas texture and saltiness scores were decreased non significantly ($P>0.05$) and the scores for all the sensory attributes were comparable upto 5% incorporation of sorghum, oat and ragi flours in milk slices. The best variant from each flour viz. sorghum (S1), 5% oat (O1) and 5% ragi flour (R1) were taken for storage studies along with T2 which served as control. The estimated pH, TBA and FFA values increased significantly ($P<0.05$) with the progression of storage. Total Plate Counts, Psychrophilic counts and Yeast & mold counts were highest in T2. *Coliform* count was not detected during whole storage period in any of the variants. There was no significant difference between control and treatments for all sensory attributes throughout the storage period, however scores for all attributes decreased significantly ($P<0.05$) at later stage of storage. The overall acceptability scores were the highest for $T2=R1>S1>O1$. The product was acceptable for 9 days under refrigeration temperature (4 ± 2 °C). The overall cost for the production of 250g of low fat fibre fortified milk slices was Rs 87.50 for T2, 84.50 for S1, 86.75 for O1 and 86.50 for R1.

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5. Quality and safety assessment of milk from different milch animals

Sadhana Ojha and Vikas Pathak

The present study was carried out for quality and safety assessment of cow, goat and buffalo milk collected from five different regions of Mathura city viz. Holigate, Sadar, Aurangabad, Chungi and Township. The milk samples were collected from dairy shops, vendors and milk producers and evaluated on the basis of various organoleptic tests, physico-chemical properties, proximate estimation and microbiological studies following the standard procedures. The milks samples of Township and Chungi areas had more clear appearance, normal texture/consistency, normal odor and color than other three areas. No cow milk sample was observed with pure white color, however 74% samples had normal light yellow color. Goat and buffalo milk had normal white color in 86 and 70% samples, whereas some samples were also observed with abnormal pale yellow or dark yellow color. No milk sample

had rancid/oxidized odor for any species in any region, however few milk samples of cow and buffalo contained weedy or absorbed odor. Watery consistency was observed in 50, 18 and 42% of cow, goat and buffalo milk samples respectively, whereas thick, ropy or slimy consistency was observed only in 4, 4 and 20% of cow's milk only. The COB test was positive in 38, 12 and 20% of cow, goat and buffalo samples respectively. The temperature, pH and specific gravity of cow, goat and buffalo milk collected from different regions were lower than normal prescribed range, however titratable acidity was higher than normal value in all three species. The moisture content of all the samples was higher however other proximate parameters like fat, solid not fat, protein, ash and total solids showed quite variable values than normal range. The higher moisture content and lower specific gravity of milk were probably because adulteration of milk with water. Out of total, 28 and 20% samples of cow and buffalo milk were positive for formalin, however there was no adulteration in goat milk. The adulteration of other preservatives i.e. boric acid and hydrogen peroxide, neutralizers and adulterants like starch, cane sugar and urea was not detected in any milk sample. . The malpractices like skimming of fat, adulteration of water and formalin etc. were less observed in goat milk as compared to other two species, however the microbial load of all milk samples was quite higher than normal prescribed limit in terms of DMC, SPC as well as *Coliform* and *Staphylococcus* count due to poor hygienic condition and sanitation during the milking, handling practices and transportation as well as use of uncleaned utensils.

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6. Status and quality assessment of meat from different food animals

Sushant Sharma and Vikas Pathak

The present study was conducted to appraise the status of meat workers and quality of meat produced in the city. The work included three different aspects viz. status of meat trade and socioeconomic status of respondents, quality of fresh meat produced in city and suitability of chicken, chevon and carabeef for development of meat nuggets. The locations of meat shops were mapped. Survey revealed that 91.17 percent of meat business was owned by Muslim community persons aged between 35-50 yrs. The respondents were mostly functionally literate and 70.59 percent were in opinion to carry forward the meat business to next generation. Persons carrying the meat business were very experienced (10-20yrs) and 47.06 percent were earning more than two lakh annually. Seventy percent of the butchers were in possession of meat shops. There were no facilities of lairage, AM and PM inspection in all of the meat shops though 38.23% meat shops were having the facility of refrigeration. Halal was the common practice of animal slaughter and source of animals for slaughter was nearby markets. The major by-products were processed and sold while rests were disposed directly as waste material. Survey divulged that 97 percent respondents were satisfied with meat business while only 88 percent expressed economical satisfaction. The major constraints in business included high competition, deficit of healthy and cheap raw materials and lack of government financial assistance.

The fresh meat produced in city was of satisfactorily quality. The pH, WHC and drip loss of meat samples ranged 5.33 to 5.42, 97.33 to 99.03 percent and 0.96 to 2.66 percent respectively. The estimated mean TBA values in meat of different food animals were 0.25 mg malonaldehyde/kg. The ERV and FFA values of samples were 31.80 to 35.0 ml and 0.05 to 0.14 percent oleic acid respectively. The mean WHC value was significantly higher in buffalo meat while no significant differences were observed in TBA, ERV and FFA values among chicken, chevon and carabeef. The respective percent moisture, fat, protein and ash content in

meat samples ranged between 72.10 to 75.70, 2.22 to 3.25, 19.12 to 21.54, 0.96 to 1.02. The crude protein content in fresh chicken meat was significantly ($P<0.05$) higher than chevon and buffalo meat while the fat content was maximum in carabeef. The mean TPC conformed to standards recommended by FSSAI while no *Salmonella* was detected in any meat sample. The mean *Coliforms* count was found to be very high and exceeded prescribed standards. The emulsion stability was found highest for chicken meat whereas minimum values were observed for chevon. The cooking yield of meat nuggets varied between 82.03 to 87.42 percent. The percent moisture, fat, protein and ash content in meat nuggets ranged between 63.28 to 63.91, 12.92 to 14.16, to 19.22 and 1.92 to 1.93 respectively. Significantly ($P<0.05$) higher fat values were recorded in nuggets made from buffalo meat whereas significantly ($P<0.05$) lower protein content was recorded in chevon meat nuggets. The nuggets made by chevon meat were adjudged best by sensory panelists.

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7. Standardization and shelf life assessment of functional chevon sausages incorporated with essential oils

Karunakara K.N. and Vikas Pathak

The present study was conducted to extend the shelf life of low salt fibre enriched functional chevon sausages with incorporation of essential oils. First experiment was attempted to standardize the optimum level of salt replacer mixture in chevon sausages. Preliminary trials were conducted to standardize the formulation of different salt replacers combinations using potassium chloride and herbs. The best herb blends as salt replacers were incorporated in chevon sausages under three sub experiments replacing 25, 50 and 75% salt i.e. blend-1 as T1, T2 and T3; blend-2 as M1, M2 and M3; blend -3 as L1, L2 and L3 respectively. For blend-1, pH of emulsion and ash content increased, however cooking yield and emulsion stability decreased significantly ($P<0.05$) with incorporation of salt replacer. Among the sensory attributes, color and appearance, flavor, juiciness, saltiness, mouth coating, meat flavor intensity and overall acceptability scores had no significant difference between control and T1, hence found optimum. For blend-2, pH of emulsion and product increased, whereas cooking yield and emulsion stability decreased significantly ($P<0.05$) in M2 and M3. Ash content, lightness and yellowness values increased significantly ($P<0.05$) in treatments. Among the sensory attributes, flavor, juiciness, saltiness, mouth coating, meat flavor intensity and overall acceptability scores were comparable upto M2 and found optimum. For blend-3, pH of emulsion, product pH and yellowness increased, whereas cooking yield and emulsion stability decreased significantly ($P<0.05$) in treatments. Among the sensory attributes, flavor, saltiness, meat flavor intensity and overall acceptability scores were comparable up to L2 and found optimum. On comparison of selected treatments, L2- cheovn sausages incorporated with 1.0% herb blend-3 replacing 50% salt was selected and was taken as control in next experiment. Second experiment was carried out to improve the functionality of chevon sausage by incorporation of different natural dietary fibres i.e. wheat bran at 5% (W1), 10% (W2) and 15% (W3); rice bran at 5% (R1), 10% (R2) and 15% (R3) and oat bran at 5% (O1), 10% (O2) and 15% (O3) levels separately. For wheat bran, all physico-chemical properties, moisture and content increased significantly ($P<0.05$) with wheat bran incorporation. Lightness and redness values had significant ($P<0.05$) effect, but no significant difference was observed in yellowness value. All sensory attributes including overall acceptability scores were comparable up to W2 and found optimum. For rice bran, all physico-chemical properties and fat as well as ash content among proximate parameters increased significantly ($P<0.05$) with rice bran incorporation. Lightness and redness values decreased significantly ($P<0.05$),

whereas there was no significant difference in yellowness value. All sensory attributes including overall acceptability scores were comparable up to R1 and found optimum. For oat bran, among physico-chemical properties and proximate parameters, cooking yield, emulsion stability, moisture and ash content increased significantly ($P<0.05$) with oat bran incorporation. There was no significant difference in color values. Various sensory attributes including overall acceptability had no significant difference between L2 and O2, hence found optimum. On comparison of selected treatments, O2- low salt chevon sausage incorporated with 10% oat bran was selected and was taken as control in next experiment. Third experiment was carried out to optimize the levels of essential oils in functional chevon sausages by incorporation of thyme at 0.1% (A1), 0.2% (A2) and 0.3% (A3), caraway at 0.1% (B1), 0.2% (B2) and 0.3% (B3) and cinnamon at 0.1% (C1), 0.2% (C2) and 0.3% (C3) levels separately. A1- functional chevon sausages incorporated with 0.1% thyme essential oil; B1- functional chevon sausages incorporated with 0.1% caraway essential oil and C1- functional chevon sausages incorporated with 0.1% cinnamon essential oil were selected on the basis of physico-chemical properties and sensory evaluation. Fourth experiment was carried out to assess the storage stability of functional chevon sausages at refrigeration temperature. A1, B1 and C1 along with control (O2) were stored at $4\pm 20^{\circ}\text{C}$ and evaluated for physico-chemical, microbiological and sensory properties at every 3 days interval. Overall highest treatment pH mean was observed in C1 followed by $A1>B1>O2$. The highest TBARS treatment mean were observed in $A1>B1>C1$, however highest FFA treatment mean were observed in $B1>A1>C1$ among the treatments. pH, TBARS and FFA values of control as well as treatments increased significantly ($P<0.05$) with progression of storage period. Total plate count, psychrophilic count and yeast and mould count of control (O2) were significantly ($P<0.05$) higher than treatments. Overall highest microbial count was observed in $O2>A1>B1>C1$. There was no Coliform count throughout the storage in control and treatments. All sensory attributes decreased significantly ($P<0.05$) in control and treatments with progression of storage. The control (O2) was not evaluated after 15th day due to microbiological spoilage and rejection by sensory panellists, whereas treatments were acceptable up to 24th day. Thereafter, treated products were not evaluated further due to presence of slime on surface and foul smell of products. Among the treatments, C1 had significantly ($P<0.05$) higher overall acceptability scores till the end of storage. It can be concluded that low salt fibre enriched functional chevon sausages may be prepared with incorporation of herb blend salt replacer containing KCl, herbs and spices at 1% level replacing 50% salt and 10% oat bran as natural fibre source. The shelf life of functional chevon sausages may be extended up to 24 days under refrigeration with incorporation of cinnamon essential oil at 0.1% level with well acceptability on the basis of microbiological studies and sensory evaluation.

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8. Development and quality assessment of chicken meat spread

Tanveer Khanam and Meena Goswami Awasthi

The present study was attempted to develop and to assess the quality characteristics of chicken spread. First experiment was carried out to optimize the processing technology of chicken spread using different cooking methods with three cooking times *viz.* Braising for 10 (B1), 15 (B2) and 20 (B3) minutes; microwave cooking at 540 MHz for 3 (M1), 5 (M2) and 7 (M3) minutes and steam cooking without pressure for 25 (S1), 30 (S2) and 35 (S3) minutes. For braising, pH, protein, fat and ash content increased significantly ($P<0.05$), whereas cooking yield and moisture content decreased significantly ($P<0.05$) with increase in cooking time. Redness value increased significantly ($P<0.05$) among color parameters. Among the sensory attributes, flavor, texture, juiciness and overall acceptability scores were significantly ($P<0.05$)

higher in B2 and found optimum. For microwave cooking, cooking yield and moisture content decreased significantly ($P<0.05$) however fat and ash content increased significantly ($P<0.05$) with time. Lightness (L^*) and yellowness (a^*) values decreased, however redness (b^*) value increased significantly ($P<0.05$) with increased time of cooking. Among the sensory attributes, color and appearance, flavor, texture, mouth coating and overall acceptability scores of M3 were significantly ($P<0.05$) higher and found optimum. For steam cooking without pressure, pH and cooking yield increased significantly ($P<0.05$) with increased time, however there was no significant difference in proximate parameters and color values. Among the sensory attributes, color and appearance, flavor, texture, mouth coating and overall acceptability scores were significantly ($P<0.05$) higher in S3 and found optimum. On comparison of selected treatments, B2- chicken meat spread prepared by braising for 15 minutes was selected and taken as control in next experiment. Second experiment was carried out to improve the spreadability of chicken spread by incorporation of different plasticizers/humectants *i.e.* sorbitol at 0.5% (SB1), 1.0% (SB2) and 1.5% (SB3), pectin at 0.5% (PC1), 1.0% (PC2) and 1.5% (PC3) and glycerol at 0.5% (GL1), 1.0% (GL2) and 1.5% (GL3) levels separately. For sorbitol incorporation, cooking yield and moisture content of chicken spread decreased significantly ($P<0.05$) in SB3. The ash content of treatments were significantly ($P<0.05$) higher than control. The lightness (L^*) value of SB3 was significantly ($P<0.05$) higher than control. The redness (a^*) and yellowness (b^*) values of treatments were significantly ($P<0.05$) lower than control. Among the sensory attributes, flavor, saltiness, spreadability and overall acceptability scores of SB3 were significantly ($P<0.05$) higher and found optimum. For pectin incorporation, there was no significant difference in physico-chemical properties except cooking yield and moisture content which decreased significantly ($P<0.05$) in PC2 and PC3. Lightness and yellowness values increased however redness values decreased significantly ($P<0.05$) with increased level of pectin. Flavor, spreadability and overall acceptability scores were significantly ($P<0.05$) higher in PC2 and found optimum. For glycerol incorporation, cooking yield and moisture content decreased whereas redness value increased significantly ($P<0.05$) with glycerol incorporation. Among the sensory attributes, color and appearance, texture, spreadability and OA scores were significantly ($P<0.05$) higher in GL2 and found optimum. On comparison of selected treatments, SB3-chicken spread incorporated with 1.5% sorbitol was selected and used as control in next experiment. Third experiment was carried out to optimize the levels of essential oils in chicken spread by incorporation of anise at 0.1% (AN1), 0.2% (AN2) and 0.3% (AN3), clove at 0.1% (CL1), 0.2% (CL2) and 0.3% (CL3) and oregano at 0.1% (OR1), 0.2% (OR2) and 0.3% (OR3) levels separately. AN2-chicken spread incorporated with 0.2% anise essential oil; CL1- chicken spread incorporated with 0.1% clove essential oil and OR1- chicken spread incorporated with 0.1% oregano essential oil were selected on the basis of physico-chemical properties and sensory evaluation. Fourth experiment was carried out to assess the shelf life of developed chicken meat spread at refrigeration temperature. AN2, CL1 and OR1 along with control (SB3) were stored at $4\pm 2^\circ\text{C}$ and evaluated for physico-chemical, microbiological and sensory properties at every 5 days interval. Overall highest treatment mean pH was observed in $\text{OR1} > \text{CL1} > \text{AN2}$, however highest TBARS and FFA treatment mean were observed in $\text{OR1} > \text{CL1} > \text{AN2}$ among the treatments. pH, TBARS and FFA values of control as well as treatments increased significantly ($P<0.05$) with progression of storage period, however TBARS and FFA values of AN2 were significantly ($P<0.05$) lower than control and other treatments throughout the storage. Total plate count, psychrophilic count and yeast and mould count of control were significantly ($P<0.05$) higher than treatments, whereas AN2 had significantly ($P<0.05$) lower microbiological count among the treatments throughout the storage period. There was no *Coliform* and *Salmonella* count detected throughout the storage in control and treatments. All sensory attributes decreased

significantly ($P < 0.05$) in control and treatments with progression of storage. The control (SB3) was not evaluated after 25th day due to microbiological spoilage and rejection by sensory panelists, whereas treatments were acceptable upto 35th days from microbiological and sensory point of view. Among the treatments, AN2 had significantly ($P < 0.05$) higher overall acceptability scores till the end of storage. It can be concluded that well accepted chicken spread may be prepared by braising for 15 minutes with incorporation of 1.5% sorbitol and 0.2% anise essential oil and this product may be acceptable under refrigeration storage upto 35 days on the basis of microbiological studies and sensory evaluation.

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9. Study on development and shelf life assessment of functional smoothie using milk of indigenous cows

Brajesh Kumar and V.P. Singh

The present investigation was carried out to enhance the functional quality of milk smoothies and to enhance its shelf life. Total six experiments were conducted on various formulations of smoothies using banana and red plum/kiwi/cherry in different proportions (75:25, 50:50 and 25:75) along with milk of Haryana, Cross breed and Sahiwal cows. In initial experiment, screening of milk was done for physico-chemical characteristics. Most of the physico-chemical characteristics showed non significant ($P > 0.05$) variations among all three breeds. However, significant ($P < 0.05$) difference was observed in percent mean value of fat in the milk of these breeds. In second experiment, banana based milk smoothies were prepared using milk of Haryana, Cross breed and Sahiwal cows. Three sugar levels were tried to optimize the formulation and four percent sugar level was found best on the basis of sensory evaluation. On physico-chemical characteristics basis, non significant ($P < 0.05$) differences were observed in the smoothies of Haryana, Cross breed and Sahiwal milk smoothies except in mean values of moisture, protein and solid-not-fat. In experiment three banana and red plum were used in all three proportions as mentioned above along with milk of all three breeds. Overall, 50:50 banana and red plum with milk of Sahiwal breed smoothie was selected on the basis of sensory evaluation. Physico-chemical characteristics indicated non significant differences in mean values of ash, fiber, pH, titrable acidity, specific gravity and vitamin-C among different breeds. However, significant ($P < 0.05$) differences were observed in percent mean values of moisture, sugar, total solids and solid-not-fat. In experiment four, banana and kiwi were used in all three proportions as mentioned above along with milk of all three breeds. Overall, 50:50 banana and kiwi with milk of cross breed smoothie was selected based on sensory evaluation. Physico-chemical characteristics wise selected variants of smoothies of various breeds showed significant ($P < 0.05$) differences in moisture, fat, sugar and solid-not fat. The significantly ($P < 0.05$) higher mean values of protein and vitamin-C were observed in Sahiwal milk based smoothies while higher mean values of ash, solid-not-fat and pH were in Haryana milk based smoothies. In fifth experiment, banana and cherry were used in all three proportions as mentioned above along with milk of all three breeds. Overall, 50:50 banana and cherry with milk of cross breed smoothie was selected based on sensory evaluation. The physico-chemical characteristics of smoothies prepared with different breeds of cow showed non significant ($P > 0.05$) differences in mean values of moisture, ash, fiber, pH, titratable acidity and specific gravity. However, significant ($P < 0.05$) differences were observed in sugar, total solids and solid-not-fat among the breeds of smoothies. In shelf life assessment, the smoothies made with banana, milk and sugar were in good condition only for two days while these smoothies treated with Tulsi, Lemon grass and Alovera showed four days shelf life under refrigeration. Similarly smoothies made with banana and red plum/kiwi/cherry was

in good condition for four days and treated smoothies for six days. The microbial profile of stored smoothies showed significant ($P<0.05$) increase in SPC and Psychrophilic counts on advancement of storage days up to last day of storage. However, no Coliform and Yeast and mould were detected in all variants of smoothies during storage. TBA values also showed same trend during storage. But microbial counts and TBA both were under the prescribed limit as described by various organizations.

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10. Development of shelf stable chicken pickle incorporated with humectants and acidulants as hurdles

Shubha Singh and Meena Gosawami Awasthi

The present study was conducted to develop and assess the quality characteristics of hurdle technology based shelf stable chicken pickle incorporated with acidulants and humectants as hurdles. First experiment was carried out to optimize the formulation and processing technology of chicken pickle. Three different cooking methods viz. steam cooking without pressure (S1, S2, S3), frying (F1, F2, F3) and microwave cooking at 540 MHz (M1., M2, M3) were used for pre-cooking of marinated meat for 10, 15 and 20 minutes separately. For steam cooking without pressure, pH, moisture content and water activity (a_w) values increased whereas titrable acidity, protein, L^* and shear force values decreased significantly ($P<0.05$) with increase in cooking time. Among the sensory attributes, flavor, texture, juiciness and overall acceptability scores were significantly ($P<0.05$) higher in S2, hence selected as the best treatment. For frying, pH, fat, ash content and a^* values increased significantly ($P<0.05$) whereas titrable acidity, moisture, L^* and shear force values decreased significantly ($P<0.05$) with increase in frying time. Among the sensory attributes, flavor, texture, juiciness, sourness and overall acceptability scores were significantly ($P<0.05$) higher in F2, hence selected as the best treatment. For microwave cooking, pH, ash content and a^* values increased significantly ($P<0.05$) whereas titrable acidity, moisture content, L^* , b^* and shear force values decreased significantly ($P<0.05$) with increased cooking time. Among the sensory attributes, color and appearance, flavor, texture, juiciness and overall acceptability scores of M1 were significantly ($P<0.05$) higher, hence selected as the best treatment. On comparison of selected treatments, S2- steam cooking (without pressure) of marinated chicken meat for 15 minutes was selected and taken as control in next experiment. Second experiment was carried out to optimize the level of different acidulants *i.e.* acetic acid (AC1, AC2, AC3), citric acid (CA1, CA2, CA3) and lactic acid (LA1, LA2, LA3 at 0.5, 1.0 and 1.5% separately. For acetic acid, pH, ash content, water activity (a_w), b^* , shear force values and sensory scores decreased whereas titrable acidity, moisture content and a^* values increased significantly ($P<0.05$) with incorporation of acetic acid. AC1 and AC2 had no significant difference for any sensory attribute; therefore AC2 was selected among the treatments. For citric acid, pH, water activity (a_w), L^* , shear force values and sensory scores decreased whereas titrable acidity, moisture content and a^* values increased significantly ($P<0.05$) with incorporation of citric acid. CA1 had significantly ($P<0.05$) higher texture, sourness and overall acceptability scores than CA2 and CA3; therefore, CA1 was selected among the treatments. For lactic acid, pH, ash content, water activity and shear force values decreased whereas titrable acidity and moisture content increased significantly ($P<0.05$) with incorporation of lactic acid. Among the sensory attributes, flavour, juiciness and overall acceptability of S2, AC1 and AC2 had no significant difference; however decreased significantly ($P<0.05$) in LA3. Therefore, LA2 was selected among the treatments. On comparison of selected treatments, LA2-chicken pickle incorporated with 1.0% lactic acid was selected and used as control in next experiment. Third

experiment was carried out to optimize the level of humectants *viz.* glycerol (GL1, GL2, and GL3), honey (HY1, HY2, HY3) and sorbitol (SB1, SB2, SB3) at 3, 6 and 9% level separately. For glycerol, pH, moisture, water activity and shear force values decreased whereas titrable acidity and a^* values increased significantly ($P<0.05$) with incorporation of glycerol. GL1 had significantly ($P<0.05$) higher sensory scores including overall acceptability than GL2 and GL3 and was selected among the treatments. For honey, pH, moisture content, water activity, L^* and shear force values decreased; however titrable acidity, ash content and a^* values increased significantly ($P<0.05$) with increased level of honey. Flavour, texture, juiciness, sourness and overall acceptability scores of LA2, HY1 and HY2 had no significant difference, but decreased significantly ($P<0.05$) in HY3. Therefore, HY2 was selected among the treatments. For sorbitol, pH, moisture content, water activity and shear force values decreased; however titrable acidity and ash content increased significantly ($P<0.05$) with increased level of sorbitol. There was no significant difference between SB1 and SB2 for any sensory attribute; however decreased significantly ($P<0.05$) in SB3. Therefore, SB2 was selected among the treatments. Fourth experiment was carried out to evaluate the storage stability of hurdle technology based shelf stable chicken pickle at ambient temperature. HY2, GL1 and SB2 along with control (S2) were stored at ambient temperature and evaluated for physico-chemical, microbiological and sensory properties at every 10 days interval for 60 days. The pH, TBARS, FFA values and microbial count of HY2, GL1 and SB2 were significantly ($P<0.05$) lower whereas titrable acidity was significantly ($P<0.05$) higher than control during storage. The values of titrable acidity, TABRS, FFA values and microbiological count of control as well as treatments increased significantly ($P<0.05$) whereas pH and scores of all sensory attributes decreased significantly ($P<0.05$) with progression of storage period. However all products were well accepted upto 60th day of storage. Among the treatments, HY2 had higher oxidation stability and lower microbiological count along with significantly ($P<0.05$) higher overall acceptability scores of storage till the end of the storage. It can be concluded that well acceptable hurdle technology based chicken pickle may be prepared by precooking of marinated meat under steam without pressure for 15 minutes with incorporation of 1% lactic acid as acidulant and 6% honey as humectants. This product may be at least well acceptable at room temperature for 60 days on the basis of physico-chemical properties, microbiological studies and sensory evaluation.

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Ph.D. Degree Programme

1. Development and quality assessment of functional chevon patties

Narendra Kumar Nayak and Vikas Pathak

The study titled, "Development and quality assessment of functional chevon patties" was carried out with the prime objective of reducing fat and sodium content and simultaneously preserving its quality by incorporating natural antioxidants. Efficacy of fat replacers viz carrageenan (0.3%, 0.6% & 0.9), poppy seed (1%, 3% & 5%) and sago flour (1%, 3% & 5%) were assessed for development of low fat chevon patties. Emulsion stability and cooking yield increased with the increasing levels of each fat replacer. Significantly ($P < 0.05$) lower fat and cholesterol contents and higher moisture and fat retention were observed in formulation with various fat replacers. Patties with 5 percent poppy seed had significantly ($P < 0.05$) higher potassium, calcium, iron, manganese and zinc content. Incorporation of fat replacer in chevon patties demonstrated significant ($P < 0.05$) effect on the textural parameter except adhesiveness in carrageenan and poppy seed incorporation. Results of color value illustrated that lightness (L^*) value significantly ($P < 0.05$) increased on addition of either of fat replacers. Sensory panelist rated higher or comparable scores for CG_2 , PS_3 and SF_2 compared to control within the individual treatments of fat replacer. These were compared to determine best variant. Lowest fat and cholesterol contents were recorded in CG_2 . Moisture and fat retention was also significantly ($P < 0.05$) higher in CG_2 . The flavor score was significantly ($P < 0.05$) higher for CG_2 compared to PS_3 and SF_2 . The carrageenan added low fat chevon patties (CG_2) were found superior and widely acceptable among the sensory panelists. Hence, 0.6 % carrageenan added chevon patties were selected to develop low fat chevon patties.

Selected variant from previous experiment was incorporated with various salt substitutes (NaCl, KCl, $CaCl_2$ & Mushroom extract) in different combination. Significantly ($P < 0.05$) lower sodium and higher potassium content was observed in chevon patties substituted with different salt blends compared to control. Sodium content was reduced from 35.86% to 38.07% in the salt substituted chevon patties. Calcium content was significantly ($P < 0.05$) higher in LS_2 , LS_3 and LS_4 salt substituted chevon patties. Hardness, gumminess and chewiness values were significantly ($P < 0.05$) higher and springiness value was significantly ($P < 0.05$) lower in control chevon patties compared to treatments. Mean score for flavor and saltiness were reduced significantly ($P < 0.05$) in LS_2 , LS_3 and LS_4 compared to control. However, flavor, juiciness and saltiness as well overall acceptability scores for LS_2 were comparable to control. Hence, treatment LS_2 was selected as most suitable salt blend to develop chevon patties enriched with antioxidant.

Three different natural antioxidants viz; noni juice, plum puree and pomegranate rind extract at 1%, 3% and 5% each were attempted in the formulation. The pH value was gradually decreased with the increasing levels of natural antioxidant and differed significantly ($P < 0.05$) in noni juice and pomegranate rind extract incorporated chevon patties. Potassium and iron content was increased significantly ($P < 0.05$) with the increasing level of pomegranate rind extract. Texture profile analysis demonstrated that the springiness and cohesiveness value was differ significantly ($P < 0.05$) at 5% added level of noni juices and hardness value differed significantly ($P < 0.05$) in pomegranate rind extract added chevon patties. Lightness (L^*) value differed significantly ($P < 0.05$) between treatments of all three natural antioxidants. However, yellowness (b^*) value increased significantly ($P < 0.05$) in pomegranate rind extract and plum puree incorporated chevon patties. Sensory attributes were comparable and most acceptable

for NJ₂, PP₂ and PE₂ variants within each treatment group and compared among each other. The cooking yield, moisture retention, general appearance and juiciness differed significantly (P<0.05) between different natural antioxidants and highest for NJ₂. Sensory panelists also rated highest scores for NJ₂ and widely accepted. Therefore, chevon patties with 3% noni juice was chosen as functional patties and evaluated for storage stability under refrigeration.

The pH, TBA, FFA, peroxide value and microbial counts of functional chevon patties was significantly (P<0.05) lower as compared to control during storage. With the advancement of storage time these were increased significantly (P<0.05). However, rate of oxidation in functional chevon patties were considered lower than control as evidenced in estimated value of FFA and PV. Functional chevon patties showed significantly (P<0.05) higher scores for flavor, juiciness and texture from 12th day of storage period. Gradual decreases in all sensory attributes were noticed during storage, however, significantly (P<0.05) higher scores in functional chevon patties as compared to control was observed throughout the storage. From the study it was concluded that the developed low fat low sodium enriched with antioxidants chevon patties may be considered as health full functional product which was very well accepted up to 15 day under refrigeration.

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2. Comprehensive study on differentiation in meat quality of indigenous chicken breeds

V.P. Singh and Vikas Pathak

A comprehensive study was undertaken to evaluate the quality variations in meat of indigenous chicken breeds like Vanraja, Aseel and Kadaknath in comparison to Cobb-400. The carcass values recorded for Cobb-400 were significantly (P<0.05) higher than those of all indigenous chicken breeds. In indigenous chicken breeds, Aseel exhibited higher carcass among indigenous breeds. The total by-products yield was significantly (P<0.05) higher in Vanraja as compared other breeds of chicken while Cobb-400 recorded significantly (P<0.05) energy requirement was highest for Aseel and lowest for Vanraja in both raw as well cooked meat. L* value of Cobb-400 meat was higher than indigenous breeds while a* and b* values were higher in Aseel for raw as well as cooked meat. Kadaknath revealed lowest values of L*, a* and B*. Breast muscle fibre and bundle diameter was noticed highest in Cobb-400 while thigh muscle fibre diameter was highest in Vanraja, however, the highest thigh muscle bundle diameter was seen in Kadaknath. Endomysium diameter of both breast and thigh muscle was higher in Cobb-400 than all other breeds while perimysium diameter of breast and thigh was highest in Aseel and Kadaknath respectively. Collagen was found to be highest in thigh muscle of Vanraja and breast muscle of Cobb-400 while minimum collagen was seen in Cobb-400 thigh and Kadaknath breast muscle. Elastic fibre and reticular fibre was higher in thigh than respective chicken breasts. Breed wise Cobb-400 was having highest elastic fibres and lowest content was observed in Kadaknath. Reticular fibres were highest in Vanraja thigh and Cobb-400 breast while lowest content was seen in Aseel in both breast as well as thigh. Moisture was non significantly and fat was significantly (P<0.05) higher in Cobb-400 while protein was significantly higher in native chickens than Cobb-400. Drip loss, pH and ash content values were higher in Vanraja than all studied chicken breeds. Sodium and potassium content was non significantly higher in Cobb-400 while iron and zinc were significantly (P<0.05) higher in Vanraja and manganese in Aseel. Indigenous breeds were having higher contents of MUFA and PUFA than Cobb-400 while later was found richer in SFA. On PCR-RFLP with MspI Aseel was showing heterozygote with Cyt-b while other RE was showing monomorphic patterns.

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3. Development of starch based edible film incorporated with essential oil for enhancing shelf life of chicken nuggets

Sanjay Kumar Bharti and Vikas Pathak

The present study was undertaken to develop biodegradable edible film from sweet potato, arrowroot and tapioca starch using casting technique for preserving chicken nuggets. Preliminary trails were carried out to standardize the ingredients and processing conditions for edible film production. On the basis of physico-chemical properties, 4% (sweet potato) & 3.5% (arrowroot and tapioca) starch and 1.5% carrageenan were found optimum. Film forming solution (FFS) was incorporated with glycerol at 5-15% level separately and formed films were characteristically analyzed. The mean pH, moisture content and tensile strength decreased significantly ($P<0.05$) with increasing concentration of glycerol. The films thickness measurement exhibited uniformity. The moisture absorption of the films increased highly significantly ($P<0.01$). The *L* (lightness parameter) and transparency value significantly ($P>0.05$) decreased with increasing glycerol concentration. On the basis of physico-chemical, mechanical, barrier and optical properties, 3.5% tapioca starch, 1.5% carrageenan and 10% glycerol were found optimum. Three essential oils (EO) viz. anise, caraway and nutmeg were selected and optimized at 0.5%, 1%, 2% and 3% on the basis of minimum inhibitory concentration by tube dilution method. Dynamic oscillatory measurements of FFS showed 'weak gel' like behavior. The film's moisture content, moisture absorption, solubility, transparency and haze value shown highly significant ($P<0.01$) difference. The film thickness, pH, swelling measurement showed non-significant difference ($P>0.05$), however tensile strength, *L* values decreased significantly ($P<0.05$) with increased EO concentration. Based on the results of antimicrobial activity and sensory scores, three films containing EOs viz. anise 0.5, caraway and nutmeg 1% were selected for detailed storage stability studies of chicken nuggets for 15 days at refrigeration temperature ($4\pm 1^\circ\text{C}$). The pH, peroxide, FFA and TBA value of treatments were significantly ($P<0.05$) lower than control. The treated products revealed significantly ($P<0.05$) higher DPPH activity. The total plate count, psychrophilic and yeast and mold count were significantly ($P<0.01$) lower in treatments, whereas, coliforms were not detected throughout the storage period. All sensory attributes except saltiness of samples were significantly influenced by the storage time ($P<0.05$). The treated samples were found well acceptable during whole storage period of 15 days however the control group showed to be the most perishable group during the storage. Reduction in overall acceptability with storage time was much pronounced in control than C1, T1, T2 and T3. Retail cost of chicken nuggets was estimated to be R 242 per kg of product with packaging by sweet potato starch R 274, arrowroot R 276 and tapioca starch film R 280.

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4. Development and quality assessment of functional chicken patties

Chappalwar Anita Maroti and Vikas Pathak

The present study was envisaged to develop and assess the quality characteristics of functional chicken patties. Optimization of cooking and formulation was done. Low fat chicken patties were prepared by replacing 50% fat in the formulation by incorporating three fat replacers viz. lemon albedo, mango peel and banana peel powder at 1.0, 2.0 and 3.0% level. The values of pH, emulsion stability, fat and cholesterol content decreased, however moisture and fat retention values increased significantly ($P<0.05$) in treatments. Mineral content, textural and color parameters showed significant ($P<0.05$) effect on addition of different fat

replacers. Chicken meat patties incorporated with lemon albedo at 1%, mango peel at 2% and banana peel powder at 2% level were found optimum and these variants were further compared to select the best variant. Patties containing with 1% lemon albedo were adjudged best and used as control in further studies. Further, low fat chicken patties were fortified with green banana pulp, apple pomace and pine apple pomace at 2, 4 and 6% level. Mineral content, textural and color parameters showed significant ($P<0.05$) effect on addition of different fruit pulp. Chicken meat patties incorporated with green banana pulp at 2%, apple pomace at 4% and pine apple pomace at 4% level were found optimum on the basis of sensory evaluation, however, low fat fiber fortified chicken patties containing 2% green banana pulp were finally selected after comparing optimal variants. Functional chicken meat patties were further incorporated with natural antioxidants viz pomegranate rind powder, orange rind powder and grapefruit rind powder each at 0.50%, 0.75% and 1% level in the following experiment. Pomegranate rind powder had highest phenolic activity and DPPH values followed by grapefruit rind powder and orange rind powder respectively. Chicken meat patties incorporated with pomegranate rind powder at 0.75%, orange rind powder at 0.75% and grapefruit rind powder at 0.50% level were found optimum on the basis of sensory evaluation, however, functional chicken patties incorporated with pomegranate rind powder at 0.75% were selected after comparing the three variants. The selected variant was further incorporated with extracts of natural antimicrobials viz, aloe vera gel, lemongrass and lime peel at 0.25%, 0.50% and 0.75% after scrutinizing antimicrobial activity against various pathogenic organisms. Aloe vera gel extract exhibited significantly ($P<0.05$) higher antimicrobial activity followed by lemongrass and lime peel extract respectively. Functional chicken patties incorporated with 0.75% aloe vera, 0.75% lemon grass and 0.5% lime peel extract were selected on the basis of antimicrobial and sensory properties. The selected variants were kept under refrigeration along with control to assess storage stability. The values of pH, peroxide, TBA, FFA and microbial count of functional chicken patties were significantly ($P<0.05$) lower than control during storage. Control patties were not evaluated after 12th day due to incipient spoilage, whereas treatments were acceptable upto 28th days from microbiological and sensory point of view. Among the treatments, functional chicken patties treated with 0.75% aloe vera gel extract had significantly ($P<0.05$) higher overall acceptability scores till the end of storage. It can be concluded that well accepted functional chicken meat patties may be prepared with incorporation of 1% lemon albedo powder as fat replacer replacing 50% added fat in formulation; 2% green banana pulp as natural fiber; 0.75% pomegranate rind powder as natural antioxidant and 0.75% aloe vera gel extract as natural microbial and this product may be acceptable under refrigeration storage upto 28th day on the basis of microbiological studies and sensory evaluation.

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DEPARTMENT OF POULTRY SCIENCE

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M.V.Sc. Degree Programme

1. Effect of *Azolla* feeding on the performance of growing turkeys

Mayank Shukla and Amitav Bhattacharyya

A study was conducted to evaluate the utilization of dried *Azolla pinnata vis-a-vis* raw *Azolla* as choice feeding for turkeys under intensive system. A total of (n=72), 8 weeks old grower turkey poultts were randomly distributed into three dietary treatments having three replicates each with eight birds. The birds of the control group (T1) were fed a basal diet (CP- 19.71% & ME-2789.79 Kcal/ kg), while the other group (T2) and choice feeding group (T3) were fed 5% of basal diet replaced by dry azolla powder on DM basis and ad libitum azolla along with the basal diet respectively. There was no significant difference ($p>0.05$) in body weight and body weight gain among the treatment group during 8-16 weeks of age. Feed consumption was significantly lower in T2 & T3 compared to control group at 9th & 11th weeks of age. Similarly, average weekly feed consumption on DM basis was significantly lower ($P<0.01$) in both the azolla fed groups compared to the control group at 9th and 11th week of age. Feed consumption was numerically lower in the choice feeding group compared to the other two treatment groups during 8-12 weeks, 12-16 weeks and 8-16 weeks of age. The total dry matter intake was significantly higher ($P<0.05$) in the T3 group compared to the T2 & T1 group at 10th week of age. Further, dry matter intake was apparently higher in the choice feeding group compared to the other two treatment groups during 8-12 weeks, 12-16 weeks and 8-16 weeks of age. In addition, it was observed that 18.61%, 11.02% and 14.01% of feed was replaced by azolla on DM basis by the birds themselves during 8-12 weeks, 12-16 weeks and 8-16 weeks of age respectively in the choice feeding group. FCR was significantly better ($P<0.01$) in the choice feeding group compared to the other two experimental groups during 8-12 weeks of age (2.34 vs 2.86 & 2.64). Further, FCR was numerically better in the choice feeding group compared to the other two experimental groups during 12-16 weeks of age. In addition, over all FCR was significantly better ($P<0.05$) in the choice feeding group compared to the other two experimental groups during 8-16 weeks of age (2.83 vs 3.21 & 3.28). HA and IgM response to 1% SRBC (log₂ titre) was comparatively better in the choice feeding group compared to the 5% azolla fed group and control group. There was no significant difference in the cell mediated response to PHA-P among the treatment groups. There was no significant difference among the treatment groups in the carcass quality characteristics and cut-up-parts group. Percent small intestine weight was significantly higher ($P<0.01$) in both azolla fed groups compared to control group at 16 weeks of age. Feed cost for 1 kg gain in live weight of the bird was comparatively lower in the azolla fed groups compared to the control group. Further, the decrease in feed cost was more pronounced in the choice feeding group compared to the 5% azolla fed group. Thus, it may be concluded that azolla feeding did not have any adverse effect on the growth, immunocompetence and carcass quality characteristics of grower turkeys. Further, cost of production of turkey grower was comparatively lower in the choice feeding group followed by other two treatment groups. Thus, choice feeding with azolla and basal diet may be economic for profitable turkey farming.

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2. Influence of graded levels of Shatavari root meal on performance of coloured chicken

Binay Kumar Yadav and P.K. Shukla

Present study was conducted to evaluate the efficacy of Shatavari root meal as a dietary feed supplement in coloured chicken. Day old coloured chicken (Chabro) were distributed into seven dietary treatments having three replicates each with ten birds. The study was conducted in coloured chicken during 0-8 weeks of age. During the experiment, the birds were fed basal ration, (control) T1 - (broiler starter diet till 4 weeks and there after broiler finisher diet till eight weeks), T2- basal ration was supplemented with Shatavari root meal @ 0.25%, T3- basal ration was supplemented with Shatavari root meal @ 0.5%, T4- basal ration was supplemented with Shatavari root meal @ 0.75%, T5- basal ration was supplemented with Shatavari root meal @ 1%, T6- basal ration was supplemented with Shatavari root meal @ 1.25%, T7- basal ration was supplemented with Shatavari root meal @ 1.5%. There was no significant difference in the weekly body weight among the treatment groups. However, T2 birds had apparently higher body weight compared to other treatment groups at 2nd week of age and this trend was maintained there after throughout the experiment. T2 coloured chicken had a significantly higher ($P<0.05$) body weight gain than T1 and T6 at 2nd week of age (129.67 vs. 101.73 and 101.40g). Further, T2 coloured chicken had an apparently higher body weight gain compared to the other treatment groups throughout the experiment. T1 group chicks had significantly higher ($P<0.05$) weekly feed consumption than T2, T3, T5 and T6 group chicks at 2nd week of age (231.67 and 233.20 vs. 215.47, 215.47, 210.27 and 213.73g). T2 coloured chicken had a significantly better ($P<0.05$) feed conversion ratio than T1, T6, T7 during 2nd week. FCR was comparatively better in T2 as compared to other treatment groups during 0-4 wks, 4-8 wks and 0-8 wks of growth phase. There was no significant difference in HA, IgG and IgM response to 1% SRBC (log 2 titre) among the different treatment groups at 8 weeks of age. T3 coloured birds had significantly better ($P<0.01$) cell mediated immune response than T1, T4, T5 and T7 and comparatively better immune response than the other treatment groups at 8 weeks of age. Plasma protein was significantly higher ($P<0.01$) in T4, T5, T6 and T7 than T1, T2 and T3 at 8 weeks of age. T1 and T2 had significantly higher ($P<0.01$) plasma cholesterol than the other treatment groups. T1, T2, T3, T4 and T5 had significantly higher ($P<0.01$) plasma ALP values than T6 and T7. However, no such difference was observed in plasma uric acid, ALT and AST among the different treatment groups. No significant difference was observed in the development of digestive organs among the different treatment groups. No significant difference was observed on the carcass quality parameters and cut up parts among the different treatment groups. However, Percent heart weight was significantly higher ($P<0.05$) in T3 than other treatment groups (0.55 vs. 0.44, 0.44, 0.47, 0.45, 0.46 and 0.49). T1, T2, T3, T5, T6 and T7 had significantly higher ($P<0.01$) protein and Ca percent in breast meat as compared to T4. Similarly, T1, T2, T3, T5, T6 and T7 had significantly higher ($P<0.05$) P percent in breast meat as compared to T4. T1, T2, T3, T4, T6, T7 thigh meat had significantly higher ($P<0.01$) moisture as compared to T5. EE percent of thigh meat of T1, T2, T3 was significantly higher ($P<0.01$) as compared to T5. Similarly, protein percent in thigh meat of T1, T2, T3, T6, T7 was significantly higher ($P<0.01$) compared to T4 and T5. Total ash percent in thigh meat of T7 was significantly higher ($P<0.01$) as compared to other treatment groups. Thigh meat of T1, T2, T3, T4, T7 had significantly higher ($P<0.01$) P percent as compared to T5. Thus, it may be concluded that supplementation of dietary supplementation of Shatavari root meal @ 0.25% although not significant but increased the growth performance and improve feed conversion ratio in coloured chicken. Dietary supplementation of Shatavari root meal did not have any adverse effect on the immunocompetence traits of coloured chicken. Further, dietary

supplementation of Shatavari root meal @ 0.5% and above may reduce plasma cholesterol in chicken.

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3. Effect of feeding Sea Buckthorn and Giloe leaf meal on the performance of turkey poults

Aditya Sharma and P. K. Shukla

Present study was conducted to evaluate the efficacy of SBT and giloe leaf as a dietary feed supplement in turkey poults. A total of (n=84) day old turkey poults were distributed into four dietary treatments having three replicates each with seven birds. The study was conducted in turkey poults during 0-8 weeks of age. During the experiment, the poults were fed basal ration, (control) T1- CP-28%, ME-2800 Kcal/kg, T2- basal ration was supplemented with sea buckthorn leaf meal powder @ 0.5%, T3- basal ration was supplemented with giloe leaf meal powder @ 0.5% and T4- basal ration was fed along with supplementation of both sea buckthorn @ 0.5% and giloe leaf meal powder @ 0.5%. T2 group birds had an apparently higher body weight compared to the other treatment groups throughout the experiment. T2 turkey poults had a significantly higher ($P<0.01$) body weight gain than T3 and T4 at 7th week of age. Weekly body weight gain was significantly better ($P<0.05$) in T2 than T3 during 5th-8th weeks and 0-8th weeks of growth phase. T2 poults had a significantly better ($P<0.05$) feed conversion ratio than T3 at 1st week and at 4th week. FCR was significantly better ($P<0.05$) in T2 than other treatment groups during 5th-8th weeks phase of growth (2.08 vs 2.24, 2.27 & 2.34). Overall, FCR was significantly better ($P<0.01$) in T2 group as compared to other treatment groups (1.96 vs 2.18, 2.21 2.26). The HA and IgM response to 1% SRBC was comparatively better in the T3 group as compared to the other groups. Plasma uric acid was found significantly increased ($P<0.05$) in T1 than T3 and T4 and ALP value was significantly higher ($P<0.05$) in T1 and T3 than T2. Percent shrinkage in live weight was significantly higher ($P<0.05$) in T1 than T2 and T3. Percent liver weight was significantly higher ($P<0.05$) in T3 than T1 and T4. Percent yield of drumstick was significantly higher ($P<0.05$) in T1 and T2 as compared to T3 group. Percent small intestine weight was significantly higher ($P<0.05$) in T3 and T4 groups as compared to T1 group. Zinc level of breast (*pectoralis major*) muscles were significantly higher ($P<0.05$) in T2 and T4 as compared to T1, while ether extract in thigh (*ilio tibialis*) muscles were significantly higher ($P<0.05$) in T2 as compared to the other treatment groups. The economics of turkey poults revealed that total feed cost per kg live bird was significantly lower in T2 group than other treatment groups (62.62 vs 68.97, 69.99 and 70.11). Thus, it may be concluded that supplementation of sea buckthorn leaf meal @ 0.5% may elicit production performance of turkey poults. Further, there was no adverse effect on the blood biochemical attributes of turkey poults subjected to SBT and giloe leaf meal supplementation @ 0.5%. Further, supplementation of sea buckthorn and giloe leaf meal may have hepatoprotective effect in turkey poults. Supplementation of 0.5% sea buckthorn leaf meal may result in higher levels of zinc and ether extract in the breast and thick cuts of turkey poults. In addition, supplementation of 0.5% sea buckthorn leaf meal in turkey poults may be economical due to decreased feed cost per kg gain in body weight.

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4. Effect of Zinc Oxide nano particles on the performance of turkey poults

Upendra Kumar and Amitav Bhattacharyya

The present study was conducted to assess the effect of supplementation of zinc oxide nanoparticles in turkey poults. Day old turkey poults (n= 84) were distributed into four dietary treatment groups, having three replicates of 7 birds each. The study was conducted in turkey poults during 0-8 weeks of age. During these phase, poults were fed basal ration, T1 (control), supplemented with 60 mg/kg inorganic ZnO, T2- basal ration, supplemented with 20 mg/kg ZONPs, T3- basal ration, supplemented with 60 mg/kg ZONPs, T4- basal ration supplemented with 100 mg/kg ZONPs. T2 and T3 poults had significantly higher ($P<0.05$) body weight compared to T1 and T4 treatment groups at 2nd and 3rd week of age respectively. T2 and T3 poults had a significantly higher ($P<0.05$) body weight gain than T1 and T4 treatment groups at 1st week of age. T2 and T3 poults had a significantly higher ($P<0.05$) feed consumption than T4 at 2nd week of age. The feed conversion ratio of turkey poults supplemented with 20 and 60 mg/kg ZONPs had significantly better ($P<0.01$) at 1st week and 3rd week ($P<0.05$) as compared to the other treatment groups. Further, 20 and 60 treatment groups had a significantly better ($P<0.05$) FCR than control at 4th week. Similarly, 60 mg/kg treatment groups had a significantly better ($P<0.05$) FCR at 6th week as compared to control and 20 mg/kg treatment groups respectively. Over all FCR was significantly better ($P<0.05$) in T3 as compared to T1 and T4 and comparatively better than T2 during the experimental period. The humoral immune response (HA) was significantly higher ($P<0.05$) in T4 as compared to T2 and T3. Further HA titre was comparatively higher in T4 than T1. IgG response was significantly higher ($P<0.01$) in T1 than T2 and T3. Plasma cholesterol was significantly higher ($P<0.05$) in T3 and T4 as compared to the control group. Plasma uric acid was significantly higher ($P<0.01$) in T1, T2 and T4 than T3. Further, AST was significantly higher ($P<0.05$) in T1 and T2 than T3 and T4. The Cu-Zn-SOD value was significantly higher ($P<0.01$) in T3, T4 than T1 and T2. Similarly, LPO value was significantly higher ($P<0.01$) in the control (T1) group as compared to the nano zinc supplemented groups (T2, T3 and T4). Nano zinc supplemented groups (T2, T3 and T4) had significantly higher ($P<0.01$) percent lymphocytes than control (T1). Further, T1 had significantly higher ($P<0.01$) percent heterophils than T2, T3 and T4 groups respectively. In addition, the heterophil-lymphocyte ratio was significantly higher ($P<0.01$) in the T1 compared to the T2, T3 and T4 groups. Percent liver weight was significantly higher ($P<0.05$) in T4 group (3.69) as compare to T1 (1.66) and T2 (2.34) groups. Further, calcium level in breast muscle of turkey poults was significantly higher ($P<0.01$) in T4 as compared to T1, T2 and T3 and Zinc level was significantly higher ($P<0.01$) in T2 and T3 as compared to T1 and T4. Zn level in thigh (*ilio tibialis*) meat was significantly higher ($P<0.01$) in T2, T3 and T4 groups as compared to the control (T1) group. It may be concluded that nano zinc supplementation @ 20 mg /kg or 60 mg/ kg may elicit growth performance and improve feed conversion efficiency in turkey poults, reduce the adverse effects of stress as depicted by a decreased heterophil lymphocyte ratio in the nano zinc supplemented groups and dietary supplementation of nano zinc in turkey poults @ 20 mg/kg or 60 mg/ kg may lead to increase deposition of zinc in breast and thigh meat cuts.

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5. Effect of organic, inorganic and nano Selenium particles on the performance of turkey poults

Abhishek Verma and Amitav Bhattacharyya

The present study was designed to assess the effect of dietary supplementation of organic, inorganic and nano-selenium particles (SeNPs) on the performance of turkey poults. A total no. of (n=84), day old turkey poults were randomly distributed into four dietary treatments having three replicates each with seven turkey poults. The study was conducted in turkey poults during 0-8 weeks of age. During this phase, poults were fed with a basal diet, control group (T₁), while T₂ group was supplemented with inorganic selenium @ 0.3 mg/kg basal diet, T₃ group was supplemented with organic selenium @ 0.3 mg/kg basal diet and T₄ group was supplemented with nano-selenium @ 0.3 mg/kg basal diet. T₄ poults had significantly higher (P<0.05) body weight compared to T₁ and comparatively higher than T₂ and T₃ treatment groups at 1st week of age respectively. T₄ poults had a significantly higher (P<0.05) body weight gain than T₁, T₂ and T₃ treatment groups at 1st week of age. Further, the nano selenium supplemented group poults had a comparatively higher average weekly body weight than the other treatment groups till 6th week of age. Further, the nano selenium supplemented group poults had a comparatively higher average weekly body weight than the other treatment groups till 6th week of age. However, weekly body weight gain in the control group (T₁) was significantly higher (P<0.05) compared to other treatment groups at 7th week of age. T₂ poults had a significantly higher (P<0.05) feed consumption than T₃ and T₁ and comparatively higher than T₄ at 3rd week of age. FCR was significantly better (P<0.05) in T₁, T₃ and T₄ than T₂ during 0-4th week of growth phase. The humoral immune response (HA) titre was comparatively higher in T₄ than other treatment groups. IgG response was significantly higher (P<0.05) in T₃ than T₁ and T₂ and comparatively higher than T₄ after 8 weeks of age. HDL and SOD levels were significantly higher (P<0.01) in T₄ than T₁, T₂ and T₃ groups. The Nano-selenium supplemented group T₄ had significantly higher (P<0.05) percent lymphocytes than other treatment groups. Further, T₁ and T₂ had significantly higher (P<0.01) percent heterophils than T₃ and T₄ groups respectively. In addition, the heterophil lymphocyte ratio was significantly lower (P<0.05) in T₄ compared to other treatment groups. In histological study T₄ showed highest amount of lymphoid cell proliferation in bursa and caeca, as compared to other treatment groups. Percent protein of breast muscle (*pectoralis major*) was significantly higher (P<0.01) in T₂, T₃ and T₄ than T₁ and Se level was significantly higher (P<0.01) in T₄ as compared to T₁, T₂ and T₃. Further, percent protein of thigh muscle (*ilio tibialis*) was significantly higher (P<0.01) in T₃ and T₄ as compared to T₁ and T₂ and Se level was significantly higher (P<0.05) in T₃ compared to T₁ and T₂ and comparatively higher than T₄. Further, Se level was significantly higher (P<0.05) in T₄ as compared to T₁ and comparatively higher than T₂. Thus, it may be concluded that supplementation of nano selenium particles @ 0.3 mg/ kg diet may reduce the adverse effects of stress as depicted by decreased heterophil lymphocyte ratio, increased lymphocytic proliferation in lymphoid organs and enhanced immunity of turkey poults. Further, dietary supplementation of nano selenium particles @ 0.3 mg/ kg diet lead to increase in percent protein in breast and thigh meat cuts and increase in selenium concentration in breast and thigh meat cuts.

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6. Effect of organic, inorganic and nano Chromium particles on the performance of turkey poults

Deshmukh Abhishek Avinashrao and Amitav Bhattacharyya

The present study was conducted to assess the effect of supplementation of organic, inorganic and nano particles of chromium on performance of turkey poults. Day old turkey poults (n= 120) were distributed into four dietary treatment groups, having three replicates of 10 birds each. The study was conducted in turkey poults during 0-8 weeks of age. Poults were fed T1 (control) basal ration, T2- basal ration supplemented with 0.5 mg/kg diet chromium chloride(inorganic chromium), T3- basal ration supplemented with 0.5 mg/kg diet chromium picolinate (organic chromium), T4- basal ration supplemented with 0.5mg/kg diet nano particles of chromium picolinate. No significance difference was observed in average weekly body weight of birds during entire experimental period. No significance difference was observed in average weekly body weight gain in birds during entire experimental period except at 6th week where birds in T3 group had significantly higher (P<0.01) body weight gain than T1, T2 and T4. The results indicated that there was no significant difference in the average weekly feed intake of birds among various treatment groups throughout the experimental period. However, T2 birds had significantly higher (P<0.05) feed intake than T4 birds during 0-4 weeks. No significance difference was observed in feed intake during 4-8 weeks and 0-8 weeks. There was no significant difference recorded in FCR of birds during the entire experimental period except at 6th week where FCR was significantly better (P<0.05) in T3 as compared to T4 and comparatively better than other treatment groups. However, when FCR during different phases of growth during 0-8 weeks of age was calculated, significantly better (P<0.05) FCR was observed in T3 than T2 and comparatively better than T1 and T4 during 0-4 week. There was no significant difference in FCR of birds during 4-8 weeks and during 0-8 weeks of growth phase. There was no significant difference in total immunoglobulins, IgG and IgM values among treatment groups. Although numerically higher values of total immunoglobulins, IgG, IgM were observed in T4 as compared to the other treatment groups. There was no significant difference observed in the foot web index of birds though the cell mediated immune response measured as foot web index was comparatively better in the nano chromium supplemented group compared to other treatment groups after 8 weeks of age. There was no significant difference observed in serum cortisol, IgG and IgM concentration among treatment groups although apparently higher values were recorded in T4 as compared to other treatment groups. There was no significant difference in plasma total proteins, plasma uric acid, ALT, AST, ALP, plasma total cholesterol and HDL cholesterol values among different treatment groups. SOD values were significantly higher (P<0.05) in T4 than T1 and comparatively higher than T2 and T3. LPO values were significantly higher (P<0.05) in T1 as compared to T2, T3, T4. Histological segments of bursa, thymus, spleen and liver showed mild changes in histoarchitecture in organic chromium supplemented group as compared to control group. Significantly higher (P<0.05) dressing percentage was observed in T3 than T1, T2 and T4. There was no significant difference in percent shrinkage and percent ready to cook yield. There was no significant difference in yield of cut up parts among different treatment groups except percent neck yield, Percent neck yield was significantly higher (P<0.05) in T3 than T1 and comparatively higher than T2 and T4. There was no significant difference recorded in development of the digestive organs among the treatment groups except small intestine weight and cecal length. Small intestine weight was significantly higher (P<0.05) in T1 than T2. Cecal length was observed significantly higher (P<0.05) in T4 than T2. Significantly higher weight (P<0.05) of thymus was observed in T4 than T1 and comparatively higher than T2 and T3. Similarly significantly higher (P<0.05) weight of bursa was observed in T4 than T1,

T2 and T3. The data obtained on proximate analysis of breast (*pectoralis major*) and thigh (*iliotibialis*) muscle showed that there was no significance difference observed in moisture, dry matter, crude protein, ether extract, total ash and calcium content of the muscle. Chromium content was significantly higher ($P<0.05$) in T4 than T1 and comparatively higher than T2 and T3 in both breast (*pectoralis major*) muscle and thigh (*iliotibialis*) muscle. Hence dietary supplementation of nano chromium @0.5mg/kg diet may reduce adverse effect of heat stress as depicted by decreased LPO and increased SOD values. Further, supplementation of nano chromium @0.5mg/kg diet resulted in increased deposition of chromium in breast and thigh meat cuts.

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7. Effect of dietary supplementation of Glutamine on the performance of turkey poults

Dheeraj Kumar Singh and P.K. Shukla

The present study was conducted to assess the effect of supplementation of glutamine in turkey poults. Day old turkey poults ($n=84$) were distributed into four dietary treatment groups, having three replicates of 7 birds each. The study was conducted in turkey poults during 0 - 8 weeks of age. The birds of the control group (T1) were fed a basal diet, while T2 group was supplemented with 0.5% glutamine along with the basal diet, T3 group was supplemented with 1% glutamine with the basal diet and T4 group was supplemented with 1.5% glutamine along with basal diet. Data on body weight changes indicated that T2 and T3 poults had higher body weight compared other treatment groups in the entire experimental period. The T3 poults had a significantly higher ($P<0.05$) body weight gain and better FCR than other treatment groups. HA titre and IgM response was significantly higher ($P<0.05$) in T3 as compared to other treatment groups. Similarly, IgG response was comparatively better in T3 group as compared to the other treatment groups after 8 weeks of age. However, foot web index value was comparatively better in the 0.5% Gln supplemented group compared to the control group after 8 weeks of age. Serum IgG, IgM and cortisol level was significantly higher ($P<0.05$) in T3 as compared to T1 and comparatively higher than other treatment groups. Total plasma protein and total plasma ALP values was significantly higher ($P<0.05$) in T2 as compared to T1 and comparatively higher than T3 and T4. The SOD value was significantly higher ($P<0.05$) in T2 than other treatment groups. LPO value was significantly higher ($P<0.05$) in the control group as compared to the glutamine supplemented groups. Supplementation of glutamine at different levels showed normal histoarchitecture of both liver and spleen in all the groups. The changes in group T2 and T4 showed more prominent structures in tissue sections in both liver and spleen such as hepatocytes in liver and germinal centers with red pulp in spleen as compared to control group. Percent shrinkage was significantly higher ($P<0.05$) in the T3 group as compared to T4. Bursa weight was significantly higher ($P<0.05$) in T3 group. Breast percent was significantly higher ($P<0.05$) in T2 group as compared to T4 groups. The percent crude protein level in breast muscle of turkey poults was significantly higher ($P<0.05$) in T3 as compared other treatment groups. It was concluded that Gln supplementation @ 1% elicited the growth performance and immunocompetence traits of turkey poults. Further, supplementation Gln @ 1% resulted in significantly higher SOD and lower LPO values in the serum which reflected better antioxidative status of the turkey poults.

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DEPARTMENT OF VETERINARY ANATOMY

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3	Anatomical observations on the centers of ossification in the long bones of appendicular skeleton in prenatal goat (<i>Capra hircus</i>)	Avnish Chaudhary	Dr. Archana Pathak	2017	65
4	Observations on the gross anatomical, histomorphological and certain histochemical change in foetal goat (<i>Capra hircus</i>) spleen	Nishant	Dr. Ajay Prakash	2017	66
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7	Gross, histological and certain histochemical studies on the pancreas of Chabro chicken	Renu Yadav	Dr. Ajay Prakash	2018	69
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M.V.Sc. Degree Programme

1. Gross, histological and certain histochemical studies on the uterus in different stage of pregnancy in goat (*Capra hircus*)

Vijay Kumar and Shri Prakash Singh

The present study was conducted on the 18 healthy and normal goat uteri of non-descript breed (*Capra hircus*) varying from day old to 150 days of gestation. The uteri were assigned in to three groups according to their gestational age; Group I (0-50 days), II (51 -100 days) and III (101-till term). After recording gross morphological feature and measurements, the tissue were fixed in one of the fixative for studying the histological and histochemical studies. Histological and Histochemical study of cotyledon was not noticed in early pregnancy period due to very loose attachments between caruncle and cotyledon.

The length, diameter and thickness of gravid horn, non gravid horn, body and cervix were significantly increased with the advancement of pregnancy. Weight of uterus was also significantly increased as pregnancy progressed. The thickness of endometrium, myometrium, perimetrium and total thickness of uterine wall in gravid horn was significantly increases with advancement of pregnancy.

During early pregnancy the surface epithelium was denuded from most of the area of endometrium. During mid pregnancy surface epithelium was reappearing in discontinuous manner. During late pregnancy the surface epithelium reappeared in most of the areas. It was simple columnar to pseudostratified.

During early pregnancy uterine glands was appeared simple tubular with occasional branching towards their base. The epithelium of different part of uterine glands varies from simple columnar to pseudostratified ciliated but mostly simple columnar and some places simple cuboidal epithelium are also present during different stage of pregnancy. During late pregnancy the epithelium of neck, middle and basal segment of uterine glands were simple columnar. The epithelial height of the uterine gland in gravid and non- gravid horn range increases significantly with advancement of pregnancy. The size of sinus was increases as pregnancy progressed and during late stage of pregnancy sinuses of endometrial glands were merged to form large size sinuses. Between the endometrial glands the collagen and reticular fibers decreased and elastic fibers increased as pregnancy progressed. Every segment of uterus the outer and inner lumen diameter and epithelial height of neck, middle and basal segments was significantly increased as pregnancy progressed.

The diameter of the individual muscle cells in myometrium were increased several times as pregnancy advanced. Particularly the thickness of tunica muscularis layer increases due to hypertrophy and hyperplasia

The thickness of perimetrium not varied as pregnancy progressed.

Surface epithelium eroded from maximum place during early stage of pregnancy. During mid stage of pregnancy was observed that the epithelium was simple columnar, pseudostratified columnar and stratified columnar type. But mostly pseudostratified columnar and stratified columnar type was present. During late stage of pregnancy was observed that the surface epithelium was simple columnar to pseudostratified columnar but mostly simple columnar type. Stratified columnar epithelium observed very few place

The lamina propria was consisted of densely arranged collagenous fibers and also contains with few reticular and elastic fiber. As pregnancy progressed the number of collagen and reticular fibers were increased and elastic fibers were decreased.

The fibro-muscular layer of the cervix consisted of circularly arranged smooth muscle. It's comprised of fine collagenous bundles and the elastic fibers were rarely present. The muscular core highly developed in the primary fold towards the external os.

The numbers of placentomes range from 92 to 153 which were significantly increased from early to mid pregnancy and subsequently significantly decrease during late pregnancy. Placentome appeared oval shape with convex surface during early pregnancy and these became concave with a thick margin during mid pregnancy. In the late pregnancy the placentome were disc like with a shallow depression and relatively thinner margin.

The length, width and thickness of the placentome were significantly increases with the advancement of pregnancy.

Crypt formation first appeared at 23 days of pregnancy. Microscopically, cotyledon was first appeared at 43 days of pregnancy. The attachment between two components of placentome very much loosens in early pregnancy, firm attachment during mid pregnancy and again gradually became looser during late pregnancy for impending parturition.

The caruncular epithelium appeared extensively denuded. The caruncular stroma comprised of superficial and deeper connective tissue zone, with the development of cryptal zone there was reduction of the superficial and deeper connective tissue zone. With the development of cryptal zone there was a reduction of superficial and deeper connective tissue zone.

Cryptal epithelium occurred in small patches during mid pregnancy while it was present in larger area during late pregnancy. The cryptal epithelium comprised of simple squamous to cuboidal and rarely columnar cells with numerous giant cells. The latter increased in the subsequent stages.

Symplasmic bodies increased with the advancement of mid pregnancy and were gradually reduced in the late pregnancy. The symplasmic body would be representing the worn out tissue of the intercrypt columns during the process of their remodeling. These would also be a source of histotroph during the maximal nutritional requirement of the conceptus.

The villi consistently branched with the progressed of gestation. The trophoblast lining the villi appeared cytotrophoblastic to syntrophoblastic in nature. The latter was relatively more evident with the advancement of pregnancy

The villus epithelium comprised of cuboidal to columnar cells with the abundant giant cells. Binucleate giant cells predominated during mid pregnancy while during late pregnancy multinucleate giant cell increased in number. Giant cells particularly binucleate types were actively phagocytic.

Arcade epithelium in the region of extensive erythrophagocytosis was frequently detached, degenerated and was probably phagocytosed by the adjoining cells. On this basis it appeared that arcade epithelium was concerned mainly with the histotrophic nutrition while villus epithelium provided haemotrophic nutrition to the foetus.

Accumulation of increasing amount of hematoma during mid pregnancy was considered to act as a source of histotrophic nutrition and late gestation assisted in the process of separation. Further histochemical reaction revealed that this mass provided a rich source of nutrition, particularly iron in to the foetus. The placentome in goat appeared syndesmochorial to epitheliochorial in most areas.

The cytoplasm of surface epithelium of endometrium and endometrial glands showed mild to intense reaction during different stage of pregnancy with Periodic acid Schiff's reaction. The cytoplasm of surface epithelium, stratum compactum of endometrial layers showed very mild activity in early and late pregnancy where as during mid pregnancy showed moderate activity towards acid mucopolysaccharides. The cytoplasm of epithelial cells of endometrium and endometrial glands showed mild to intense activity towards alkaline phosphatase during different pregnancy. Surface epithelium of

endometrium layers endometrial glands were mild activity during different stages of pregnancy towards lipid and acid phosphatase activity.

The cytoplasm of epithelial cells of in endometrial glands were showed mild to intense reaction during different stage of pregnancy towards Sudan black B and nuclei of different segments of endometrial glands varied between mild to intense Feulgen reaction.

The syncytial mass of inter crypt columns was mild PAS positive while cytoplasm of binucleate giant cells were strongly PAS positive. Reaction of PAS positive substances, acid mucopolysaccharides and alkaline phosphatase in the arcade zone epithelium varied from negative to moderate. Cytoplasm of cuboidal to columnar cells of villus epithelium exhibited trace to mild reaction for PAS positive material. It occurred mild to moderate amounts in uninucleate and multinucleate cells while the binucleate giant cells revealed moderate to intense concentration.

There was intense reaction for alkaline phosphatase in the caruncular area during early pregnancy while it was mild to moderate during mid and late pregnancy. Binucleate giant cells showed greater concentration of this enzyme other than other cells. Increased alkaline phosphatase activity in the caruncular area during early pregnancy. A faint reaction with acid phosphatase activity was notice in the villous epithelium while cytoplasm of binucleate giant cells exhibited larger amounts.

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2. Gross histological and histochemical studies on the pancreas in prenatal goat (*Capra hircus*)

Dharmendra Singh and Ajay Prakash

The present study was conducted on the 24 goat foeti of either sex ranging from 42 days to full term gestation. The material was divided into early prenatal (0 to 50 days), mid prenatal (51 to 100 days) and late prenatal (101 to till term) periods. After recording morphometrical parameters the tissue were fixed in various fixative for histological and histochemical studies. Morphometry was not noticed in early prenatal period due to very small size of pancreas. In all goat foetuses the pancreas lied in the abdominal cavity partly on right and partly on left side of median plane. Its left and right lobes were creamish white in color and were fused to form an irregularly quadrilateral mass. From 53 days the topographic location of foetal goat pancreas to adjacent organs was changes. The biometric parameters via; weight, length and width of the pancreas were significantly increased with the advancement of foetal age.

At 42 and 44 days gestation the primordium of foetal goat pancreas lied in close vicinity of developing duodenum and abomasum. Initially the parenchyma consisted of pancreatic tubules lined by simple cuboidal, columnar and stratified epithelium with spheroid or spherical nuclei. At various places, particularly the end tubules formed tubular buds. In addition to these free bud cells were also found in the intertubular areas. From 69th day of gestation the parenchyma began to divide in to lobes and lobules. With the advancement of foetal age occurrence of tubules, buds and free buds cells gradually decreased. Up to 59 days gestation the reticular fibers, collagen fibers and elastic fibers were absent in foetal goat pancreas. Fine reticular fibers along with fibroblasts were first observed at 69 days of gestation. From 76 days onwards reticular fibers and fibroblast were more pronounced in capsule, interlobar and interlobular areas. Moreover, these encircled the tubules, buds, acini, islets and blood vessels. Beyond this the reticular fibers gradually became coarser with the increase in age of foetus. Fine collagen fibers were seen around vicinity of blood vessels at 115 days and distinct collagen fibers were observed at 118 days of gestation.

The beginning of the process of acini formation in the foetal goat pancreas was first observed at 76 days of gestation where cells of several buds became loosely arranged and their peripheral cells began to arrange in the form of either a complete or a part of circle to form a central lumen. From 115 days onwards the cytoplasm of few acinar cells around the nucleus had a little bluish tinge. From 132 to full term foetus the pancreas was chiefly formed by developed acini, however at few places some immature acini were also found. From 115 days centroacinar cells were seen in few acini. At 69 days of gestation it was observed that the ducts were originated by the branching of tubules. The differentiation of the duct from tubules gradually became more pronounced with the advancement of foetal age. The lining epithelium of the smaller ducts was simple cuboidal having spherical and spheroidal vesicular nuclei with eosinophilic cytoplasm. However, larger ducts were lined by a single layer of low columnar or cuboidal cells. Occasionally at few places, the ducts were either lined by two layers of cuboidal cells or by pseudo stratified epithelium.

At 42 and 44 day of gestation the endocrine part of foetal goat pancreas was seen in the close vicinity of tubules in the mesenchyme. The cytoplasm of the cells of these developing islets of Langerhans was relatively darker eosinophilic than the cytoplasm of the mesenchymal and tubular epithelial cells and gave them muddy appearance. The endocrine part was developed by two methods, the first was direct proliferation of tubular bud cells, second tubules enveloped the blood vessels and whole mass proliferated and differentiated into endocrine cells. Up to 56 days the islets were generally found in apposition with the tubular parenchyma but at some places these began to separate from the tubules and partially or fully encircled by the blood capillaries. Distinct capsule around the islets was observed at 76 days onwards which was made up of reticular fibers, fibroblasts and mesenchymal cells. From this stage an empty space was usually found between its capsule and islets. The average diameter and occurrence of islets gradually increased with the advancement of foetal age. Generally central cells of islets were loosely arranged than peripheral parts. Up to 69 days the blood vessels were found within the islets as well as at its periphery beyond this day of gestation the blood vessels were absent but free erythrocyte were observed in some stages. From 87 days the islets chiefly consisted of larger dark eosinophilic spheroid and polyhedral cells with large spherical or oval vesicular nuclei. In addition to these the islets contained some smaller light colored cells which had relatively darker spheroid and oval nuclei. These dark and light color cells were alpha and beta cells, respectively. As age advanced the percentage of beta cells were increased and scattered in the whole islets.

In all the structural components (Tubules cells, bud cells, islets cells, acinar cells, pancreatic duct cells, mesenchymal cells, stromal tissue and wall of blood vessels) of foetal goat pancreas showed positive reaction for glycogen, mucopolysaccharides, acid mucopolysaccharides and lipids in mid and late prenatal period except in the stromal tissue where lipids were almost absent. In both groups the reaction for alkaline phosphate in mesenchymal cells, islets, acinar cells, stromal tissue and wall of blood vessels showed positive reaction for this enzyme. The nuclei of the cells of all structural components in goat foetal pancreas showed weak reaction for DNA whereas these showed negative reaction of acid phosphatase.

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3. Anatomical observations on the centres of ossification in the long bones of appendicle skeleton in prenatal goat (*Capra hircus*)

Avanish Chaudhary and Aarchana Pathak

The study was conducted on the long bones of fore and hind limbs of apparently healthy embryos/ fetuses of non-descript goats irrespectively of sex from 1 to 150 days of gestation, divided into three groups viz; Group I (≤ 50 days of gestation), Group II (>50 days to ≤ 100 days of gestation) and Group III (> 100 days till parturition) comprising of 8 embryos/ foetuses in each group. The study revealed that in early prenatal goats the fore limb bud was laid as a conspicuous extension from the cranio-lateral aspect of trunk region on 35 days of gestation. It became more elongated and divided into two segments on 37 days, three segments on 38 days and four segments on 39 days of gestation to form shoulder, arm, forearm and manus regions. The bud for the hind limb was seen as a paddle shaped out growth from the caudal aspect of trunk region on 35 days of gestation. It became more elongated on 37 and 38 days of gestation. On 39 days three distinct segments were present which would form thigh, leg and pes regions. On 44 days miniature fore and hind limbs were seen in cartilaginous form. Histologically, on 35 days of gestation, the primordium comprising of mesenchymal cells surrounded by several blood vessels was laid. At 37 days, the anlagen were formed of early stages of developing cartilages. On 39 days they presented typical chondrocytes in the capsules surrounded by a dense layer of matrix. The peripheral mesenchymal cells formed the perichondrium. At 46 days of gestation the centres of ossification were observed for the diaphyses of humerus, radius and ulna (Alizarin Red-S stained specimen). The centre of ossification for the shaft of metacarpals, appeared on 55 days of gestation and for I phalanx and II phalanx it appeared on 62 days of gestation. The total length and ossified length of various long bones of fore limb increased with the increase in age (from 46-148 days of gestation). The centres of ossification for the proximal and distal extremities of humerus appeared at 119 and 135 days of gestation respectively. In radius, the centres of ossification for proximal and distal extremities were exhibited on 135 days of gestation. The centre of ossification for olecranon tuberosity of ulna appeared on 129 days of gestation. The centres of ossification for the distal and proximal extremities of metacarpus were exhibited on 135 and 148 days of gestation, respectively. The centres of ossification for the diaphyses of femur, tibia and fibula, appeared on 46 days and for metatarsus on 55 days of gestation (Alizarin Red-S techniques). The centres of ossification for the shaft of I and II phalanges were exhibited on 62 days of gestation. The centres of ossification for the head of femur and distal extremity were observed on 129 days of gestation but for trochanter major it appeared on 135 days of gestation. In tibia the centres of ossification for proximal and distal extremities appeared on 135 days of gestation. The shaft of fibula was not traceable after 55 days of gestation. The centre of ossification for the distal extremity of metatarsus was seen on 135 days of gestation. Radiographically, the centres of ossification for the diaphyses of humerus, radius, ulna and metacarpals were exhibited on 71 days of gestation. The centres of ossification for distal and proximal epiphyses of humerus were detected on 129 and 135 days of gestation, respectively. The centres of ossification for proximal and distal epiphyses of radius exhibited on 129 and 135 days of gestation. The centre of ossification for olecranon tuberosity of ulna appeared on 148 days of gestation. The centre of ossification for distal extremity of metacarpals was exhibited on 135 days of gestation. The centres of ossification for the shaft of I and II phalanges in fore limb appeared on 71 and 82 days of gestation and for proximal extremity of both phalanges it appeared on 148 days of gestation. The centres of ossification for the diaphyses of femur, tibia and metatarsals were detected on 71 days of gestation. The centres of

ossification for the proximal and distal epiphyses of femur were detected on 129 days of gestation, but for trochanter major was appreciated on 135 days. The centres of ossification for proximal and distal extremities of tibia appeared on 129 and 135 days, respectively. For the distal extremity of metatarsals, the centre of ossification was exhibited on 135 days of gestation. The centres of ossification for the diaphyses of I and II phalanges appeared on 71 and 82 days of gestation, respectively. For the proximal extremity of both the phalanges the centre of ossification appeared on 148 days of gestation. It is concluded that, the early centre of ossification can be detected quite satisfactorily by Alizarin Red-S technique. Radiographically these can be detected either on the same day or after that. Thus among the two methods, the staining methods is more of academic importance while the radiographic procedure is more practical and can be effectively used for diagnostic purposes.

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4. Observations on the gross anatomical, histomorphological and certain histochemical change in foetal goat (*Capra hircus*) spleen

Nishant and Ajay Prakash

Gross anatomical, histomorphological and certain histochemical studies were conducted on the spleen of 24 healthy and normal goat embryos/ foeti of either sex at different gestation. Approximate age of embryos/ foeti was estimated and the material was grouped in to I (up to 50 days), II (51- 100 days) and III (101- till term). The primordium of spleen in goat foetus was first observed at 32 days gestation. Up to 50 days gestation it was in the form of white thickening on the dorso medial aspect of the stomach tube near its cranial end. It became nearly triangular between 51 to 58 days gestation and quadrilateral in outline from 70 days gestation. Between 51 days to 58 days gestation the splenic primordium changed its position from dorso medial aspect to dorso lateral aspect of the developing rumen. The visceral surface was toward the developing rumen and parietal surface was towards the ribs. The hilus became distinct near the cranio dorsal angle on the visceral surface at 81 days of gestation. At 32 days gestation the primordium consisted of densely packed mesenchymal cells in the dorsal mesogastrium. The dorsal surface of the spleen in goat foetus up to 41 days gestation was covered by a layer of simple squamous epithelium, the mesothelium. At 46 days gestation the fibroblasts began to appear. The capsule was distinctly demarcated from the parenchyma at 70 days gestation and was more cellular. From 83 days gestation the capsule was divided in two distinct layers whereas, from 101 days onwards it was distinctly divided in to three layers. In 148 days old goat foetus the smooth muscle cells formed a continuous and dense layer in the capsule close to the parenchyma. At age of 76 days gestation trabeculae were observed for the first time in the spleen of goat foetus. From 135 days gestation onwards trabeculae became highly pronounced which at some places completely divided the parenchyma in to the compartments. Fine but short reticular fibers were observed for the first time in the spleen of 46 days old foetus; these were sparsely distributed in the parenchyma but were relatively dense towards the surface of the organ. From 55 days gestation the reticular fibers were around the developing splenic sinuses and around the developing blood vessels. From 76 days gestation these fibers were condensed to form capsule and trabeculae. In goat foetus fine collagen fibers were observed for first time in the splenic capsule and trabeculae at 76 days gestation. At 148 days gestation reticular, elastic and collagen fibers were found in the splenic nodules around the nodular artery. At 32 days gestation the parenchyma of foetal goat splenic primordium consisted of condensed mass of densely packed and irregularly arranged mesenchymal cells along with few blood islands with nucleated

erythrocytes. The red and white pulps were first time observed in goat foetal spleen at 55 days and 70 days gestation, respectively. From 70 days gestation the major part of splenic parenchyma had rich network of sinuses which were filled with densely packed erythrocytes. From 83 days of foetal age thin bundles of smooth muscle cells were noticed in the parenchyma and from 89 days gestation onwards aggregated plasma cells were observed. From 107 days gestation ill developed network of splenic cords was observed which became distinct at 121 days gestation. The megakaryocytes were observed for the first time in the splenic red pulp at 63 days gestation and their occurrence gradually increase from 81 to 121 days gestation beyond this their occurrence was greatly reduced. The blood vessels were fewer in the splenic parenchyma at 51 days gestation but these became distinct and numerous from 70 days gestation onwards. The trabecular arteries were observed from 104 days gestation onwards. The nodular arteries were observed from 107 days gestation and were found usually in the center of the nodules. In late prenatal period large sinuses were formed by the confluence of small sinuses. In 70 days old foetus the beginning of initial stages of periarteriolar lymphatic sheath (PALS) differentiation was observed as concentrically arranged mesenchymal cells and fibroblasts around small arteries. The lymphoblasts and lymphocytes in PALS were observed first time at 81 days and 101 days gestations, respectively. The splenic nodules in the white pulp of goat foetus were first observed from 107 days gestation. The mesenchymal cells of splenic parenchyma and mesothelial cells that covered the spleen showed mild to moderate reaction for PAS positive substances whereas, the stromal tissue showed intense reaction for PAS. The mesenchymal cells, stromal tissue and wall of blood vessels showed mild to moderate reaction for bound lipids. In early prenatal period the nuclei of mesenchymal cells showed moderate to intense Feulgen's reaction. In mid and late prenatal goat the nuclei of mesenchymal cells, lymphoblasts, lymphocytes exhibited mild to moderate reaction. It was concluded that up to 55 days gestation the foetal goat spleen was engaged in the erythropoiesis. Between 63 to 121 days it was active in thrombopoiesis. From 70 days to 135 days gestation it had storage function and the spleen in goat foetus started worked as lymphoid organ from 83 days onwards but it became a proper lymphoid organ from 107 days gestation onwards, however, the germinal centers were not observed during the entire study. It might be due to lack of exposure of foetus to the antigen.

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5. Gross, histological and certain histochemical studies on the thyroid gland of Chabro chicken reared in summer and winter seasons

Amit Singh Vishen and Varsha Gupta

Gross, histological and certain histochemical studies were conducted on thyroid gland of eight to ten weeks old 24 apparently healthy Chabro chickens procured from Poultry Farm, DUVASU, Mathura after approval of CPCSEA. For this study the chickens were divided into two groups consist of 12 chickens in each group (six male and six female) reared in summer and winter season. The thyroid gland of chabro chicken was light pink coloured two oval bodies i.e. right and left thyroid located on the ventral surface of the base of the neck just caudal to the junction of subclavian and common carotid arteries. The left thyroid gland was situated more cranially than the right thyroid. The length and thickness of right thyroid were more than left. Weight of the thyroid gland was more in male in summer and almost equal in winter. Length and width in male and thickness in female was more in summer while in winter length in male and width and thickness in female was more. There was 1.8 and three times increase in carcass weight of chicken and weight of thyroid gland, respectively from

summer to winter season. The average weight of chicken and thyroid, all biometrical parameters of thyroid viz. length, width and thickness were more in winter. Histologically, the thyroid gland was composed of stroma (capsule and interfollicular connective tissue) and parenchyma (follicles and 'C' cells). The capsule had outer thick adipose and inner thin fibrous layers. The follicles were filled with colloid produced by the cells lining the follicles. On the basis of diameter of follicles were classified into small, medium and large follicles. The percentage of small follicle was more followed by medium and large follicles. The follicles were lined by simple squamous epithelium in summer and cuboidal epithelial cells in winter. The colloid was less in amount and was lightly eosinophilic in active follicles. Bound lipids, protein and alkaline phosphatase reaction was intense in follicular cells. The nuclei of follicular cells exhibited intense positive Feulgen's reaction for DNA. The colloid showed strong reaction for PAS in both seasons. All micrometrical parameters, amount of reticular fibers, percentage of large and active follicles and concentration of T3, T4 and TSH were higher in winter. The thyroid of the chabro chicken was structurally and functionally more active in winter season as compared to summer season.

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6. Morphological, histological and histochemical studies on the post hatch development of the bursa of fabricius in Chabro bird

Anand Singh and Shri Prakash Singh

The present study was conducted on the bursa of Fabricius of Chabro bird. For this study 30 healthy birds were selected after the hatching irrespective of the sex and were divided into five groups; group 1st (0 day), 2nd (30 day), 3rd (60 day), 4th (90 day) and 5th (150 day) of age. The bursa of Fabricius was located on the dorsal aspect of cloaca and connected to the dorsal wall of the proctodeum by small stalk. The shape of the bursa was oval and it was creamy white colour in all groups. The luminal surface of the bursa of Fabricius was occupied by number of large and small folds called primary and secondary plicae, respectively. Biometrical studies revealed the significant increase in the body weight with advancement of age. The weight of bursa significantly increased upto 4th group then significantly decreased. The bursal index significantly increased in 1st and 2nd group and then significantly decreased in 4th and 5th group. The bursal length, width and thickness, plical length and height significantly increased upto 4th group and significantly decreased after 4th group which plical width start decreased from 3rd group. Histologically, the wall of the bursa of Fabricius was comprised of three basic layers from inwards to outward, tunica mucosa, tunica muscularis and tunica serosa. There was significant increase in thickness of all tunics. Length of primary and secondary plicae significantly increased from group 1st to 4th group and significantly decreased from 4th to 5th group. The lining epithelium was made up of pseudostratified columnar epithelium while it was simple columnar in crypts. The pseudostratified columnar epithelium was consisted of three types of cells. Type I cells were columnar cells with elongated nucleus. Type II cells were basally placed with round nucleus. Type III cells were goblet cells found among the columnar cells. The epithelium covering the plicae were divided into two types viz, follicle associated epithelium (FAE) or epithelial tuft with pale columnar cells which was in direct contact with medulla of the lymphoid follicles and the interfollicular epithelium (IFE) covering the remaining part of the plicae consisting of darkly stained columnar cells i.e. between the follicles. The height of IFE was more than FAE in all group. Length and width of primary and secondary plicae and length of FAE and IFE significantly increased upto 4th group and significantly decreasing from 4th to 5th group. The lamina propria was consisted of connective tissue framework filled with lymphoid follicles of different shape and size in all age groups. The connective tissue consisted of network of fine collagen and numerous reticular fibres surrounding the follicles and scanty elastic fibers especially around blood vessels. The amount of collagen and reticular fibres increased with the

advancement of the age. Each plica was completely filled with follicles separated by interfollicular connective tissue. Each lymphoid follicle was comprised of outer dark cortex and inner pale large medulla separated by a cortico-medullary junction. In 1st group the cortex and medulla of follicles could not be differentiated. The middle tunica muscularis layer consisted of an outer circular and inner longitudinal layers of smooth muscle fibres and longitudinal layer. A thin serosal layer was composed of connective tissue fibres. Thickness of tunica muscularis and tunica serosa increased significantly up to 4th group then it significantly decreased. After 3rd group relatively less increment in the weight of bursa, bursa length, width, height, plical length, width and thickness and from 4th group to 5th group these observations were significantly decreased showed the involuntary changes started from the 3rd group. Histologically, depletion of lymphocyte population from the periphery of cortex, medulla gives the acinar structure of the follicles, separation of cortex from adjacent follicles, vacuolation in the medulla, formation of cyst in epithelium and medulla of follicles, size of the bursal follicles was found to decreased, height of epithelium decreased, epithelial attenuation, vacuolation folding and detachment were noted over the plical surface and interplical area. With the decreased in size of follicles the amount of collagen and reticular fibers increased. The tunica muscularis layer deorganised. Histochemically, the epithelial lining, showed mild to moderate PAS positive reaction, mild PAS positive reaction were observed in the cortex, medulla, corticomedullary junction which increased as age advanced. The epithelial lining showed moderate to intense activity of acid mucopolysaccharides which decreased with the advancement of age. The FAE and IFE corticomedullary junction showed mild activity in 1st group and increased with advancement of age. Alkaline phosphatase activity were observed in lining epithelium bursal follicles from group 2nd onwards the activity of alkaline phosphatase decreased with advancement of age. The acid phosphatase activity was mild in epithelial lining and follicles and moderate activity from group 2nd onwards.

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7. Gross, histological and certain histochemical studies on the pancreas of Chabro chicken

Renu Yadav and Ajay Prakash

Gross, histological and histochemical studies were conducted on the pancreas of twenty four apparently healthy chabro chickens irrespective of sex. The birds were divided into 0 day, 30 days, 60 days and 150 days age groups. The pancreas was located in the abdominal cavity on the right side of median plane within the duodenal loop. It was elongated and was creamy white in group-1 and pale pink in other groups. It consisted of dorsal, ventral and splenic lobes which were drained by dorsal and ventral pancreatic ducts; these entered obliquely in the distal part of ascending limb of duodenal loop. Weight and length of pancreas increased significantly with age of birds. The pancreas of chabro chicken was covered by thin fibrous connective tissue capsule. Septae extended from the capsule and divided the parenchyma into lobules and sublobules. Capsule and septae chiefly formed by reticular and collagen fibers and fibroblasts. Septae showed the presence of interlobular ducts, blood vessels and nerves along with the lymphocytes and nerve cell bodies. The interstitial tissue around the acini was formed by reticular fibers which generally encircled one third to two third part of the individual acini in group 1 and the entire acini in other groups. From group-2 onwards fine to coarse reticular fibers were observed inside the islets of Langerhans. The lymphatic aggregations were relatively more in the stroma splenic lobe than the other lobes. Exocrine parenchyma consisted of serous tubuloacinar secretory acini and the duct system. Acini were elongated, oval and globoid. In group - 1 developed and developing acini were observed but in other groups most of acini were fully developed. The acinar epithelium consisted of single layer pyramidal cells but often columnar cells were also found. The

cytoplasm of acinar cells had bipolar staining which became prominent from group- 2 onwards. The occurrence of the granules in the apical acidophilic zone varied considerably in the cell cytoplasm of different acini. The basophilic basal zone increased with the increase of age of birds. Usually one centroacinar cell was found but in some acini two cells were also observed. Duct system consisted of intercalated, intralobular and interlobular ducts. The wall of larger interlobular ducts consisted of mucosa and adventitia; the former was projected in the lumen in the form of mucosal folds. Islets of Langerhans were oval and rounded however, their shape varied considerably. Their occurrence was relatively much higher in splenic lobe. Some islets had cluster of acinar and islets cells and appeared to be formed by the detached cells of the exocrine acini. Fully developed islets were beta and mixed types were formed by the clusters of beta cells which were oval, triangular, irregular, elongated and polyhedral cells. Mixed islets were consisted of beta and alpha cells and were found only in splenic lobe. The alpha cells were spherical, elongated and irregular in shape and appeared relatively smaller than beta cells. The histochemical reactions for best carmine, acid mucopolysaccharides, bromophenol blue and Sudan black B exhibited similar reaction in the stroma and in stromal blood vessels in all age groups. The reaction for PAS was also similar in the stromal tissue in various groups but in stromal blood vessels it varied among the groups. In acinar cells the cytoplasm and in cytoplasmic granules reaction for PAS, acid mucopolysaccharides, best carmine, Sudan black B and bromophenol blue was similar in various groups. In the epithelium of various types of ducts the reaction for best carmine, Sudan black B and bromophenol blue was similar in all the groups but the reaction for acid mucopolysaccharides and PAS was different in various groups. In all the groups of present study the material in the lumen of various types of ducts exhibited similar reaction for PAS, acid mucopolysaccharides, best carmine, Sudan black B and bromophenol blue. All the cells of islets exhibited mild to moderate PAS positive reaction. The islets cells showed mild positive reaction for best carmine. The reaction for acid mucopolysaccharides was negative in the islets cells. The islets cells showed moderate positive reaction for bromophenol blue. Mild positive reaction for Sudan black B was found in the islets of Langerhans in all the groups of present study.

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8. Gross anatomical, histological and histochemical studies on the thyroid gland of prenatal goat (*Capra hircus*)

Anamika Sharma and Ajay Prakash

Gross anatomical, histomorphological and histochemical studies were conducted on thyroid gland of 18 healthy and normal goat embryos/foeti of either sex at different age of gestation. Material was divided into group I (0-50 days), group II (50-100 days) and group III (100-150 days). Gland was situated ventro-laterally on cranial part of neck close to the junction of trachea and larynx. Isthmus was connection between left and right lobes of gland and lied on ventral aspect of trachea. Thyroid was reddish-brown in colour. Lobes were obovate in shape. Cranial end of lobes extended up to cricoid cartilage but caudal end of lobes and position of isthmus varied on trachea. Length, thickness and weight of both lobes and width of right lobe and length and weight of isthmus were highly significantly correlated with age of foeti. Thyroid gland capsule was formed by a thin layer of reticular fibers in 31 days old foetus. Collagen fibers in capsule and elastic fibers in blood vessels of capsule first appeared at 73 and 97 days of gestation, respectively. Adipose tissue first appeared in 115 days old foetus. With advancement of age in foeti these fibers became coarser and thicker and capsule showed a gradual increase in the thickness. In 31 days old foetus parenchyma of

thyroid gland was chiefly formed by cords and clumps of polyhedral and spheroidal cells. From 43 days onwards more fenestrated capillaries of varying shapes and sizes were observed and parenchymal cells lied as one to two cell layer thick cords or cell clusters along their walls. At 82 days of gestation follicles appeared first time in thyroid gland. Most follicles up to 97 days of gestation were smaller in size. From 105 days gestation lumen of some empty follicles contained spheroidal parenchymal cells having spherical vesicular nuclei. From 124 days of gestation occurrence of medium and large sized follicles was greatly increased. At 134 days of gestation few follicles had simple columnar epithelium and few follicles had vacuolated colloid. In 31 days old foetus ultimobranchial bodies were found close to thyroid, these became part of the gland in 38 days old foetus and it's remnants were found upto 73 days of gestation. From 38 days of gestation the process of erosion in the epithelium of ultimobranchial bodies and delamination of epithelial cells was observed; the latter cells were found among the thyroid parenchymal cells in the vicinity of the bodies. It indicated the process of differentiation of parafollicular cells in the thyroid gland of goat foeti. Capsule in all foeti showed mild reaction for the PAS. Parenchymal cell cords, palisade like structures and follicular cells showed moderate reaction for PAS. Endothelial lining of the blood vessels showed moderate reaction for PAS but erythrocytes showed intense reaction. Colloid showed moderate to intense reaction for PAS. Capsule and fibroblasts were moderately reactive for AMPS. Parenchymal cell cords, parafollicular cells and ultimobranchial bodies showed mild reaction for AMPS. Nuclei of fibroblasts and nucleated erythrocytes showed intense Feulgen reaction, whereas, nuclei of parenchymal cells, parafollicular cells and the cells of ultimobranchial bodies showed moderate Feulgen reaction. Capsule exhibited mild to moderate reaction for bound lipids. Parenchymal cell cytoplasm and ultimobranchial bodies cells cytoplasm showed moderate reaction for bound lipids. Colloid showed moderate to intense bound lipids reaction. Fibroblasts, endothelial lining of blood vessels and parenchymal cell margins showed intense reaction for bound lipids, whereas, erythrocytes showed mild to highly intense reaction for bound lipids.

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Ph.D. Degree Programme

1. **Cyomorphological, cyochemical, cyoenzymic and ultrastructural studies on the blood cells of race horses**

Keshav Kumar Sharma and Ajay Prakash

Cytomorphological, cytochemical, cytoenzymic and ultrastructural study was conducted on blood cells of 15 healthy race horses of either sex ranged from 3-7 years of age. The erythrocytes were anuclear, spherical, ovoid, irregular in shape and its average diameter was 5.2 ± 0.12 (am. The rouleaux formation was a common phenomenon. The cytoplasm of erythrocytes exhibited moderate reaction for PAS and alkaline phosphatase and slight reaction at the periphery for lipid. Under SEM erythrocyte were biconcave, rounded or irregular in shape with variable degree of concavity in the centre. Under TEM the erythrocytes were biconcave, round elongated in shape and showed variable degree of electron dense area. The neutrophils were oval or round in shape, composed of 2-7 nuclear lobes and its average diameter was 11.31 ± 0.28 pm. The nuclei showed moderate to intense Feulgen reaction. The cytoplasm of neutrophils showed intense reaction for PAS and slight reaction for acid phosphatase Under SEM, it showed mushroom like cytoplasmic processes of different size, shape and number. Under TEM, neutrophils were oval or rod shape with different size of cytoplasmic processes, and different size of cytoplasmic granules. The eosinophils were oval, rounded in shape and had 2-4 nuclear lobes. Its average diameter was 12.07 ± 0.31 pm. The cytoplasmic granules were homogenously distributed throughout cytoplasm and gave the eosinophils mulberry appearance. The cytoplasm of eosinophils showed slight to moderate reaction for PAS, intense reaction for lipid and slight reaction for iron. Under TEM, eosinophils were irregularly round in shape and had processes. Electron dense granules of various size were scattered throughout the cytoplasm. The basophils were the second largest cell among the leucocytes and its average diameter was 12.22 ± 0.22 pm and its nucleus composed of 2-3 lobes. Cytoplasmic granules were more concentrated towards the periphery and stained violet in colour. The cytoplasm of basophils showed slight reaction for lipid and acid mucopolysaccharides. Under TEM, the basophils were roughly round to oval in shape with 1-2 cytoplasmic processes and eccentric nucleus. Electron dense and electron lucent area was observed at the periphery. Cytoplasmic granules were electron dense and on the basis of their size, five types of granules were noticed. The lymphocytes were round to oval in shape and on the basis of their size, these were classified as small, medium and large and measured 4.70 ± 0.18 pm, 6.93 ± 0.23 pm and 9.22 ± 0.21 pm respectively. Under TEM, lymphocytes were round in shape and had cytoplasmic processes. Small, medium and large size of lymphocytes was noticed. The monocytes were round in shape and their average diameter was 12.58 ± 0.23 pm. The eccentric nuclei had varied shapes, ranging from ovoid to kidney having a notch. The cytoplasm of monocytes showed slight reaction for iron and cytochrome oxidase, slight to moderate reaction for PAS and moderate reaction for acid mucopolysaccharides. Under TEM, monocytes were irregular in shape and cytoplasmic processes were noticed. Nucleus was deeply indented. The platelets were roughly round or irregular in shape and their average diameter was 2.16 ± 0.12 pm. The platelets showed slight to negative reaction for PAS and slight to moderate reaction for acid mucopolysaccharides and alkaline phosphatase. Under SEM, platelets were irregular in outline and had cytoplasmic processes. Under TEM, platelets were elongated and oval in shape and had 1-2 pseudopodia and microtubules and mitochondria observed.

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2. Morphological, histological and histochemical studies on the stomach of prenatal goat (*Capra hircus*)

Varsha Gupta and M.M. Farooqui

On perusal of literature it was found that very meager attention has been paid on the sequential changes in the development of ruminant stomach during various stages of gestation. Therefore, the present study was designed. Macro and microscopic studies were conducted on the stomach of 36 healthy and normal goat embryos/ foeti of either sex. Age of the embryos/foeti was estimated by using formula derived by Singh et al., (1979). Embryo/foeti were grouped into I (0-50 days), II (51-100) and III (101-till term). At 38 days, all the four compartments of ruminant stomach were clearly discernible. Undifferentiated stratified epithelium changed into stratified squamous and simple columnar epithelium at 102 and 76 days of gestation in forestomach and abomasum, respectively. Microscopically, the rumen, reticulum and omasum were distinguished by the appearance of papillae, crests and laminae at 51, 60 and 38 days of gestation, respectively. Corial papillae first came in sight in reticulum and omasum at 121 and 51 days of gestation, respectively. Muscularis mucosae could not be observed in rumen till term. The orientation of smooth muscle fibers of tunica muscularis was inconstant throughout the gestation in forestomach. Earliest appearance of reticular fiber was noticed in blastemic tissue of fore stomach (omasum) and abomasum at 44 and 38 days of gestation, respectively. Collagen fibers embarked in lamina propria and submucosa of rumen and fundic part of abomasum at 60 and 76 days of gestation, respectively. At 100 days of gestation, the elastic fibers were first debuted concomitantly in the wall of the blood vessel of omasum and abomasum. Four different types of cells were identified in fundic region of abomasum viz. undifferentiated, chief, parietal and mucous neck cells. The number of undifferentiated cells was predominant. Parietal and chief cells emerged concurrently at 70 day and mucous neck cells at 82 days of gestation. The cytoplasm was eosinophilic and basophilic in parietal and chief cell, correspondingly. Mucous neck cells were largest and confined to neck region only. The pyloric gland contained undifferentiating, mucous secreting and sporadic parietal cells. Well differentiated mucous secreting cells were noticed at 121 day of gestation. Ganglionic and supporting cells of nerve elements were found either in the subserosal tissue or in between the muscle bundles of stomach throughout the gestation. Basal zone of epithelium showed intense activity for PAS and lipid in forestomach. AMPS reaction was pronounced in most of the epithelial cells of superficial zone. Intense Feulgen reaction was detected in nuclei of the epithelial cells of basal zone. Smooth muscle cells and blood vessels exhibited weak reaction for alkaline phosphatase enzyme. Surface epithelium and glandular blind ends of pyloric abomasum revealed intense reaction for PAS and AMPS. Cell boundaries, parietal cells, smooth muscle cells, blood vessels and nerve cells showed intense reaction for lipid. At term abomasum was largest and omasum was the smallest compartment. Macroscopically and microscopically the wall of omasum was thickest among all the compartments. Statistical analysis of data revealed that all biometrical parameters of fore stomach increased significantly with the advancement of age and were highly significantly correlated with each other. Micrometrical analysis of different strata of fore stomach showed that the thickness of propria submucosa, tunica muscularis and serosa was highest in omasum followed by reticulum and lastly the rumen. However, the thickness of epithelium was found to be maximum in reticulum, followed by rumen and lastly the omasum. From the above study it can be concluded that the organogenesis and histogenesis of non glandular as well as glandular stomach was almost completed in prenatal life. However, to become functional the stomach still required more time as the process of keratinization in forestomach and differentiation of different cells of fundic and pyloric parts of abomasum were yet to be completed. □□□

3. Anatomical studies on the development of heart in prenatal goat (*Capra hircus*)

Sunil Kumar Gupta and Archana Pathak

The gross, histological and histochemical studies were conducted on the developing heart of 36 prenatal goat (*Capra hircus*) divided into three groups; group-I (early prenatal period ≤ 50 days), group-II (mid prenatal period ≥ 51 days to ≤ 100 days) and group-III (late prenatal period ≥ 101 days till parturition) with 12 embryos / fetuses in each group. In early prenatal period, on 23 days of gestation, the primitive heart of goat embryo was present caudal to the mandibular arch and cranial to the liver in the cervical flexure region. It lay in the ventral part of thoracic cavity on 48 days of gestation. On 34 days, four chambers of heart were found as indicated externally by transverse and longitudinal grooves. These grooves were occupied by blood vessels in the embryo of 42 days and onwards. On 76th day of gestation the heart was covered with pericardium and it was attached with sternum by sternopericardiac ligament. On 79th day, the fat deposition started in transverse groove which increased with the advancement of age. On 82nd day of gestation the anastomosis of right and left longitudinal arteries took place. The weight and volume of heart increased from 23rd to 148th days of gestation continuously in a co-linear fashion, however maximum gain in weight and volume occurred during late prenatal period. The heart weight percentage of total body weight goes on reducing with the advancement of age. Internally on 51 days of gestation, the left and right atria were divided by incomplete interatrial septum forming foramen ovale. Among the three papillary muscles of right ventricle, the anterior papillary muscle was the largest and septal papillary muscle was the smallest. The wall of left atrium and left ventricle was thicker than the wall of the right atrium and right ventricle, respectively. The left ventricular wall was about 1.2 times thicker than the wall of right ventricle. Histologically on 23 days of gestation the heart was tubular, comprised of 4 segments; truncus arteriosus, bulbus cordis, ventricle and atrium. On 26th day the sinus venosus was distinguished. On 23rd day of gestation, the septum primum and septum secundum were present between right and left atria. The interventricular septum leaving the interventricular foramen was present between bulbus cordis and ventricle. Upto 32 days of gestation the atrial and ventricular walls were made up of two layered tissues i.e. outer epimyocardial layer and inner endocardial layer. The epimyocardial layer was made up of multi-layers of cuboidal to polyhedral myocardial cells (myocytes) with spherical to oval nuclei. The endothelial layer was made up of a single layer of spindle shaped cells with flattened nuclei. In 34 days goat embryo, the four chambered heart appeared. The interventricular canal was completely obliterated by interventricular septum, the later was made up of the septum musculare (cardiac myocytes) and septum membranaceum (mesenchymal tissue). The epimyocardium of the tubular heart differentiated into an outer layer of epicardium and inner layer of myocardium. Thus the atrial and ventricular walls of the heart consisted of three layers i.e. epicardium, myocardium and endocardium with out inwards. A-V valves, chordae tendineae and sporadic Purkinje fibers in subendocardium of ventricles were formed on 38th day of gestation. On 42 days of gestation, striations were found in the cardiac myocytes of the ventricles. On 46th day the foetal heart attained the general structural features similar to the adult heart. Ganglionated and non-ganglionated nerve plexuses were found in the deeper part of epicardium in the fetuses of 47 days and more. In mid prenatal period the Purkinje fibers became multinucleated. Intercalated disc and muscle bundles lined with perimysium were present on 71 days of gestation. The amount of collagen, reticular and elastic fibers increased in the heart with the advancement of age. In late prenatal period the histological features of the heart was same but the thickness of the wall and amount of fibers increased more significantly resulting

into increase in the thickness of epicardium, myocardium and endocardium of atrial and ventricular walls. Histochemically, in the walls of atria and ventricles the PAS, AMPS and lipid activity was more in early prenatal period. The DNA reactivity, alkaline phosphatase and acid phosphatase was more in late prenatal period. In the major blood vessels the activity for AMPS was more in early prenatal period, but PAS and alkaline phosphatase activity was more in late prenatal period. The activity for lipid, DNA and acid phosphatase was nearly the same in all the major blood vessels of all the three groups of goat embryo/foetuses.

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4. Histological and histochemical studies on the skin of prenatal goat (*Capra hircus*)

Prabhakar Kumar and Ajay Prakash

The histological and histochemical studies were conducted on the skin of 36 healthy and normal goat embryos/ foetuses of either sex at different stages of gestation. The approximate age of each embryos/ foetuses was estimated and the material was grouped into I (0-50 days), II (51-100) and III (101-till term). Small pieces of skin tissues were collected and processed from eight different body regions namely chin, face, neck, shoulder, back, abdomen, flank and thigh for histological and histochemical studies. Paraffin sections were stained by various staining methods to study histoarchitecture and histochemical reactions of the tissues. The epidermis of goat embryo appeared as a single layer of spindle shaped cells at 23 days of gestation which had undergone the stratification process. The periderm, stratum spinosum, stratum granulosum, stratum corneum and stratum disjunction appeared first on 32, 51, 56, 70 and 106 days gestation, respectively. The melanocytes and melanin granules (56 days), Langerhans cells (114 days) and Merkel cells (118 and 94 days) were found in stratum basale and follicles, stratum spinosum and stratum basale and sinus hair follicles, respectively. At 23 days of gestation the subepithelial tissue contained undifferentiated mesenchymal cells with scanty ground substance. In later stages of gestation the mesenchymal cells differentiated into fibroblast (38 days). The mast cells were first appeared at 43 days of gestation in the dermal tissue. The reticular, collagen and elastic fibers were first observed at 42, 75 and 87 days of gestations, respectively in chin region and subsequently became matured and found in abundance in other regions. The arrector pili muscle started appearance in face region at 87 days gestations and they were fully developed at 118 days of gestation. Hair follicles first noticed in the head region epidermis at 46 days of gestation and at 56 days in chin region. Hair follicles observed in all regions of the study from 70 days gestation. These passed through pregerm, hair placode, hair plug, hair papilla, early hair cone, hair cone, hair formation, hair in hair canal and emerging hair stages. Differentiation of inner and outer root sheaths of hair follicles began at 102 days when the follicles were in hair cone stage. At 106 days the inner root sheath was well differentiated consisting of inner cuticle layer, middle Huxley's layer and outer Henle's layer. From 131 days gestation hair began to emerge out and near term fully formed hairs from the hair follicles projected over the epidermal surface. Sinus hair follicles were confined to chin region and noticed first time at 75 days; these contained blood sinuses in their dermal sheath. Encapsulated nerve endings and Merkel cells were encountered in external dermal sheath and outer root sheath, respectively. Only sebaceous glands and skeletal muscles were observed in association with these follicles. The sweat glands primordia were observed at 84, 87 and 94 days in chin, face and all body regions, respectively. Canalization of these glands started in chin and face regions at 94 days and in all body regions from 106 days gestation. The coiling of the terminal part of these glands began at 102 days gestation in chin region and from 145 days gestation in

all body regions. Myoepithelial cells in the terminal part of gland were noticed from 102 days gestation. Sebaceous glands arose from peripheral parts of ordinary hair follicles, outer root sheath of sinus hair follicles and also directly from epidermis at 102, 102 and 108 days gestations, respectively. Epidermis showed moderate to intense PAS and AMPS reactions. Intense reaction for bound lipids was limited to stratum corneum. The nuclei of the cells of epidermis showed intense reaction for DNA. The epidermis revealed mild acid phosphatase reaction near term. The dermal tissue showed faint to moderate reaction and moderate to intense reaction for PAS and AMPS, respectively. The dermal tissue showed intense reaction for glycogen in early stages of gestation. The glycogen granules were observed in the skeletal muscle of chin region from 118 days gestation. The dermal tissue showed mild to moderate acid and alkaline phosphatase reactions. Peripheral part of hair follicles showed PAS positive granules in mid and late gestation periods. The dermal sheath of hair follicles showed moderate to intense reactions for AMPS during late stages of gestation. The glassy membrane of sinus hair follicles showed moderate to intense reaction for PAS and their dermal sheath showed moderate to intense reactions for AMPS. The margin of the cells of sebaceous glands showed highly intense reaction for bound lipids. The averages thickness of skin, epidermis and dermis were $477.49 \pm 8.06 \mu\text{m}$, $26.64 \pm 0.38 \mu\text{m}$ and $450.5 \pm 7.95 \mu\text{m}$, respectively. The single cell layered epithelial covering of goat embryo differentiated and attained structural complexities to form well differentiated epidermis with the advancement of gestation age. The maximum growth of epidermis took places in the mid prenatal period and all the four strata got differentiated in this period. The dermal tissue was delicate and cellular type in early gestation. In mid prenatal period it had areolar and dense irregular connective tissues. Finally it transformed into dense irregular connective tissue in late gestation.

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DEPARTMENT OF VETERINARY AND ANIMAL HUSBANDRY EXTENSION

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M.V.Sc. Degree Programme					
1	Impact assessment of livestock services delivery among livestock owners in Mathura district of Uttar Pradesh	Dharmendra Kumar Gupta	Dr. Sanjeev Kumar Singh	2015	78
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3	Impact study on livestock insurance in Mathura district	Jitender Kumar	Dr. Sanjeev Kumar Singh	2016	79
4	Entrepreneurial and adoption behaviour of rural youth in animal husbandry practices	Sandeep Sharma	Dr. Sanjeev Kumar Singh	2018	80
5	Study on dairy production scenario in the urban and peri urban areas: A exploratory study	Shivani Singh	Dr. Amit Singh	2018	80
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M.V.Sc. Degree Programme

1. Impact assessment of livestock services delivery among livestock owners in Mathura district of Uttar Pradesh

Dharmendra Kumar Gupta and Sanjeev Kumar Singh

Livestock services play a central role in livestock production and in animal husbandry development. But in terms of efficiency and cost effectiveness, it is becoming increasingly clear that many of the existing livestock service delivery systems are hardly sustainable in the long run and in future. Livestock owners must have to contribute more and pay not only for a service as such, but also for the quality of service as well. The present study was carried out in eight villages of four blocks of Mathura district of Uttar Pradesh. Two villages were selected randomly from each block and twenty respondents' were selected from each village among which 10 beneficiaries' respondent and 10 non-beneficiaries from each villages and was thus total sample size of 160. A pretested interview schedule was used for collection of data from respondents. India is an agricultural developing countries getting attention from the past decade. However, continuous increases in livestock population therefore increase in demand of livestock services. Hence, there is an urgent need to study delivery livestock service. Keeping this in view, an earnest effort was made to study the delivery of livestock services by various organizations under six heads, viz. feeding, breeding, treatment, management and extension services also constraints in livestock services. The study of the result revealed that majority of livestock farmers were middle age, mostly illiterate, agriculture as main education and livestock rearing as sub-occupation. They mostly lived in medium size family possessed medium land holding and had medium experience in dairy farming. The respondents belongs to medium group of training in livestock rearing whereas majority of non- beneficiaries belongs to lower group of training in livestock rearing. And It is further revealed that majority of beneficiaries' livestock farmers belongs to high group of total income, knowledge and gathering more information from formal source of information than non-beneficiaries'. The respondent stated non-availability of land for green fodder cultivation as the major constraint among beneficiaries as well as non- beneficiaries. Apart from this lack of knowledge and scientific practices were other important constraints identified by the respondents.

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2. A Comparative study to assess role of information and communication technology (ICT) among livestock owners

Rajiv Nayan and Sanjeev Kumar Singh

The present study was conducted to assess role of ICT with special reference to enhanced livestock production, disease control, entrepreneurial development in two districts Mathura and Gautam Buddha Nagar in Uttar Pradesh. Eight villages were selected randomly from four blocks of two districts. Out of total respondents (200), twenty five respondents were selected from each village. Respondents were selected from each selected village and detailed information was collected through interview schedules as per the objectives of the study. Data collected through personal interviews were analyzed using appropriate statistical tools. Majority of respondents belongs to middle aged group have nuclear family, literate, possessed fair number of ICT tools. Majority of the respondents have primary occupation small and medium size land holding, small to medium size herd size with high milk production, total annual income and spent 1- 2 hrs on ICT tools. Regarding the role of ICT in enhancing

livestock production, majority of respondents in Mathura and Gautam Buddha Nagar reported the information of galactagogue information of balanced ration, production and management in disaster provides major role of ICT in enhancing livestock production. However role of ICT in disease control, seasonal management of cross breed dairy animals, information of disease control were important roles in Mathura and Gautam Buddha Nagar respectively .However respondents at Mathura and Gautam Buddha Nagar reported the information on export import of livestock products like milk, meat etc and government policies placed important role of ICT in marketing, price information, rural credit, supply chain management. Majority of respondents accepted role of ICT in enterpreunal development, rural governance, forest governance, land administration. Thus it reflects effectiveness of role of ICT in various aspects to farmers. In respect to constraints, educational illiteracy of respondents, complex nature of content, frequent fault in computer, electricity availability and trained staff illiteracy of respondents, language dominance of English in ICT contents were major constraints. The result of this study will be helpful in starting various programmers for farmers and policy makers.

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3. Impact study on livestock insurance in Mathura district

Jitendra Kumar and Sanjeev Kumar Singh

Livestock Sector is an important sector of national economy, especially rural economy. Livestock comprise an important productive asset and source of income for about two-third of India's farm households. The supplemental income derived from rearing of livestock is a great source of support to the farmers facing uncertainties of crop production, apart from providing sustenance to poor and landless farmers. In the event of an animal disease outbreak, the livestock sector suffers large or even catastrophic economic losses. Hence in order to check economic losses of the livestock sector, the Government launched a centrally sponsored scheme on Livestock Insurance which was initially implemented on pilot basis during the 10th Plan. The centrally sponsored livestock insurance scheme was implemented on a pilot basis during 2005-07 and in 2007-08 on a regular basis in selected 100 districts of the country including Mathura district of Uttar Pradesh. The present study was carried out in ten villages of five blocks of Mathura district of Uttar Pradesh. Two villages were selected randomly from each block and twenty respondents' were selected from each village thus comprising a total 100 respondents for this study. Data was collected through pre- designed interview schedules as per the objectives of the study and the results were analyzed using suitable statistical tools. The study revealed that majority of farmers was middle age group, possessing secondary level of education and having medium family size. The 51.0% of the respondent were having high experience of livestock rearing, having herd size of 2-4 animals and having low information source. The result further reveals the awareness for livestock insurance indicates that 89.0% livestock holders were not aware with process of livestock insurance, 87.0% were unaware about regular premium compensation, 87.0% were not aware to inform the insurance agent in case of casualty of their animals respectively. Adoption of insurance requires motivation by friends and community as well as high risk of mortality due to increase the adoption percentage. The important constraint in adoption of livestock insurance were inadequate information about livestock insurance, inadequate information for applying livestock insurance as well as inadequate awareness programme by Govt. Animal Husbandry department, and less coverage of small animal in the insurance policies and past experience of farmer reveals unsatisfactory payment of insurance claim respectively.

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4. Entrepreneurial and adoption behaviour of rural youth in animal husbandry practices

Sandeep Sharma and Sanjeev Kumar Singh

The youth represent the most dynamic and vibrant segment of the population and India is one of the youngest nations in the world in respect to age group of youth population. Approximately 65 percent of Indian population is under 35 years of age. Today the Government of India is presently taking significant steps towards entrepreneurship development of youth through various ministries/ departments in various sectors including animal husbandry. Government of India has also launched several programmes & scheme launched for youth entrepreneurship viz., Intensive Dairy Development Programme (IDDP), Strengthening infrastructure for quality and clean milk production, Cooperative Dairy Development, KVK (Krishi Vigyan Kendra), ARYA (Attracting and Retaining Youth in Agriculture, Rural and Entrepreneurship Awareness Development Yojana" (READY), NABARD, Micro Units Development Refinance Agency(MUDRA), Dairy Entrepreneurship Development Scheme is a part of creating productive youth workforce for sustainable development of the country. The main aim of Government of India behind these schemes to promote the entrepreneurship and retaining youth in dairy, agriculture and its allied sectors. The present study was conducted in ten villages of five blocks of Dholpur district of Rajasthan. Two villages were selected randomly from each block and twelve respondents' were selected from each village thus comprising a total 120 respondents for this study. Data was collected through pre- structured interview schedules as per the objectives of the study and the results were analyzed using suitable statistical tools. The study revealed that majority of respondents belongs to 24 - 26 year age group, possessing secondary level of education and having medium family size. Most of the respondents were having medium experience of livestock rearing, having herd size of 5-6 animals. The result further reveals that nearly 55.00 percent of the respondents possessed medium level of entrepreneurial behavior may be due to their medium financial condition and economic status, education level, medium economic motivation and scientific orientation. Adoption index of housing management practices has positive and significant correlation with education of farmers. Information seeking behaviour has negative and significant relation with age of respondents. Majority of respondents (52.50) percent belongs to medium level adoption. Major constraint faced by rural youth is that fear of failure because more risk & uncertainty, difficulties in getting loan, non-remunerative price for milk, high cost of concentrate and lack of experts advice etc.

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5. Study on dairy production scenario in the urban and peri urban areas: A exploratory study

Shivani Singh and Amit Singh

Urbanization is an index of transformation from traditional rural economies to modern industrial one. With the rural-urban distribution in India had increased to 68.84% and 31.16%, the population and economic growth has fostered urbanization in the country and the numbers of urban towns and cities have drastically increased. The growing population is putting tremendous pressure on the agriculture as there is sudden need to double the agriculture production to feed the growing population. Though India stands first in the production of milk, It is expected that demand of milk will increasing day by day due to rapid increase in demand of milk and milk products and it might be dream for anyone to capitalize this fact growing milk and its product market. This situation creates a prospectus for local

dairy production in urban and peri-urban area and to enhance the income. The present scenario is giving a chance to the emergence of market-oriented commercial large scale and smallholder dairying. With these aspect the present study on “Study on the dairy production scenario in the urban and peri urban areas: A exploratory study” was planned. The present study was carried out in Firozabad and Mainpuri districts of Agra Division. Two blocks were randomly selected from each district and forty respondents selected from each district that considered as urban areas and twenty respondents from each block that considered as peri urban areas. To know the perception of consumers towards milk that procured from urban and peri urban areas forty respondents randomly selected from both districts thus comprises total 160 respondents for this study. Data was collected through pre-designed interview schedule as per the objectives of the study and the results were analyzed by using suitable statistical tools. The result revealed that majority of respondents (45.83%) was middle age group, possessing middle level of education (37.50%) and having high level of experience (46.67%), large herd size (47.50%). The respondents were having large herd size that is more than 7 animal and milk production ranges from 5 to 41 liters. The result further reveals the knowledge level of respondents that there is an significant difference between the knowledge level of urban and peri urban farmers. The Urban farmer (45.72%) posses more knowledge the peri urban farmers (37.50%). It was observed that the consumer who are procuring milk from these dairy gave first preference to quality of milk (53.39 %) followed by impact on public health (45.71 %) and other consumer need (39.82 %). Remunerative prices of milk in and high demand of milk during festival season in urban & peri urban areas is the opportunity that is motivating for the farming. Major challenges that were faced by these farmers includes limited availability of credit for establishment of dairy farms, higher frequency of disease incidence in dairy animals and limited availability of land for establishment of dairy farms.

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6. Analytical study of women entrepreneurial activities involved in dairying enterprise

Deepanka and Amit Singh

India has a population of about 1210.19 million, comprising 586.47 million women which constitute about half of the total population. The empowerment of women is fundamental for the progress of the country. The net productivity of the women is definitely much more than the men, as they manage both the home and outside work in a well-balanced way. Women have made their presence felt in all fields be it Business, Education, Fashion, Entertainment, Finance, Information Technology and has flourished throughout the world with flying colours. Social and economic scenario of India has undergone a huge change and Women are seen as the engines of the growing Indian economy. Women despite of inadequate education, proper facilities and with other problems still engage themselves in various activities such as agriculture or livestock rearing. Thus it was seen that women had played a remarkable role in dairy enterprise by managing and organizing the activity. Women can be regarded as an entrepreneur. Thus keeping the above facts the present study was designed to study the activity profile of women, extent of adoption of practices, their entrepreneurial behaviour and the constraint face by them. The present study was carried out in eight villages of four blocks of Mathura district of Uttar Pradesh with a total sample size of 120. A pretested interview schedule was used for collection of data. The study of the result revealed that majority of livestock farmers were middle age, mostly primary to middle level educated, agriculture as main occupation, livestock rearing as sub-occupation. They mostly possessed small to medium land holding and had good experience in dairy farming, they posses large

herd size, majority of respondents had high milk production, sale and low milk consumption, majority of livestock farmers belongs to high group of total income, with no social participation, knowledge and gathering more information from formal source of information. In activity profile of respondent's majority of women were involved in feeding practices. They had adequate control and asses to the items like refrigerator, television, fan, cell phone but less control on vehicles. In decision making ability decision regarding feeding, management were generally taken by women. Majority of women were adopting scientific dairy farming in feeding practices. Constraints perceived by the respondents were highest in institutional category. In entrepreneurial behaviour index EBI was found highest in self confidence and then achievement motivation. It is noticed that dissatisfaction with functioning of government functionaries and departments among the dairy entrepreneurs and rural masses in general. A comprehensive Entrepreneurship Development training programme (EDP) model or strategy should be developed through collaboration of various institutions for developing dairy entrepreneurs.

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DEPARTMENT OF VETERINARY BIOCHEMISTRY

S. No	Title	Author/ Student	Guide	Year	Page No.
M.V.Sc. Degree Programme					
1	Studies on circulating leptin level, polymorphism of LEP and LEPR genes in Haryana cows	Ram Bachan	Dr. Rajesh Nigam	2016	84
Ph.D. Degree Programme					
1	A study on milk proteome and biochemical analysis of milk and blood of mastitic and healthy Sahiwal cows	Pawanjit Singh	Dr. Rajesh Nigam	2016	85
2	Studies on influence of certain blood adipokines and their genetic polymorphism on lactation of sahiwal cows	Vijay Pandey	Dr. Rajesh Nigam	2018	86
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M.V.Sc. Degree Programme

1. Studies on circulating leptin level, polymorphism of LEP and LEPR genes in Haryana cows

Ram Bachan and Rajesh Nigam

Leptin is one of the polypeptide hormones secreted from white adipose tissues which regulate feed intake, energy metabolism, lactogenesis, reproduction and immune functions. The present study was executed to elucidate the trend of plasma leptin hormone and some other biochemical indices levels during periparturient period and genetic polymorphism by PCR-RFLP assay using *Bsa*AI and *Bse*GI restriction enzymes in LEP and LEPR genes, respectively and their associations with production and reproduction traits in Haryana cows. Blood samples were collected from 6 pregnant Haryana cows from -30 days prepartum to +90 days postpartum at 15 days interval for biochemical study and from 62 lactating cows for studying genetic polymorphism in LEP and LEPR genes. Biochemical study in periparturient cows revealed sharp reduction in plasma leptin and glucose concentration at calving and then gradual increase during lactation whereas plasma urea remains low during pregnancy and then rose after calving. Lipid profile remains normal during prepartum period and increases near term except triglycerides which remains high during pregnancy and become lowest just after calving. The PCR-RFLP analysis using *Bsa*AI and *Bse*GI found capable of revealing genetic polymorphism in LEP and LEPR gene in Haryana cattle. LEP/*Bsa*AI assay exhibited AA, AB and BB genotypes with genotypic frequencies 9.67, 54.8 and 35.5 % respectively and its association study revealed significant influence of these genotypes on gestation period, dry period, lactation period, total milk yield, milk yield at 300 days, peak yield and days to reach peak yield. Besides, LEPR/*Bse*GI assay indicated CC, CT and TT genotypes with genotypic frequencies 8.06, 87.09 and 4.83 % respectively and revealed significant influence of these genotypes on gestation period, lactation period, total milk yield and milk yield in 300 days.

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Ph.D. Degree Programme

1. A study on milk proteome and biochemical analysis of milk and blood of mastitic and healthy Sahiwal cows

Pawanjit Singh and Rajesh Nigam

The study was performed at Department of Veterinary Biochemistry, College of Veterinary Sciences and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go- Anusandhan Sansthan, (DUVASU), Mathura, Uttar Pradesh, India. The study was carried out on lactating Sahiwal cows maintained at ILFC of the College of Veterinary Science, Mathura. Milk samples used in this study were of control/apparently healthy group, subclinical mastitis and clinical mastitis group. The animals were classified into three stages of lactation namely, early lactating group (up to 100 days), mid lactating (from 101 to 200 days) and late lactating (from 201 to 280 days). These groups were further divided in to three subgroups, namely control/healthy group subclinical mastitis and clinical mastitis group on the basis of screening by using somatic cell count (SCC), California mastitic test (CMT) and electrical conductivity test (ECT) on the milk samples. Biochemical analysis of milk samples revealed significant increase in sodium, copper, MDA, Lactose, LDH, AST and ALP and decrease in magnesium, calcium, phosphorus, potassium, iron, zinc, total protein and albumin compared to healthy animals. Milk of healthy animal showed significant decrease in sodium and LDH whereas Subclinical mastitic animals revealed significant fall in copper and LDH and rise in copper levels in mid and late lactation compared to early lactation. Biochemical study of serum of healthy, SCM and CM cows showed significant increase in sodium, potassium, total protein, globulin LDH and AST while significant decrease in calcium, copper, albumin, A/G ratio, FFA, MDA and cholesterol. The stage of lactation found to affect biochemical profile in both serum and milk and revealed significant increase in calcium, phosphorus, copper, glucose, cholesterol, LDH and AST and decrease in total protein in mid and late lactation compared to early lactation in healthy animals. Similarly cows having SCM showed significant increase in calcium, phosphorus, sodium, glucose and cholesterol and decrease in Total protein and MDA in mid and late lactation.

Bacterial isolation and multiplex PCR performed on milk samples of SCM/CM revealed infection of *Staphylococcus*, *Pseudomonas* and *E. coli* in the studied cows. SDS PAGE analysis of pooled milk samples healthy milk revealed 23 protein bands where as both subclinical and clinical mastitic milk 27 protein bands. The 4 protein bands which were not found in the healthy milk revealed 14, 56, 160 and 168 kDa molecular weight proteins. 2-D gel electrophoresis was carried out by using isolated whey proteins of healthy SCM and CM sahiwal cows. IEF and I-D gel analysis revealed expression of large set of proteins but on gel analysis revealed 3 differentially expressed protein spots in CM group of proteins. Those spots were identified, digested and sent for MALDI analysis. MALDI analysis revealed DNA-dependent protein kinase catalytic subunit protein, Rho-specific guanine nucleotide exchange factor (RhoGEF), acyl-CoA-binding domain-containing protein 6 isoform X4 [*Bubalus bubalis*], Ankyrin repeat domain-containing protein [*Homo sapiens*] and Chordin-like protein 1 f) kielin/chordin-like (KCP) protein.

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2. Studies on influence of certain blood adipokines and their genetic polymorphism on lactation of Sahiwal cows

Vijay Pandey and Rajesh Nigam

Adiponectin and resistin are very vital adipokines which influence the energy homeostasis, insulin sensitivity and mobilization of body fat stores. The present investigation was performed to elucidate the influence of periparturient period (-30 to +90 days), stage of lactation (early, mid and late lactation) and parity (first, second and third) of animals on circulating levels of adipokines and other biochemical indices (hormones and metabolites) and genetic polymorphism in adiponectin (ADIPOQ) and resistin (RETN) genes and their association with production and reproduction traits in Sahiwal cows. Blood samples were collected from 6 pregnant Sahiwal cows from -30 days prepartum to +90 days postpartum at 15 days interval and from six animals each of first, second and third parity in their early (30th day), mid (90th day) and late (200th day) lactation for biochemical study while from >70 lactating cows for studying genetic polymorphism in ADIPOQ and RETN genes. Biochemical study in periparturient cows revealed sharp decrease in plasma levels of ADIPOQ, LEP, progesterone, TG, cholesterol, HDL, proteins and Ca from pregnancy to parturition and thereafter gradually increased to prepartum levels. The circulating levels of RETN, estrogen, urea and NEFA increased from pregnancy to parturition and subsequently reduced to prepartum levels. During periparturient period, ADIPOQ levels showed positive association with LEP and lipid profile while negative association with estrogen, NEFA and creatinine while levels of RETN revealed positive association with estrogen, NEFA and creatinine while negative association with LEP and lipid profile. Association studies of ADIPOQ/*TasI* PCR-RFLP assay revealed significant influence of genotypes on Calving Interval (CI), Lactation Period (LP), Total Milk Yield (TMY) and Days to Reach Peak Yield (DRPY). ADIPOQ/*RsaI* PCR-RFLP assay showed monomorphic pattern in Sahiwal cattle due to absence of restriction site for *RsaI* restriction enzyme at specific location in ADIPOQ gene. Association studies of RETN /SSCP assay revealed significant influence of genotypes on LP, Average Milk Yield (AMY), Milk Yield in 300 Days (MY300) and Peak Yield (PY). Association studies of RETN/SSCP assay revealed significant influence of genotypes on Birth Weight (BW), Dry Period (DP) and LP. In conclusion, SNP identified in ADIPOQ and RETN genes suggests that these genes might serve as candidate genetic marker for selection of Sahiwal cattle with better milk yield. However further studies are needed to explore SNPs in other regions of these genes, and validation of these markers in another breed and population and their association with other production traits required to be verified.

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3. A Study of purification and characterization of urinary antimicrobial peptides in different seasons from indigenous and crossbred cows

Ambika Sharma and Rajesh Nigam

The present study was undertaken to extract and characterize urinary antimicrobial peptides from healthy indigenous and crossbred cows in three different seasons. In this study, fresh urine samples were collected from 10 healthy, non-pregnant cows of both the breeds in three different seasons winter, summer and rainy and physicochemical analysis of urine samples was carried out for assessing any abnormality in the urine samples. The urine samples were pooled together and protease inhibitor was added, vacuum filtered using 0.2 μ

membrane filter and stored at 4°C till further analysis. The samples were dia-filtered and concentrated using 10 kDa Amicon-4 ultra-centrifugal filters. After dia-filtration, proteins were extracted by ion-exchange chromatography. Cationic peptides were extracted from dia-filtered urine using weak cationic exchanger CM Macrorep resin. Both the anionic and cationic fractions were quantified in terms of protein recovery using Bradford method. Both the fractions were then subjected to two dimensional gel electrophoresis (2-D GE), gel image analysis revealed 40 and 15 protein spots in anionic and cationic fractions, respectively. Upon gel matching, 12 spots were unique to both the gel. Urinary cationic fractions of both the breeds in different seasons were analyzed by acid urea polyacrylamide gel electrophoresis (AU-PAGE) using HBD-1 as standard. Fractions which migrated similarly to HBD-1 standard were pooled together and further purified by RP-HPLC. A total of sixty fractions after HPLC were collected. RP-HPLC purified fractions were again analyzed by AU-PAGE with a standard 10 kDa HBD-1. AU-PAGE gel image analysis using ImageLab 5.1 revealed a single band for each sample. A total of 14 bands were excised and subjected to MALDI-TOF coupled with PMF. Peptides or proteins were identified using SWISSPROT protein database. Of 14 excised and digested bands, 12 were successfully identified as antimicrobial peptides and proteins. Gene Ontology (GO) by PANTHER 11.0 bioinformatics software platform was used for functional characterization of identified peptides which showed that all the identified peptides belonged to the defense/immunity protein class. Cytoscape along with ClueGO used to create protein-protein interaction network identified pathways for all peptides linked to the antimicrobial/antibacterial humoral response and innate mucosal response in mucosa mediated by AMPs. Four bands from AU-PAGE gels were analyzed by Q-TOF MS/MS for amino acid sequencing. These four bands match with three different antimicrobial peptides, namely neutrophil defensin-4, beta defensin-127 and cathelicidin-4 as 97, 99 and 144 amino acids chain. The RP-HPLC fractions were also assessed for their antimicrobial and cytotoxic potential. The antimicrobial potential of urinary peptides was determined using RDA and microtitre broth dilution assay to determine minimal inhibitory concentration (MIC). The cytotoxic potential was tested using MTT assay with human embryonic kidney cell line (HEK cells). Antibacterial activities of urinary peptides were tested against five strains of microbes namely *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus agalactiae*, *E. coli*, and *Pseudomonas aeruginosa*. Urinary peptides exhibited significant activity against these microbes. It exhibited high activity index (AI) against both groups of pathogens viz., Gram negative bacteria (AI-3.3), and Gram positive bacteria (AI-2.5). Lowest MIC was recorded against the fungi *Aspergillus niger* at 28 µg/ml. The results of the study clearly concluded cow urine as an effective antimicrobial and cytotoxic agent and a promising source of highly potent antimicrobial peptides for therapeutic applications.

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DEPARTMENT OF EPIDEMIOLOGY AND PREVENTIVE MEDICINE

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2	Comparative studies on serological and molecular diagnosis of bovine brucellosis	Neha	Dr. Amit Kumar Verma	2015	90
3	Sero-epidemiology and comparison of a PCR assay in whole blood, milk and serum specimens for brucellosis diagnosis in bovines with reproductive disorders	Krishna Govind Bohrey	Dr. Barkha Sharma	2019	91
Ph.D. Degree Programme					
1	Development of marker vaccine and attempt to develop diagnostic ELISA kit for detection of BHV-1 infections	Rashmi Singh	Dr. Amit Kumar Verma	2014	93

M.V.Sc. Degree Programme

1. Molecular epidemiology of *Campylobacter* spp. in dogs

Iftehar Ahemad and Amit Kumar Verma

Campylobacteriosis is a major cause of gastroenteritis in humans and some studies have suggested that dog ownership is a risk factor for the condition. To determine the prevalence, species distribution, antibiogram and risk indicators for *Campylobacter* spp. infecting dogs attending veterinary practice in Mathura, India, faecal samples were collected in a cross – sectional study from 134 dogs with and without clinical signs. The prevalence of *Campylobacter* spp. was 28.36% (38/134). Twenty six (68.42%) of the 38 *Campylobacter* strains were classified as *C. jejuni* by PCR, no strains (0.00%) were classified as *C. upsaliensis* and twelve strains (31.57%) were classified as other species in the *Campylobacter* genus. Susceptibility of the isolates to 19 antibiotics was determined by disc diffusion technique. Of the 19 antibiotics, amoxycillin, ampicillin, aztreonam, cefotaxim, lincomycin, oxytetracycline, penicillin, streptomycin and tetracycline revealed no zone of inhibition suggestive of resistance against these nine drugs, while high rate of resistance was observed against pefloxacin (92.11%), chloramphenicol (86.84%), ciprofloxacin (84.21%), nitrofurazone (78.94%), ofloxacin (76.32%), norfloxacin (73.68%) and cefaclor (73.68%) based on zone of inhibition as per manufacturer guidelines. Only few antibiotics viz., enrofloxacin (31.58%), gentamicin (23.68%) and amikacin (18.42%) revealed zone of inhibition suggestive of sensitivity. The high prevalence rate of resistance to these drugs among campylobacters from dog faeces is of public health significance. Risk factor analysis indicated that non-descript dogs and younger dogs were more likely to carry campylobacters and the high prevalence of this pathogen supports the hypothesis that dogs, particularly younger animals, may be an important source of campylobacter infection for humans. Association of campylobacteriosis and health status of dogs revealed that prevalence of campylobacteriosis was significantly higher in diarrheic dogs in comparison to non-diarrheic dogs. The prevalence of campylobacteriosis was significantly higher in dogs, which shared their habitat with other dogs in comparison to those of which do not share their habitat with other dogs. The study also revealed that need for further studies to determine role of dogs in campylobacteriosis-a zoonosis. In further studies, dog owners may be sampled for excretion of campylobacters. Dog, their owners and human population in general should also be screened for presence of campylobacters, to detect correlation (if any) in the prevalence of infection in dogs and their contacts.

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2. Comparative studies on serological and molecular diagnosis of bovine brucellosis

Neha and Amit Kumar Verma

In this cross-sectional study, out of 924 serum samples from dairy animals (cattle and buffaloes) of different age, sex at districts place (districts), rearing pattern and health status were screened for seroprevalence of brucellosis using Rose Bengal Plate Test (RBPT) and Indirect ELISA. An overall sero-prevalence of brucellosis in dairy animals was found to be 8.55% and 20.45%, respectively. Risk factors such as species, age, sex, place, rearing practice and health status of dairy animals were assessed. The study indicated that the species, age, sex, districts, rearing practice and health status especially reproductive disorders had significant effect on seropositivity of brucellosis. The most crucial risk factor which facilitates spreading of brucellosis is abortion/delivery of an infected animal on the farm. The infected animals contaminate the environment following abortion/delivery. Improper disposal of placenta and uterine discharges is

a common practice which results into poor sanitary conditions on dairy farms. In addition, high animal density is commonly seen on dairy farms which provide opportunity of close contact between animals and helps in transmission of disease from infected to uninfected animals. A total of 89 animals showing symptoms of reproductive disorders were tested by three different diagnostic techniques, i.e. RBPT, I-ELISA and PCR. Out of 89 samples, 44 were positive by ELISA, 18 by RBPT and amplicons of 498bp were detected in 21 samples by polymerase chain reaction PCR. Different tests viz., RBPT and PCR were compared and sensitivity, specificity, predictive value (positive) and predictive value (negative) of different tests when compared with i-ELISA considering it as "Golden Standard technique" were calculated. Sensitivity of PCR was the higher (47.73%) than that of RBPT (29.55%). Specificity of PCR was 100%, while the specificity of RBPT was 88.89%. RBPT, ELISA and PCR were compared against each other by applying kappa statistic and concordance percentage. There seems to be fair agreement between PCR and ELISA, while a slight agreement was observed between RBPT and ELISA; and RBPT and PCR (Table 15). Analysis of concordance percentage indicated a higher concordance percentage of 77.6% between PCR and ELISA. From the present study, it can be concluded that I-ELISA can be routinely used for an accurate and efficient diagnosis of brucellosis, because the chances of non-detection of an infected animal in I-ELISA are minimal. PCR alone cannot be used for routine diagnosis of brucellosis using serum as starting material. However, PCR can be used in combination with I- ELISA to complement the serological diagnosis for detection of bovine brucellosis.

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3. Sero-epidemiology and comparison of a PCR assay in whole blood, milk and serum specimens for brucellosis diagnosis in bovines with reproductive disorders

Krishna Govind Bohrey and Barkha Sharma

In this study, a total of 470 samples from 203 cattle and 59 buffaloes were collected Including 262 serum samples from 203 cattle and 59 buffaloes which were screened for seroprevalence of bovine brucellosis using RBPT, STAT and I-ELISA. Further, 108 whole blood samples and 100 milk samples were also collected and processed for molecular detection of *Brucella*. This study also considered the effect of various epidemiological parameters like place or geographical area, species, age, rearing practice and health status with reproductive problems on the occurrence of the disease in the population. RBPT, STAT and I-ELISA tests used for diagnosis of brucellosis were compared for sensitivity and specificity of the diagnostic test. The overall seroprevalence was found 9.92%, 16.03%, 6.48% by RBPT, STAT and IELISA respectively. Species wise seroprevalence of brucellosis found in cattle 11.82%, 16.25%, 8.37% by RBPT, STAT and I-ELISA respectively while in buffaloes seroprevalence were found 3.38%, 15.25% and 0% by RBPT, STAT and I-ELISA respectively. On the other hand age wise seroprevalence in young animals were 7.76%, 13.04% and 4.34% by RBPT, STAT and I-ELISA respectively while in adult animals 11.17%, 17.64% and 7.64% by RBPT, STAT and I-ELISA respectively. Unorganized rearing of animals were show seroprevalence 8.68%, 5.43% and 6.52% by RBPT, STAT and I-ELISA while the seroprevalence of organized rearing of animals were 10.58%, 21.76%, 6.47% by RBPT, STAT and I-ELISA respectively. RBPT, STAT and I-ELISA were compared against each other by applying Kappa Statistics and concordance percentage. According kappa statistics, moderate agreement (0.5547) was present between RBPT and I-ELISA while STAT and IELISA showed fair agreement (0.3061). Similarly concordance between RBPT and I-ELISA was more (91.98%) as compared to STAT and I-ELISA (85.87%). Molecular detection of Brucellosis by PCR assay from serum (100 samples),

whole blood (108 samples) and milk (100 samples) revealed the total positive samples 5 out of 308 (1.62%). None of the sera samples were found positive by PCR whereas 3(2.8%) and 2(2%) from whole blood and milk samples, respectively were found positive and showed amplicons of 223bp confirming *Brucella* genus and amplicon of 498bp confirming *Brucella abortus*. So among samples processed for PCR, whole blood was found to be better than milk and serum. Hence, a combination of RBPT, STAT and I-ELISA were found to be the most suitable serological tests for the confirmation of Brucellosis and in case where infection is not established or pronounced, confirmation by PCR using whole blood might be most suitable for the diagnosis of bovine brucellosis.

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Ph.D. Degree Programme

1. Development of marker vaccine and attempt to develop diagnostic ELISA kit for detection of BHV-1 Infections

Rashmi Singh and Amit Kumar Verma

Infectious bovine rhinotracheitis (IBR) is a highly infectious and contagious disease of animals including cattle, buffalo, pig and other wild ruminants and worldwide in distribution. The greatest economic impact comes from losses resulting from IBR abortions, which occur chiefly during the last half of gestation, often without evidence of other clinical signs. In India, seroprevalence of IBR in cattle and buffaloes ranges from 14.75% to 73.00%. The disease is caused by Bovine Herpes Virus-I (BHV-1), a member of the genus *Varicellavirus* in the sub-family *Alphaherpesvirinae* of family *Herpesviridae*. BHV-1 is an enveloped virus having an icosahedral nucleocapsid consisting of 162 capsomeres. The BHV-I genome is double stranded DNA molecule of about 140kb. It is composed of a unique long (UL) region and a unique short (US) region that are flanked by internal and terminal inverted repeat sequences (IR and TR, respectively). The viral genome encodes approximately 70 different proteins, of which eleven are glycoproteins. Genetic analyses of various clinical isolates have found at least three distinct BHV-1 subtype: a respiratory subtype, a genital subtype and an encephalitic subtype designated as BHV-1.1, BHV-1.2, and BHV-1.3, respectively. In our country, no suitable vaccine against IBR is available for preventing the disease, while good vaccines viz., Bovilis IBR marker (Intervet), IBR marker (Bayer) etc are available in foreign developed countries. Therefore, in this study an attempt was made to construct a suitable vaccine for effectively control of IBR. In the present study, IBR virus was isolated from an aborted foetus (7 months) collected from Government livestock farm, Hastinapur, Meerut, Uttar Pradesh. Virus was isolated and grown on MDBK cells, characterized and identified as BHV-1. Further, the result was validated from OIE referral laboratory, which confirmed the virus as BHV 1.1. Large scale isolation of BHV-1 was done in MDBK cell line. PCR was carried out to amplify both 3' and 5' flanking regions of gE. Both PCR products were cloned in pUC18 vector in JM104 (*E. coli*) competent cells and both clones are ligated to obtain gE deletion fragment. Co-transfection was performed by calcium phosphate method with gE deleted virus and wild virus. Mutant virus was selected by indirect immunoperoxidase monolayer assay and purified by plaque purification method. Virus was grown in large amount in large size glass bottles. Viral suspension was centrifuged and supernatant collected. Virus was inactivated by heat treatment two times. Freund's incomplete adjuvant was added in equal amount. Sterility and safety was checked. In laboratory trial on rabbits, the immune response was found low in comparison to that of wild virus, so there is further need to be revalidate the result using different approach.

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DEPARTMENT OF VETERINARY GYNECOLOGY AND OBSTETRICS

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3	Studies on effect of additives on cryopreservation of Haryana bulls semen	Ajendar Singh Pal	Dr. Vijay Singh	2014	99
4	Studies on effect of butylated hydroxyl toluene and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation	Akhil Patel	Dr. Atul Saxena	2014	100
5	Studies on effect of bovine serum albumin and identification of capacitation like changes in bhadawari bull spermatozoa during cryopreservation	Abishek Kumar	Dr. Atul Saxena	2014	100
6	Studies on effect of iodixanol and identification of capacitation like changes in Bhadawari bull spermatozoa during cryopreservation	Sanjay Singh Yadav	Dr. Vijay Singh	2014	101
7	Studies on effect of glutathione and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation	Dushyant Yadav	Dr. Vijay Singh	2014	101
8	Studies on assessment of capacitation like changes in the cry preserved sperms in Haryana bull	Meena Verma	Dr. Vijay Singh	2015	102

9	Studies on cystic ovarian follicles of bovine	Pramod Kumar	Dr. Ram Sagar	2015	102
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12	Effect of addition of glutathione on tyrosine phosphorylation and apoptosis like changes in cryopreserved Haryana bull semen	Nadeem Shah	Dr. Vijay Singh	2016	104
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15	A Study on Hsp expression and sperm quality following cryopreservation of Haryana bull semen supplemented with sericin	Chhote Lal Yadav	Dr. Atul Saxena	2017	106
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2	Comprative studies on effect of antioxidants on plasma membrane integrity of cropreserved bovine spermatozoa	Akhil Patel	Dr. Atul Saxena	2017	113
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M.V.Sc. Degree Programme

1. Studies on effect of different concentration of egg yolk and glycerol in tris based extender on cryopresercation of Bhadawari bull semen

Vipin Sonker and Atul Saxena

The present experiment was design to study the effect of seasonal variations on semen quality, freezability and semen biochemical indices in Hariana bull. Study was accomplished with the participation of four fertile, healthy adult Hariana bulls aged between 5.5-6.5 years and weighing more than 450-550 kg. The study was conducted over a period of four months and divided into two seasons as winter season (December-January) and summer season (May-June). Twenty-four semen samples (n=24) were collected in each season (six ejaculates from each bull) by using Artificial Vagina. Each ejaculate was divided into two aliquots; one was assessed for physico- morphological and functional attributes, while other was used for evaluation of seminal biochemical and enzymatic profile. The results of the study showed physico-morphological characteristics of semen *viz.* reaction time, volume (ml), mass activity (0-5 scale), pH, sperm concentration (million/ ml), progressive motility (%), sperm livability (%), HOST (%), total sperm abnormal morphology (%) and acrosomal integrity (%) were estimated at different stage in the different seasons. The reaction time, volume of ejaculate and pH was significantly ($p<0.05$) higher in summer than winter season. Percentage of progressively motile spermatozoa were significantly ($p<0.05$) higher in winter than summer season at post-thaw stage. Live (%) spermatozoa were significantly ($p<0.05$) higher in winter than summer season at fresh and pre-freeze stage. HOST positive and acrosomal integrity was significantly ($p<0.05$) higher in winter season than summer at all three stage. Total morphologically abnormal spermatozoa % were significantly higher in summer than winter season.

In the present study showed seminal biochemical and enzymatic profile of semen *viz.* SOD, GST, MDA, GOT and LDH were evaluated. Among the seminal enzymatic profile significant ($p<0.05$) difference were observed for SOD and GST enzymes activity. Hariana bull seminal plasma contains high activities of SOD and GST enzymes activity in winter season that have an influence on the functional competence of cryopreserved spermatozoa.

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2. Studies on certain minerals, antioxidant and hormonal profiles in anoestrus buffaloes

Surya Kumar Maurya and Ram Sagar

This research was designed to study the alteration in the hormonal, biochemical and mineral profile in the anoestrus rural buffaloes and how it varies with cyclic estrus counterpart and further attempt were made to study the microenvironment of the follicles especially two important biochemical molecules i.e. ascorbic acid and nitric oxide which are closely associated with follicular remodelling, oxidant-antioxidant system and ovulation. Anestrous buffaloes were selected from clinical camps and blood sample were collected for mineral, hormonal and biochemical studies. The rural anoestrus buffaloes were divided into three groups and treated for a definite period with antioxidants (Treatment 1), minerals (Treatment 2) and untreated control group (Treatment 3): following the treatment blood samples were also collected for aforesaid mentioned parameters similar pattern were adopted

for natural estrus buffaloes. In phase 2, slaughter house organs (especially ovaries) were collected and classified for cyclic and acyclic animals, determined for numbers of surface follicle and biochemical parameter especially ascorbic acid and nitric oxide were estimated from the aspirated follicular fluid.

In postpartum anoestrus animals the pregnancy percentage in control group was recorded 10% where as in natural oestrus animals it was recorded as 50%. In treatment group 1 (antioxidant group) pregnancy percentage is recorded 43.75% were as in natural oestrus animals it was 50%. In treatment group 2 (mineral mixture) of postpartum anoestrus animals pregnancy rate was 52.94% were as the natural oestrus animals have shown 60%. Comparison of mineral profile of postpartum anoestrus animals with natural oestrus animals did not reveal any significant difference for phosphorus, magnesium, copper, zinc and cobalt, however, significant difference was observed for calcium. Similarly, comparison of hormonal profile (Progesterone, Oestradiol and Insulin) revealed a significant difference whereas for biochemical profile difference was observed for glucose and cholesterol. No difference was observed for total protein concentration. The concentration of ascorbic acid in the two conditions was also found to be significant. In follicular fluid the concentration of ascorbic acid and nitric oxide was also found to differ significantly in acyclic and cyclic ovaries with lower levels in cyclic ovaries indicating their role in steroid production.

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3. Studies on effect of additives on cryopreservation of Haryana bulls semen

Ajendra Singh Pal and Vijay Singh

Oxidative damage to sperm resulting from reactive oxygen species generated by the cellular components of semen is one of the main causes for the decline in motility and fertility of sperm during the freeze-thawing process. The aim of this study was to determine the effects of additives like cysteine hydrochloride and vitamin-E on standard semen parameters of Haryana bull. For this purpose, ejaculates were collected from four Haryana bulls using artificial vagina at twice weekly. Eight ejaculates from each Haryana bull were diluted with Tris extender. The diluted semen sample without any additives was considered as control (group I), samples with incorporation of cysteine hydrochloride @ 5.0 mM and vitamin-E @ 2.5 mM in extender were considered as group II and group III, respectively. Diluted semen was filled in straws, equilibrated for 5 hrs. at 4°C, kept in biological freezer for 7.25 minutes and then stored in liquid nitrogen. Thawing was performed after 24 hrs. of storage, at 37°C for 45 seconds. Progressive motility (%), live spermatozoa (%), abnormal spermatozoa (%), percent HOS responsive spermatozoa and acrosomal integrity (%) were accessed at different stages of cryopreservation (start of equilibration, end of equilibration) and post-thaw. Semen extender supplementation with cysteine (5mM) and vitamin-E (2.5 mM) caused significant ($P<0.05$) increase in all sperm attributes such as percent progressive motility, percent live spermatozoa, percent HOS responsive spermatozoa, percent acrosomal integrity while significant ($P<0.01$) decrease was observed in total sperm abnormality rates in comparison to control group at post thaw semen. The results indicated that supplementation of additives like cysteine hydrochloride (5 mM) and vitamin-E (2.5 mM) improves quality of post thaw semen.

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4. Studies on effect of butylated hydroxyl toluene and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation

Akhil Patel and Atul Saxena

This experiment was designed to study the effect of BHT and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation. For this purpose, ejaculates were collected from two Haryana bulls using artificial vagina at biweekly interval. The semen sample which possesses more than 70 % progressive motility and above 600 million/ ml spermatozoa concentration was subsequently subjected to processing for LN₂ vapour freezing. Semen samples were extended in GEYT extender and split into 3 parts. One part was left as such (control) while the other two parts contain 0.5mM BHT (T1) and 1.0 mM BHT (T2) were considered as treatment 1 and 2 respectively. At different stages of freezing (After dilution, Pre-freezing) and following thawing, semen was also evaluated for per cent live spermatozoa, per cent progressive motility, per cent HOST positive spermatozoa, per cent live spermatozoa with intact acrosome, per cent dead spermatozoa with intact acrosome, per cent live spermatozoa with lost acrosome, per cent dead spermatozoa with lost acrosome, per cent uncapacitated spermatozoa, per cent capacitated spermatozoa and per cent acrosome reacted spermatozoa. Addition of BHT improves most of the parameters at after dilution and pre-freezing stages while more pronounced results were found after post thaw and give a significant effect at the concentration of 1.0 mM in comparison to control and 0.5 mM concentration. Addition of BHT maintains the acrosomal integrity of spermatozoa and lowers the capacitation like changes during cryopreservation.

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5. Studies on effect of bovine serum albumin and identification of capacitation like changes in Bhadawari bull spermatozoa during cryopreservation

Abhishek Kumar and Atul Saxena

This experiment was designed to study the effect of bovine serum albumin (BSA) and identification of capacitation like changes in Bhadawari bull spermatozoa during cryopreservation. For this purpose, ejaculates were collected from two Bhadawari bulls using artificial vagina at biweekly interval. Following evaluation of neat semen and those which confirmed the standard, the semen sample of each bull were split into three equal parts and extended with three different combinations of extenders i.e. GEYT (control), GEYT with 5 mg/ml BSA (T1) and GEYT with 10 mg/ml BSA (T2). The semen sample which possesses more than 70 % progressive motility and above 600 million/ml spermatozoa concentration was subsequently subjected to processing for LN₂ vapour freezing. At different stages of cryopreservation (after dilution and pre-freezing) and following thawing, semen samples were evaluated for per cent live spermatozoa, per cent progressive motility, per cent HOST reactive spermatozoa, per cent live spermatozoa with intact acrosome, per cent dead spermatozoa with intact acrosome, per cent live spermatozoa with lost acrosome, per cent dead spermatozoa with lost acrosome, uncapacitated, capacitated and acrosome reacted spermatozoa. Additional of BSA improves most of the parameters at post thaw stage as compared to control group, however, there was a significant ($P < 0.05$) difference between the two concentrations (5 mg/ml and 10 mg/ml) of BSA for most of the parameters at post thaw

stage. Addition of BSA reduces capacitation like changes during cryopreservation of spermatozoa.

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6. Studies on effect of iodixanol and identification of capacitation like changes in Bhadawari bull spermatozoa during cryopreservation

Sanjay Singh Yadav and Vijay Singh

This experiment was designed to study the effect of iodixanol and identification of capacitation like changes in Bhadawari bull spermatozoa during cryopreservation. For this purpose, ejaculates were collected from two Bhadawari bulls using artificial vagina at biweekly interval. Following evaluation of neat semen and those which confirmed the standard, the semen sample of each bull were split into three equal parts and extended with three different combinations of dilutors i.e. GEYT (control), GEYT with 1.25% (v/v) Iodixanol (T1) and GEYT with 2.5% (v/v) Iodixanol (T2). The semen sample which possesses more than 70 % progressive motility and above 600 million/ml spermatozoa concentration was subsequently subjected to processing for LN₂ vapour freezing. At different stages of freezing (after dilution, pre-freezing) semen samples were evaluated for per cent live spermatozoa, per cent progressive motility, per cent HOS positive spermatozoa, per cent acrosome intact live, acrosome intact dead, acrosome lost live, acrosome lost dead, uncapacitated, capacitated and acrosome reacted spermatozoa. Additional of Iodixanol improves most of the parameters at post thaw stage as compared to control group, however no significant difference was found in between the two concentrations (1.25% and 2.5%) of Iodixanol for most of the parameters. Addition of Iodixanol reduces capacitation like changes during cryopreservation of spermatozoa.

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7. Studies on effect of glutathione and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation

Dushyant Yadav and Vijay Singh

This experiment was designed to study the effect of Glutathione and identification of capacitation like changes in Haryana bull spermatozoa during cryopreservation. For this purpose, ejaculates were collected from two Haryana bulls using artificial vagina at biweekly interval. Semen ejaculates were splitted into 3 equal parts and extended with three different combinations i.e. GEYT (control), GEYT with 0.5 mM Glutathione (treatment 1) and 1.0 mM Glutathione (treatment 2). The semen sample which possesses more than 70 % progressive motility and above 600 million/ ml spermatozoa concentration was subsequently subjected to processing for LN₂ vapour freezing. At different stages of freezing (After dilution, Pre-freezing) and following thawing, semen was also evaluated for per cent live spermatozoa, per cent progressive motility, per cent HOST positive spermatozoa, per cent acrosome intact live, per cent acrosome intact dead, per cent acrosome lost live, per cent acrosome lost dead, per cent uncapacitated spermatozoa, per cent capacitated spermatozoa and per cent acrosome reacted spermatozoas. Addition of Glutathione improves most of the parameters after dilution and pre-freezing stages while more pronounced results were found after post thaw and gives a significant effect at the concentration of 0.5 mM in comparison to control and 1.0

mM concentration. Addition of Glutathion maintained the acrosomal integrity of spermatozoa and reduces the capacitation like changes during cryopreservation.

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8. Studies on assessment of capacitation like changes in the cry preserved sperms in Haryana bull

Meena Verma and Vijay Singh

The present study was accomplished with the participation of four Haryana bulls aged between 5.5 - i. years and weighing more than 450 - 500 kg. Forty ejaculates were collected from four bulls during the period of study with the use of artificial vagina. The study was designed with two objectives firstly to cryopreserve Haryana bull spermatozoa in 0.25 ml straws using Tris egg yolk glycerol extender and secondly to study the protein tyrosine phosphorylation in the fresh and cryopreserved Haryana bull spermatozoa. Percent live spermatozoa, progressive motility, HOS reactive spermatozoa, CTC positive spermatozoa and acrosome intact spermatozoa significantly reduced with the stages of freezing (fresh with initial dilution, semen with final dilution and post thaw). Capacitation and acrosome reaction in post thawed spermatozoa were significantly higher as compared to fresh and finally diluted semen. SDS-PAGE of fresh semen samples showed 84, 82, 80, 78, 76, 75, 72, 60, 58, 54, 50, 45, 42, 40, 38, 20 and 14 kDa protein bands when resolved in SDS- PAGE. Similar pattern of bands were also shown by the proteins of finally diluted semen samples. The resolved gel showed protein bands of molecular weight (150, 140, 120, 110, 90, 84, 82, 80, 78, 76, 75, 72, 60, 58, 54, 50, 45, 42, and 40 kDa) with in thawed semen. Five high molecular weight proteins having molecular weight 150,140,120,110 and 90 kDa were found only in thawed semen samples. Protein of 38kDa was absent in the thawed semen samples. Fresh and finally diluted semen samples revealed 5 tyrosine specific phosphorylated proteins as evident from immune-blotting. The proteins identified were p40, p42, p48, p68 and p70. Immuno- blotting of post thawed semen samples exhibited nine proteins which were appeared to be tyrosine phosphorylated. The proteins identified were p28, p42, p44, p48, p50, p68, p78, p84 and p94. Tyrosine specific phosphorylated proteins were found to be localized on the sperm flagella and in specific, tyrosine phosphorylated proteins were confined to middle and principal piece of sperms. The study concluded that with freezing capacitation of sperms occur and it is associated with phosphorylation of tyrosine containing proteins. Further studies are required to identify these specific proteins and their role in sperm function and capacitation.

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9. Studies on cystic ovarian follicles of bovine

Pramod Kumar and Ram Sagar

This research was designed to study the incidence of cystic condition in cows and buffaloes as one of the cause of infertility in the present scenario, their association with biochemical, mineral and hormonal profile and the effectiveness of hormone GnRH and progesterone towards treatment of cystic condition.

Cystic animals were selected on the basic of history of infertility followed by confirmation with ultrasonography examination. Selected animals belong to University farm (ILFC) and daily OPD of University clinics (TVCC). The cystic animals were divided into two groups and treated with GnRH (Receptal, 0.02 mg I/M, Treatment 1) & progesterone (Triu B, 948 mg Intra-vaginally, Treatment 2). Beside, normal cyclic cows and buffaloes were kept as control. Blood sample from these animals were collected for biochemical, mineral and hormonal studies on

three occasions i.e on the day of detection of cyst (day 0), 14th after treatment and at induced estrus. For normal cyclic animals blood collection schedule was on the day of estrus and 14 day post estrus .

The study reveals the incidences of cystic condition in cows as 22.29% (37/166) and in buffaloes' as 23.15% (22/95).

Comparison of biochemical parameters of cystic animals (cows and buffaloes) with normal cyclic animals (cows and buffaloes) reveal significant difference for glucose, cholesterol, total protein , albumin, AST and ALT however, no significant difference was observed for blood urea nitrogen, creatinine . For mineral profile significant difference was observed for zinc, manganese and magnesium, however, no significant difference was observed for copper, iron, calcium and phosphorus.

Concentration of hormone progesterone differs significantly however, estrogen was found to differ significantly in buffaloes only.

Cystic cows treated with GnRH (T1) responded 100% to treatment and resulted in 60% pregnancy taking 2.50+0.37 service per conception. The average duration from induction to conception was 53.4+7.77 days. In progesterone treated group (T2), 95% animals responded to treatment and resulted in 63.16% pregnancy taking 2.00+0.23 service per conception. The average duration from induction of estrus to conception was 41.62+5.63 days.

In cystic buffaloes for GnRH treatment group (T1), 100% responded to treatment resulting in 66.66% pregnancy taking 3.16+0.27 service per conception. The average duration from induction of estrus to conception was 78.50+21.28 days. In progesterone treated group (T2) 90% animals responded to treatment resulting in 77.78% pregnancy taking 2.29+0.22 service per conception. The average duration from induction of estrus to conception was 24.30+9.25 days.

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10. Study on effect of cooling and freezing rate on cryopreserved Haryana bull spermatozoa

Alok Kumar and Atul Saxena

This experiment was designed to study the effect cooling and freezing rate in Haryana bull spermatozoa during cryopreservation. For this purpose, ejaculates were collected from four Haryana bulls using artificial vagina at biweekly interval. The semen sample which possesses more than 70 % progressive motility and above 500 million/ ml spermatozoa concentration was subsequently subjected to processing for LN2 vapour freezing. Semen samples were extended in GEYT extender and split into 3 parts. For these parts temperature reduced from 35 oC to 4oC by means of rapid (@ 2.060C/min), moderate (@ .480C/min) and slow cooling (@ .250C/min). Each cooled part again given three freezing protocols i.e. rapid (@ 200C/min), moderate (@ 100C/min) and slow (@ 50C/min). Samples were evaluated at pre-freeze and post-thawing stage for per cent live spermatozoa, per cent progressive motility, per cent HOST positive spermatozoa, per cent spermatozoa with intact acrosome (FITC-PSA) and different category of plasma membrane integrity as shown by AN/PI assay and reduction of each parameter was observed as the stage of cryopreservation advanced. Maximum reduction observed in rapid cooling-rapid freezing whereas damage found to be minimal in slow cooling- slow freezing protocol.

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11. Studies on effect of cholesterol loaded cyclodextrin on freezability and capacitation status of Haryana bull spermatozoa

Hanuman Prasad Yadav and Anuj Kumar

The present study evaluated the beneficial effects of incorporation of Cholesterol loaded cyclodextrin (CLC) as an additive in Tris egg yolk based extender in Haryana bull semen opted for ultralow freezing. The study evaluated physical seminal attributes (motility, livability and membrane integrity), cryocapacitation like changes, process of tyrosine phosphorylations, and apoptosis like changes in terms of mitochondrial transmembrane potential and DNA integrity after equilibration and thawing. Ten ejaculates from four Haryana bulls were divided into four aliquots: One aliquot diluted with egg yolk tris citrate (EYTG) extender Group I (control, without CLC), second aliquot was diluted with EYTG but supplemented with 0.5mg CLC/120x10⁶ spermatozoa Group II, 1.0 mg CLC/120x10⁶ spermatozoa Group III and the fourth aliquot was diluted with EYTG and supplemented with 2.0 mg CLC/120x10⁶ spermatozoa Group IV and were cryopreserved. Semen evaluation after equilibration and post-thaw showed supplementation of 0.5mg CLC/120x10⁶ spermatozoa to EYTG extender significantly ($P < 0.05$) increased motility, viability and membrane integrity of spermatozoa. The degree of cryocapacitation was significantly ($P < 0.05$) decreased in 0.5mg CLC/120x10⁶ spermatozoa supplemented group. Immunoblot revealed seven proteins which were tyrosine phosphorylated and protein of 32kDa (p32) showed differential variation in intensity in the four groups. There was significant reduction in band intensity of 32kDa in Group II as compared to other three groups. Immunolocalisation studies revealed localisation of tyrosine phosphorylated proteins at mitochondria (high fluorescence), post-acrosomal region (medium fluorescence), principal piece (low fluorescence) and neck (high fluorescence) of spermatozoa. Addition of 0.5mg CLC/120x10⁶ spermatozoa significantly decreased percentage of spermatozoa showing fragmented DNA after thawing as compared to control. Along with this, CLC significantly increased the percentage of spermatozoa with high transmembrane mitochondrial potential. The result of the present study clearly demonstrated beneficial effects of CLC supplementation on post thaw cryocapacitation and apoptosis like changes in spermatozoa and it can be suitably incorporated for long term cryopreservation of spermatozoa.

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12. Effect of addition Of glutathione on tyrosine phosphorylation and apoptosis like changes in cryopreserved Haryana bull semen

Nadeem Shah and Vijay Singh

The present study evaluated the beneficial effects of incorporation of Glutathione as an additive in Tris egg yolk based extender in Haryana bull semen opted for ultralow freezing. The study evaluated physical seminal attributes (motility, livability and membrane integrity), process of cryocapacitation, immunoblotting for identification of tyrosine phosphorylated proteins, immunolocalization of tyrosine phosphoproteins in spermatozoa, and apoptosis like changes in terms of mitochondrial transmembrane potential and DNA integrity during after equilibration and thawing. Ten ejaculates from four Haryana bulls were divided into three aliquots: One aliquot diluted with egg yolk tris citrate (EYTC) extender (Control), second aliquot was diluted with EYTC but supplemented with 0.5Mm Glutathione (T1) and the third aliquot was diluted with EYTC and supplemented with 1.0Mm Glutathione

(T2) and were cryopreserved. Semen evaluation at equilibration and post-thaw showed supplementation of Glutathione (0.5Mm) to EYTC extender significantly ($P < 0.05$) increased motility, viability and membrane integrity of spermatozoa. The degree of cryocapacitation was significantly ($P < 0.05$) decreased in Glutathione supplemented group. Immunoblot revealed six proteins which were tyrosine phosphorylated and protein of 30kDa (p30) showed differential variation in intensity in the three samples. There was significant reduction in band intensity of 30kDa in T1 as compared to control and T2. It was also found that tyrosine phosphorylated proteins were differently located during different stages of semen preservation. Addition of GSH significantly decreased percentage of spermatozoa showing fragmented DNA after thawing as compared to control. Along with this, GSH supplementation significantly increased the percentage of spermatozoa with high transmembrane mitochondrial potential. The result of the present study clearly demonstrated beneficial effects of Glutathione supplementation on post thaw cryocapacitation and apoptosis like changes in spermatozoa and it can be suitably incorporated for long term preservation of spermatozoa.

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13. Studies on cryopreservation of spermatozoa in relation to seasonal variation

Vipin Singh and Anuj Kumar

The present experiment was design to study the effect of seasonal variations on semen quality, freezability and semen biochemical indices in Haryana bull. Study was accomplished with the participation of four fertile, healthy adult Haryana bulls aged between 5.5-6.5 years and weighing more than 450-550 kg. The study was conducted over a period of four months and divided into two seasons as winter season (December-January) and summer season (May-June). Twenty-four semen samples ($n=24$) were collected in each season (six ejaculates from each bull) by using Artificial Vagina. Each ejaculate was divided into two aliquots; one was assessed for physicomorphological and functional attributes, while other was used for evaluation of seminal biochemical and enzymatic profile. The results of the study showed physico-morphological characteristics of semen viz. reaction time, volume (ml), mass activity (0-5 scale), pH, sperm concentration (million/ ml), progressive motility (%), sperm livability (%), HOST (%), total sperm abnormal morphology (%) and acrosomal integrity (%) were estimated at different stage in the different seasons. The reaction time, volume of ejaculate and pH was significantly ($p<0.05$) higher in summer than winter season. Percentage of progressively motile spermatozoa were significantly ($p<0.05$) higher in winter than summer season at post-thaw stage. Live (%) spermatozoa were significantly ($p<0.05$) higher in winter than summer season at fresh and pre-freeze stage. HOST positive and acrosomal integrity was significantly ($p<0.05$) higher in winter season than summer at all three stage. Total morphologically abnormal spermatozoa % were significantly higher in summer than winter season. In the present study showed seminal biochemical and enzymatic profile of semen viz.SOD, GST, MDA, GOT and LDH were evaluated. Among the seminal enzymatic profile significant ($p<0.05$) difference were observed for SOD and GST enzymes activity. Haryana bull seminal plasma contains high activities of SOD and GST enzymes activity in winter season that have an influence on the functional competence of cryopreserved spermatozoa.

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14. Functional characterisation of voltage gated potassium channels in bull spermatozoa

Rishi Kumar Gupta and Vijay Singh

In the current study, molecular and functional characterisation of Kv 1.1 was carried out in spermatozoa of Haryana bulls. Sixty four ejaculates were collected from four Haryana bulls and were used for series of experiments. Immunoblotting and immunocytochemistry were employed for the Molecular characterisation of Kv 1.1. Immunoblotting identified a single band of 56 kDa corresponding Kv1.1 in Haryana bull spermatozoa. Positive immunoreactivity was seen in head, middle piece and in principal piece of the spermatozoa for Kv 1.1. Functional study was carried out using antagonist 4-aminopyridine and two agonists were used namely cromakalim and pinacidil during the entire study, the study, three groups were used namely, control (100 μ L of sperm dilution medium (SDM) containing 10×10^6 cells), vehicle (99 μ L of SDM containing 10×10^6 cells, DMSO- 1 μ L) and drug (99 μ L of SDM containing 10×10^6 cells, drug 1 containing drug). Blocking of Kv using 4-AP resulted significant ($p < 0.05$) decrease in sperm progressive motility, capacitation (B-pattern) and acrosome action (AR-pattern), however, significant ($p < 0.05$) increase in per cent swollen spermatozoa was served. Activation of Kv channels using both cromakalim and pinacidil resulted in significant ($p < 0.05$) increase in sperm motility, capacitation (B-pattern) and acrosome reaction (AR-pattern), were, significant ($p < 0.05$) decrease in per cent swollen spermatozoa was seen. Treatment of spermatozoa with both agonist and antagonist resulted in capacitation like changes in spermatozoa and these changes were confirmed by immunoblotting and immunolocalisation. Blotting of proteins confirmed the presence of p78, p110 and p115 tyrosine phosphorylated proteins and these were found be localized at middle piece of the spermatozoa. Protein p115 showed highest band intensity indicating the significant protein involved in the process of capacitation after modulation of Kv channels. Computer assisted semen analysis of motion and kinematic parameters in 4-AP treated spermatozoa indicated reduction in sperm motion parameters like LIN, STR, VSL and VAP and higher LH, VCL, and BCF indicating sperm hyperactivity and reverse trend was seen in agonist cromakalim treated samples. In conclusion- Kv 1.1 was found to be present in bull spermatozoa and was selectively associated with regulation of functional dynamics of spermatozoa. It was not possible to deduce from the study that how it is associated with induction of hyperactivity and further studies are warranted to undermine its mechanistic involvement in regulation of sperm function.

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15. A Study on HSP expression and sperm quality following cryopreservation of Haryana bull semen supplemented with sericin

Chhote Lal Yadav and Atul Saxena

The present study evaluated the beneficial effects of incorporation of Sericin as an antioxidant in Tris egg yolk based extender in Haryana bull semen opted for ultra low freezing. The study evaluated seminal attributes (motility, liveability, acrosomal integrity and host reactive spermatozoa) and expression of Heat Shock Protein (HSP70 and HSP90). Six ejaculates from four Haryana bulls were divided into three aliquots: One aliquot diluted with egg yolk tris citrate (EYTG) extender (Control), second aliquot was diluted with EYTG but supplemented with Sericin @ 0.25% (T1) and the third aliquot was diluted with EYTG and

supplemented with Sericin @0.50%(T2) and were cryopreserved. Semen evaluation at equilibration and post-thaw showed supplementation of Sericin @0.25% to EYTG extender significantly ($P < 0.05$) increases motility, viability and spermatozoa with intact acrosome and HOS positive spermatozoa.

The expression of HSP70 and 90 mRNA were found to be significantly lower in cryopreserved semen compared to fresh semen. The concentration of HSP 70 and 90 expression were significantly higher in sericin treated samples and 0.25% sericin was found to be significantly superior compare to 0.50% concentration.

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16. Functional characterisation Of TRPV1 channel in bull spermatozoa

Akashay Kumar and Vijay Singh

Calcium influx in flagellated cell like spermatozoa plays critical role in regulation of sperm motility, capacitation, hyperactive motility and acrosome reaction. Calcium influx is primarily mediated by CatSper channels and transient receptor potential (TRP) channels. TRP Vanilloid 1 is considered as most versatile thermosensitive, pH sensitive and chemosensitive ion channel found in spermatozoa. Little information is available with contrasting reports regarding the role of TRPV1 in regulation of sperm function. Molecular and functional characterisation of TRPV1 was carried out in spermatozoa of Harijana bulls. Sixty four ejaculates were collected from four bulls and were used for series of experiments. Immunoblotting and immunocytochemistry were employed for the molecular characterisation of TRPV1. Immunoblotting identified a single band of 104 kDa corresponding to TRPV1 in Harijana bull spermatozoa. Positive immune-reactivity was seen in acrosomal, preacrosomal, post acrosomal and flagellar regions corresponding to TRPV1. Functional study was carried out using TRPV1 blocker namely Capsazepine (Cp) @ 10 μ M and one activator was used namely Anandamide (AEA) @ 0.3 μ M. In the study, three groups were used namely, control (100 μ L of sperm dilution medium (SDM) containing 1 \times 10⁶ cells), vehicle (3 μ L) and drug (Cp, AEA and their combinations). Different time of incubations was used depending on the experiments. Blocking of TRPV1 resulted in significant ($P < 0.05$) till 1h and after that PSM was sustained as compared to control. However, both during blocking and activation of TRPV1, per cent spermatozoa showing hyperactive motility was increased (20-30%) ($P < 0.05$). Evaluation of Cp and AEA treated spermatozoa stained with CTC revealed significant ($P < 0.05$) increase in B-pattern of spermatozoa indicating induction of capacitation. Spermatozoa treated with different pH gradients showed significant ($P < 0.05$) reduction in motility as compared to control both with and without drugs modulating TRPV1. Functions of TRPV1 were found to be mediated through cAMP and PKA pathway in the induction of hypermotility in sperm cells as evident from inhibition of sAC and PKA. Both L- and T- type of calcium channels were found to be associated with TRPV1 function as evident from their respective blocking and its effect on PSM. Blocking as well as activation of TRPV1 showed significant ($P < 0.05$) reduction in sperm livability, per cent spermatozoa having intact membrane, per cent spermatozoa having intact acrosome, per cent spermatozoa showing high mitochondrial transmembrane potential indicating the involvement of TRPV1 in the process of regulation of sperm functional dynamics. From the study, it was concluded that TRPV1 channels are found in bull spermatozoa and are pH dependent. These channels mediate number of sperm functions like hyper motility, capacitation and acrosome reaction through complex interacting pathways through calcium and pH dependent mechanisms. Further studies are required to find out the possible relationship between TRPV1 channels and other

channels in regulating spermatozoa function and possible mechanisms associated with TRPV1 activation as well as its role in sperm function regulation.

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17. Studies on effect of melatonin on cryopreservation of Haryana bull spermatozoa

Dilip Kumar Yadav and Anuj Kumar

The present study evaluated the beneficial effects of Melatonin as an additive in Tris egg yolk based extender in Haryana bull semen opted for cryopreservation. The study evaluated physico-morphological seminal attributes (motility, livability, HOS response and membrane integrity), cryocapacitation like changes and process of tyrosine phosphorylation after equilibration and thawing. Post-thaw seminal plasma enzymes activities (SOD, LPO and GST) was also evaluated. Eight ejaculates collected from four Haryana bulls were divided into four aliquots: One aliquot diluted with egg yolk tris glycerol (EYTG) extender Group I (control, without melatonin). Group II was diluted with EYTG and supplemented with - i. 5mM Melatonin/100x10⁶ spermatozoa, Group III supplemented with 1.0mM Melatonin /100x10⁶ spermatozoa, Group IV was diluted with EYTG and supplemented with 2.0mM Melatonin/100x10⁶ spermatozoa were cryopreserved. Semen evaluation after equilibration and post-thaw stage showed supplementation of 2.0mM Melatonin/100x10⁶ in semen significantly ($P < 0.05$) increased motility (%), sperm livability (%), HOST (%), and acrosomal integrity (%) of spermatozoa. In the present study, seminal plasma enzymatic profile of semen v/z.SOD, GST and MDA were evaluated at post-thaw stage of semen cryopreservation. Among the seminal enzymatic profile significant ($p < 0.05$) difference was observed for MDA and GST enzymes activity. The degree of cryocapacitation was significantly ($P < 0.05$) decreased in group supplemented with 2.0mM Melatonin/100x10⁶ spermatozoa at post-thaw stage of semen cryopreservation. However no significant difference was observed in degree of cryocapacitation at pre-freeze stage of semen cryopreservation between the groups. Immunoblot revealed seven proteins which were tyrosine phosphorylated and protein of 48kDa (p48) showed differential variation in intensity in the four groups. There was significant reduction in band intensity of 48kDa in Group IV as compared to other groups. Immunolocalisation studies revealed localization of tyrosine phosphorylated proteins at middle piece (high fluorescence), anterior part of head (high fluorescence) and post acrosomal region (medium fluorescence) at post-thaw stage of semen cryopreservation. The result of the present study clearly demonstrated beneficial effect of Melatonin @2.0mM on cryopreservation of Haryana bull spermatozoa.

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18. Studies on effect of trehalose on cryopreservation of Haryana bull spermatozoa

Gyanesh Kumar and Atul Saxena

The present experiment was design to study the effect of supplementation of ose on freezability and antioxidant activity of Mariana bull semen. Semen sample were in TFYG extender containing different Trehalose concentrations (1 OmM, 30mM. M. 100mM. 150mM). The control samples were extended with TFYG alone. Extended samples were packed into 0.25 mL polyvinyl french straws and kept at 4°C for 5 hours libration. The straws were cryopreserved in the vapours of liquid nitrogen (LN2) for 7 then dipped in liquid LN2. Frozen straws were thawed at 37°C for 45 sec and were evaluated for semen characteristics at various

stages (after dilution, after thawing and Post-Thawing). Results clearly indicated that 30 mM Trehalose group had significantly ($P < 0.05$) higher percentage of individual motility, sperm viability, HOST sperm and acrosomal integrity in comparison to the control and other Trehalose extended groups. Trehalose @30mM supplemented group had significantly ($p < 0.05$) the percentage of capacitated spermatozoa compared to control and other Trehalose extended groups. In biochemical assays, no clear cut demarcation for antioxidant was found for Trehalose supplementation. However, Trehalose supplementation has increased activity for Glutathione reductase. In conclusion extender supplemented with @ 30 mM and 10mM concentration improve the post thaw semen quality and was to be having more beneficial effect on freezability of Haryana bull spermatozoa as testified by post-thaw seminal parameters.

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19. Studies on effect of soya milk based extenders on cryopreservation of Haryana bull spermatozoa

Manoj Kumar Yadav and Atul Saxena

The present experiment was designed to study the effect of Soya milk based extenders on freezability of Haryana bull semen in order to have a better substitute for egg yolk based extenders following issues of xenobiotic contamination. For the experiment, four adult Haryana bulls were taken as semen donor. Twenty-eight semen samples were collected ($7 \times 4 = 28$) by using Artificial Vagina. Semen samples were extended with TEYG and Tris glycerol extender supplemented with 5%, 15% and 25% soya milk. Extended semen samples were packed into 0.25 mL polyvinyl french straws and kept at 4°C for 5 hours of equilibration. The straws were cryopreserved in 3* vapours of liquid nitrogen (LN2) for 7 minutes then dipped in liquid LN2. Frozen straws were thawed at 37°C for 45 sec and samples were evaluated for semen characteristics at various stages (after dilution, after equilibration and Post-Thawing) for the fresh ejaculates of Haryana bulls, no significant ($P > 0.05$) difference was observed for semen volume (ml), mass motility (0-5 scale), pH, sperm concentration (million/ml) in the fresh ejaculate of the four Haryana bulls. A significantly (< 0.05) higher value was observed in the percentage of progressive motility, live sperm, host tractive sperm, intact acrosome at all the stages (post dilution, pre-freeze and post thaw) in egg yolk based (control) extender as compared to soya milk (5%, 15% and 25%) based extenders, however, the cryocapacitation was significantly ($P < 0.05$) reduced in soya milk based extenders. The seminal enzymatic profile study showed significant ($p < 0.05$) difference for SOD (super oxide dismutase), MDA (malondialdehyde) and GR (glutathione reductase) enzymes activity with higher activity of SOD in control (egg yolk based) and lower activities of MDA and high GR activity in soya milk based extender. The experiment revealed role of soya milk concentration in cryopreservation of Haryana bull semen however, a detail study is needed to further select a proper concentration of soya milk.

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20. Studies on effect of egg yolk powder on freezability of Haryana bull spermatozoa

Arun Kumar and Anuj Kumar

The present study was to evaluate the effect of Egg yolk powder as a substitute in Tris egg yolk base extender in Haryana bull semen opted for cryopreservation. The study evaluated physico-morphological seminal attributes (motility, livability, HOST response and acrosomal integrity). Post-thaw seminal plasma enzymes activities (SOD, LPO and GST) was also

evaluated cryocapacitation like changes and tyrosine phosphorylation. Eight ejaculates collected from four Hariana bulls were divided into seven aliquots. One aliquot diluted with egg yolk tris glycerol (EYTG) extender Group I (Control without egg yolk powder), Group II was diluted with tris glycerol and supplemented with 5% egg yolk powder/ 100* 10⁶ spermatozoa, Group III was diluted with tris glycerol and supplemented with 10% egg yolk powder/100x10⁶ spermatozoa, Group IV was diluted with tris glycerol and supplemented with 15% egg yolk powder/100x10⁶ spermatozoa, Group V was diluted with tris glycerol and supplemented with 20% egg yolk powder 100x10⁶ spermatozoa were cryopreserved. Semen evaluation after equilibration and post-thaw stages showed supplementation of with 5% egg yolk powder/ 100x10⁶ spermatozoa in semen significantly ($p < 0.05$) increased motility (%), sperm livability (%), HOST (%), and % acrosomal integrity of spermatozoa. In the present study, seminal plasma enzymatic profile of semen viz. SOD, GST and MDA were evaluated at post-thaw stage of semen cryopreservation and significant ($p < 0.05$) difference was observed between estimated seminal plasma enzymatic profile. The degree of cryocapacitation was ($p < 0.05$) decreased in the group supplemented with 5% egg yolk powder/100x10⁶ spermatozoa at post-thaw stage of cryopreservation. Immunoblots revealed five proteins which were tyrosine phosphorylated proteins at middle piece, principal piece, acrosome and post acrosome regions at post-thaw stages of cryopreservation. The result of the present study clearly demonstrated beneficial effect of egg yolk powder @5% on cryopreservation of Hariana bull spermatozoa.

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21. Studies on effect of taurine on cryopreservation of Hariana bull spermatozoa

Alok Kumar Rathor and Atul Saxena

The present investigation was planned to evaluate the beneficial effect of Taurine on different seminal attributes (liveability, progressive motility, HOST reactivity, acrosomal integrity, capacitation status) and antioxidant enzymes (SOD, LPO [MDA], GST) during freeze-thaw process of Hariana bull spermatozoa. Seven ejaculates from four Hariana bulls (7x4=28) were evaluated for volume (ml), pH, mass motility, concentration (millions/ml), progressive motility and live percentage of spermatozoa and upon evaluation of their parameters in the prescribed range, were used for further processing. Semen samples were extended with EYTG and EYTG + taurine @ (25mM, 50mM, 100mM and 200mM). The control (EYTG) and treatment (EYTG + taurine @ 25, 50, 100, 200) groups were subjected to the process of cryopreservation and evaluated for various parameters during different stages (post dilution and pre-freeze) of freeze-thaw process. In the neat semen of Hariana bulls, no significant ($P > 0.05$) difference was observed among seminal pH, concentration (millions/ml), progressive motility and live sperm percentage, however, significant difference was observed for semen volume (ml) and mass motility (0-5 scale).

After dilution, pre-freeze and post thaw stage, all parameters i.e. live sperm percentage, HOS positive spermatozoa, sperm with intact acrosome, *F\ 'B', 'AR' pattern spermatozoa (CTC assay) and antioxidant enzymes (SOD, LPO[MDA] and GST) did not found to differ significantly ($P > 0.05$). However, the percentage of progressively motile spermatozoa differ significantly ($P < 0.05$) between control and treatment groups. Taurine between 25 to 50 mM behave similar to control, however, at 100 and 200 mM suppresses the progressive motility. In conclusion, Taurine (25 to 200 mM) did not improve the freezability of Hariana bull spermatozoa based on the parameters covered under this study. However, its higher concentration (100mM and 200mM) inhibited the progressive motility of spermatozoa.

Key words: Bull semen/Cryopreservation/Haryana /Taurine/Antioxidant.

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22. Studies on effect of quercetin on freezability of Haryana bull spermatozoa

Amit Kumar Yadav and Atul Saxena

The present investigation was planned to evaluate the beneficial effect of Quercetin on different seminal attributes (progressive motility, liveability, HOST reactivity, acrosomal integrity, capacitation status) and antioxidant enzymes (SOD, LPO [MDA]) during freeze-thaw process of Haryana bull spermatozoa. Eight ejaculates from four Haryana bulls (8x4=32) were evaluated for volume (ml), mass motility, seminal pH, concentration (millions/ml) and progressive motility of spermatozoa and upon evaluation of their parameters in the prescribed range were used for further processing. Semen samples were extended with EYTG and EYTG + Quercetin @ (20^μM, 40^μM and 60^μM). The control (EYTG) and treatment (EYTG + Quercetin @ 20, 40, 60,) groups were subjected to the process of cryopreservation and evaluated for various parameters of freeze-thaw process.

In the neat semen of Haryana bulls, no significant (P>0.05) difference was observed among volume (ml), mass motility, seminal pH, concentration (millions/ml) and progressive motility.

After dilution, pre-freeze and post-thaw stage, all parameters i.e. progressively motile spermatozoa, live spermatozoa, membrane integrity (HOST), spermatozoa with intact acrosome (Giemsa), capacitation status of spermatozoa along with antioxidative enzymes (SOD, LPO [MDA]) were found to differ significantly (P<0.05). The per cent progressive motility, live sperm percentage, per cent HOS positive spermatozoa, per cent spermatozoa with intact acrosome, uncapacitated spermatozoa were significantly (P<0.05) higher in T2 group as compared to control and other treatment groups (T1 and T3). For antioxidant enzymes, the significantly higher values were found with T3 group (60^μM quercetin).

Thus, Quercetin supplementation at concentration of 40 μM produce better results in the terms of sperm per cent progressive motility, live sperm percentage, per cent HOS reactive spermatozoa, per cent spermatozoa with intact acrosome, Quercetin at the concentration of 40 μM can be added to the Tris extender for its positive effect on sperm progressive motility, live %, membrane integrity, acrosomal integrity.

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23. Studies on effect of Igf-1 on cryopreservation of Haryana bull spermatozoa

Gyaan Veer Singh and Anuj Kumar

This experiment was designed to study the effect of insulin like growth factor (IGF-1) on cryopreservation of Haryana bull spermatozoa. For this purpose, semen ejaculates were collected from four Haryana bulls using artificial vagina at biweekly interval. The semen sample which possess more than 70% initial progressive motility and above 500 million/ml spermatozoa concentration was subsequently subjected to processing for experiment. Semen samples were extended in egg yolk tris glycerol (EYTG) extender and split into four parts. For these parts IGF-1 added @ 50 ng/80 x 10⁶ spermatozoa, 100 ng/80 x 10⁶ spermatozoa and 150 ng/80 x 10⁶ spermatozoa in group II, III, IV respectively and group I taken as control (without IGF-1). Semen samples were evaluated at pre-freeze and post-thaw stage for per cent live

spermatozoa, percent individual progressive motility, percent HOS positive spermatozoa, percent spermatozoa with intact acrosome and seminal plasma enzyme (SOD,MDA) activity. A significant ($P<0.05$) higher individual progressive motility, viability, HOS response and acrosomal integrity observed at pre-freeze and post-thaw stage by using 150 ng/ml IGF-1. No significant ($P<0.05$) difference was observed in seminal plasma enzymatic (SOD, MDA) activity both at pre-freeze and post-thaw stages. However, the SOD and MDA activity in seminal plasma was non significantly lower in group IV (supplimented with IGF-1 @ 150 ng/ 80×10^6 spermatozoa).

Concentration of 150 ng/ml IGF-I was found to be more beneficial in cryopreservation of Haryana bull spermatozoa as evidenced by post-thaw seminal parameter.

Study suggests that IGF-1 can be added to extender for improving cryosurvival of Haryana bull spermatozoa.

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Ph.D. Degree Programme

1. To study development a potential of goat tetraploid embryos derived from in-vitro fertilization

Jitendra Kumar Agarwal and Atul Saxena

The present study was designed to evaluate the effects of different electro-fusion field strength (kV/cm) and pulse duration (μ s) on development of goat tetraploid embryos. The effect of different O₂ concentrations was also evaluated on the production of tetraploid embryos. For production of tetraploid embryo, 2-cell embryos were obtained from two sources; first by the method of activation of oocytes using calcium ionophores and second by the method of in-vitro fertilization. The results of the present study revealed that the cleavage rate from the parthenogenetic activation was recorded 61.87%. Out of total cleavage, the embryos which developed upto the stage of morula and blastocyst were 11.99% and 2.49%, respectively. The cleavage rate of in-vitro fertilized oocytes was 14.8%. Out of total cleavage, the embryos which developed upto the stage of morula and blastocyst were 4.29% and 0.92%, respectively. The produced 2-cell embryos were exposed to different field strength and pulse duration and their fusion rate and subsequent developmental stages were compared. Optimum cleavage was obtained at the 1.2 kV/cm for 4 μ s duration, from activation derived 2-cell embryos (58.97%) as well as from IVF derived 2-cell embryos (33.33%). When the effect of different oxygen concentrations was evaluated, it was observed that maximum cleavage (82.35%) was recorded at the 5% oxygen concentration and 4.76% of the embryos developed upto the blastocyst stage. It was concluded that 1.2 kV/cm field strength and 4 μ s pulse duration and 5% oxygen concentration were the most suitable treatment for production of tetraploid embryos.

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2. Comparative studies on effect of antioxidants on plasma membrane integrity of cryopreserved bovine spermatozoa

Akhil Patel and Atul Saxena

This experiment was designed to compare the effect of two different antioxidants i.e. (a) 2.50 % Iodixanol (v/v) and (b) 1 mM BHT on plasma membrane and mitochondrial integrity of cryopreserved bovine spermatozoa. For this purpose, ejaculates were collected from four Haryana bulls using artificial vagina at biweekly interval. The semen sample which possesses more than 70 % progressive motility and concentration above 600 millions spermatozoa/ml, was subsequently subjected for processing to LN₂ vapour freezing. The fresh ejaculate was divided into three parts. The first part was extended with EYTG (control), the second part with EYTG + 2.50 % Iodixanol (v/v) (Treatment 1) and third part with EYTG + 1mM BHT (Treatment 2). At different stages of freezing (After dilution, Pre-freezing) and following thawing, semen was evaluated for per cent live spermatozoa, per cent progressive motility, per cent spermatozoa with intact acrosome, per cent HOS reactive spermatozoa, per cent spermatozoa with high mitochondrial membrane potential, percent uncapacitated spermatozoa, per cent capacitated spermatozoa and percent acrosome reacted spermatozoa. Addition of antioxidants had resulted in a significant improvement in all the parameters under study compared to control group. Amongst the antioxidants, BHT proved significantly better compared to Iodixanol in preserving the different parameters under study. Thus use of

2.5% Iodixanol & 1 mM BHT can be successfully used in cryopreservation of Hariana bull semen.

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3. Studies on Apoptosis like changes during cryopreservation in Hariana and Murrah bull semen

Chetna Gangwar and Atul Saxena

The present study was conducted to evaluate the effect of semen cryopreservation on the various cryodamages or apoptotic changes that occur in sperm plasma membrane, acrosomal membrane (capacitation like changes) and mitochondrial membrane. For the study four Hariana and two Murrah bulls were used as semen donor, and semen was collected twice a week interval through artificial vagina, A total of 8 (n=32) ejaculate from each Hariana bull and 10 (n=20) ejaculate from each Murrah bull were collected. The study evaluated the physical, functional and structural attributes of semen like motility, viability, membrane integrity, acrosomal integrity, mitochondrial activity, capacitation like changes, apoptotic changes and sperm morphometry in fresh and frozen thawed semen. Semen evaluation at fresh, post equilibration and post-thaw showed that there was significant ($P < 0.05$) decrease in progressive motility, viability, acrosomal integrity, plasma membrane integrity and head size and a significant ($P < 0.05$) increase in capacitated, acrosome reacted and moribund spermatozoa after cryopreservation in Hariana bull. These parameters were more pronounced in Murrah bull semen. Supplementation of reduced Glutathione in Murrah bull semen revealed a significant ($P < 0.05$) increase in motility, viability, membrane integrity, acrosomal integrity and significant ($P < 0.05$) decrease in capacitated, acrosome reacted and apoptotic sperm in Glutathione treated group as compared to control group at post thaw stage. Further, GSH supplementation significantly increased the percentage of spermatozoa with high transmembrane mitochondrial potential. The result of the present study clearly demonstrated that freeze thaw inflicts apoptotic changes in Hariana and Murrah bull spermatozoa and supplementation of GSH in the semen of Murrah bull reduces the apoptotic changes, which will be helpful for long term preservation of semen.

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DEPARTMENT OF VETERINARY MEDICINE

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M.V.Sc. Degree Programme

1. Metabolic profiling of buffaloes suffering from mastitis and its management using adjective remedial

Krishan Veer Singh and Shanker Kumar Singh

In the present study a total of 500 lactating buffaloes were screened and high prevalence (18.2%) of SCM was recorded animal. Those buffaloes that were in early lactation and positive for subclinical mastitis were allocated into two groups and one group was administer with nutritional supplements, while other was kept as negative control. Additionally buffaloes with clinical mastitis were also divided into two group and one group was treated alone with cefquinome while other with cefquinome and nutritional supplements. Milk composition of subclinical mastitic milk, metabolic and biochemical status of affected buffaloes were evaluated at day 0 and day 7 post-therapy. On day 0, remarkable alteration in milk composition of subclinical mastitis buffaloes and significant alteration in metabolic status of clinical as well as subclinical mastitic buffaloes was recorded in comparison to the healthy controls. The altered nutritional panels as well as milk compositions were ameliorated toward normalcy at day 7 post-therapy in subclinical mastitic buffaloes administered with nutritional supplements. Additionally clinical mastitic buffaloes treated with antibiotic and nutritional supplements also revealed remarkable improvement in clinical score as well as in metabolic panels towards normalcy in comparison with the antibiotic alone treated buffaloes. At day 7 post-therapy, remarkable improvements in SCC was also recorded in theses buffaloes when compared with day 0 values of same group, but the values were still significantly higher than the healthy controls. Thus, remarkable alterations in milk compositions of subclinical mastitic buffaloes and metabolic status of subclinical as well as clinical mastitic buffaloes are implicated. These altered metabolic panels and milk components can be ameliorated toward normalcy by administering of nutritional supplements in mastitic buffaloes.

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2. Metabolic profiling of cattle suffering from mastitis and its management using adjective remedial

Deepak Sharma and Shanker Kumar Singh

In the present study a total of 521 lactating cattle were screened and very high prevalence (26.42%) of SCM was recorded animal. Those cattle that were in early lactation and positive for subclinical mastitis were allocated into two groups and one group was administer with nutritional supplements, while other was kept as negative control. Additionally cows with clinical mastitis were also divided into two group and one group was treated alone with cefuroxime while other with cefuroxime and nutritional supplements. Milk composition of subclinical mastitic milk and metabolic status of affected cows were evaluated at day 0 and day 7 post-therapy. On day 0, remarkable alteration in milk composition of subclinical mastitis cows and significant alteration in metabolic status of clinical as well as subclinical mastitic cows was recorded in comparison to the healthy controls. The altered nutritional panels as well as milk compositions were ameliorated toward normalcy at day 7 post-therapy in subclinical mastitic cows administered with nutritional supplements. Additionally clinical mastitic cows treated with antibiotic and nutritional supplements also revealed remarkable improvement in clinical score as well as in metabolic panels towards normalcy in comparison with the antibiotic alone treated cows. At day 7 post-therapy, remarkable improvements in

SCC was also recorded in these cows when compared with day 0 values of same group, but the values were still significantly higher than the healthy controls. Thus, remarkable alterations in milk compositions of subclinical mastitic cows and metabolic status of subclinical as well as clinical mastitic cows are implicated. These altered metabolic panels and milk components can be ameliorated toward normalcy by administering of nutritional supplements in mastitic cows.

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3. Clinico-epidemiological diagnostic and therapeutic studies on trypanosomiasis in buffaloes

Ashish Pratap Singh and A.K. Tripathi

Trypanosomiasis, an arthropod born blood protozoan disease commonly known as Surra is caused by *Trypanosoma evansi* and transmitted mainly by *Tabanus* spp. The present study was conducted to investigate the various diagnostic techniques (viz. giemsa stained thin blood smear, buffy coat method and TE-LAT) used in the diagnosis of trypanosomiasis in buffaloes, clinical epidemiology of trypanosomiasis in buffaloes and comparative efficacy of various anti-trypanosomal drugs (viz. isometamidium chloride hydrochloride, diminazene aceturate and quinapyramine sulphate).

The order of decreasing diagnostic efficacy during present investigation was found as: TE-LAT (52.02%) > Buffy Coat method (45.62%) > Giemsa stained thin blood smear (38.20%). The maximum prevalence was recorded during the month of September followed by the month of October and the least prevalence was recorded in the month of June. It has been found that prevalence of trypanosomiasis in buffaloes does not vary with the sex, breed and pregnancy status. However, higher prevalence was observed with the increase in the age and parity of the animals. Predominant clinical signs observed in trypanosomiasis with highest frequency were anorexia, fever, depression, reduced milk yield, congested mucous membrane, nasal discharge, emaciation, respiratory distress, pallor mucous membrane, anaemia and edema of dependent parts, with moderate degree of frequencies were superficial lymph node swelling, salivation, ocular discharge, Ileus, head pressing and with lowest frequencies were frequent urination, excitement, circling, abortion, abdominal pain, muscular twitching, diarrhoea, bloat, corneal opacity, localized paralysis, generalized paralysis and exophthalmia. There was a reduction in Hb, PCV, TEC and lymphocyte count and increase in TLC and neutrophil counts in all the positive cases before treatment, however, no significant variation in basophils, monocyte and eosinophils percentage were recorded. In biochemical study the values of serum globulin, creatinine, BUN, ALT, AST and ALP in all the positive cases were found significantly higher while blood glucose and serum albumin values were found lower, however, no alteration in total protein was recorded before treatment. In terms of improvement in hematological and biochemical values after treatment of positive cases, best recovery was assessed in treatment with isometamedium followed by diaminazine and quinopyramine. On the basis of clinical improvement in terms of disappearance of clinical signs & parasitological examination, isometamidium chloride hydrochloride was found as most effective anti-trypanosomal drug followed by diminazine aceturate and quinapyramine sulphate was found to be least effective.

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4. Effects of prepartum supplementation of antioxidants and anionic salt on metabolic and oxidative status of transition Sahiwal cattle

Brajesh Kumar Yadav and Shanker Kumar Singh

In the current study apparently healthy twenty four Sahiwal cows in the last trimester of pregnancy were enrolled and randomly allocated into four groups (group 1, 2, 3 and 4) having six cows in each one. The cows of group 2 were supplemented with 25 g of antioxidants mixture daily from 21 days before parturition to till day of parturition. Cows of group 3 were supplemented with 60 g of anionic salt with same regimen. Cows of group 4 were supplemented with the both 25 g of antioxidants and 60 g of anionic salt with same regimen. However, cows of group 1 were not supplemented with any supplements and were kept as standard control. Remarkable alterations in serum metabolites, lipid dynamics and oxidant/antioxidant balance in control cows were recorded during the transition period. The control cows suffered from production diseases and their milk quality was also differed from the supplemented cows. The cows prepartal supplemented with antioxidants and/or ammonium chloride revealed remarkable amelioration of metabolites and oxidative damage and have superior milk quality. These cows have a smooth transition period and were free of production diseases. Among these groups the cows supplemented with the both antioxidants and anionic salt revealed the best mitigating potential over the transition stress and metabolomic changes. Therefore, prepartal supplementation with antioxidants and anionic salts could be one of the best strategies to curb transition induced physiological aberrations and thus production diseases in *Sahiwal* cows.

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5. Phytochemical and clinical evaluation of plant extracts and its fractions against goat tick (*Rhipicephalus* spp.)

Mahendra Kumar Chaudhary and Ashok Kumar

The present study was conducted to test the acaricidal efficacy of plant extracts and its fractions against goat tick *Rhipicephalus* spp. The acaricidal property of *Annona sqamoa* leaves, *Lantana camra* leaves, *Melia azedarach* leaves, *Embelia ribes* seed, *Calatropis procera* leaves *Azadirachta indica* leaves, *Pongamia glabra* seed, *Vitex negundo* leaves, *Butea frondosa* seed *Nicotiana tabacum* leaves and *Eucalyptus* leaves were studied by using methanolic crude extract and subsequently with solvent fractioned. Percent yield of crude extract were found 21, 36.5, 27/14, 8, 12, 31, 13.5, 26.5, 8, 15, 40 % respectively. LC50 of *Annona sqamoa*, *Lantana camra*, *Calatropis procera* and *Vitex negundo* in BSLT Test were 4.17, 14.24, 19.64 and 14.15 mg/ml respectively. On adult mortality test we found LC50 of *Annona sqamoa*, *Lantana camra*, *Calatropis procera* and *Vitex negundo* were 11.58, 43.66, 30.62, 29.84 mg/ml respectively. In egg hatch assay *Annona sqamosa* was most effective with LC50 value of 7.31 mg/ml. The other LC50 were *Calatropis procera*{ 9.96mg/ml}, *Vitex negundo*(9.96mg/ml) & *Lantana camara*(11.93 mg/ml). In larval mortality assay 4 plant showed excellent efficacy with LC50 of *Annona sqamosa* (0.26 mg/ml), *Calatropis procera*(0.32 mg/ml), *Lantana camra*{0.41 mg/ml}, *Vitex negundo*(1.24 mg/ml). On fecundity test *Annona sqamosa* and *Calatropis procera* reduced egg reduction. On phytochemical analysis, The *annonna sqamosa* was positive for alkaloids, glycodides, fixed oil and fats, *negundo* was positive for flavinoids, saponins, glycodides. *Lantana camra* was positive for flavinoids, alkaloids, saponins, steroids, tannins. *Calatropis procera* was positive for alkaloids, glycodides. In clinical studies. On clinical evaluation the

mean percent reduction of tick was observed 18.99%, 39.24%, 58.23%, 100% at 10mg/ml concentration and at 50 mg/m the mean mortality percentage was recorded 23.46%, 40, 74%, 69.14%, 100% at 1, 2, 4, & 24 hrs post spray. 1.

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6. Clinico-epidemiological diagnostic and therapeutic studies on *Trypanosoma evansi* infection in equine

Rakesh Kumar Singh and A.K. Tripathi

Trypanosomiasis, an arthropod born blood protozoan disease commonly known as Surra is caused by *Trypanosoma evansi* and transmitted mainly by *Tabanus* spp. The present study was conducted to investigate the various diagnostic techniques (viz. giemsa stained thin blood smear, buffy coat method, TE-LAT and Ab-ELISA) used in the diagnosis of trypanosomiasis in equines, clinical epidemiology of trypanosomiasis in equines and comparative efficacy of various anti-trypanosomal drugs (viz. diminazene aceturate, quinapyramine sulphate and isometamidium chloride hydrochloride) alone and along with vitamin-E as an antioxidant.

The order of decreasing diagnostic efficacy of various methods during present investigation was found as: Ab-ELISA (22.33) > TE-LAT (21.16%) > Buffy Coat method (9.90%) > Giemsa stained thin blood smear (6.99%). The maximum prevalence was recorded during the month of September followed by the month of October and the least prevalence was recorded in the month of June. It has been found that prevalence of trypanosomiasis in equines does not vary with the sex, age, species pregnancy status and months of pregnancy. Predominant clinical signs observed in trypanosomiasis with highest frequency were reduced performance (92.83%), fever (82.61%), petechial hemorrhages (76.52%), anorexia (75.65%), depression (72.17%), pallor of mucous membrane (70.43%), congested mucous membrane (69.57%), emaciation (67.83%), anemia (61.74%), with moderate degree of frequencies were respiratory distress (34.78%), circling (33.04%) and localized paralysis (posterior paresis) and with lowest frequencies were ocular discharge (27.83%), nasal discharge (25.22%), superficial lymph node swelling (24.35%), excitement (21.74%), frequent urination (19.13%), head pressing (18.26%), ileus (16.52%), diarrhoea (14.78%), colic/ abdominal pain (13.04%), corneal opacity (07.83%), abortion (5.88%) and generalized paralysis (1.74%). There was a reduction in Hb, PCV, TEC and lymphocyte count and increase in TLC and neutrophil counts in all the positive cases before treatment, however, no significant variation in basophils, monocyte and eosinophils percentage were recorded. In biochemical study the values of serum globulin, creatinine, BUN, ALT and LPO in all the positive cases were found significantly higher while blood glucose, serum albumin, SOD and catalase values were found lower, however, no alteration in total protein, AST and ALP was recorded before treatment. In terms of improvement in hematological and biochemical values after treatment of positive cases, best recovery was assessed in the treatment with diaminazine aceturate along with vitamin-E followed by isometamedium chloride HCl with vitamin-E and least with quinopyramine sulfate alone or along with vitamin-E. On the basis of clinical improvement in terms of disappearance of clinical signs & parasitological examination, diminazine aceturate alone or along with vitamin-E was found as most effective anti-trypanosomal drug followed by isometamidium chloride hydrochloride alone or along with vitamin-E and quinapyramine sulphate alone or along with vitamin-E was found to be least effective.

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7. Studies on the effects of prepartum supplementation of ammonium chloride along with immunomodulators on metabolomics and immunodynamics of periparturient indigenous cows

Vivek Kumar Singh and Shanker Kumar Singh

In the current study apparently healthy sixteen indigenous cows in the last trimester of pregnancy were enrolled and randomly allocated into two groups (group 1 and 2) having eight cows in each group. The cows of group 1 were supplemented with one bolus of immunomodulator and 50g of ammonium chloride daily from 14 days before parturition to till day of parturition. However, cows of group 2 were not supplemented with any supplements and were kept as negative control. A group of eight cows in mid lactation (>90 days) were taken as standard control. Remarkable alterations in serum metabolites, lipid dynamics and immunodynamics in control cows, who were not fed prepartum with the supplements (Group 2), were recorded during the transition period. The control cows suffered from clinical and subclinical production diseases. The cows prepartal supplemented with immunomodulators and ammonium chloride (Group 1) revealed remarkable resistance in metabolomic and immunodynamic alterations and were capable to maintained immuno-metabolomic status towards normalcy during the transition period. These cows have a smooth transition period and were free of production diseases. Therefore, prepartal supplementation with immunomodulators and anionic salts could be one of the best strategies to curb transition induced physiological aberrations and thus production diseases in indigenous cows.

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8. Studies on add-on effects of a polyherbal formulation on clinical recovery and immuno-competence of dogs with demodicosis

Alok Singh and Shanker Kumar Singh

A breach in the immune status of the young dogs may results into the occurrence of generalized demodicosis. Cytokines secretions from T lymphocytes have played important roles in the immune response of the dogs against generalised demodicosis. Therefore, the present study aimed to evaluate the effects of a polyherbal formulation on clinical recovery and immuno-competence of dogs with generalised demodicosis. Total 19 client-owned dogs with generalised demodicosis of juvenile onset were allocated into two groups. Control group (n=9) were treated with conventional miticide (Amitraz), whereas other 10 demodicosed dogs were supplemented with a polyherbal formulation (Pyodermacare-G) adjunct with Amitraz regime. Clinico-haemato-biochemical and circulatory cytokines (TNF- α , IFN- γ and IL-10) were estimated pre- and post-therapies. Demodicosed dogs of the control group have not revealed remarkable amelioration in most of the altered serum haemato- biochemical and immunological panels at day 60 post-therapy. Remarkable parasitological and clinical recovery could not be achieved by the dogs of control group at day 60 post-therapy. Whereas, the demodicosed dogs supplemented with Pyodermacare-G revealed remarkable amelioration in haemato- biochemical and immunological panels (TNF- α , IFN- γ and IL-10) at day 60 post-therapy. Remarkable improvements in clinical and parasitological recovery were also revealed by the demodicosed dogs of Pyodermacare-G supplemented group. Therefore, it can be concluded that miticidal therapeutic regimens of canine generalised demodicosis warrants supplementary medicines

having immunomodulatory potential for the holistic management and to get rid of the wretched clinical condition. The polyherbal formulation (Pyodermacare-G) could be promising candidate for the holistic managements of immuno-clinico-pathological anarchies of canine generalised demodicosis.

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9. Clinico epidemiological, diagnostic and therapeutic studies on bovine trypanosomosis

Hemant Kumar and Mukesh Kumar Srivastava

The study was performed at TVCC, DUVASU Mathura from June, 2016 to May 2017. The epidemiological data was recorded on the basis of month, sex, age, breed, parity and pregnancy status. Hospital based prevalence of trypanosomosis was found to be 11.41% and 22.90 % in cattle and buffaloes, respectively. Highest prevalence of trypanosomosis in cattle and buffaloes was recorded during the rainy season and lowest in summer. It has been found that prevalence of trypanosomiasis in cattle and buffaloes does not vary with the sex and breed. Maximum occurrence of trypanosomiasis is found in the age group of more than 5 years and more than, three parity and the lowest in the age group of less than 2 years and zero parity. The important clinical signs exhibited by the trypanosome positive cattle and buffaloes having highest frequency were anorexia, fever, depression and reduced milk yield. Out of 50 blood samples screened, significantly higher percentage of animals were diagnosed positive for *T. evansi* with PCR followed by BCT and minimum with blood smear. Sensitivity and specificity of BCT (75.0% and 100%) and blood smear (37.5% and 100%) of *T. evansi* was reported, respectively. and it was found that PCR is more sensitive than the conventional method of examination. There was a significant reduction in hemoglobin concentration, packed cell volume and total erythrocyte count in all the positive cases. There was an increase in the hemoglobin concentration, packed cell volume and total erythrocyte count at the day 7th and day 14th after the treatment in all treatment groups. There was an increase in serum ALT, AST and ALP concentration in the positive cases of cattle and buffaloes. On the basis clinical improvement in terms of disappearance of clinical signs & parasitological examination in present study, the percent recovery shown by the isometamidium chloride along with enrofloxacin and diminazine acetate along with enrofloxacin on day 7th and day 14th post-treatment was found to be 100 percent. Animals treated by diminazine acetate on day 7th and day 14th posttreatment was found to be parasitologically cured however moderate and mild clinical sign were present on day 7th and day 14th respectively. Animals treated by the isometamidium chloride on day 7th and day 14th post-treatment was found to be parasitologically cured however mild clinical sign were present on day 7th and on day 14th 100% recovery observed. Therefore, isometamidium chloride along with enrofloxacin and diminazine acetate along with enrofloxacin is found as most effective and among isometamidium and diaminazine acetate, isometamidium chloride is found better than the diaminazine acetate during present investigation.

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10. Diagnostic relevance of bovine specific cardiac biomarkers and associated electrocardiographic features of theileriosis in cattle calves

Kapil Kumar Gupta and Mukesh Kumar Srivastava

Bovine tropical theileriosis is an important tick born disease causing widespread alteration in physical, haemato-biochemical, electrocardiographic and cardiac biomarkers parameters. For the study, total seventy one calves were screened and out of them twelve were confirmed for theileriosis by PCR, and taken for further investigation. Classical symptoms of theileriosis affected calves were fever followed by anorexia, enlargement of lymph node, pale mucous membrane, ocular and nasal secretions, exophthalmoses, petechial hemorrhage on conjunctival mucous membrane along with melena and hemoglobinuria with least occurrence. Physical parameters like rectal temperature, respiration rate and pulse rate were significantly elevated with simultaneous fall in mean value of hemoglobin concentration, packed cell volume, total erythrocyte count and total leucocyte count. However the mean value of neutrophils, monocytes, eosinophils was considerably decreased with increase in lymphocytes. The mean values of AST, ALT, creatinine, urea and total bilirubin were elevated, while ALP, albumin, globulin and total protein, were decreased in theileriosis affected calves. Electrocardiographic findings revealed presence of sinus tachycardia, atrial premature complex, bilateral atrial hypertrophy and first degree AV block in few theileriosis affected calves. Results of cardiac biomarkers analysis showed significant increase in mean value of both the cardiac biomarkers (cTnI and CPK-MB), which indicate cardiac involvement in pathogenesis of theileria with subsequent damage of myocardial cell membrane resulting into leakage of cardiac biomarkers like cardiac troponin-I (cTnI) and creatinine phosphokinase (CPK-MB). Conventional treatment was applied to theileriosis affected calves and all the data except electrocardiography were re-evaluated after 15 days of treatment. Conventional treatment of present investigation included buparvoquone, long acting oxytetracycline, iron dextran (20%) along with vitamin and other supportive therapy as per need according to clinical signs, which resulted in recovery of 8 calves out of 12 theileriosis affected calves. Primary outcome variable in post treatment group include clear improvement in 50%, improvement and insufficient improvement in 8.33% and failure in 33.33% after 14 days of therapy. Cardiac biomarker analysis in recovered calves showed 87.5% have concentration of cardiac troponin lower than pretreatment value, while 100% of the calves had concentration of creatinine phosphokinase-MB lower than their pre-treatment value. Significantly reduced levels of these biomarkers in post treatment calves were indicative for halt of further myocardial damage after conventional treatment described above. From above discussion it can be concluded that the conventional treatment, used in present study, has good efficacy in term of improvement of cardiac health in theileriosis affected calves.

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11. Evaluation of ameliorative effects of herbal drugs on oxidative stress and thrombocytopenia in canine ehrlichiosis

Amangeet Parashar and Mukesh Kumar Srivastava

In present investigation total 80 dogs were screened for ehrlichiosis, which was based on observation of 2-3 classical clinical symptoms of ehrlichiosis in dogs. All the screened dogs underwent for blood smear examination to confirm the disease status. Blood smear examination results showed 6 dogs positive for ehrlichiosis; primary PCR revealed 23 positive

dogs, however nested PCR confirmed 79 dogs positive for ehrlichiosis of all screened dogs. Out of 79 positive dogs for ehrlichiosis, 8 dogs were found positive for Babesia spp as co-infection as evidenced by simple PCR examination test. Important clinical signs exhibited by the ehrlichia positive dogs in decreasing frequency were high fever and mucosal pallor followed by lymphadenomaegaly, melena, depression and splenomegaly. Clinical signs with moderate frequency were ecchymotic and petechial hemorrhages, weight loss, vomiting, epistaxis, ascites, hind limb/scrotal edema/facial edema. Signs with least frequency were ocular abnormalities, icterus, hematuria and hematemesis. Ocular abnormalities include corneal opacity, uveitis, scleral bleeding, and corneal ulcer. Vital parameter like rectal temperature, heart rate, respiration rate were significantly elevated with simultaneous fall in hemoglobin, total erythrocyte count, packed cell volume, leucocyte count, platelet count however the mean values of neutrophil was considerably increased with decreased in lymphocytes. While mean values of basophil, monocytes, eosinophil, MCV, MCH, MCHC were non significant from the control. The mean values of ALT, AST, ALP, BUN were elevated while albumin, total protein, glucose were decreased in ehrlichiosis affected dogs. While mean values of globulin, serum creatinine, triglycerides, cholesterol were non significant from the control. Weekly percentage enhancement of platelet count in dogs of group 1 with conventional treatment was compared with the other two groups with additional antioxidants and herbal platelet enhancer. Percentage positivity of ehrlichiosis was recorded on the basis of age, sex, breed and season, respectively, Age group with highest positivity was category 3 (> 1 year) with a positivity of 65.82 %, sex with highest positivity was male (70.88 %), German Shepherd breed (50.63%) and summer season (79.74%), respectively. In present study it was found that there was significant increase in total oxidative status and decrease in total antioxidant capacity in all the treatment groups of dogs in comparison with control. There was significant decrease in the total oxidative status in all the treatment groups with highest recovery is seen in group 3rd and 2nd and minimum in group 1st. Significant increase of total antioxidant capacity is seen in the groups 3rd followed by in group 2nd and minimum in group 1st. Therefore in terms of improvement in treatment groups of dogs, best recovery was assessed in treatment with conventional treatment with *Tinospora cordifolia* and *Carica papaya* extract and conventional treatment along with polyherbal preparation and minimum recovery assessed in group 1st in which no herbal drugs were prescribed.

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12. Evaluation of oxidant and antioxidant status of dogs affected with sarcoptic mange and its amelioration by using antioxidant

Ankur Upadhayay and Mukesh Kumar Srivastava

Present study describes the oxidant/antioxidant status of dogs affected with sarcoptic mange and ameliorative effects of antioxidant supplementation on oxidant/antioxidant status of dogs affected with sarcoptic mange. Study was undertaken on twelve client owned dogs confirmed to be suffering from sarcoptic mange, which were randomly divided in two groups (group 1 and 2) irrespective of age, sex and breed, comprising of six dogs each, while group 3 comprised of six healthy dogs. Group 1 dogs were treated with only Doramectin @ 0.4 mg/kg subcutaneously weekly for 5 treatments, whereas group 2 were additionally given Sadenosylmethionine (SAME) @ 20 mg/kg/d PO for 28 days. Blood samples were collected on day 0, 14 and 28 post therapy. The mean values of haemato-biochemical parameters viz. total leucocyte count, eosinophil counts, mean corpuscular volume, aspartate aminotransferase, alanine aminotransferase were significantly higher in scabetic dogs than in control while haemoglobin, total erythrocyte count, haematocrit, lymphocyte counts, glucose,

cholesterol total protein, albumin, albumin/globulin ratio and blood urea nitrogen showed significantly lower mean values in scabetic dogs than in control. In the present investigation dogs with sarcoptic mange were found in a state of oxidative stress as indicated by significantly elevated Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) values and significantly reduced Total Antioxidant Capacity (TAC) values as compared to healthy dogs. The dogs of group 2 showed better clinical recovery and marked ameliorations in TOS, TAC and OSI values in comparison to group 1 at the end of therapy. On the basis of findings of present investigation, it is concluded that administration of SAME in addition to standard therapy can mitigate these alterations expediting the clinical recovery of diseased dogs and therefore can be recommended as an adjunct therapy with miticides for management of canine sarcoptic mange.

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13. Evaluation of therapeutic potential of certain homeopathic preparations in canine atopic dermatitis

Sujit Kumar Chaudhary and Shanker Kumar Singh

Canine atopic dermatitis (CAD) in dogs is a multifactorial genetically predisposed inflammatory and pruritic skin disease with characteristic clinical features that is associated with IgE antibodies. A dysregulation of cutaneous immune system which favours an acute T-lymphocyte helper type-2 (Th2) pro-inflammatory reaction to allergens has recently been documented. The release of many pro-inflammatory cytokines after allergen exposure is now considered to be the key to the allergic response. Glucocorticoids (GCs) (prednisone, prednisolone, methylprednisolone) are considered to be the first line of sole therapy to manage CAD. However, GCs have potential detrimental side effects on the health of treated dogs and are not considered to be safe on prolonged use. The dog-owning public is now more interested in “natural” and “alternative” methods of disease management. Albeit, homeopathic preparations are being used as alternative medicines since long, the scientific validation of their efficacy and mechanism of action in animal’s skin diseases is still in infancy. Hence, the present study aimed to evaluate the efficacy of homeopathic preparations for clinical recovery and immuno-regulatory potential in dogs with AD. The dogs with atopic dermatitis were allocated into four groups of six dogs in each one. Dogs of Group 1 were treated with prednisolone; Group 2 were treated with Sulphur 200 C; Group 3 were treated with Psorinum 200 C and Group 4 were treated with the combination Sulphur 200C and Psorinum 200 C. All homeopathic medicines were given at a dose rate of 3-5 drops, three times in a day orally for 60 days. Dogs with AD revealed remarkable alterations in the leukograms in comparison to healthy controls. Remarkable elevations in circulatory inflammatory cytokines (IL-17 and IL31) and total Ig-E levels were observed in dogs with AD. Atopic dogs treated with prednisolone and Psorinum + Sulphur revealed significant amelioration of the altered haemato-biochemical panels. Similarly, these dogs also found to have significant reduction in circulatory pro-inflammatory cytokines (IL-17 & IL-31) and Ig-E levels at day 60 post-therapy as compared with their own day 0. Marked reduction in clinical scores (CADLI and VAS) of atopic dermatitis were revealed by the atopic dogs when treated with Sulphur alone and in combination with Psorinum at days 30 and 60 post-therapy as compared with day 0 scores. No adverse effects were observed in any of the homeopathy treated dogs. Therefore, it can be concluded that dogs with AD have marked alterations in leukograms with eosinophilia. Remarkable elevation in circulatory pro-inflammatory cytokines (IL-17 and IL-31) and Ig-E might be associated with immuno-pathogenesis of canine atopic dermatitis. Combination of Psorinum 200 C and Sulphur 200 C could be a novel potent therapeutic agent

for management of CAD. These preparations also have potential to heal the skin lesions of dogs with AD and have almost equivalent therapeutic potential to that of standard glucocorticoid. The homeopathic preparations; Psorinum 200 C and Sulphur 200C are quite safe and superior over prednisolone for long term therapy and have excellent potential to ameliorate the immunological aberration of dogs with AD and can cure the dermatological lesions of AD from the root in diseased dogs.

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14. Studies on coagulation profile, clinical relevance of canine C-reactive protein and oculoopathies related to ehrlichiosis in dogs

Jai Deep Singh and Mukesh Kumar Srivastava

In present investigation total 79 dogs were screened for ehrlichiosis, which was based on observation of 2- 3 classical clinical signs of ehrlichiosis in dogs. Blood smear examination results showed 7 dogs positive for ehrlichiosis, primary PCR revealed 21 positive dogs; however nested PCR confirmed 64 dogs positive for ehrlichiosis of all screened dogs. Important clinical symptoms exhibited by the ehrlichia positive dogs in decreasing frequency were fever and mucosal pallor. In the present study, out of 42 dog, which were studied to the recovery state, ocular lesions were observed in 28.57% dogs only. Major ophthalmic signs in dogs of present study were corneal opacity, uveitis with corneal ulcer, conjunctivitis, bilateral corneal opacity, corneal ulcer, scleral bleeding, dermoid growth over cornea, corneal opacity with glaucoma. Hametological and biochemistry parameter like mean values of hemoglobin, total erythrocyte count, packed cell volume, leucocyte count was significantly decrease however the mean values of neutrophil was considerably increased with decreased in lymphocytes. While mean values of basophil, monocytes, eosinophil, MCV, MCH, MCHC were non significant from the control. The mean values of ALT, AST, ALP, BUN were elevated while albumin, total protein, glucose were decreased in ehrlichiosis affected dogs. Present study revealed significant increase in APTT, FIB and D-Dimer concentration in the dogs of treatment group in comparison to DOGS OF control group; however, significant decrease was recorded in the PLT, PCT value in the dogs of treatment group in comparison to control group. Analysis of PT, MPV, and PDW showed non-significant changes in between treatment and control group. Significant increase in CRP concentration was recorded in the treatment groups (2a, 2b) of dogs in comparison with control. Within subgroups of treatment group, more and significant increment in CRP was seen in group 2and (acute) when compared with group 2bnd (chronic).

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15. Evaluation of ameliorative potential of a polyherbal formulation on toll-like receptors and cytokines expression in peripheral blood mononuclear cells and lesional skin of dogs with generalised demodicosis

Sandeep P Somen and Shanker Kumar Singh

The generalized demodicosis can be a serious dermatological condition in dogs if not managed well and the exact pathogenesis of generalized canine demodicosis is yet to be unravelled; however, an aberration in host immunity is considered one of the most significant factors apart from the perpetuating parasite. The therapeutic management of canine

demodicosis remains one of the main challenges in veterinary dermatology. Traditional medicines hold a great promise as a source of easily available effective therapy for skin diseases to the people particularly in tropical developing countries including India. Therefore, the present study aimed to evaluate the effects of a polyherbal formulation on clinical recovery and immuno-competence of dogs with generalised demodicosis. The dogs diagnosed with generalised demodicosis were categorised into two groups; nine demodicosed dogs were treated with 0.0375% solution v/v of amitraz rinse along with Polyherbal formulation and another six were treated with amitraz alone. Marked alterations in the haemograms and leukograms were observed in dogs with GD as compared to healthy dogs. The dogs supplemented with Pyodermacare-G were found to have marked amelioration in the altered leukograms on day 60 post-therapy. Marked upregulation of TLR-2 in systemic as well as local lesion skin of the dogs with GD. Whereas, marked downregulation of TLR-6 in systemic as well as local lesion skin was observed in dogs with GD. Marked systemic up-regulation of immunosuppressive cytokines, IL-10 and TGF- β , in dogs with GD. While changes in the expression of these immunosuppressive cytokines genes could not be detected in the skin lesions of dogs with GD. A marked amelioration of immunosuppressive cytokines in PBMCs of demodicosed dogs adjunctly supplemented with Pyodermacare-G capsules on day 60 post-therapy was detected. On day 30 and 60 post-therapy, the per cent reduction in total mites counts in Pyodermacare-G supplemented dogs were remarkably higher as compared with the same day values of the non-supplemented group. The clinical recovery e.g. improvement in Demodex-induced skin lesions score (DSLs) of both the studied group of demodicosed dogs revealed that on day 30 and 60 post-therapy, the per cent improvement in DSLs in Pyodermacare-G supplemented dogs were remarkably higher as compared with the same day values of non-supplemented dogs. Therefore, it can be concluded that marked alterations in haemato-biochemical panels and immunological dysregulation in including both the innate and adaptive immune systems are attributed to generalised demodicosis in dogs. Pyodermacare-G capsule have potential to improve the immunological dents of canine demodicosis, and can hasten the clinical and parasitological cure when adjunct supplemented with amitraz.

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16. Studies on ameliorative potential of polyherbal formulation on expression of skin barrier proteins and cytokines in dogs with atopic dermatitis

Sarita Kanwal and Shanker Kumar Singh

Canine atopic dermatitis (AD) is one of the most frequent skin diseases of dogs and skin barrier functions and misbalance of inflammatory cytokines are considered to have a paramount role in the pathogenesis AD. The protein expression of FLG and INV in canine AD is still unclear. A major determinant of which antibody class predominates is which one of two subsets of T-helper lymphocytes (designated Th1 and Th2) is dominant. The factors that determine whether a Th1 or a Th2 response will predominate are complex but include both genetic and environmental influences. The management of canine AD is multifaceted, and usually combines interventions such as allergen avoidance, allergen-specific immunotherapy, antimicrobial and anti-inflammatory pharmacotherapy. Glucocorticoids (GCs) (prednisone, prednisolone, methylprednisolone) are considered to be the first line of sole therapy to manage canine AD. Owing to the wide prevalence of skin diseases, increasing incidence detrimental side effects with the most of allopathic drugs, there is a quest for the safe alternative medicines. Traditional medicines hold a great promise as a source of easily

available effective therapy for skin diseases to the people, particularly in tropical developing countries, including India. In celebration of these facts, the present study was aimed to evaluate the therapeutic potential of Polyherbal formulation in dogs with atopic dermatitis, and to evaluate the ameliorative potential of polyherbal formulation on the expression of skin barrier proteins and Th1/Th2 cytokines in dogs with atopic dermatitis. The dogs which fulfilled any five of the Favrot's AD inclusion criteria were included in the present study. The dogs with atopic dermatitis were allocated into two groups. Dogs with AD were treated with Prednisolone) and kept as standard positive controls. In another group, dogs with AD were treated with the Polyherbal capsule. Before the start of therapy, the dogs with AD revealed significantly higher expression of filaggrin and lower expression of involucrin genes. On day 60 post-therapy, atopic dogs treated with Polyherbal capsule revealed a significant reduction in filaggrin and significant increase involucrin genes expressions as compared to their day 0 expressions. However, on day 60 post-therapy, atopic dogs treated with prednisolone revealed significant reductions in both filaggrin and involucrin genes expressions. Before the start of therapy, dogs with AD revealed significantly higher expression of TNF- α , IL-31, and IL-13. On day 60 post-therapy, the atopic dogs treated with Polyherbal capsule revealed significant downregulation of TNF- α and IL-31 genes. On day 60 post-therapy, the atopic dogs treated with prednisolone have not revealed significant reduction in TNF- α . On the contrary, the atopic dogs treated with prednisolone revealed a significantly higher expression of IL-31 genes on day 60 post-therapy as compared to their day 0 values. Remarkable alterations were observed in the leukograms and haemograms of dogs with AD as compared to healthy dogs. Remarkable alteration in some biochemical panels was observed in dogs with AD as compared with healthy dogs. The atopic dogs treated with Polyherbal capsule revealed $55.55 \pm 5.84\%$ and $75.46 \pm 7.61\%$ improvements in CADLI on day 30 and day 60 post-therapy, respectively. Whereas, the atopic dogs treated with prednisolone revealed $29.67 \pm 3.34\%$ and $46.50 \pm 5.52\%$ improvements in CADLI on day 30 and day 60 post-therapy, respectively. An appreciable improvement in body coat lustre, hair re-growth, healing of skin lesions and food intake was revealed by the atopic dogs treated with Polyherbal capsule. The result of the present study indicates that the Polyherbal capsule might have the therapeutic effects against atopic dermatitis via regulation of the barrier proteins expression in the skin as well as via mitigating the immunological deregulation in dogs with AD. Therefore, it can be concluded that the Polyherbal capsule could be a better therapeutic agent against canine AD and can cure atopic dermatitis in dogs not only at the symptomatic echelon but also at the immunopathological points.

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Ph.D. Degree Programme

1. **Effect of Mannan oligosaccharide (MOS) and medicinal plant supplementation on immune status of peri- parturient goats and neonatal goat kids**

Ashish Srivastava and Ashok Kumar

The present work was designed to study the following aspects of preweaning mortality in goat kids. First one is influence of genetic and nongenetic factors on preweaning mortality of goat kids. Second one is to observe the effects of supplementing prebiotic and immunomodulatory polyherbal formulation to pregnant goats on their immunity and metabolism, immune quality of their colostrum and ultimately on immunity and growth of their kids. Lastly, effects of direct supplementation of these formulations were observed on immunity, disease susceptibility and growth of neonatal goat kids. Epidemiological study was conducted on three breeds maintained at CIRG Mathuraviz. Barbari, Jamunapari & Jakhrana whereas supplementation trial was conducted on pregnant Jamunapari goats and neonatal kids. Overall preweaning mortality rate of 2.59 % was recorded in kids of all the three breeds. Kids birth weight, sex or age had no effect on mortality. Highest mortality was observed in kids born as triplet/quadruplet (4.49 %) followed by that in twins (2.88 %) and singlets (2.01 %). Dam's parity was found to have significant effect on mortality with highest mortality observed in kids born to primiparous dams (3.95 %) followed by kids born to dams having \geq 5th parity (3.01 %) and least in 2nd to 4th parity (1.82 %). The goats were supplemented 06 weeks prepartum to 04 weeks postpartum with yeast origin prebiotic (MOS + β -glucan) or polyherbal formulation (combination of crude powder of *Withania somnifera* roots, *Tinospora cordifolia* stem, *Asparagus racemosus* roots and *Punica granatum* fruit rind in ratio of 3:3:3:1 by weight) @ 100 mg/Kg body weight/day. Goats supplemented with polyherbal formulation had significantly higher increase in serum IgG after 2 weeks of feeding. Both type of supplements induced more tolerogenic type of immune response in goats which was characterised by less elaboration of IFN- γ compared to control animals in in vitro IGRA. Both supplements resulted in significantly higher specific gravity of first drawn colostrum and kids born to prebiotic supplemented goats had higher plasma IgG post suckling and these kids. Kids born to prebiotic supplemented goats were more tolerant to LPS induced sepsis and natural infections during 1st month of life. No effect was observed on birth weight and growth rate of the kids. In another experiment, goat kids were directly fed daily from day 03 to day 30 of life with extract of the polyherbal formulation or prebiotic. Supplementation with prebiotic or polyherbal formulation resulted in significantly lower LPS induced release of cytokines - TNF- α as well as IL-10 compared to unsupplemented goat kids and better resistance to infections during 1st month of life. However, protective effect of the prebiotic on cumulative disease incidence lasted while it was being fed regularly and then gradually waned off after the feeding was ceased. Here again, no positive effect was observed on growth rate of the kids.

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2. Effect of maternal supplementation of organic trace minerals (OTM) on immune status of per parturient goats and neonatal goat kids

Arvind Kumar Tripathi and Ashok Kumar

The present study was designed to evaluate the effect of maternal supplementation of organic trace mineral on immune parameters of periparturient goats and neonatal goat kids. In the first step of the study comparative variations of trace minerals status in blood at different stages of periparturient periods and colostrum and milk, colostrum immunoglobulin's and disease incidence/morbidity and mortality were also evaluated in various recognised breeds of goats maintained at organized farms of CIRG in comparison to the non-descript goats maintained at farmers field flock . The results reveal that blood, colostrum and milk levels of zinc (Zn), copper (Cu), iron (Fe) manganese (Mn) and cobalt (Co) were found to be significantly lower at different stages of periparturient period in the Nondescript breed of goats. The colostrum immunoglobulin's (IgG, IgM and IgA) were also found to be lowest in the non-descript breed of goats than recognized breeds of goats. The morbidity and mortality were found to be highest in goat kids born of non-descript breed of goats during pre weaning period in comparison to the recognized breeds. To evaluate the effect of maternal supplementation of OTM the organic salts of Cu and Zn were supplemented at minimum and mean level dose as recommended by NRC. The effect of maternal supplementation of OTM on periparturient goats, trace minerals (Cu and Zn) levels, immune parameters such as humeral and cell mediated immune status and biochemical parameters viz. total proteins, albumins, globulin, A/G ratio and total antioxidant capacity (TAC) were also estimated in Jamunapari goats of various treated groups. The values of Cu and Zn in the goats of treatment group II found to be significantly high in comparison to control and treatment group-I at the time of parturition and one month post-partum stages of periparturient period. Humeral immunity in terms of the levels of IgG, IgM and IgA and cell mediated immunity in terms of the T-lymphocyte stimulation/ blastogenesis were found to be significantly higher in the treatment group II during periparturient period than control and treatment group I. The level of serum total protein and serum globulin, serum insulin like growth factor (ILGF) and total antioxidant capacity (TAC) were found to be significantly high in treatment group II goats during periparturient period in comparison to the control group and treatment group I. It was also observed that the serum cortisol level of treatment group II goats was found to be significantly low at periparturient period under study in comparison to the control group and treatment group I goats. In another study the effect of selected organic trace minerals (Cu and Zn) in pregnant does on quality of colostrum and immune status, disease susceptibility, birth weight and overall growth and vitality of full term kids were evaluated and results reveal that trace minerals viz. Cu and Zn levels, colostrum immunoglobulin's and colostrum antimicrobial proteins were found to be significantly high in treatment group II in comparison to control and treatment group I. the passive transfer of immunity was very efficient in goat kids born from goats of treatment group II as levels of various serum immunoglobulins, serum total protein, globulin, insulin like growth factor and total antioxidant capacity and blood levels of Cu and Zn were found to be significantly higher in the goat kids born from treatment group II in comparison with control and treatment group I, however the level of cortisol was found to be significantly low. Lowest degree of sepsis was observed in the goat kids born from goats of treatment group II than control and treatment group I kids, the cytokines viz. TNF- α , IFN- γ level (pro-inflammatory cytokines) and IL-10 (anti-inflammatory cytokine) were found to be significantly low in treatment group II kids both pre LPs and post LPS (02 hrs) in the kids of treatment group II goats in comparison to

the control and treatment group I. Average daily weight gain (ADWG) was found to be significantly higher in the treatment group II kids in comparison to control and treatment group I. Lowest disease incidence/morbidity and mortality was recorded in kids born of treatment group II goats in first month, second month and third month in comparison to the control group and treatment group I kids.

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3. Studies on immuno-ophthalmopathy of bovine tropical theileriosis in calves with special reference to immunomodulatory effect of injectable trace mineral complex supplementation

Pradeep Kumar Ram and Shanker Kumar Singh

The calves detected positive for *Theileria* were categorised into two groups, eg. Calves with theileriosis and absence of exophthalmia (n=30) and calves with theileriosis and the presence of exophthalmia (n=13). Sixteen healthy, free from any haemoprotozoal infection, have served as healthy controls. For therapeutic regimens plan, the calves with theileriosis were allocated into Group 2 (BUPA) (n=9), Group 3 (BUPA+OXY) (n=10), Group 4 (BUPA+ITM) (n=10), and Group 5 (BUPA+OXY+ITM) (n=14). The clinical manifestation of exophthalmia was observed in approximately every third calf with theileriosis. In the present study the results of haemograms, and serum biochemical panels were found to be significantly altered in calves with theileriosis as compared to healthy calves. *Theileria* infected calves with- and without exophthalmia had marked elevation in circulatory content of both proinflammatory cytokine, TNF- α , and immunosuppressive cytokine, IL-10 than that of healthy controls. Interestingly, the *Theileria* infected calves having exophthalmia had also revealed significantly elevated levels of TNF- α and IL-10 as compared to the *Theileria* infected calves having no exophthalmia. Moreover, the immunostimulatory cytokine, IFN- γ , was markedly lowered in *Theileria* infected calves with- and without exophthalmia as compared to healthy controls. The combination BUPA+OXY was found to have a better therapeutic potential than that of BUPA alone. However, the calves treated with BUPA+ITM revealed the highest per cent improvements in the haemograms followed by the calves treated with BUPA+OXY+ITM. On day 14 post-therapy, the calves treated with BUPA+ITM and BUPA+OXY+ITM had revealed a marked reduction in IL-10 levels. Moreover, the calves treated with BUPA+ITM and BUPA+OXY+ITM had revealed a marked elevation in circulating level of immuno-stimulatory cytokine, IFN- γ , levels. The calves treated with BUPA+OXY and BUPA+OXY+ITM had revealed a marked reduction in TNF- α , while the other two groups could not achieve significant reduction. In conclusion, tropical theileriosis results in major dents over the haemograms of newborn calves, but, no alterations in the leukograms, except to the eosinophilia. Exophthalmia is a paramount clinical manifestation of theileriosis and could be the result of an overproduction of TNF- α , rather, the increased IOP. Reduced systemic production of IFN- γ and overproduction of IL-10 could be the strategies of the parasite to escape the host-immunity. Injectable trace mineral (ITM) has the potential to augment the circulatory contents of IFN- γ (immuno-stimulatory) and curb the circulatory contents IL-10 (immuno-suppressive) in calves with theileriosis. BUPA+OXY+ITM can be recommended for the holistic therapeutic management of tropical theileriosis in newborn calves.

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DEPARTMENT OF VETERINARY MICROBIOLOGY

S. No	Title	Author/ Student	Guide	Year	Page No.
M.V.Sc. Degree Programme					
1	Studies on detection and prevalence of rotavirus infection in lambs and kids.	Uttam Singh	Dr. Rashmi Singh	2014	134
2	Comparison of immunogenicity of FMD vaccines in sheep and goat.	Yogesh Kumar	Dr. Rashmi Singh	2015	134
3	<i>mecA</i> and <i>vanA</i> gene based molecular characterization of methicilin resistant <i>Staphylococcus aureus</i> .	Ritika Yadav	Dr. S. K. Yadav	2015	135
Ph.D. Degree Programme					
1	Development and evaluation of formalized killed adjuvant <i>Brucella melitensis</i> biovar 3 vaccine.	Amit Kumar	Dr. V.K. Gupta	2016	137

M.V.Sc. Degree Programme

1. Studies on detection and prevalence of rotavirus infection in lambs and kids

Uttam Singh and Rashmi Singh

Rotavirus is an important cause of severe, acute, dehydrating diarrhea in neonates of caprine and ovine. Group A rotavirus (RVA) are most commonly associated with gastroenteritis. Recently, *Picobirnavirus* (PBV) has been associated with diarrhea. A total of 225 fecal samples were collected from 0-3 months old diarrheic and non-diarrheic sheep lamb (n=100) and goat kids (n=125) during July, 2013 - Feb, 2014 from unorganized and organized farms in the Mathura region. Ten percent fecal suspension was used for extraction of viral RNA by phenol: chloroform or TRI reagent. The extracted RNA was subjected to RNA-PAGE. All caprine and ovine fecal samples failed to reveal clear 11 segmented banding pattern of RV and a truncated banding pattern were observed. One sample (K23) showed presence of faint 4-6 bands that was identified in RVA specific ELISA and was further typed for RVA and PBV. Other 12 samples showing truncated banding pattern with faint one or two bands were positive in diagnostic RVA (n=9) and PBV (n=4). Detection of rotavirus by antigenic ELISA Kit was performed for RVA antigen and a prevalence of 0.8% caprine RVA was found. For ovine RVA, none of the sample was positive in ELISA. With VP6 gene based diagnostic PCR, a prevalence of 8% was obtained for caprine RVA (10.66% in diarrheic kids and 4% in non-diarrheic kids) and 5% was obtained for ovine RVA (8% in diarrheic lambs and 2% in non-diarrheic lambs). On genotyping, VP7 gene amplicon size of 746 bp and 590 bp corresponding to G6 and G8 genotypes was obtained in one caprine sample (K23). On P typing for VP4 gene, amplicon size of 334 bp corresponding to P[11] was obtained in K23 caprine isolate. Fourteen samples remained untypeable. In the multiplex-nested PCR assay for genogrouping of PBV, three caprine samples (2.4%) and three ovine samples (3.0%) were positive for amplicon size of 201 bp for genogroup I (GGI). Two ovine samples (2.0%) also showed the presence of amplicon size of 369 bp for genogroup II (GGII). Three ovine and one caprine (K23) sample showed mixed infection of RVA and PBV. K23 sample was positive for G6G8P[11] on RVA typing and GGI genogroup for PBV. One ovine sample untypeable for RVA was positive for GGI and two ovine samples untypeable for RVA were positive for GGI and GGII. The K23 sample was collected in the month of August 2013 and was from kid below 1 month of age. In the present study, the prevalence of caprine RVA and age of infection is similar to what have been reported earlier. Typing results in the present study indicate the presence of already reported G6 and G8. P[11] has not been reported earlier. G6 and G8 have not been reported earlier as mixed infection. These mixed infections most likely represent naturally occurring reassortance of rotavirus strains. In the present study, it appears that ovines are less frequently infected than caprines. The presence of a large number of samples as untypeable in the present study needs further investigation.

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2. Comparison of immunogenicity of FMD vaccines in sheep and goat

Yogesh Kumar and Rashmi Singh

FMD is a contagious disease of cloven footed animals including cattle, sheep, goat and others. FMD is caused by virus of family *Picornaviridae*, genus *Apthovirus*. FMDV has seven

serotypes O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3. In India, most of the outbreaks are due to serotype O, A, C and Asia 1. Since last 20 years there is no report of FMD outbreak due to serotype C. The disease is very severe in cattle and buffalo while in sheep and goat it is subclinical in nature. But small ruminants have epidemiological importance in spreading the disease. In the present study, a total 43, sheep (n=16) and goat (n=27) were included. They were divided in three groups separately. Group 1 received combined triovac vaccine (FMD, HS and BQ), group 2 received monovac (FMD only) vaccine and group 3 were used as control. The serum samples were collected on day 0, 30, 60 and 90. NSP based 3 AB3 DIVA ELISA was performed for day 0 samples. A total 12.5% of sheep and 0% of goat were found positive suggesting a mild viral activity. Serum samples were also evaluated by LPB- ELISA to know the humoral immune response. Both monovac and combined vaccines showed peak immune response on day 30 (except for serotype O in combined vaccine) in goat. In sheep, serotype O and Asia 1 in monovac vaccine and serotype O in combined vaccine got peak immune response on day 30. While serotype O in monovac and serotype A and Asia 1 in combined vaccine showed peak immune response on day 60 in sheep. No significant difference was found between the monovac and combined vaccine in both sheep as well as in goat ($p < 0.05$) There was also no significant difference between sheep and goat for combined as well as for monovac vaccine ($p < 0.05$) It can be concluded that combined vaccines can replace the monovac vaccine but further study for antigens other than FMD, is needed in evaluation of immune response. It can be concluded that small ruminants should be brought under FMD surveillance and control programme to prevent FMD dissemination from them.

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3. *mecA* and *vanA* gene based molecular characterization of methicillin resistant *Staphylococcus aureus*

Ritika Yadav and Sharad Kumar Yadav

The commensal presence of *S. aureus* to reside in the anterior nares of wild and domestic animals and of humans worldwide and its ability to overcome the antimicrobial effects it always remains the centre of attraction for the researchers. Non pathogenic as well as non resistant *S. aureus* can acquire resistant form against various antibiotics like methicillin and vancomycin (MRSA, VRSA). The presence of such pathogens in the environment is posing a great challenge to the Veterinary profession, particularly in India, where animals are reared in the close vicinity of animal owners with minimum hygienic measurements. Moreover, the report of Increasing trend in prevalence of MRSA in cattle in India and also the presence of VRSA in Kolkata (India) in 2005 are of great concern not only for veterinarians but also for medical field so the present study was planned to establish status of MRSA as well as VRSA from the pyogenic clinical cases in animals and also the presence of *mecA* gene and *vanA* gene in respective isolates. 100 pus samples from cattle, buffalo (Wound, Abscess, Gangrene, Otitis, Uterine discharge, tail amputation, Pus in teat) and dogs (wound and post operative wound samples) were examined for the presence of *S. aureus* bacteria. Forty isolates of *S. aureus* were found out of 100 pus samples. On the basis of species the prevalence of *S. aureus* in cattle, buffalo and dog pus samples are 38.0%, 38.09% and 50% respectively and the species based prevalence of MRSA among the *S. aureus* isolates are found to be 50%, 58.3% and 62.5% in cattle, buffalo and dog, respectively. The antibiotic sensitivity reveals that in bovines the *S. aureus* isolates are most sensitive for chloramphenicol, streptomycin, gentamicin, cefoxitin, and most resistant for cefotaxime and amoxicillin while in case of dogs most sensitive is gentamicin, amikacin, and most resistant for amoxicillin, *nuc* gene based molecular characterization confirmed prevalence of 38.09% and 50% *S. aureus* in Bovines and dogs,

respectively. *mecA* gene based molecular characterization confirmed the prevalence of 50%, 58.2% and 62.5% MRSA among *S. aureus* in cattles , buffaloes and dogs, respectively. The amplification of *coa* gene revealed the polymorphism with the amplification of more than one amplicon. Out of 40 isolates of *S. aureus* only 19 showed the presence of *coa* gene. Thus this study provides us the evidence that *S. aureus*, particularly MRSA is present in high proportion in the pyogenic samples specially in dogs and buffaloes.

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Ph.D. Degree Programme

1. Development and evaluation of formalized killed adjuvant *Brucella melitensis* biovar 3 vaccine

Amit Kumar and V.K. Gupta

The present study was conducted to develop a stable, safe and effective vaccine against caprine brucellosis. For that three well approved and recognized adjuvants viz., MontanideTM IMS, MontanideTM GEL 1 and MontanideTM ISA61VG were incorporated with virulent *Brucella melitensis* biovars 3 IND1 (Accession no. VTCCBAA228) bacterial strain, isolated from stomach content of aborted fetus of infected goat to develop three different formalized killed adjuvant *B. melitensis* biovar 3 IND1 vaccines viz. NPV (Nano particle based vaccine), PGV (Polymer gel based vaccine) and OAV (oil adjuvant based vaccine), respectively to make it 1.47×10^8 and 1.47×10^{10} CFU per shot in mice and goats vaccine. These vaccines were tested for sterility and then safety in adult female inbred BALB/c mice and were kept at different temperature to assess its stability. Sterile, safe and stable all three vaccines were inoculated 10 μ l vaccine with 1.47×10^8 CFU of *Brucella* in per dose intra nasally (NPV & PGV), Subcutaneously (PGV) and intra muscularly (OAV) in adult female BALB/c mice in the group of 10 for efficacy. 50% of mice were vaccinated with single vaccination where as remaining 50% mice were given booster on 21st day of initial vaccination. Vaccinated mice were challenged on 28th day of vaccination and booster vaccination, respectively with live virulent *B. melitensis* biovar 3 cultures (10^9 CFU) through I/P route and sacrificed on 7th day of challenge. During mice experiment, blood was collected at 7th, 14th, 28th and 35th days of booster vaccination (28th, 35th, 49th and 56th day of first vaccination) for serum as well as whole blood. The blood erythrocytes were used for the estimation of oxidative stress biomarker parameters, plasma for plasma cytokine level whereas serum was used for the status of serum antibodies against Brucellosis by RBPT and indirect ELISA. After sacrifice, mice organs were collected for live weight whereas spleen and liver were also used for live *Brucella* count and molecular confirmation of *B. melitensis* by amplification of 16S rRNA and Omp31 genes. Splenocytes proliferation and expression of cytokines in spleen by Real time PCR were done. Analysis of all the parameters revealed all the vaccines produced efficacy as desired in OIE guidelines and European pharmacopeia. The sterile and stable vaccines which were found to be safe and effective in inbred BALB/c mice (NPV, PGV, OAV) were further used in homologous host (three pure bred apparently healthy non pregnant *Brucella* free Jamunapari adult female goats in each group) with 1.47×10^{10} CFU per shot with intra nasal, subcutaneous and intra muscular routes, respectively and compared with standard Rev.1 (IIL, Hyderabad). Vaccinated animals were subjected to blood collection on 0, 14th and 28th day of vaccination for serum as well as whole blood. On 28th day, animals were challenged with live virulent *B. melitensis* biovar 3 cultures (10^9 CFU) through subcutaneous route and monitored for physical, physiological and other adverse reactions and blood samples were collected 14th, 28th, 60th and 90th days post challenge till animals were sacrificed on 90th day of challenge. Serum separated from blood samples were used for detection of serum antibodies by RBPT, STAT and indirect ELISA along with serum enzyme chemistry. The whole blood was used for blood hematology, plasma for cytokine levels and estimation of oxidative biomarker parameters was done in erythrocytes. The vital organs collected immediately after sacrifices were subjected to histopathology to observe the changes produced by challenge, estimation of oxidative biomarker parameters, live *Brucella* load in spleen and liver and molecular confirmation of *B. melitensis* by amplification of 16S rRNA and Omp31 genes. The splenocyte proliferation and expression of TLR by Real time PCR in spleen, liver, supra mammary lymph node and uterus

tissues were also examined. On the basis of the findings of present study we can conclude the following: 1. *Brucella melitensis* biovar 3IND1 can be used as a vaccine candidate for the control of caprine Brucellosis in India. 2. Three vaccines developed with formalized killed *Brucella melitensis* biovar 3IND1 and nano particle (NPV), polymer gel (PGV) and oil adjuvant (OAV) were found stable for the duration of 12 months under refrigeration temperature (4-8°C). 3. *Brucella melitensis* biovar 3IND1 based formalized killed vaccines (NPV, PGV and OAV) confer good serological as well as cell mediated immune response in mice and goats. 4. Nano particle, polymer gel and oil adjuvant can be used as adjuvants to improve immunogenicity in caprine. 5. The persistence of antibodies due to killed vaccination is for shorter duration in comparison to Rev.1 in caprine. 6. The protection against virulent *Brucella melitensis* biovar 3IND1 in goats vaccinated with killed vaccines was comparable to Rev.1 for the duration of 90 days. 7. Among three killed vaccines attempted in present study, OAV revealed better efficacy and safety in comparison to other two vaccines (NPV & PGV). 8. The OAV was followed by PGV in efficacy and safety parameters. Based on laboratory findings as well as trial on homologous host OAV can be further recommended for field trial.

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DEPARTMENT OF VETERINARY PARASITOLOGY

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2	Epidemiological and phytotherapeutic studies on gastrointestinal parasites of large ruminants	Amreesh Sachan	Dr. Daya Shanker	2014	140
3	Prevalence of trypanosomiasis its clinico-haemato-biochemical impact and PCR based detection in buffaloes	Rahul Parashar	Dr. Daya Shanker	2014	141
4	Classico -molecular studies and characterization of Rotat 1.2VSG of <i>Trypanosome evansi</i> in equines	Anjali Devi	Dr. Daya Shanker	2017	142
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1	Studies on susceptibility pattern in response to natural coccidial infection in goats of semi-arid region	Alok Kumar Singh	Dr. Daya Shanker	2015	143
2	Immunomolecular studies for diagnosis and characterization of <i>Sarcocystis</i> species in buffaloes	Amit Singh	Dr. Daya Shanker	2017	143
3	Evaluation of some biochemical parameters and TLR expression in <i>Haemonchus contortus</i> resistant and susceptible goats	Jitendra Tiwari	Dr. Daya Shanker	2017	145
4	Evaluation of excretory secretory antigen as a candidate for serodiagnosis of caprine amphistomosis	Amit Kumar Jaiswal	Dr. Daya Shanker	2018	146
5	Genotyping and characterization studies on sarcocysts of buffalo origin	Vikrant Sudan	Dr. Daya Shanker	2019	147

M.V.Sc. Degree Programme

1. Epidemiological and phytotherapeutic studies on gastrointestinal parasites of small ruminants

Kanojiya Dharmendra Hariprasad and Daya Shanker

Out of 1869 animals (939 sheep and 930 goats) examined, 1606 animals were found to be affected with either one or more helminthes infection. Goats were found to be more affected (86.34% with 803 positive cases) than sheep (84.45% with 793 positive cases). Adults (87.08% adult sheep and 85.55% adult goats) were found to be more prone (78.19% young sheep and 80.50% young goats). Highest endoparasitic prevalence was seen in August month in both sheep and goat. The highest and lowest EPG values were observed in May and February months for both sheep and goat species, respectively. The highest EPG values for adult, young one and male sheep were observed in May, May and July months, while lowest EPG output was observed in February month. However, adult, young and male goat showed highest EPG values during May, June and June month where as lowest EGP were seen in February months, respectively. Regarding season wise prevalence, maximum and minimum EPG were observed during summer and winter seasons, respectively in both species regardless of the age and sex. Coproculture revealed *Haemonchus* spp. to be the most prevalent species affecting both sheep and goat in the studied area followed by *Trichostrongylus* spp., *Strongyloides* spp. and *Oesophagostomum* spp. Regardless of individual species, OPG revealed highest incidence of coccidiosis during August months for both sheep and goats, respectively, while the lowest incidence was observed during February months.

The anthelmintic potential of three medicinal plants viz *Eucalyptus globulus*, *Ocimum sanctum* and *Allium sativum* was evaluated in the present study using both *in vitro* and *in vivo* tests employing egg hatch test, larva development test, larval paralysis test and faecal egg count reduction tests, respectively. Aqueous extracts of all these plants were found to be better than methanolic counterparts. The aqueous extract of *E. globulus* was most effective amongst all and showed cent percent inhibition in egg hatch assay even at lower concentration of 6.25%. The aqueous and methanolic extracts of *A. sativum* were most effective and showed cent arrest of larval development at 25 % However, in Larval paralysis test both aqueous and methanolic extracts of *E. globulus* and *O. sanctum* were found to be equally effective at 100% concentration levels. In general, *E. globulus* was found to be more efficient in all the concentration levels compared to other plant extracts. FCERT results revealed *E. globulus* (66.00% efficacy) and *A. sativum* (56.86% efficacy) to be more effective in controlling parasitism than *O. sanctum* 39.41% efficacy) during the trial period of three weeks compared to albendazole (90.20% efficacy). Significant changes were observed in SGPT, RBCs, Hb, and RDWc while non significant changes were observed in SGOT, WBCs, HCT, MCV, MCH, MCHC, PLT, MPV, PCT, Lymphocyte%, Monocyte% Granulocyte% and FRAP.

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2. Epidemiological and phytotherapeutic studies on gastrointestinal parasites of large ruminants

Amreesh Sachan and Daya Shanker

Out of 2151 animals (1186 cattle and 965 buffaloes) examined, 1602 animals were found to be affected with either one or more helminthes infection. Cattle were found to be more affected (77.81% with 925 positive cases) than buffaloes (70.08% with 677 positive cases). Young ones (78.92% cattle calves and 72.40% buffalo calves) were found to be more prone

(77.54% adult cattle and 69.37% adult buffaloes). Highest endoparasitic prevalence was seen in September month in both cattle and buffaloes. The highest and lowest EPG values were observed in September and February months for cattle and September and March months for buffaloes, respectively. The highest EPG values for adult, calves and male cattle were observed September months, while lowest EPG output was observed in February, April and April month. However, adult, calves and male buffalo showed highest EPG values during September month where as lowest EGP were seen in March, April and February months, respectively. So far as season wise prevalence is concerned, overall higher prevalence of gastrointestinal parasitism in cattle and buffaloes was observed in rainy season. Coproculture revealed *Strongyloides* spp. to be the most prevalent species affecting both cattle and buffalo in the studied area followed by *Toxocara* spp., *Haemonchus* spp., *Oesophagostomum* spp. *Trichostrongylus* spp. and *Trichuris* spp.

The anthelmintic potential of three medicinal plants viz *Cucurbita maxima*, *Carica papaya* and *Saraca indica* was evaluated in the present study using both aqueous and methenolic extracts of *Cucurbita maxima* (seeds), *Carica papaya* (seeds) and *Saraca indica* (leaves) were *in vitro* tested for anthelmintic properties using Egg hatch test. Larval paralysis test and Larva development test. Aqueous extracts of all these plants were found to be better than methanolic extracts. The aqueous extract of *C. maxima* was most effective amongst all and showed cent percent inhibition in egg hatch assay even at lower concentration of 12.5%. In Larva development test, the aqueous and methenolic extracts of *C. papaya* were found most effective and showed cent percent arrest of larval development at 12.5 % concentration However, in Larval paralysis test methenolic extracts of *C. maxima* and *C. papaya* were found to be equally effective at 50% concentration levels while, both the extracts of *S. indica* and Aqueous extracts of both *C. maxima* and *C. papaya* showed cent percent inhibition at 100 % concentration.

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3. Prevalence of trypanosomiosis, its clinico-haemato-biochemical impact and PCR based detection in buffaloes

Rahul Parashar and and Daya Shanker

Prevalence of trypanosomiosis in 2880 (720 cattle and 2160 buffalo) large ruminants of Mathura district was studied. A total of 154 (21.38%) cattle and 428(19.81%) buffaloes were found positive for trypanosomiosis. There was higher prevalence of trypanosomiosis among cattle in comparison to buffaloes. Among buffaloes, heifers (6 to 2 years) (27.23%) were found to be more affected than young ones (<2 year)(21.99%) and adult (>2 year)(16.47%). Suggesting that, trypanosomiosis was more likely to affect the animals which were of young and heifer group. Amongst cattle, adult (> 2year) (26.11%) were found to be more affected than heifer (6 to 2 years)(18.18%) and young ones(<2 year) (14.81%), suggesting that trypanosomiosis was more likely to affect the animals which was of more than two year old/adult .The rainy season had the maximum prevalence of trypanosomiosis followed by summer and winter. By PCR study total 80 samples were examined and 49 samples were found positive for trypanosomiosis, while by blood smear examination 38 samples were found positive. A total 11 samples were found negative by blood smear examination which were found positive by PCR assay. The sensitivity (95%CI) and specificity (95%CI) of PCR method was 77.55% and 100% in comparison to Giemsa staining method with kappa value of 0.728.

Score card method of clinical examination revealed various pathological signs and symptoms amongst trypanosomiosis affected cattle and buffaloes. Study and analysis of various haemato- biochemical indices revealed significant alterations among MCH, MCHC , glucose,

direct bilirubin , FRAP values in buffaloes and for cattle significant changes were noticed in MCH, MCHC , platelets. PCT, AST, urea, glucose, total bilirubin, direct bilirubin , FRAP values while rest all the parameters showed non significant variation and were within normal range in comparison to normal healthy controls.

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4. Classico molecular studies and characterization of Rotat 1.2 VSG of *Trypanosoma evansi* in equines

Anjali Devi and Daya Shanker

Trypanosomiasis (Surra), caused by the *Trypanosoma evansi* is a major constraint in the health and productivity of domestic animals throughout the tropics and subtropics. The disease is mainly transmitted mechanically through the bite of blood sucking flies. Widespread morbidity in the form of progressive emaciation, anaemia, oedema, pyrexia, lowered weight gains, lowered milk yield, lowered work capacity and abortions, results in heavy economic losses to the livestock owners. The Office Internationale des Epizooties listed it as the list B disease among notifiable diseases of significance. In present study, prevalence of trypanosomiasis was screened in equines from clinical camps organized by Brooke Hospital for Animal in Mathura, Unnao and Raebareli alongside those animals which are brought to Teaching Veterinary Clinical Complex (TVCC), DUVASU, Mathura. A total of 86 animals were screened for trypanosomiasis using Giemsa stained thin blood smears out of which 3 were found positive for trypanosomiasis. Trypanosomiasis was found to be more likely affecting the animals which were of adult age group So far as sex wise distribution of prevalence rates is concerned, males were found to be more infected (5.55%) than females (2.00%). DNA was isolated from these samples by phenol chloroform method. The primer used in the study was RoTat 1.2 VSG F/R. These primers target the most commonly conserved VSG repertoire in *T. evansi*. Out of 86 samples tested, 3 were found to be positive by blood microscopy and 5 by PCR. Again 3 samples were found positive both with microscopy as well as PCR. There was no such sample which was positive by blood smear and negative by PCR. The sensitivity (95%CI) and specificity (95%CI) of PCR method was 100% and 93.98% in comparison to giemsa staining method with kappa value of 0.521.

The study characterized two RoTat 1.2 VSG repertoires, viz., VSG 1 and VSG 2. The RoTat 1.2 VSG 1 *T. evansi* horse isolates (KY457408) used in the present study showed cent percent homology with that from camel isolates from Egypt and yielded comparatively lesser homologies with that of isolates of camel and buffalo origin from India. RoTat 1.2 VSG 2 (KY457409) revealed 100.0% homology with other isolates of buffalo, camel and horse isolates from Karnataka and Bikaner, India; Kenyan isolate; camel and cattle isolates from Egypt.

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Ph.D. Degree Programme

1. Studies on susceptibility pattern in response to natural coccidial infection in goats of semi-arid region

Alok Kumar Singh and Daya Shanker

The present study was designed to analyze susceptibility to coccidiosis in kids of Barbari and Jamunapari maintained semi intensive system in semi-arid region. During study period, a total of 1422 faecal samples (777 from autumn season and 645 from spring season) were collected at different age group; 3.5, 6.0 and 7.5 from Central Institute for Research on Goat, Farah, Mathura, U.P. Oocyst per gram (OPG) was used to find out the prevalence of coccidian infection in goats. The overall coccidian oocysts were found in 94.37% population. Age wise average prevalence of OPG was found 1426.79, 563.97 and 1227.64 at 3.5, 6.0 and 7.5 month of age, respectively. The sex wise average prevalence were found higher in female (1145.44) as compared to male (998.29). The average seasonal prevalence of OPG was highest in autumn season (1235.93) as compared to spring season (876.28).

During the year 2012-2015 average temperature ranged from 20.42 - 46.31 (minimum-maximum) in summers and (4.60 - 31.58) in winters. The average rainfall was 38.60 mm. The relative humidity ranges between 21.67- 84.06 (%).

Five *Eimeria* species were identified viz, *Eimeria arloingi*, *E. ninakohlyakimovae*, *E.hirci*, *E.christenseni* and *E.caprina*. *Eimeria arloingi* was the predominant species in semi-arid region in response to natural coccidian infection in semi intensive management. Jamunapari goat observed to be more resistance (10.43%) than Barbari (6.32%). There was no significant differences observed in haemato-biochemical parameters in response to natural coccidian infection in Barbari and Jamunapari goat.

The challenge study was performed to use a standardized dose of 3×10^4 sporulated oocysts. The first oocysts were observed on the day 8th after the inoculation of the sporulated oocyst. Faecal score also observed i.e. diarrhoea (foul smelling diarrhoea, shooting diarrhoea, watery diarrhoea, bloody diarrhoea and mild diarrhoea) along with clinical symptom like, cough, nasal discharge, fever. The highest oocysts were observed on day 23rd.

Different anticoccidial treatment such as Toltrazuril, Amprolium, Sulphadimidine and combination of herbal were used to test their efficacy. Toltrazuril was found higher efficacious than other combination. A rise in WBC, Hct and Hb and fall in, ALP, Na⁺, Cl⁻ and K⁺ were observed in infected kids. The super oxide dismutase (SOD) level was decreased in infected kids whereas, the level of Interferon- γ and Cortisol were increased in infected kids. Body weight and gain was observed higher in treatment groups as compared to control.

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2. Immunomolecular studies for diagnosis and characterization of *Sarcocystis* species in buffaloes

Amit Singh and Daya Shanker

A study was conducted for the diagnosis and molecular characterization of *Sarcocystis* species in buffaloes. Representative tissue samples (oesophagus, tongue, heart and diaphragm) and blood samples were collected from each of the 160 buffaloes slaughtered at local slaughter house of Mathura. Sera was extracted from the blood samples for western blot analysis and antibody detection enzyme immunoassay whereas tissue samples were

subjected to macroscopic and microscopic examination (muscle squash method and pepsin digestion method) for the detection of *Sarcocystis* infection. Isolated cysts and bradyzoites were stored in alcohol for DNA isolation to be used later for molecular identification of species. Cysts and bradyzoites were also stored in phosphate buffered saline for antigen preparation. For histopathological observations, positive tissue samples preserved in 10% neutral buffered formalin were used. Overall prevalence, pattern of distribution of infections in organs as well as sex wise occurrence of disease was recorded. Tissue examination revealed overall prevalence of 56.25% as 90 out of 160 buffaloes were detected with cysts/bradyzoites in tissues. Microscopic examination detected more number of animals (54.37%) than the macroscopic examination (33.75%) positive for *Sarcocystis* infection. No significant difference in the occurrence of the sarcocystosis was found between males (53.84%) and females (56.71%). Organ wise, maximum infection was in oesophagus (56.25%) followed by tongue (14.32%) and diaphragm (4.3%). However, no cysts were detected in cardiac tissues. Species identification was done by morphometry and micrometry measurement. Macroscopic size cysts were fusiformis, spindle and globular in shape. The mean (Mean \pm S.E.) length and width of the macroscopic size cysts was 6.6mm \pm 0.70mm and 1.75mm \pm 0.10mm, respectively whereas that of of bradyzoites liberated from the crushed macroscopic cysts, mean length and width was 13.35 μ m \pm 0.34 μ m and 4.17 μ m \pm 0.06 μ m, respectively. Mean length and width of the microscopic size cysts and the bradyzoites released from it was 811.55 μ m \pm 14.10 μ m \times 79.8 μ m \pm 4.17 μ m and 12.55 μ m \pm 0.26 μ m \times 3.88 μ m \pm 0.047 μ m, respectively. Bradyzoites recovered by pepsin digestion measured 11.68 μ m \pm 0.22 μ m in length and 4.05 μ m \pm 0.066 μ m in width. By morphometry and micrometry analysis, macroscopic and microscopic like cysts appeared to be *Sarcocystis fusiformis* which later got confirmed by molecular study. Histopathological analysis showed thin walled cysts with mild infiltrations of the inflammatory cells with mild loss of striations of muscle fibrils. For genetic confirmation, 18S rRNA gene was amplified by species specific set of primers and the amplified products (approximately, 900bp) of one macroscopic cysts, two microscopic cysts and one minute bradyzoites pellet were subjected to gene sequencing and RFLP for species identification. Two *S. fusiformis* specific fragments of approximately 768bp and 99bp were seen in all the four PCR products after digestion with RE (Dra1). Out of four, gene sequencing results obtained successfully for one macroscopic, one microscopic and one pellet of bradyzoites which were then submitted in NCBI to receive accession number (Mathura 1 isolate: accession no. MF508604; Mathura 3 isolate: accession no MF508605; Mathura 4 isolate: accession no: MF508606). Genes of Mathura isolates were aligned with seven published sequence of *Sarcocystis* spp. taking *Neospora caninum* and *Theileria annulata* as outgroups. Three isolates of Mathura showed more than 99% homology in nucleotide sequence among themselves. Comparison of Mathura isolates with other strains of *S. fusiformis* showed 97-98% similarity in nucleotide sequences with Indian strain (accession number: JQ713824), Egyptian strain (Accession number- KR186117), Sweden strain (accession no- U03071) and China strain (accession number- U03071). Multiple alignments of genes of all the isolates of *S. fusiformis* including three of Mathura origin showed that the 18S rDNA gene sequence varied by single nucleotide polymorphisms and indels. Phylogenetic study showed Mathura isolates of *S. fusiformis* closer to five previously reported *S. fusiformis* strains (accession number-JQ713824, KR186117, KR 186130, U03071, AF 176927). Hence, the molecular study showed the presence of only *S. fusiformis*. After species confirmation, WCL antigen of *S. fusiformis* was prepared for protein profiling and antibody detection enzyme immunoassay. Hyperimmune serum was raised against this WCL antigen in Wistar rats. Out of 12 major polypeptides (78, 66, 53, 50, 42, 39, 32, 29, 27, 24, 19 and 15 kDa) resolved by SDS-PAGE, six (32, 39, 42, 53, 66, 78kDa) with HIS and only two (53 kDa and 66 kDa) with pooled positive serum were found

immunoreactive by western blot analysis. None of the polypeptides showed reactivity with pooled known negative serum. Whole cyst lysate antigen based ELISA detected antibodies of sarcocystosis in 102 (63.75%) serum samples with 88.88% sensitivity and 68.57% specificity. Therefore, antibody ELISA employing WCL antigen was found with moderate sensitivity and specificity despite detecting more number of positive animals than tissue examination. In the present study, *S. fusiformis* was the only species encountered as confirmed by molecular and morphological examination. So, molecular characterization tools were found useful in identifying or confirming the species.

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3. Evaluation of some biochemical parameters and TLR expression in *Haemonchus contortus* resistant and susceptible goats

Jitendra Tiwari and Daya Shanker

Haemonchosis represents primary constraint to profitable production of sheep and goats around the World. Sole dependence over few available anthelmintics for the control makes the parasite resistant against nearly all of them. The present study was carried out at Department of Parasitology, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura and at Central Institute for Research on Goats (CIRG), Makhdoom, Farah, Mathura with the objectives to detect the resistance pattern in Jamunapari goats against *H. contortus* and to compare TLR expression in resistant and susceptible animals. In the study, a resource population of 344 Jamunapari goat kids with complete pedigree records has been analysed for resistance pattern detection. The animals were characterized for their resistance to natural challenge of GINs on the basis of FEC. Also, FEC of sires was included in the final selection for genetic resistance in the animals.

Coprological examination was done in Jamunapari goat kids of 3-6 months of age group born during three kidding seasons under the study period of 18 months from February 2013 to August 2014. Under natural infection a significantly ($P<0.05$) higher incidence of strongyle worms were observed during monsoon-autumn season (14.23%) as compared to spring-summer season (8.13%). Larva culture of the positive samples revealed a significantly ($P<0.01$) higher incidence of *H. contortus* (90%) as compared to other strongylid worms. In the study, a non-significantly higher infection was observed in female kids (39.14%) as compared to male kids (31.95%).

The haematological and biochemical changes occur due to damage caused by *H. contortus* in selected resistant and susceptible animals were evaluated in controlled study after challenge infection with 30,000 L3 larvae of the parasite. In the study, resistant animals remained apparently healthy with low FEC whereas susceptible animals showed significantly ($P<0.05$) higher FEC. A range of haematological measures were significantly different between the susceptible group and the resistant and uninfected controls at one or more time points. Statistical analysis revealed significant ($P<0.05$) decreases in Hb, PCV, TEC, serum glucose, total protein and albumin and a significant ($P<0.05$) increases in serum ALP level in susceptible group as compared to resistant and control groups at different time intervals after the infection. This concluded that experimental *H. contortus* infection causes disturbances to the haemopoietic system resulting in anaemia and severe damage to abomasal mucosa with lower serum protein and higher ALP enzyme activity. It was concluded that decreased Hb, PCV, TEC, serum glucose, total protein and albumin levels were important indicators of haemonchosis in goats.

To understand the molecular basis of genetic resistance against *H. contortus*, expression profiles of TLR1-10 genes were investigated in animals demonstrating resistance or susceptibility against natural infection, using quantitative real-time PCR. The animals were treated with broad spectrum anthelmintics to remove existing parasitic load and were then experimentally challenged with L3 larvae of *H. contortus*. The mRNA expression profiles of these TLRs in abomasal mucosa and abomasal lymph node were compared between resistant and susceptible animals. In the study, resistant animals exhibited significantly ($P < 0.05$) increased expression of TLR2 (2.35 fold), TLR4 (3.24 fold) and TLR6 (4.22 fold) in both the tissues three days post infection. This elevated expression indicated the role of these three TLRs in providing resistance against *H. contortus* in Jamunapari goats. This is for the first time that this study has established the role of TLRs in the resistance mechanism against *H. contortus* in goats. Overall the results have enriched the information on genomic basis of resistance against GI nematodes in this important livestock species.

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4. Evaluation of excretory secretory antigen as a candidate for serodiagnosis of caprine amphistomosis

Amit Kumar Jaisawal and Daya Shanker

A study was conducted for the molecular characterization and development of serodiagnosics for caprine amphistomosis. A total of 92 rumens of goats naturally infected with amphistome species were collected from Army Slaughter House, Mathura (U.P). Beside rumen, blood samples were also collected from 11 newly born kids of age upto 1 week having stall feeding history of their dam and 240 goats by random sampling from nearby villages of Mathura district. Prevalence of amphistomosis by rumen examination was divided in three major groups viz. heavy, moderate and light infection. Month wise prevalence observed that the total maximum prevalence was found in month of July (21.87%) followed by September (19.15%) and May (17.18%). The minimum prevalence of amphistomosis was observed in month of December (8.73%) followed by November (9.44%) and January (11.60%). Years of study were also divided into three seasons viz. winter (Nov–Feb), summer (March–June) and monsoon (July–October). The highest overall prevalence of amphistomosis on the basis of rumen examination was reported in summer season (17.83%) followed by monsoon season (16.64%) and winter season (10.53%). The prevalence of amphistomosis on the basis of faecal examination (n= 240) revealed 17.08% during August to October 2016. Female goats (19.20%) were found more susceptible to amphistomosis in comparison to male goats (11.11%). On the basis of morphology and molecular technique using amplification of ITS2 gene, three species of amphistomes viz. *Paramphistomum epiclitum* (95.60%), *Gastrothylax crumenifer* (3.20%) and *Fischoederius* spp. (1.08%) were identified from rumen. The amplicons of about 400bp was obtained by amplification of ITS2 gene with used primer. A total of 04 representative amplicons were sequenced by outsourcing. The sequences were submitted in NCBI and the accession number (*Paramphistomum epiclitum* isolate mtjamp1.1: KX657873; *Paramphistomum epiclitum* isolate mtjamp1.3: KX657875; *Fischoederius* sp.: KX639720 and *Gastrothylax crumenifer*: KY889141) were received. Genes of Mathura isolates were aligned with thirteen previously published sequences of amphistome species. Multiple sequence alignment report suggested that there was little difference between Mathura isolate with other Indian isolates, while somewhat higher nucleotide substitution with foreign isolates. Percent identity matrix of Mathura isolates with other known isolates suggested more than 95% homology between Mathura isolates. *P. epiclitum* Mathura isolates showed 100% homology with each other without showing any difference of buffalo or goat origin. Phylogenetic

analysis indicated that *P. epiclitum* of Mathura origin were came in same clad without showing any difference. The nucleotide sequence of *Fischioederus* spp. of Mathura isolate showed similarity with *G. crumenifer* of Indian isolate. The 75% alcoholic fractionation of ES antigen was isolated from 200 to 300 live *P. epiclitum* for protein profiling and antibody detection enzyme immunoassay. Protein concentration of 75% alcoholic fractionation of ES antigen was found to be 2mg/ml. Hyperimmune serum was raised against this ES antigen in wistar rats. Electrophoresing of ES antigen through 12% separating and 5% stacking SDS-PAGE gel resolved 8 major polypeptides of size 56, 31, 27, 25, 22.5, 12, 11 and 10 kDa. On western blot analysis, five polypeptides of size 11, 12, 25, 27 and 31 kDa were found immunoreactive when probed with homologous hyperimmune serum. However, probing with pooled known positive serum revealed only two immunoreactive polypeptides (11 kDa and 12 kDa). None of the polypeptides showed reactions with pooled known negative serum. The working dilutions of antigen, sera and conjugates were determined by checkerboard titration for employing ELISA. Cut-off O.D. was calculated by taking the mean absorbance value of 11 negative kid sera added by three time standard deviation and was calculated 0.616. Antibodies to 75% alcoholic fractionation of ES antigen was detected in 32 (20.00%) goats of either sex serum samples by ELISA. The sensitivity and specificity of ELISA was found to be 100.00% and 86.76%, respectively. As per kappa value estimation, the strength of agreement was found to be good. The study confirmed that molecular characterization tools using ITS2 gene was very useful for the identification of the amphistome species and 75% alcoholic fractionation of ES antigen of *P. epiclitum* based ELISA may be used for serodiagnosis of caprine amphistomosis.

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5. Genotyping and characterization studies on *Sarcocysts* of buffalo origin

Vikrant Sudan and Daya Shanker

Sarcocystosis is a common parasitic entity affecting wide range of ruminants with cosmopolitan distribution. Tissue samples from oesophagus, tongue and heart were collected from buffaloes slaughtered at the local slaughter house of Mathura, organized slaughter houses at Unnao and Aligarh alongside those brought for post mortem at Department of Pathology, DUVASU, Mathura. Tissue samples were screened for the presence of sarcocysts. The average size of the macroscopic sarcocysts varied from 5.0-29.5 x 3.1-7.3 mm. The cysts were thin walled (2.0-2.5 μ m) with average size of bradyzoite ranging from 15.0-16.5 x 3.5-4.3 μ m. The average size of microscopic cysts in the heart varied from 0.2-1.0 x 0.15-0.5 mm. The micro sarcocysts found in heart were also thin walled with average size of bradyzoite ranging from 9.3-10.8 x 3.0-4.1 μ m. Based on morphological features, the macroscopic sarcocysts were identified as *S. fusiformis* while the microscopic sarcocysts were identified as *S. levinei*. On histopathological examination, both the macroscopic as well as microscopic cysts were found to be having characteristic thin wall structure. Histopathological findings revealed muscle fiber degeneration with mild degenerative changes like loss of striations of muscle fibers around all those macrosarcocysts. None of inflammatory cells were observed around the intact cysts. No hyalinization of muscle fibrils was seen. Three DNA isolation protocols were compared to find out the most suitable protocol for DNA isolation from sarcocysts. All the three protocols showed good results with regards to DNA extraction yield as well as DNA purity. However, 18S based PCR was able to detect *Sarcocystis* spp. DNA from maximum number of samples isolated with kit with modification. A total of 88 sequences were generated in the present study. Wide nucleotide variations were

noticed within the studied isolates of various *Sarcocystis* spp. as well as with other isolates across the globe for all the studied genes. Studied sequences of *S. fusiformis* showed 99.1-100.0%, 99.2-100.0%, 99.2-100.0% & 99.1-100.0% nucleotide homology within themselves and 44.6-95.5%, 77.9-99.9%, 97.3-99.9% & 98.2-99.8% nucleotide homology with isolates across the globe for 18S, cox 1, 28S and ITS 1 genes, respectively. Alongside, the studied sequences of *S. buffalonis* showed 99.3-99.9 %, 99.8-100.0 %, 99.9-100.0 % & 100.0 % nucleotide homology within themselves for 18S, cox 1, 28S and ITS 1 genes, respectively. They also showed 97.6-100.0 %, 99.7-99.9 %, 95.4-99.9 % & 93.1-99.8 % nucleotide homology with isolates across the globe for 18S, cox 1, 28S and ITS 1 genes, respectively. So far as studied sequences of *S. levinei* are concerned, they showed 99.8-100.0 %, 99.7-100.0 %, 99.7-99.9 % & 99.8-99.9 % nucleotide homology within themselves for 18S, cox 1, 28S and ITS 1 genes, respectively. However, they showed 99.6-100.0 %, 99.8-100.0 % & 95.3-99.9 % nucleotide homology with isolates across the globe for 18S, cox 1 and 28S genes, respectively. So far as phylogenetic analysis of various *Sarcocystis* spp. is concerned, *S. fusiformis* was found close to *S. cafferi* for 18S gene while rest three genes showed separate clade for *S. fusiformis*. *S. buffalonis* was found close to *S. hirsuta* in phylogenetic trees for all the four genes. *S. levinei* was found close to *S. cruzi* in phylogenetic trees for all the four genes. Despite the occurrence of some genetic variations in the hypervariable regions of the 18S rDNA in *Sarcocystis* spp., restriction sites of the studied enzymes were found conserved amongst all studied sarcocysts. *Dra* I gave uniform restriction digestion pattern with *S. fusiformis*. However, it was not able to cut *S. buffalonis* and *S. levinei*. *Ssp* I gave similar sized digestion products with *S. fusiformis* and *S. buffalonis* but failed to digest *S. levinei*. *Fok* I was able to digest all the three *Sarcocystis* spp. giving characteristic restriction patterns. Hence, *Fok* I was found best to differentiate these three *Sarcocystis* spp. on PCR-RFLP basis.

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DEPARTMENT OF VETERINARY PATHOLOGY

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M.V.Sc. Degree Programme

1. **Status of pathological lesions vis a vis diagnostic efficacy of different tests for *Mycobacterium avium* subspecies *paratuberculosis* (Map) infection in adult goats**

Shivam Chaturvedi and A.K. Srivastava

Understanding pathogenesis during progressive stages of infection by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and finding suitable methods for its diagnosis is the key to the control of Johne's disease in animals. In this study, the pathological lesions and the diagnosis of Johne's disease in goats is investigated using histopathological (HP) examination, Acid Fast Staining (AFS) of tissue sections, immunofluorescent test, tissue PCR and culture. These tests were carried out on 74 paired tissue samples of intestine and mesenteric lymph nodes. The gross lesions were mainly observed at the terminal ileum especially at ileo-caecal valve and the mesenteric lymph nodes. Histopathological examination of routine stained tissue sections from the 74 ilea and corresponding lymph nodes revealed variable grade of lesions of JD in 37 (50%) ilea and 21(28.40%) mesenteric lymph nodes. In general, the affected part of the intestine revealed degeneration and partial to complete denudation of lining epithelial cells forming naked villi. The villi exhibited variable changes that included dilated lacteals, villous distortion and thickening and fusion of villi. At places, the villi were shortened, thin and atrophied. Histologic lesions were classified into four grades from grade 1 (least severe) to grade 4 (most severe) on the basis of types and density of cellular infiltrate (lymphocytes, macrophages and epithelioid cells). In AFB staining, 16 (21.60%) and 10 (13.50%) out of 74 paired cases of intestine and MLN respectively, revealed presence of acid fast pink colour bacilli individually or in clusters in the epithelioid cells indistinguishable from *Mycobacterium avium* paratuberculosis. Thirteen (17.56 %) and 9 (12.16%) out of 74 paired tissue samples of intestine and MLN respectively were found positive in fluorescent antibody test. Eleven (14.86%) and 7 (9.40%) cases of intestine and mesenteric lymph nodes were found positive by PCR (IS900). Culture was positive in 7 (9.50%) and 8 (10.81%) cases out of 74 paired tissue samples of intestine and MLN respectively. In present study, sensitivity of histopathology (H&E staining) was found to be more than the any other test for diagnosis of MAP infection in goats. Sensitivity of ZN staining was 100% in comparison to PCR (69.23%). The strength of agreement the ZN staining and FAT was considered to be very good. Sensitivity of FAT was 90.91% in comparison to ZN staining (76.92%). The strength of agreement between the FAT and PCR was considered to be very good. Sensitivity of FAT was 100% in comparison to PCR (81.82%). Bacterial culture showed poor sensitivity. There was no significant difference between the sensitivity of ZN staining, FAT and PCR in grade II, III and grade IV lesions. In grade I lesions, histopathologic H&E staining based diagnosis was found to be more sensitive followed by ZN staining. Histopathology was found to be a better indicator of paratuberculosis infection in goat.

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2. **Pathological studies on the co-exposure of cadmium and chlorpyrifos in broilers with ameliorative effect of Vitamin E**

Raghavendra Singh and A.K. Srivastava

In the present study entitled "Pathological studies on the co-exposure of cadmium and chlorpyrifos in broilers with ameliorative effect of Vitamin E" was investigated. For this

purpose a total of 48 chicks were randomly divided into eight equal groups. The cadmium chloride was given @ 50 PPM (50 mg/lit) in drinking water in bird of groups-III, V, VII and VIII and Chlorpyrifos was given @ 4 mg/kg body weight by oral route in bird of groups-IV, VI, VII and VIII and Vitamin E was administered @ 150 mg/kg body weight by oral route in bird of groups-II, V, VI, and VIII daily for the period of 45 days. The birds of the group-I were kept as control. At the intervals of 15, 30 and 45 days post feeding various parameters of study were carried out.

Clinical signs of reduced appetite, listlessness, diarrhoea, gasping, inability to stand, difficult breathing, inco-ordination in movement, stiffness, muscle twitching, dry oral mucous membrane with mucous like substances present in the oral cavity, enlarged joints, scaly skin, reduced growth, ruffled feathers, dull, depressed and paresis of left leg were observed after 15 days post feeding in the toxicity groups. The body weight of the bird of groups-III, IV, V, VI, VII and VIII revealed significant reduction from 15 days to end of the experimentation. The weight of liver, lungs, kidneys, heart, brain, spleen and bursa of Fabricius was found to be significantly lower in bird of toxicity groups.

Hematological observations revealed significant decrease in the values of Hb, PCV and TEC. The mean values of TLC and lymphocyte significantly decreased and heterophils increased significantly in toxicity groups. The mean values of AST, ALT and ALP were significantly increased in the toxicity groups. There was significant increase in the level of glucose, urea, creatinine and decrease in total proteins, albumin, and globulins in toxicity groups. The mean values of LPO and SOD depicted significant increase while the mean values of GSH revealed significant decrease in the bird of toxicity groups. The values of various biochemical attributes were less severe at different intervals in the bird of ameliorative groups-V, VI and VIII.

Pathomorphologically, the toxicity groups showed degenerative changes, cholangio-hepatitis, coagulative necrosis and lymphoid nodules in the liver; moderate to marked congestion in the blood vessels with extravasation of erythrocytes, oedema formation in the air vesicles, degeneration and desquamation of bronchial epithelium, and presence of a mass of cartilage in the parabronchi surrounded by air vesicles of the lungs; degeneration and desquamation of lining epithelium of renal tubules and extravasation of erythrocytes, shrunken and degenerated glomeruli, focal areas of sub-acute interstitial nephritis, sub-acute glomerular nephritis, hyperplasia and degeneration of podocytes and parietal layer of epithelium in the kidneys; congestion and extravasations of large number of erythrocytes in myofibres, vacuolization, focal areas of sub-acute myocarditis with infiltration of mononuclear cells viz. lymphocytes, plasma cells and macrophages in the heart; congestion and extravasation of erythrocytes, presence of shrunken and small neurons having triangular and eccentric nucleus, neuronophagia, hyperplasia of ependymal cells and separation of molecular and granular layers in the cerebellum of the brain; degeneration and necrosis of mucosal epithelium with infiltration of mononuclear cells in the proventriculus; degeneration and necrosis of lining epithelium especially at tip of the intestine; hyperplasia of bursal epithelium, forming numerous acini like structures depletion of lymphoid tissue and presence of fragmented cellular debris in the bursa of Fabricius.

Similar pathomorphological lesions but of mild in nature were also recorded in the ameliorative groups (V, VI and VIII) suggested the protective action of vitamin E on biological tissue.

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3. Pathological studies on the co-exposure of cypermethrin and sodium fluoride in rats with ameliorative effect of vitamin E

Renu Singh and A.K. Srivastava

In the present study entitled "Pathological studies on the co-exposure of Cypermethrin and fluoride in experimental rats with ameliorative action of vitamin E" was investigated. For this purpose, a total of 48 rats were randomly divided into eight equal groups. The rats of group-I were kept as control. The Cypermethrin was given @ 25 mg/kg body weight (corn oil) orally in the rats of group- i. III, IV and group-VIII. The rats of group-III, V, VI and VIII were administered with Sodium fluoride @ 8 mg/kg body weight (distilled water) orally. Vitamin E was given @ 100 mg/kg body weight (com oil) orally in the rat of group-IV, VI, VII and group-VIII. The clinical symptoms observed daily in each group and body weight and haematobiochemical were carried out at 15, 30 and 45 days of intervals. The organ weight, oxidative stress and pathomorphological studies were carried out on day 45 of experiment.

Clinical signs of reduced appetite, less water intake, decrease in body weight, dry skin, rough hair coat, dullness, depression, emaciation and decrease in growth rate were observed after 15 days post feeding in the toxicity groups (II, III & V) but of milder intensity in the rat of ameliorative groups (IV, VI & VIII). The body weight gain revealed onset of significant reduction in the body weight from 15th day in the rat of toxicity groups (II, III & V) and ameliorative groups (IV, VI & VIII). The weight of liver, lungs, kidneys, heart, brain, spleen and testes was found to be significantly lower in toxicity groups (II, III & V). Hematological observations revealed significant anemia in toxicity groups (II, III & V) and significant improvement was found in ameliorative groups (IV, VI & VIII). The mean values of TLC revealed significant decrease at all the intervals with significant increase in neutrophil and significant decrease in lymphocyte count in toxicity groups (II, III & V). The mean values of AST, ALT, ALP creatinine, urea, uric acid and glucose was significantly increased in toxicity groups (II, III & V) as compared to group-I (control) and VII at all the intervals of the experimentation. The mean values of various biochemical attributes were less severe and low frequency at different time intervals in the rat of ameliorative groups (IV, VI & VIII). The mean values of total proteins were significantly decreased in toxicity groups (II, III & V) as compared to group-I and VII at all intervals. The mean values of albumin was significantly decreased in toxicity groups (II, III & V) as compared to group-I and VII (vitamin E) at 30 and 45 days intervals of experimentation and significant improvement in the mean values of total proteins and albumin in the rat of ameliorative groups (IV, VI & VIII) at all intervals. The mean values of SOD, GSH and catalase significantly decrease and LPO significantly increased in liver and kidney homogenate in toxicity groups (II, III & V).

The rat of toxicity groups (II, III & V) grossly revealed pale with occasional of presence of pinpoint haemorrhages and mottling on the dorsal surface of liver; congested and edematous lungs; mild congestion in kidneys, heart and testes. Microscopically, congested and distended sinusoids with hyperplasia of kupffer cells, sub acute hepatitis characterized by area of necrosis of hepatocytes along with infiltration of mononuclear cells and degenerative changes ranging from cellular swelling to vacuolization in liver; mild congestion of alveolar capillaries with or without oedema formation in the alveolar spaces, formation of lymphoid nodules in the peribronchiolar regions, lesion of steatitis characterized by infiltration of mononuclear cells in the interstitium of adipose tissue in lungs; mild to moderate congestion in glomeruli and intertubular blood vessels with degenerative changes in tubules, vacuolization in the lining epithelium of tubules and sub acute interstitial nephritis in kidneys; degenerative changes, vacuolization and separation of cardiac myofibres and extravasation of erythrocytes

in heart; mild to moderate congestion in choroid plexus of ventricles with or without extravasation of a few erythrocytes, perineural and perivascular edema, neuronophagia, spongiosis and separation of molecular and granular layers with depletion of purkinje cells in brain; mild depletion of lymphoid tissue in the malpighian corpuscles of white pulps with accumulation of large number of erythrocyte in the red pulp in spleen; hyperplasia of goblet cells, diffuse degeneration and necrosis of villous epithelium in intestine; accumulation of pink watery fluid in the interstitial spaces with depletion of spermatogonial layers in the seminiferous tubules in testes of toxicity groups (II, III & V) were observed. The lungs of the rat of group-III also revealed the lesions of pneumocyto-carcinoma featured by presence of large, flat, pleomorphic squamous type of cells filling the alveolar lumen having hyperchromatic nuclei. Similar but mild morbid lesions except pneumocyto-carcinoma was also recorded in the rat of ameliorative groups (IV, VI & VIII) treated with vitamin E @ 100 mg/kg body weight orally as compared to the rat of toxicity groups (II, III & V).

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Ph.D. Degree Programme

1. Molecular pathogenesis of enterotoxaemia in neonatal goat kids

Desh Deepak Singh and Raj Veer Singh Pawaiya

In this study, a total population of 1156 neonatal goat kids was examined for clinical diarrhoea and 134 necropsied were examined for enteritis, pneumo-enteritis, tapeworms/round worm infestations and septicemia, suspectedly died due to enterotoxemia from January, 2015 to December, 2016. A total of 238 diarrhoeic fecal and 84 intestinal loop samples were collected from ICAR-CIRG, Makhdoom, Mathura (UP) and spontaneous outbreaks of Uttar Pradesh and Rajasthan States of India. Isolation, identification and molecular detection of toxinotypes of *Clostridium perfringens* were performed in 238 diarrhoeic samples and 84 intestinal loop samples for confirmatory diagnosis of enterotoxemia. Diarrhoeic samples and intestinal contents were used for isolation in Robertson cooked meat media (RCMM) and subsequently inoculated in 5% defibrinated sheep blood brucella agar with vitamin K1, hemin and selective clostridial supplements (CLS-BBA) and egg yolk agar (EYA) under anaerobic conditions. Toxinotyping were done by multiplex PCR using toxin genes viz., cpa, cpb, cpb2, etx and iap. After confirmatory diagnosis, molecular characterization for different isolates was done by PCR cloning and gene sequencing. The etx-full gene cloned in TA vector for various field isolates was sequenced by using Bigdye® terminator v1.1 cycle sequencing kit (Applied biosystems). Phylogenetic analysis was conducted by minimum evolution tree analysis. Experimental study using characterized isolate (CIRG-1816) was undertaken to study molecular and gene expression profiles. The inoculum was prepared in RCMM and culture supernatant (CS) containing epsilon toxin was trypsinized and titrated in mouse. Twenty male Jakhra goat kids of 0-3 month's age weighing 9-12 kg were divided in four equal groups (n=5). Group I was treated with CS, group II with washed cells (WAC), group III with whole culture (WC) and group IV with only RCM medium (control). By laparotomy, 20% starch solution was infused into abomasum and 300 ml of inoculum into duodenum. Treated animals were kept under observation for 24 hrs. All kids treated with CS, WAS and WC, showed diarrhoea. Affected kids were euthanized and necropsied to observe the gross pathological changes and suitable samples were collected to confirm pathogen's presence at predilection site. *C. perfringens* type D was further confirmed by cultural and molecular methods. Expression profile of inflammatory genes IL-1 β , IL-2 and TCF-20 was studied in intestinal tissues to understand molecular pathogenesis by implying qRT-PCR. GAPDH gene (housekeeping gene) was used as control for comparing expression. Tissue samples collected from spontaneous as well as experimental cases of enterotoxaemia were subjected to histopathology and immunohistochemistry. In RCMM, gas production was observed along with turbidity, while Gram's staining showed stumpy or slender Gram positive rods with truncated or plummy ends. On CLS-BBA, greyish, rounded raised or flat colonies with double zones of hemolysis and on EYA, opalescence indicative of lecithinase activity were observed. The incidence percentage (%) of *C. perfringens* in diarrhoeic samples and necropsied kids was 15.13% and 27.38%, respectively. In clinically ill animals, 75% isolates of *C. perfringens* were toxinotype A and 25% were toxinotype D. In necropsied cases, 47.83% isolates were toxinotype A and 52.17% were toxinotype D, indicating that the epsilon toxin was principal virulence factor for pathogenesis of enterotoxaemia in goats. The gene encoding β 2-toxin (cpb2) was present in 61.11% of diarrhoeic and 30.43% of necropsied kids suggesting its virulent association with clinical diarrhoea. Pathologically in goat kids, lesions occur mostly in distal portion of ileum with additional involvement of lung, kidney and brain to variable extend. The etx full length

gene cloning and sequencing revealed point mutation (silent) CIRG 1816 and CIRG 3716 isolates compared to IVRI Vac1 reference strain. No antigenic (phenotypic) changes were found in our strains in comparison to IVRI Vac1 reference strain. Culture supernatant containing activated ϵ toxin experimentally induced diarrhoea in kids in the shortest period (12-14 hrs. post inoculation) followed by whole culture containing live bacteria and ϵ toxin (18-19 hrs. post inoculation) and live bacteria (washed cells) (21-24 hrs. post inoculation). Experimentally induced ET in kids of all three groups showed major pathological lesions of congested ileum, necrosis of villi epithelium and emphysema of lungs. Presence of epsilon toxin was demonstrated by immunohistochemistry. Gene expression study revealed the highest expression of IL-1 β in spontaneous enterotoxaemia followed by experimental ET produced by activated ϵ toxin which suggested severe inflammatory process of the disease and may act as a promising biomarker of acute ET disease. The mRNA expression of IL-2 gene was highest in field outbreaks followed by washed bacterial cells which indicated significance of the bacterial moiety in the clinical infection. Down regulation of TCF20 gene in experimental and natural ET suggested that the toxin or other component of *C. perfringens* is involved in damaging the normal cellular functions during the ET pathogenesis.

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2. Molecular Pathology and Transcriptional Profiling of Enterotoxaemia in Post-Weaned Goats

Neeraj Kumar Gangwar and Raj Veer Singh Pawaiya

In current study, a total of 168 diarrhoeic fecal and 189 intestinal loop samples were collected from ICAR-CIRG, Makhdoom, Mathura (UP) and spontaneous outbreaks of Uttar Pradesh, Haryana and Rajasthan States of India. Toxinotyping were done by multiplex PCR using toxin genes viz., *cpa*, *cpb*, *cpb2*, *etx* and *iap*. After confirmatory diagnosis, molecular characterization for different isolates was done by PCR cloning and gene sequencing. Experimental study using characterized isolate (CIRG-NK) was undertaken to study molecular and gene expression profiles. Group I was treated with CS, group II with whole culture (WC), group III with washed cells (WAS) and group IV with only RCM medium (control). Treated animals were kept under observation for 72 hrs. Affected goats were euthanized and necropsied to observe the gross pathological changes and suitable samples were collected to confirm pathogen's presence at predilection site. *C. perfringens* type D was further confirmed by cultural and molecular methods. Expression profile of inflammatory genes IL-1 β , IL-2, IL-8 and Cat-L was studied in intestinal tissues to understand molecular pathogenesis by implying qRT-PCR. In RCM, gas production was observed along with turbidity, while Gram's staining showed stumpy or slender Gram positive rods with truncated or plumby ends. On CLS-BBA, greyish, rounded raised or flat colonies with double zones of hemolysis and on EYA, opalescence indicative of lecithinase activity were observed. The incidence percentage (%) of *C. perfringens* in diarrhoeic samples and necropsied goats was 16.07% and 22.75%, respectively. In clinically ill animals, 81.48% isolates of *C. perfringens* were toxinotype A and 18.51% were toxinotype D. In necropsied cases, 55.81% isolates were toxinotype A and 48.81% were toxinotype D. The gene encoding β 2-toxin (*cpb2*) was present in 44.44% of diarrhoeic and 44.81% of necropsied goats suggesting its virulent association with clinical diarrhoea. Pathologically in goats, lesions occur mostly in distal portion of ileum with additional involvement of lung, kidney and brain to variable extend. The *etx* full length gene cloning and sequencing revealed point mutation (silent) CIRG NK and CIRG 3716 isolates compared to IVRI Vac1 reference strain. The native CIRG, isolates were unique compared to the Indian IVRI isolates. Culture supernatant containing activated ϵ toxin experimentally

induced diarrhoea in goats in the shortest period (20-24 hrs. post inoculation) followed by whole culture containing live bacteria and ϵ toxin (20-24 hrs. post inoculation) and live bacteria (washed cells) (30-36 hrs. post inoculation) showed anorexia, depression and diarrhoea. Presence of epsilon toxin was demonstrated by immunohistochemistry. Gene expression study revealed the highest expression of IL-1 β in spontaneous enterotoxaemia followed by experimental ET produced by activated ϵ toxin which suggested severe inflammatory process of the disease and may act as a promising biomarker of acute ET disease. The mRNA expression of IL-2 gene was highest in field outbreaks followed by washed bacterial cells. The Expression of IL-8 was highest in CS group followed by field isolates explains the CS which has more toxin component has phenomenal expression of IL-8. The Expression of cat-L was highest in spontaneous group of ET suggested that the toxin or other component of *C. perfringens* is involved in damaging the normal cellular functions during the ET pathogenesis.

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DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

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M.V.Sc. Degree Programme

1. Sub-acute pulmonary toxicity of flubendiamide and its amelioration with curcumin in rat

Kuldeep Singh and Atul Prakash

Present study was undertaken to evaluate the pulmonary toxicity of flubendiamide and its possible amelioration with curcumin in wistar rats of either sex. The toxicity and its amelioration were considered on the basis of feed and water intake, weekly body weight and percentage weight gain, absolute and relative organ weight, hemato-biochemical profiles, DLC in broncho-alveolar lavage fluid, oxidative stress parameters in erythrocytes and tracheal smooth muscle responsiveness. Forty two rats were divided into seven groups each containing six rats viz. - G1: control, G2: vehicle control, G3: curcumin, G4: flubendiamide (100 ppm), G5: flubendiamide (200 ppm), G6: flubendiamide (400 ppm) and G7: flubendiamide (200 ppm) plus curcumin.

Daily feed and water intake was reduced at 200 and 400 ppm flubendiamide alone groups on last week whereas, no change was found in flubendiamide plus curcumin treated group as compared to control group. No significant change in body weight and percentage weight gain was found among all groups, however slight decrease in body wt. was found at 200 ppm and 400 ppm dose level on 14th, 21st, 28th day as compared to control group. Absolute liver weight increased significantly in 200 and 400 ppm groups whereas no significant change was found in in flubendiamide plus curcumin treated group. Adrenal gland weight was also increased in 400 ppm group significantly whereas it was increased non-significantly in 200 ppm group as compared to control group. Dose dependent significant decrease in hemoglobin and TEC level was found in 100 ppm, 200 ppm and 400 ppm groups as compared to control group, however, no significant change was found in flubendiamide plus curcumin group as compared to control group. Platelet count was also increased significantly in 400 ppm group as compared to control group. In DLC, the percentage of eosinophils and neutrophils was increased significantly in 400 ppm group, however eosinophils were also increased significantly in 200 ppm group as compared to control group. Lymphocyte percentage was decreased significantly in 400 ppm group whereas it was non-significantly in 200 ppm group as compared to control group. No significant change was found in eosinophils, neutrophils and lymphocyte percentage in flubendiamide plus curcumin treated group.

Total protein and cholesterol level in plasma were increased significantly in 400 ppm group, however total protein was also increased significantly in 200 ppm group as compared to control group. No significant change was found in total protein and cholesterol level in flubendiamide plus curcumin treated group. ALT and AST level was altered non-significantly in 200 and 400 ppm flubendiamide alone treated groups whereas no significant change was found in flubendiamide plus curcumin treated group. Lipid peroxidation (LPO) and GST level in erythrocyte was increased significantly in 400 ppm group and increased non-significantly in 200 ppm group whereas no significant change was found in flubendiamide plus curcumin treated group. The level of SOD, CAT and GPx increased non-significantly in 400 ppm group as compared to control group.

Differential leucocyte count of bronchoalveolar lavage fluid (BALF), eosinophil count increased significantly in different flubendiamide alone treated groups. Neutrophil count was increased significantly at 400 ppm dose level and non significantly at 200 ppm dose level. Alveolar macrophase count was decrease significantly in 200 and 400 ppm flubendiamide

alone treated group. No significant change was found in eosinophils, neutrophils and alveolar macrophase count in flubendiamide plus curcumin treated group.

Preliminary study on tracheal smooth muscle responsiveness against sub-acute exposure of flubendiamide exhibited no indication in alteration of basal tone of tracheal rings. There was no indication in alteration in potency and efficacy of acetylcholine and maximum response of 80 mM KCl solution on isolated tracheal smooth muscle against sub-acute exposure of flubendiamide.

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2. Pharmacological studies on patho-physiological dynamics of endometritis in murine model

Neelam Kurmi and Satish K. Garg

Present study was undertaken on pathophysiological dynamics of endometritis in murine model and evaluates the efficacy of certain plant extracts. *Eucalypts citridora* and *Moringa oleifera* leaves and *Tagetes erecta* flowers methanolic extract were subjected to phytochemical analysis using qualitative tests as well as HPLC and GC-MS analysis. All the three plant extracts were found to possess oxalic acid, salicylic acid and acetyl salicylic acids apart from normal phytoconstituents which include alkaloids, flavonoids, saponins, sugars, tannins, glycosides, fixed oil, protein and amino acids. GC-MS analysis of *E. citridora* leaves revealed 11 compounds with five major constituents while *M. oleifera* revealed 14 different compounds with three major compounds and *T. erecta* flowers revealed 45 compounds with four major marker compounds.

Uterine discharge of clinical and subclinical endometritis cases having history of anestrus (17%), repeat breeding (43%) and abortion (39%) were found to have *E. coli* (43.3%), *Staph, aureus* (30%), *Streptococcus* (16.7%), *Klebsiella* (6.67%) and *Pseudomonas* (3.33%). *Eucalyptus citridora* and *Moringa oleifera* leaves methanolic extracts (50 µl each of 200 mg/ml) exhibited promising antibacterial activity against all the clinical isolates while *Tagetes erecta* flowers extract exhibited poor antibacterial activity as determined by disc diffusion method.

Murine bacterial endometritis model was established by inoculating the mixture of *E. coli* and *Staph, aureus* in to uterine horns during diestrus stage followed by cervical ligation and the model was confirmed based on presence of visible pus in the uterus, edematous uterine horn, thinning of endometrial lining and presence of large number of PMN cells and bacterial load in uterine flushing.

Efficacy of *E. citridora* and *M. oleifera* leaves methanolic extracts was evaluated based on marked to significant reduction in uterine weight, uterine secretion index, PMN cells and bacterial load in uterine flushings, alteration in TLC and DLC, serum TNF α levels and histopathological changes. Results were compared with gentamicin and it was evident that *E. citridora* and *M. oleifera* produced curative and protective effect against endometritis. All the three test plant extracts produced inhibitory effect on myometrial activity in control, sham-operated and endometritis groups. Enrofloxacin and gentamicin also produced inhibitory effect on myometrial contractility and gentamicin was more potent than enrofloxacin but ampicillin did not produce any appreciable effect on myometrium contractility in any of the three groups. Oxytocin and PGF $_{2\alpha}$ produced contractile effect on uteri of all three groups but compared to healthy control group, oxytocin produced markedly higher contractile effect while PGF $_{2\alpha}$ produced significant higher contractile effect on endometritic rats compared to that on healthy control. Following treatment of endometritis in rats with *E. citridora* and *M.*

oleifera, there was marked alteration in sensitivity of myometria to the effect of oxytocin, PGF_{2α} and potassium chloride.

Based on these studies, it may be inferred that *E. citridora* and *M. oleifera* leaves extracts possess promising antibacterial activity and efficacy against experimental endometritis and, therefore, can be exploited in drug development program for treatment of endometritis in human beings and animals.

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3. Studies on biomarkers of mercury induced alterations in activity of rat myomerium with particular reference to muscarinic receptors

Swati Koli and Atul Prakash

Present study was undertaken to evaluate the toxicity of mercury (@ 5, 50 and 500 ppb) with particular reference to its effect on signal transduction mediated through muscarinic receptors in rat myometrium. The study was conducted in two phases- phase I (*In-vivo* study in rats intoxicated with mercury for a period of 28 days). Adult female Wistar rats weighing 150-200 g divided in to four groups [Control (deionized water); Group II (5 ppb Hg); Group III (50 ppb Hg) and Group IV (500 ppb Hg)] containing eight animals in each were used. Mercury was dissolved in deionized water and given through drinking water. In phase II (*In-vitro* study, a total of 30 female Wistar rats were used for isolation of myometrium and assessing the effect of different concentrations of mercury in presence of muscarinic agonists and antagonists and other signaling molecules). After 28 days of exposure period, mercury did not show any apparent sign of toxicity and body weight of rats, absolute and relative weight of organs. However, non-significantly reduced body weight was observed in 500 ppb intoxicated group and increase in absolute weight of ovary and brain was recorded at 50 and 500 ppb, respectively.

Creatinine, cholesterol, uric acid, bilirubin (direct and indirect), albumin, globulin and total protein did not differ significantly, except glucose and uric acid. Serum estradiol and progesterone levels were also not altered among groups. Residual concentration of mercury in kidney, liver, ovary & uterus and calcium level in kidney ovary and blood did not differ significantly compared to control. In uterus, dose dependent accumulation seen with significant reduction in residual calcium. In kidney, accumulation of mercury was reduced with increasing dose. In blood, highest mercury level was observed at 500 ppb dose however, it was reduced at 500 ppb. Highest concentration of mercury observed in blood compared to other tissues. In uterine myometrial study, mercury has been shown to induce myometrial contractility. Mercury at lowest dose (5ppb) significantly increased MIT and decreased frequency. Mercury at lowest dose (5ppb) markedly/significantly potentiated calcium chloride/KCl-induced myometrial contractility. Mercury decreased the contractile response to ACh, PGF_{2α}, oxytocin and Bay K8644 except potentiation of effect of Bay K8644 at 500 ppb.

In conclusion, mercury seems to have affinity for accumulation in kidney, liver, ovary, uterus and blood levels can be considered as biomarkers of exposure to mercury. Mercury-induced myometrial contractility seems to be regulated through cholinergic neurotransmission pathway/ receptors (M₂ and M₃ muscarinic receptor). Mercury-induced myometrial contractions seem to be calcium dependent and involve VDCC or Rho-kinase, PKC and PLC pathway.

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4. Studies on inflammatory biomarker of endometritis and their modulation by *Eucalyptus citriodora* leaves extract in rats

Aastha Tiwari and Atul Prakash

Present study was undertaken to investigate the modulatory effect of *Eucalyptus citriodora* leaves extract and cefixime on inflammatory biomarkers in experimentally induced endometritis in Wistar rats. Uterine discharge of clinical and subclinical endometritis cases having history of repeat breeding (43%) and abortion (57%) were found to have *E. coli* (60%) *Staphylococcus aureus* (40%). *Eucalyptus citriodora* leaves methanolic extracts exhibited promising antibacterial activity against both clinical isolates as determined by disc diffusion method. Rat endometritic model was developed by inoculating the mixture of *E. coli* (10^6) and *Staphylococcus aureus* (10^8) in to uterine horns during diestrus stage followed by cervical ligation and the model was confirmed based on presence of visible pus in the uterus, edematous uterine horn, thinning of endometrial lining and presence of large number of polymorphonuclear cells and bacterial load in uterine flushing. *Eucalyptus citriodora* leaves extract and cefixime were observed to significantly reduce the uterine weight, uterine secretion index, polymorphonuclear cells and bacterial load in uterine flushings, alteration in total leucocyte count and differential leucocyte count. Tumor necrosis factor- α (TNF- α), pro and anti inflammatory cytokines like interleukin-1 *beta* (IL-1 β) and interleukin-10 (IL-10), serum amyloid A (SAA) and intercellular adhesion molecule-1 (ICAM-1), myeloperoxidase (MPO), toll like receptor 4 and 9 (TLR-4, TLR9), cyclooxygenase-1 and 2 (COX-1, COX-2), inducible nitric oxide synthase (iNOS) and nitric oxide (NO) were found to be significantly reduced after treatment with *Eucalyptus* leaves extract. The effect was comparable with the cefixime treatment. Histopathological changes in uterus also showed efficient induction of endometritis by presence of inflammatory cells which are lessened effectively after treatment with *Eucalyptus* leaves extract. Results were compared with cefixime and it was evident that *Eucalyptus citriodora* produced curative and protective effect against endometritis. Oxidative stress parameters in vital organs like liver, spleen, kidney and brain showed decrease in reduced glutathione (GSH), catalase (CAT) while an increase in lipid peroxidation (LPO) and superoxide dismutase (SOD) after induction of endometritis. However, after treatment reduced glutathione and catalase level was significantly increased while lipid peroxide and superoxide dismutase significantly reduced. Based on these studies, it may be inferred that *Eucalyptus citriodora* leaves extracts possess promising antibacterial activity and efficacy against experimental endometritis and, therefore, can be exploited in drug development program for treatment of endometritis in animals.

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5. Studies on hydrogen sulfide-induced alterations in myometrial activity and its associated downstream signaling pathways in water buffaloes (*Bubalus bubalis*)

Sooraj V. Nair and Satish K. Garg

Hydrogen sulphide, a gas with rotten egg smell, is traditionally considered as a toxicant and an environmental pollutant but recently, it has gained attention as a mediator of physiological and biological processes. The present study was undertaken to unravel the effect of H₂S on myometrial activity and its underlining mechanism in non-pregnant buffaloes. L-cysteine is considered to be the main endogenous amino acid responsible for hydrogen sulphide formation due to the action of two cytosolic enzymes- cystathione β

synthase (CBS) and cystathione γ lyase (CSE/CGL). Sodium hydrogen sulphide and GYY4137 are two major hydrogen sulphide donors were used in this present study. *In vitro* exposure of isolated buffalo myometrial strips to L-cysteine (10 nM to 3 mM) produced concentration-dependent uterotonic effect. L-cysteine induced uterotonic action is dependent on the activity of cystathione β synthase (CBS) and cystathione γ lyase (CGL/CSE) as evidenced by rightward shift of the DRC of L-cysteine in the presence of enzyme blockers (AOAA and PAG, respectively). It is to be further noted that following blockade of CBS and CSE enzyme, possibly some relaxant mechanism may get activated by L-Cysteine to produce uterine relaxation. The existence of CBS enzyme of molecular weight, 63kDa and CSE/CGL of 45kDa was observed by Western blot. L-cysteine failed to produce any appreciable contraction in the absence of extracellular calcium. Further, in the presence of nifedipine, the uterotonic action of L-cysteine was completely abolished. Cumulative addition of sodium hydrogen sulphide (10 nM to 300 μ M), exhibited biphasic effect on isolated myometrial strips which is characterized by initial contraction (up to 10 μ M) followed by marked relaxation at higher doses (30 μ M to 300 μ M). The relaxant effect of NaHS differed in spontaneous and oxytocin precontracted myometrial strips. The DRC of NaHS was significantly shifted towards left in OT-precontracted tissues compared to the control. GYY4137 was found to produce no significant change in myometrial spontaneity compared to vehicle (DMSO) control. Thus, our findings evidently suggest that H₂S regulates myometrial spontaneity in non-pregnant buffaloes.

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6. Pharmacology characterization of calcium regulatory pathways in oviduct of water buffaloes (*Bubalus bubalis*)

Pranshu Sharma and Satish K. Garg

Present study was embarked upon to extricate the underlying calcium signaling mechanisms responsible for spasmogens (PGF_{2 α} , and oxytocin)-induced muscular contractions in oviducts of non-pregnant buffalo in oestrous stage. Isometric tension in longitudinal oviductal in both ampulla and isthmus strips was recorded under the resting tension of 1 ± 0.2 g following mounting the tissue in Ringer-locke solution. Following an equilibration period of about 120 to 150 min, oviductal strips from both ampulla and isthmus presented a consistent and rhythmic pattern of spontaneity. Mean Integral Tension (MIT) and amplitude (g) of ampulla and isthmus in normal spontaneous tissue did not differ significantly. The frequency (BPM) of ampulla and isthmus in normal spontaneous tissue differed significantly ($P < 0.05$). PG_{2 α} produced significantly ($P < 0.05$) greater contraction than oxytocin in both ampulla and isthmus of buffalo oviduct in concentration-dependent manner. In isthmus the contraction produced by oxytocin was negligible. PGF_{2 α} produced a significant change in tonic contraction between ampulla and isthmus but the change in phasic contraction between ampulla and isthmus was not significant. PGF_{2 α} produced significantly ($P < 0.05$) greater contraction in ampulla than in the isthmus of buffalo oviduct. The normal spontaneity was almost abolished in calcium free RLS and also PGF_{2 α} mediated contractile effect was also negligible in both ampulla and isthmus. Nifedipine, a L-type voltage- dependent calcium channel (VDCC) blocker completely abolished the spontaneity and DRC of PGF_{2 α} was significantly ($P < 0.001$) shifted towards right and the maximal contraction (%) was significantly ($P < 0.05$) decreased in both ampulla and isthmus. NNC55-0396, a T-type VDCC blocker, abolished spontaneity in ampulla but not in isthmus, but DRC was significantly ($P < 0.001$) shifted towards right in the presence of NNC55-0396 both in ampulla and isthmus. The maximal contraction (%) significantly ($P < 0.05$) decreased in both ampulla and isthmus.

Concurrent use of NNC55-0396 and nifedipine to determine the extent of involvement of VDCCs, the E_{max} values were almost comparable to that observed when these blockers were used alone. In presence of SKF96365, a non specific store operated calcium channel (SOCC)/ Transient receptor potential canonical (TRPC) blocker spontaneity was completely abolished in both ampulla and isthmus and DRC of $PGF_{2\alpha}$ was significantly shifted ($P<0.001$) shifted towards right in the presence of SKF96365 of both ampulla and isthmus. The maximal contraction (%) was significantly decreased in both ampulla and isthmus. Pyr3, a specific TRPC3 channel blocker did not abolish the spontaneity in both ampulla and isthmus. DRC of $PGF_{2\alpha}$ was significantly shifted towards right in both ampulla and isthmus in the presence of Pyr3. The maximal contraction (%) was significantly ($P<0.05$) decreased in both ampulla and isthmus. Histological study of buffalo oviduct showed that the muscular layer was greater in thickness in isthmus as compared to ampulla whereas mucosal layer was thicker in ampulla than in the isthmus. TRPC3 and TRPM3 protein expression was studied by immunohistochemistry and TRPC3 protein was shown to be localized in muscular layer whereas TRPM3 was localized in mucosal and muscular layer of ampulla. In isthmus TRPC3 and TRPM3 proteins were shown to be localized in both serosal and muscular layer of isthmus of buffalo oviduct. So, on the basis of present finding it can be concluded that ampulla and isthmus of buffalo oviduct exhibit spontaneous contractility which is not markedly different from each other and is dependent on extracellular calcium. Oxytocin mediated contraction does not seem to play a major role in oviductal contraction mechanism during oestrus. $PGF_{2\alpha}$ exert concentration-dependent contraction in both ampulla and isthmus which is dependent on extracellular calcium. Ampulla is more sensitive to contractile effect produced by $PGF_{2\alpha}$ than isthmus. $PGF_{2\alpha}$ -induced contraction is sensitive to both L and T type calcium channels in both ampulla and isthmus contributing equally in $PGF_{2\alpha}$ -induced contraction. SOCCs also regulate spontaneity and $PGF_{2\alpha}$ -induced contractions in ampulla and buffalo oviduct.

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7. Studies on endocannabinoid(s)-mediated lipid signaling in mouse aorta during early and late phase of sepsis

Preeti Singh and Soumen Choudhury

The present study was carried out to assess the role of endogenous cannabinoids in regulating aortic vascular response during early and late phases of sepsis. Polymicrobial sepsis was induced by caecal ligation and puncture (CLP) in mice. Besides, recording the isometric tension in arterial rings, estimation of haemato-biochemical parameters, histopathological examinations of vital organs, mRNA expression of CB1 receptor and MAGL enzyme and their role in the vasoconstrictor response to noradrenaline in the aorta of septic mouse were studied. Sepsis significantly reduced RBC, Hb and WBC counts during both early (CLP-6h) and late (CLP-20h) phases of sepsis whereas neutrophil count was increased during early phase. There was also a marked fall in lymphocyte count during late phase of sepsis indicative of immunosuppressive state. Significant rise in the plasma ALT, AST, BUN and creatinine levels during early and late phases of sepsis were suggestive of liver and kidney dysfunctions which were further substantiated by histopathological examinations of these vital organs. Sepsis produced a state of hypoproteinaemia with significant reduction in plasma albumin level. Histopathological examination of lungs, heart and intestine showed progressive degenerative changes which were more prominent with progression from early to late phase of sepsis. In addition, sepsis also impaired the vascular reactivity to noradrenaline (NA) and relaxant response to acetylcholine (indicative of endothelial damage), in the mouse aorta during both early and late phases of sepsis without affecting the relaxant

response at vascular smooth muscle cell level as evidenced by non-significant alteration in the relaxant response to sodium nitroprusside (SNP), a nitrovasodilator. Among the two endocannabinoids (2-AG and AEA) studied in the present study, 2-arachidonylglycerol (2-AG) produced concentration-dependent contractile effect on phenylephrine (PE)-induced vascular tone in sham-operated (SO) mice as well as early phase septic animal which were sensitive to indomethacin or nimesulide suggesting the role of COX-1 and COX-2-derived metabolites, respectively, in the vasoconstricting effect of 2-AG. However, 2-AG-induced vasoconstriction in late phase septic mouse aorta was resistant to indomethacin and nimesulide. Unlike 2-AG, arachidonoyl ethanolamide (AEA) did not produce any effect either on basal tone or PE-induced tone in the mouse aorta from SO mice as well as early and late phases septic animals. Significant potentiation of NA response in the presence of KT109, a specific inhibitor of DAGL, and attenuation of NA-induced vasoconstriction in the presence of JZL184, a specific inhibitor of MAGL, in SO mice suggest a functionally relevant role of endogenously produced 2-AG in controlling vascular reactivity to NA. However, in the presence of JZL 184, the vascular hyporeactivity to NA in septic mice (early and late phase) was not affected which may be due to significant attenuation of mRNA expression of MAGL in the mouse aorta from these groups of animals as compared to SO mice. Role of CB1 receptor activation in regulating vascular hyporeactivity to NA in mouse aorta during early and late phases of sepsis was evidenced by the significant reversal of NA-induced attenuated vasoconstriction in the presence of AM 251, a specific antagonist of CB1 receptor. Accordingly, we have found a significant increase in the mRNA expression of CB1 receptor in mouse aorta from early and late phases of sepsis as compared to SO mice. Unlike AM 251, rimonabant, another CB1 receptor antagonist, failed to produce any significant effect on vascular reactivity to NA in mouse aorta either from SO mice or both the phases septic mice. Interestingly, AM 251 failed to produce any effect on altered vascular contractility which are mediated by direct influx of Ca²⁺ through voltage gated Ca²⁺ channels into the cells without involving G-proteins-coupled signalling mechanism. Taken together, based on the findings of the present study, it may be inferred that caecal ligation and puncture produces time-dependent progression of sepsis in mice affecting multiple organs including cardiovascular system. 2-AG plays an appreciable role in regulation of vascular reactivity to NA in mouse aorta. Further, excess expression of CB1 receptor in mouse aorta is responsible for vascular hyporeactivity to NA during sepsis and inhibition of this receptor by AM 251 restores the vascular reactivity to NA in sepsis. However, vascular contraction unrelated to G-proteins coupled signalling mechanism remains unaltered by AM 251-induced CB1 receptor inhibition in mouse aorta.

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8. Evaluation of ameliorative potential of α -tocopherol and curcumin against cisplatin-induced nephrotoxicity in male Wistar rats

Vishwajeet Singh Chandel and Atul Prakash

Present study was undertaken to evaluate the ameliorative potential of α -tocopherol, curcumin and/or in combination with cisplatin following 28 days continuous exposure. Forty eight male wistar rats of 190-210 g were divided into eight groups group I (NSS, i.p.), group II (corn oil, oral gavage) served as vehicle control, group III received cisplatin, group IV (α -tocopherol), group V (curcumin), group VI (cisplatin + α -tocopherol), group VII (cisplatin + curcumin), group VIII (cisplatin + curcumin + α -tocopherol). Cisplatin was given by intra-peritoneal route @ 0.5mg/kg b.wt., while α -tocopherol @ 100 mg/kg b.wt. and curcumin @ 50 mg/kg b.wt. by oral gavage continuously for 28 days. Cisplatin produced

apparent signs of toxicity like rough coat, cachexia, decreased activity but no mortality in rats. Body weight and percent weight gain in rats with cisplatin alone and in combination with α -tocopherol and curcumin treated groups were significantly lower. Absolute and relative organ weight did not differ between different groups. Significant reduction in Hb on 21 day and 28 day after exposure. Marked reduction in PCV, TLC and platelet count in cisplatin alone treated group following 28 day exposure however DLC remain unaltered. Significant decrease in creatinine clearance in all cisplatin exposed groups when compared to control groups. Protein urinary creatinine ratio in cisplatin alone treated group increased significantly on 28 day of experiment, while did not divericates significantly when cisplatin given in combination with α -tocopherol and curcumin. Significant rise in serum creatinine, urea, BUN, uric acid, ALT, AST, LDH, GGT was found in rats of cisplatin alone, cisplatin + α -tocopherol and cisplatin + curcumin treated groups with maximum increase in cisplatin alone group. Exposure of rats to cisplatin alone and in combination with α -tocopherol and curcumin revealed significant increase in MDA levels, decrease in GSH level, decrease in activity of CAT and SOD. Activity of GST and GPx were significantly reduced in cisplatin alone group, but no difference in activity of GST and GPx were observed among control and cisplatin + α -tocopherol and cisplatin + curcumin treatment groups. Urinary KIM-1 expression was increased significantly on 7 day of experiment, while KIM-1 expression among cisplatin + α -tocopherol, cisplatin + curcumin treated groups and control did not differ significantly. After 28 day of experiment significant increase in urinary KIM-1 expression in cisplatin exposed groups. Compared to control groups, kidneys of cisplatin alone in combination treated groups revealed accumulation of proteinacious fluid in the renal tubules with cellular swelling, degeneration, sloughing of renal tubular epithelium and congestive changes in the renal parenchyma, but changes were less severe in cisplatin + α -tocopherol and cisplatin + curcumin treated groups.

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9. Studies on *Gymnema sylvestre* and ITK formulation for therapeutic management of cardiomyopathy in type II diabetic rats

Chandan Patil and Atul Prakash

The objective of the present study was to explore the therapeutic potential of *Gymnema sylvestre* and ITK formulation against obese streptozotocin-induced type II diabetes and diabetic cardiomyopathy in male Wistar rats. In Phase I study, 80% hot methanolic extract of *Gymnema sylvestre* leaves and hot aqueous extract of ITK formulation (ingredients gum acacia, black cumin, wheat and barley) was prepared and evaluated for in vitro antioxidant and antidiabetic activity of extracts. Extracts have potent antioxidant (studied by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay), antidiabetic (determined by in vitro α -amylase and α -glucosidase inhibitory activity) and glucose uptake potential in cultured lymphocytes and were observed to possess alkaloids, flavonoids, saponins, anthraquinones and phenols in *Gymnema sylvestre* leaves with additional resins, tanins, proteins, glycosides and fixed oils and fat in ITK formulation after screening by qualitative tests and further confirmed to possess active principles having antidiabetic, antioxidant, cardioprotective, nephroprotective, and anti-inflammatory properties. Further in Phase II study, 42 obese male Wistar rats were divided into seven groups viz. group I (Normal control), group II (Obese control), group III (Obese diabetic), group IV (Obese diabetic + Metformin), group V (Obese diabetic + *Gymnema sylvestre*), group VI (Obese diabetic + ITK) and group VII (Obese diabetic +

Gymnema sylvestre + ITK) consisting six animals in each, were experimentally-induced-diabetes with streptozotocin @ 35 mg/kg body weight, i.p. *Gymnema sylvestre* extract, ITK formulation and metformin were given @ 400 mg/kg, @ 445 mg/kg and @ 50 mg/kg body weight by oral gavage continuously for 60 days. Increase in feed and water intake, decrease in percent weight gain and anthropometric parameters, reduced percent haemoglobin and platelets count, significant increase in fasting blood glucose and per cent glycated haemoglobin (HbA1C), was recorded in obese diabetic group rats. Treatment with metformin and extracts alone and combination lowered blood glucose and percent HbA1C, where, ITK formulation was emerged as a potent hypoglycaemic formulation and was comparable to metformin, also this formulation reduced the feed and water intake towards control values. Dyslipidemia (increase in triglycerides, total cholesterol, LDL and decrease in HDL), rise in serum ALP, GGT, ALT, AST (liver injury markers), urea, creatinine, total proteins, albumin, globulin and ratio between albumin and globulin (kidney injury markers) and LDH, CK-MB, cardiac troponin-I (cardiac injury biomarkers) was observed in obese diabetic rats which were partially and significantly restored in rats treated with hot methanolic extract of *Gymnema sylvestre* leaves and hot aqueous extract of ITK formulation alone or in combination and metformin. Obese diabetic rats revealed a significant increase in MDA and decrease in GSH level, decreased activity of CAT, SOD, GST and GPX in heart whereas, improvement was observed in antioxidant enzymes in all the treatment groups. Mean arterial pressure was significantly increased and ECG indices were altered in diabetic rats and treatment with extracts alone and/or combination significantly restored the hypertension and also the ECG indices (QRS interval, R-amplitude and ST-height) were shifted towards normal control values. A significant increase in expression of cardiac tissue glucose transporter-4 (GLUT-4) was observed in both extracts alone and combination of extracts compared to obese diabetic group revealing their protective action against hyperglycemia induced cardiac injury. Histopathological findings revealed degenerated pancreatic islets, acini and collagen deposition, disrupted cardiac myofibres and infiltration of inflammatory cells in obese diabetic rats whereas, treatment groups reversed the pathological features of cardiac injury, with decrease in collagen fibre deposition and showed less degenerative changes in pancreatic architecture. Thus, it can be concluded that, both hot methanolic extract of *Gymnema sylvestre* and hot aqueous ITK formulation are comparable to control hyperglycemia in STZ-induced-obese diabetic rats and to combat free-radical mediated derangements in the body.

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10. Mechanistic study on vascular dysfunctions in septic mice with pre-existing diabetes

Manju Gari and Satish K. Garg

Present study was undertaken to unravel the influence of experimentally induced preexisting diabetes on survival time and/or mortality pattern in septic animals and how diabetes and sepsis, when occur concurrently, alter vascular reactivity of aorta. Type-1 diabetes was induced following intraperitoneal injection of streptozotocin (STZ: @ 65 mg/kg) for 5 consecutive days while sepsis was induced by caecal ligation and puncture (CLP). Streptozotocin produced a sustained hyperglycaemia in mice and there was decrease in body weight with progression of hyperglycaemic state. CLP caused hyperglycaemia followed by euglycaemia and hypoglycaemia, while in diabeto-sepsis hypoglycaemia was set earlier, leading to early mortality. Alteration in vascular reactivity and endothelial dysfunction was observed. Histopathological examination showed that STZ caused destruction of only beta cells without affecting the exocrine and other cells and sepsis mainly affected the exocrine

acinar cells without or mildly affecting the endocrine part, whereas in co-existing diabetes and sepsis, both exocrine and endocrine parts were affected. Histopathology of liver, lungs, kidney, brain and heart also showed that organ damage was more severe in co-existing diabetes and sepsis. Interestingly, histopathology of aorta showed intact endothelium in diabetes, damaged endothelium in sepsis, while in diabeto-sepsis endothelium became serrated and slightly damaged. Haematological and biochemical parameters revealed that sepsis in pre-existing diabetes shifted almost all the parameters towards healthy control. Interestingly, diabeto-septic group the platelet count was found to be almost comparable to that of control group. Functional studies revealed augmented responses of vasoconstrictors (like high K⁺ -depolarising solution, noradrenaline and calcium chloride) in diabetes, while reduced response was observed in sepsis. Sepsis in pre-existing diabetes shifted the augmented response of vasoconstrictors towards in healthy control and sham operated (SO) groups. Diabetes increased the expression of α_{1D} adrenoceptor which seems to be responsible for potentiation of NA response while in sepsis, there was decrease in α_{1D} adrenoceptor expression which may be responsible for decrease in responsiveness to NA. Sepsis in preexisting diabetes shifted the receptor expression towards normal level. Decreased potency of calcium chloride in diabetes may be due to decrease in passive leakage of intracellular calcium from endoplasmic reticulum. Vascular response to endothelium-dependent vasorelaxant (acetylcholine) did not differ in diabetes, but, was attenuated in sepsis. Sepsis in pre-existing diabetes decreased the vasorelaxant response to ACh but remained higher than in sepsis alone group. Diabetes also potentiated the ACh response, which may be due to comparatively less damage to endothelium and increased release of nitric oxide (NO) and prostacyclin. eNOS expression was decreased by 52 % in diabetes, 71 % in sepsis and 35 % in diabeto-sepsis. Maximal relaxation response of endothelium-independent vasorelaxant i.e. sodium nitroprusside (SNP) did not differ in diabetes and diabeto-septic mice, while diabetes potentiated SNP response. Potentiation of vasorelaxant-response to SNP may be attributed to enhanced production of iNOS-derived NO.

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11. Study on the effect of oleic acid in isoprenaline-induced myocardial injury in rats

Pawan Kumar Singh and Soumen Choudhury

The present study was designed to assess the cardio-protective role of oleic acid in myocardial injury. Myocardial injury was induced in rats by intra-peritoneal injection of isoprenaline (ISO; 110 mg/kg b.wt) for two consecutive days at 24 h interval. Oleic acid was administered orally (@ 5mg/kg b.wt or 10 mg/kg b.wt) for 21 days before inducing myocardial injury to evaluate its ameliorative potential. Sample (blood, heart) were collected from different groups of experimental animals 24 h after last injection of isoprenaline. Besides evaluation of heart weight to body weight (HW/BW) ratio, myocardial infarct size, oxidative stress parameters and haemato-biochemical parameters, cardio-specific biomarkers of injury, ECG, isolated right atrial response and mRNA expression of gene coding for cardiac uncoupling protein-2 (UCP-2) were quantified. Isoprenaline administration significantly increased the HW/BW ratio, myocardial infarct size, lipid profiles (total cholesterol, HDL-C, triglyceride) in ISO-induced myocardial injured rats. Further, ISO-induced myocardial injury significantly elevated the cardio-specific biomarkers (viz. LDH, CK-MB, cardiac troponin-I) suggesting the myocardial necrosis and alteration of membrane permeability. Necrosis and degeneration of cardiac myofibrils with deposition of collagen fibers were also observed in the histopathological examination of cardiac tissue sections. Further, significant increase in

the heart rate and height of ST segment whereas decrease in RR and QT intervals were observed in the ISO-induced myocardial injured rats implying the abnormality in the cardiac functionality in rats following isoprenaline administration. Oleic acid pre-exposure at higher dose significantly improved the HW/BW ratio, myocardial infarct size, lipid profiles and cardiac injury biomarkers suggesting its cardio-protective role. The ameliorative potential of higher dose of oleic acid was further substantiated by its ability to reduce the cardiac oxidative stress as evidenced by significant decrease in lipid peroxidation with corresponding increase in superoxide dismutase and reduced glutathione. Significant increase in RR interval and QT intervals in oleic acid pre-exposed rats were also observed. The mRNA expression of cardiac UCP-2 gene was significantly increased in the oleic acid pre-exposed group as observed in ISO-induced myocardial injured rats. Though UCP-2 gene is responsible for fatty acid oxidation, its potential role in modulating reactive oxygen species (ROS) is also mentioned. Thus increasing the gene expression of UCP-2 in cardiac tissue may be a protective measure against myocardial injury. Further, reduction of fatty acid oxidation is always not successful in heart failure because it may directly influence the supply of ATP to comprised heart resulting in further decrease in cardiac efficiency. Thus as an alternative measure up-regulation of glucose oxidation may be a useful measure in cardiac ischemia. Further studies are warranted to evaluate the effect of oleic acid on cardiac glucose oxidation. Based on the above findings it may be inferred that oleic acid has the potential cardioprotective action against myocardial injury due to its anti-oxidative property and its ability to modulate cardiac metabolic processes. Thus incorporation of oleic acid as a component of diet may be a useful measure against myocardial ischemia or injury.

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12. Studies on therapeutic potential of *Tribulus terrestris* on diabetic nephropathy in Wistar rats

Priyanka Rajpoot and Atul Prakash

The objective of the present study was to explore the therapeutic potential of *Tribulus terrestris* and ITK formulation against obese streptozotocin- induced type II diabetes and diabetic nephropathy in male Wistar rats. In Phase I study, 70% hot ethanolic extract of *Tribulus terrestris* fruits and hot aqueous extract of ITK formulation (ingredients gum acacia, black cumin, wheat and barley) was prepared and evaluated for in vitro antioxidant and antidiabetic activity of extracts. Extracts have potent antioxidant (studied by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay), antidiabetic (determined by in vitro α -amylase and α -glucosidase inhibitory activity) and glucose uptake potential in cultured lymphocytes and were observed to possess carbohydrates, alkaloids, proteins and amino acids, tannins, flavonoids, saponins, fixed oils and resins in *Tribulus terrestris* fruits with additional glycosides in ITK formulation after screening by qualitative tests and further confirmed to possess active principles having antidiabetic, antioxidant, nephroprotective, cardioprotective and anti-inflammatory properties. Further in Phase II study, 42 obese male Wistar rats were divided into seven groups viz. group I (Normal control), group II (Obese control), group III (Obese diabetic), group IV (Obese diabetic + Metformin), group V (Obese diabetic + *Tribulus terrestris*), group VI (Obese diabetic + ITK) and group VII (Obese diabetic + *Tribulus terrestris* + ITK) consisting six animals in each, were experimentally-induced-diabetes with streptozotocin @ 35 mg/kg body weight, i.p. *Tribulus terrestris* extract, ITK formulation and metformin were given @ 200 mg/kg, @ 445 mg/kg and @ 50 mg/kg body weight by oral gavage continuously for 60 days. Increase in feed and water intake, decrease in percent weight gain and anthropometric

parameters, reduced percent haemoglobin and platelets count, significant increase in fasting blood glucose and percent glycated haemoglobin (HbA1C) was recorded in obese diabetic group rats. Treatment with metformin and extracts alone and combination lowered blood glucose and percent HbA1C, where, ITK formulation was emerged as a potent hypoglycaemic formulation and was comparable to metformin, also this formulation reduced the feed and water intake towards control values. Dyslipidemia (increase in triglycerides, total cholesterol, LDL and decrease in HDL), rise in serum ALP, GGT, ALT, AST (liver injury markers), urea, creatinine, uric acid, total proteins, albumin, globulin and ratio between albumin and globulin (kidney injury markers) and urine output and urinary albumin (urinary biochemical parameters) was observed in obese diabetic rats which were partially and significantly restored in rats treated with *Tribulus terrestris* fruits extract and hot aqueous ITK formulation alone or in combination and metformin. Obese diabetic rats revealed a significant increase in MDA and decrease in GSH level, decreased activity of CAT, SOD, GST and GPX in liver, kidney, brain, spleen, testes and heart whereas, improvement was observed in antioxidant enzymes in all the treatment groups. A significant decrease in expression of sodium glucose co-transporter (SGLT-2) was observed in both extracts alone and combination of extracts compared to obese diabetic group revealing their protective action against hyperglycemia induced kidney injury. Histopathological findings revealed degenerated pancreatic islets, acini and severe collagen fibre deposition in kidney tubules and glomeruli in obese diabetic group rats whereas, in obese diabetic group rats, treatment groups reversed the pathological features of kidney injury and showed less degenerative changes in pancreatic architecture and restoration of kidney tubules and glomerular morphology with decrease in collagen fibre deposition. Thus, it can be concluded that, both hot ethanolic *Tribulus terrestris* extract and hot aqueous ITK formulation are comparable to control hyperglycemia in STZ-induced-obese diabetic rats and to combat free radical mediated derangements in the body.

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13. Studies on purinergic signalling in vascular hyporeactivity in septic mice

Jagadeesh T. and Satish K. Garg

Present study was undertaken to assess the effect of sepsis on purinergic signaling with special reference to P2Y₆ and P2X₇ receptors in mouse aorta; and to unravel the interplay between purinergic receptor (P2Y₆) and angiotensin II type I receptor (AT1R) in mediating vascular hyporeactivity during sepsis. Sepsis significantly decreased total erythrocytes count, haemoglobin level, total leukocytes count and differential leukocytes count compared to sham-operated mice (SO). Sepsis significantly increased plasma levels of ALT, AST, BUN, creatinine values. Similarly sepsis significantly increased total bacterial count in peritoneal lavage, blood and spleen compared to healthy control. Functional studies revealed that in mice of SO groups, high K⁺-depolarising solution produced almost equal contraction in the aortic rings having the intact-endothelium or denuded. But compared to the SO group, mice of the sepsis group, irrespective of the presence or absence of endothelium in aortic rings, exhibited significantly reduced contractile response following exposure to high K⁺-depolarising solution. UDP-induced vasoconstriction was significantly higher in endothelium-denuded mouse aorta compared to the endothelium-intact aorta and this response is mediated through P2Y₆ receptors. Bz-ATP-induced vasoconstriction was also higher in endothelium-denuded mouse aorta compared to the endothelium-intact aorta and this response is mediated through P2X₇ receptors. Ang-II also produced higher contraction in endothelium-denuded mouse aorta compared the endothelium-intact aorta and response is

mediated through AT1 receptors rather than AT2. UDP and Ang-II, but not of Bz-ATP, produced higher contractile effect in presence of LNAME, the non-specific inhibitor of nitric oxide synthase, in endothelium-intact mouse aortic rings. Sepsis resulted in contractile response following exposure to UDP, Bz-ATP, Ang-II in mouse aorta. Sepsis caused up-regulation of P2Y6 receptor mRNA expression in endothelium-intact septic mouse aorta. But sepsis caused down regulation of AT1a receptor. Ang-II produced higher contractile effect in the presence of 1400W, the specific inhibitor of iNOS, in septic mouse aorta. Ang-II produced lower contractile effect in the presence of MRS-2578, the selective antagonists of P2Y6 receptor, in SO mouse aorta but the contractile effect of UDP was not altered in the presence of losartan, the selective antagonists of AT1 receptor and also contractile effect of noradrenaline was not altered in the presence of MRS-2578.

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14. Studies on testicular activity of type 2 diabetic rats following concurrent pre-exposure to arsenic and chromium

Abhishek Pathak and Atul Prakesh

The objective of present study was to evaluate the influence of pre-exposure of chromium @ 1 mg/kg body weight and arsenic @ 38 ppb in drinking water continuously for 30 days in experimentally streptozotocin-induced type 2 diabetes in male Wistar rats and after 30 days of continuous exposure ameliorative potential of an ITK formulation @ 435 mg/kg body weight and its comparative efficacy with metformin @ 50 mg/kg body weight on diabetes-induced testicular dysfunction. Eighty four obese male Wistar rats were divided into fourteen groups viz. Normal Control, Obese, Non-diabetic + Chromium, Non-diabetic + Arsenic, Non-diabetic + Chromium-Arsenic combination, Obese Diabetic + chromium, Obese Diabetic + Arsenic, Obese Diabetic + Chromium-Arsenic combination, Obese Diabetic + Chromium + Metformin, Obese Diabetic + Arsenic + Metformin, Obese Diabetic + Chromium-Arsenic combination + Metformin, Obese Diabetic + Chromium + ITK, Obese Diabetic + Arsenic + ITK, Obese Diabetic + Chromium-Arsenic combination + ITK, consisting six animals in each group, were induced diabetes with streptozotocin @ 30 mg/kg body weight, intraperitoneally single dose after 30 days of pre-exposure to chromium and arsenic. After induction of diabetes ITK formulation and metformin were given by oral gavage continuously for 30 days. Obese diabetic rats pre-exposed to chromium and arsenic increased feed and water intake and decrease in body weight, percent weight gain and anthropometric parameters, hemoglobin, TEC and platelets count whereas significant increase in TLC, fasting blood glucose and percent HbA1c. Also increased triglyceride, total cholesterol, LDL and decrease in HDL, increased activity of liver injury markers (ALP, GGT, ALT, AST) and rise in kidney injury markers (BUN, creatinine, total proteins, albumin, globulin and ratio between albumin and globulin) was observed. Testicular injury biomarkers (ACP, SDH, LDH, GGT, 17-β HSD) were also increased along with decrease serum testosterone level. Also increase in lipid peroxidation (MDA level) and decrease in GSH content with antioxidant enzymes (CAT, SOD, GST and GPX) in testes were evident. Live and dead sperm count and HOST (+ve) sperm cells were adversely affected in pre-exposed obese diabetic rats. Treatment with ITK formulation and metformin moderately to significantly improved general parameters, hematological indices, oxidative stress markers (by increasing GSH content and activity of CAT, SOD, GST and GPx in testes and decrease in lipid peroxidation) improving antioxidant defense system. However, experimental type 2 diabetes and pre-exposure of chromium and arsenic could not have been able to induce micronuclei formation, comet formation and DNA fragmentation in sperm cell. Histopathological findings revealed relatively normal testes in chromium exposed

group whereas, degeneration (vacuolation) with presence of edema in interstitial tissue and loss of some spermatogenic cells in arsenic treated groups, combination of chromium and arsenic showed normal histoarchitecture while edema of interstitial space and hyperaemia of the parenchyma present. Histoarchitecture of testes was severely affected in diabetic pre-exposed rats, while ITK and metformin restored the normal histology of testes.

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Ph.D. Degree Programme

1. Role of α -tocopherol, copper and/or flubendiamide-induced chronic toxicity in rats with special reference to male reproductive system

Rajesh Mandil and Satish K. Garg

Present study was undertaken to evaluate the ameliorative effect of α -tocopherol (100 mg/kg) against copper (33 mg/kg) and/or flubendiamide (200 mg/kg)-induced toxicity following 90 days oral exposure. Fifty four male rats of 130-150 g were divided into nine groups of six animals each. Rats of group I served as negative control and group II as vehicle control (corn oil) while animals of groups III to IX received orally α -tocopherol (100 mg/kg), copper sulphate (33 mg/kg), flubendiamide (200 mg/kg), flubendiamide (200 mg/kg) + copper sulphate (33 mg/kg), copper sulphate (33 mg/kg) + α -tocopherol (100 mg/kg), flubendiamide (200 mg/kg) + α -tocopherol (100 mg/kg) and flubendiamide (200 mg/kg) + copper sulphate (33 mg/kg) + α -tocopherol (100 mg/kg), respectively. Both the xenobiotics failed to produce any apparent clinical sign of toxicity or mortality in rats. Body weight and per cent weight gain in rats of copper and flubendiamide alone groups and in combination treated (copper + flubendiamide) rats were markedly lower. But organ weights and relative organ weight did not differ between different treatments groups except significant increase in relative weight of lungs and brain in rats of copper and copper + flubendiamide treatment groups. No significant alterations were observed in feed and water intake in any of the treatment groups except significant reduction in feed intake during 9th and 11th weeks in copper-treated rats. Significant reduction in Hb, PCV, WBCs count and granulocytes count was observed in flubendiamide and copper + flubendiamide treated rats while lymphocytes % was significantly increased in rats of flubendiamide alone treated. RBCs, Hb, MCV, MCH, MCHC, LY%, MO%, GR% and PCT values did not differ significantly in any of the xenobiotics treatment groups except significant increase in RDWC and decrease in PLT count, respectively in copper or flubendiamide or copper + flubendiamide-treated rats. Total proteins, albumin and globulin, glucose, cholesterol and HDL-cholesterol, AST and ALT values did not differ significantly any of the xenobiotics-exposed groups except significant increase in total and direct bilirubin in flubendiamide-treated rats and creatinine and uric acid levels in copper-treated rats. LPO level significantly increased in erythrocytes, kidneys, spleen and testes while modestly in liver of copper and flubendiamide and copper + flubendiamide treated rats. SOD activity was significantly lower in RBC, kidneys and spleen of copper treated rats while significantly lower only in liver and testes of flubendiamide treated rats, and copper + flubendiamide treated rats, and moderate decrease in testes of all three xenobiotics treated groups. CAT activity significantly decreased in testes, not significant effect was seen in liver, kidneys, spleen and erythrocytes of xenobiotics exposed groups. GSH levels did not differ significantly in RBCs, liver, kidneys, spleen and testes of any of the treatment groups compared to controls except in spleen of flubendiamide-treated rats. GPx activity in copper, flubendiamide and copper + flubendiamide treated groups revealed significant to moderate decrease in testes, spleen and erythrocytes while moderately increase in liver and kidneys compared to control, significant to moderate reduction in GST activity was evident in kidneys, spleen, testes and liver while significant increase in GST activity in erythrocytes. No significant alterations were observed in ACP, ALP and SDH activities in testes of rats of any of the treatment groups, but there was significant increase in LDH, γ -GT and abnormal sperms count and depletion of 17 β -HSD, percentage of live sperms, HOST+ve sperms and testosterone level in copper and flubendiamide and copper + flubendiamide treated rats.

Compared to control, testes of copper or flubendiamide or copper + flubendiamide exposed rats exhibited severe degenerative alterations in histoarchitecture which included degeneration of germinal epithelium, nuclear pyknosis, necrotic germ cells and loss of spermatozoa, spermatocytes and spermatids and complete loss of spermatids. CYP450 and CYP b5 activities were significantly increased in flubendiamide-treated rats liver while CYP450 and CYP b5, APH, ANDM, GST and UGT activities did not differ significantly in any of the xenobiotics treatment groups except with copper-treated rats, where significant decrease in APH, GST and UDP glucuronosyltransferase activities were observed. Markedly increase in Cu and Fe levels and decrease in Zn and Mn levels in liver of copper, flubendiamide as well as copper + flubendiamide- treated rats. Phase II in vitro study was undertaken for evaluating apoptotic and genotoxicity potential of flubendiamide and copper on isolated thymocytes and splenocytes of rats and its prevention by resveratrol (5 and 10 μM), catechin (10 and 20 μM), curcumin (5 and 10 μM) and α -tocopherol (5, 10 and 20 μM) by employing by propidium iodide, TUNEL assay, micronuclei, DNA ladder and comet assays. Propidium iodide staining study revealed that flubendiamide and copper produced dose-dependent increase in percentage of dead/or apoptotic cells in thymocytes and splenocytes and 40 μM concentration was considered as the median lethal concentration of both flubendiamide and copper. Flubendiamide (40 μM) and copper (40 μM) treated thymocytes and splenocytes showed more number of Tunel+ve cells, micronuclei formation, DNA fragmentation and comets cell formation. All the four tested natural antioxidants, namely-resveratrol, catechin, curcumin and α -tocopherol were found to be effective against cytotoxicity and genotoxicity induced by flubendiamide and copper. Based on the above results, flubendiamide or copper seems too exert toxic effects on haemopoietic, renal and male reproductive systems and have cyto-genotoxic potential; and α -tocopherol was found to possesses the partial reparative potential against the copper and flubendiamide toxicity.

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2. Pharmacological and molecular characterization of TRP channels in buffalo uterine artery

Abhishek Sharma and Satish K. Garg

Present study was undertaken to unravel the underlying signaling mechanisms of GSK-induced vasorelaxation in uterine artery of non-pregnant buffaloes. GSK1016790A-induced vasorelaxation in non-pregnant buffaloes collected from the local slaughter house. Passive tension and precontractile agonists studies revealed that, endothelin 1 was the best effective spasmogen and 2 gm passive tension was optimum for uterine arterial rings in MKHS. Following an equilibration period of about 1 hr, uterine arterial rings were pre-contacted with 60 mM KDS and thereafter pre-contracted with endothelin 1 to check endothelium intactness by using acetylcholine. Acetylcholine-induced relaxation was endothelium-dependent (65-70 %) and relaxation was almost comparable both in the uterine artery of non-pregnant and early-pregnancy stage buffaloes. SNP-induced vaso-relaxation was found to be significantly different in the endothelium-intact and endothelium-denuded uterine arterial rings of non-pregnant and early-pregnancy stage buffaloes and was faster in denuded uterine artery compared to that with intact-endothelium but the effect of SNP in the presence L-NAME was similar to following denudation of endothelium. Activation of TRPV4 channels with the selective agonist GSK caused fast relaxation in endothelium-intact than in endothelium-denuded buffalo uterine artery rings and the TRPV4 channel selective antagonist HC067047 significantly attenuated the vasodilator response to GSK. Interestingly, GSK-induced maximal relaxant response (R_{max}) was almost similar in both endothelium-intact

and endothelium-denuded uterine arterial rings but the potency of GSK was significantly higher in endothelium intact uterine artery. In uterine artery of buffaloes with endothelium, inhibition of nitric oxide synthase by L-NAME or inhibition of soluble guanylate cyclase (sGC) by ODQ did not attenuate the vasodilator response to GSK. These observations suggest that NO/sGC pathway does not contribute significantly in TRPV4 channel-mediated endothelium-dependent relaxation in buffalo uterine artery. Rather, it was very interesting to note that following inhibition of NO synthase by L-NAME (300 μ M) the vasodilator response to GSK was significantly potentiated compared to that of GSK alone. Indomethacin, an inhibitor of cyclooxygenase, significantly attenuated (35 %) the endothelium-dependent vasorelaxant effect of GSK but significantly potentiated when the tissues were pre-incubated with L-NAME plus indomethacin. Compared to the DRC of GSK in the presence of L-NAME and indomethacin, the DRC of GSK was significantly shifted towards right in the presence of L-NAME + indomethacin + apamine + TRAM-34 but the DRC was significantly shifted towards left when compared with the DRC of GSK in the presence of indomethacin alone. In the presence of indomethacin + apamine + TRAM-34, DRC of GSK was significantly shifted towards right when compared to DRC of GSK in the presence of indomethacin alone and that in the presence of L-NAME + indomethacin + apamine + TRAM-34. In high KDS (60 mM), arterial rings pre-incubated with L-NAME + indomethacin exhibited significantly higher (21 %) GSK-induced vaso-relaxation compared to that of only 7 % in the absence of these blockers. Uterine artery with denuded endothelium, GSK failed to produce any appreciable decrease in the basal tone as it was only around 2 % of the 60 mM K⁺-induced contraction and GSK-induced vaso-relaxation was inhibited in endothelium-denuded uterine arterial ring of buffaloes in the presence of iberiotoxin. Molecular characterization of TRPV4, TRPC3/6/7 and TRPM3 channels using western blot and PCR methods and immune-localization of channels protein in endothelium and vascular smooth muscle cells of buffalo uterine artery and protein expressions of TRPV4 and TRPC3/6/7 channels were found to be increased during pregnancy while TRPM3 channels protein expression was decreased during pregnancy. Immuno-histochemistry revealed the expression of TRPM3 protein signals in the endothelium and smooth muscular layers of uterine artery of buffaloes while TRPC3/6/7 proteins could be appreciated mainly in endothelium layer. Presence of TRPV4 channel mRNA and TRPC3 channel mRNA in non-pregnant buffalo uterine artery revealed their presence in uterine artery of buffaloes. These findings suggest that GSK-induced vasorelaxations is mainly COX and EDHF dependent (involvement of IK_{Ca} and SK_{Ca}) in endothelium-intact uterine artery but endothelium-independent/smooth muscle dependent relaxation by GSK is mediated by activation of BK_{Ca} channels of VSMS. Augmentation of the GSK-induced vaso-relaxation response in the presence of L-NAME in uterine artery of buffaloes needs further investigation.

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3. Endothelium-dependent and endothelium-independent relaxant pathways in uterine artery of buffaloes (*Bubalus bubalis*)

Udayraj P. Nakade and Satish K. Garg

Present study was undertaken to unravel the endothelium-dependent and endothelium-independent relaxant pathways in uterine artery of buffaloes collected from local abattoir. Micrometrical measurements show that width of tunica interna, lumen and arterial diameter increased while width of tunica media decreased during pregnancy along with increase in the width of tunica externa and arterial wall thickness during early pregnancy and decrease during mid pregnancy as compared to non-pregnancy state. The isolated arterial rings were

mounted in organ bath containing MKHS maintained at 37 ± 0.5 °C. Following an equilibration period of about 1 hr, uterine arterial rings were pre-contacted with 60 mM KDS and thereafter pre-contracted with endothelin-1 to induce vasoconstriction. Endothelium intactness was ascertained by ACh-induced vasorelaxation. Uterine artery collected from the animals in late phase of diestrus with straight and smooth blood vessels responded best. Passive tension and pre-contractile agonist's studies revealed that, endothelin-1 was the best precontractile agent and 2 g passive tension was optimum for uterine arterial rings in MKHS. Uterine artery of the early pregnancy stage buffaloes was most sensitive to endothelin-1. Uterine arterial rings of non-pregnant buffaloes exhibited significantly higher basal tension or tonicity and archived lower maximal tension to 60 mM KDS than the uterine artery of pregnant buffaloes. Acetylcholine-induced relaxation in uterine artery of buffaloes was endothelium-dependent in both non-pregnant and pregnant buffaloes. Non-pregnant buffalo uterine artery was most sensitive to vasorelaxant effect of ACh. L-NAME at 300 μ M, but not at 100 μ M, blocked the release of NO in uterine artery of non-pregnant buffaloes. Both L-NMMA (100 μ M) and PTIO (100 μ M) failed to block ACh-induced vasorelaxation in non-pregnant buffalo uterine artery. Indomethacin (10 μ M) alone failed to block ACh-induced vasorelaxation but when use in combination with L-NAME at 100 μ M and 300 μ M caused significant inhibition of ACh-induced relaxation in non-pregnant buffaloes uterine artery. ACh-induced relaxation in non-pregnant buffaloes uterine artery was significantly reduced in the presence of 60 mM KDS and it was decreased as the pregnancy advanced. Both TRAM-34 (1 μ M) and apamin (100 nM), alone and in combination, significantly reduced the ACh-induced relaxation in non-pregnant buffalo uterine artery. Indomethacin (10 μ M) alone and in combination with L-NAME (100 μ M) resulted in significant rightward shift of the DRC of ACh with significant attenuation in pD_2 value of ACh while L-NAME (100 μ M) alone failed to significantly reduced ACh-induced relaxation in early-pregnancy stage uterine artery. In the presence of L-NAME (100 μ M) and indomethacin (10 μ M), respectively, the DRC of ACh was significantly shifted towards right and left with significant reduction and potentiation of the maximal responses respectively, while in the combined presence of L-NAME (100 μ M) and indomethacin (10 μ M) the DRC was superimposed over the DRC of ACh in uterine artery of mid-pregnancy stage buffaloes. SNP caused dose-dependent relaxation in buffalo uterine artery, and the DRC of SNP was significantly shifted towards left with significant increase in potency during pregnancy. In the presence of ODQ, the DRC of SNP was significantly shifted towards right with significant reduction in the potency without much change in the maximal relaxation. Amongst the different antagonists used, only combination of L-NAME (100 μ M/300 μ M) and indomethacin (10 μ M) cause marked increase in basal tone of non-pregnant buffalo uterine artery. PCR, western blot and immunohistochemistry studies revealed the presence of eNOS, COX-1, and IK_{Ca} in the endothelium, BK_{Ca} in the smooth muscle and SK_{Ca} in both the endothelium and smooth muscle of buffalo uterine artery and their expression is differentially regulated during pregnancy. Gene sequencing of eNOS, COX-1 and SK_{Ca} genes in uterine artery of buffaloes showed more than 93% structural similarity with ovine (*Ovis aries*), caprine (*Capra hircus*) and Indian cow (*Bos indicus*). From the results of present study, it is conclude that both endothelium-dependent (EDHF and EDRF) and endothelium-independent (sGC-cGMP) relaxant pathways are present in buffalo uterine artery and they differentially contribute to vasorelaxation during non-pregnant and pregnancy states.

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4. Studies on toxicodynamic of chromium with particular reference to its effect on myometrial activity in rat

Bhatiya Shirish Kumar Ishwarbhai and Satish K. Garg

Present study was conducted to evaluate the effect of chromium on myometrial activity in rats and the possible mechanistic pathways. The study was undertaken in three phases i.e. Phase I- Biomonitoring studies; Phase II-*In-vivo* study on effect of chromium exposure @ 250 ppb, 1ppm and 10 ppm in drinking water for 90 days and, also 45 days after its withdrawal; and Phase III-*In-vitro* study on effect of chromium on myometrial activity and its mechanistic pathways. For *in-vivo* studies, adult female wistar rats were divided into seven groups of ten animals each. Rats of Group-I served as control group whereas Group-II, III and IV were orally administered with potassium dichromate @ 250 ppb, 1 ppm and 10 ppm daily for 90 days while rats of Group- V, VI and VII constituted the withdrawal groups and study was undertaken after 45 days of withdrawal following 90 days exposure. Cows and buffaloes suffering with different reproductive problems had higher blood levels of chromium than the permissible value of 0.5 µg/L. Highest blood levels of 1.90 ± 0.12 and 1.76 ± 0.22 µg/L were observed in cows and buffaloes having the history of repeat breeding and abortions, respectively. Highest chromium level was observed in Ganga river water (332.7 ± 34.1 µg/L) and lowest (57.70 ± 5.32 µg/L) in the water samples collected from Mathura University. After 90 days of continuous exposure and 45 days of withdrawal period, no significant effect was observed on the body weight, relative and absolute organ weights. Chromium-exposure resulted in significant ($p < 0.05$) reduction in hemoglobin, TEC, TLC and MCV values. Plasma BUN and creatinine levels were significantly ($p < 0.05$) increased in chromium treated rats of Group-III and IV as compared to the control group. Significant ($p < 0.05$) increase in ALT activity and bilirubin levels in rats of Group-II and Group-III indicated chromium-induced hepatotoxicity. Chromium significantly ($P < 0.05$) increased lipid peroxidation, reduced catalase activity and significantly ($p < 0.05$) reduced in GSH and GPx activity in erythrocytes, liver and kidneys in rats of chromium-treated groups. Withdrawal of chromium exposure for 45 days did not have any significant effect in restoring the MDA levels, catalase and GST activities and GSH levels in rats. Thus, suggesting chromium-induced oxidative stress which is irreversible up to 45 days. Exposure of rats to chromium @ 250 ppb, 1 ppm and 10 ppm for 90 days, resulted in accumulation of significantly higher concentration of chromium in uteri and ovaries of rats of 1 ppm and 10 ppm exposure groups but chromium levels did not significantly decline even after 45 days of withdrawal. Chromium significantly ($p < 0.05$) decreased the blood levels of iron and zinc in rats of Group-II, III and IV, and also Group-V, VI and VII. Thus, suggesting that chromium exposure had long term deleterious effects on blood iron and zinc levels in rats. With the increment of chromium exposure levels, absolute tension (g), mean integral tension (g) and frequency (BPM) of chromium in rat myometria increased with increase in dose but the increase in tension was statistically significant only in 1 and 10 ppm exposure groups. CaCl_2 , KDS, oxytocin and $\text{PGF}_{2\alpha}$ -induced maximal contractions were significantly reduced in myometrium of rats of Group-II while significantly increased in rats of Group III and IV but pD_2 values in chromium exposed groups were significantly lower than in control group. Compared to the control, there was potentiation of the relaxant effect (R_{max}) of terbutaline in uteri of rats of Group-II and III while reduction in R_{max} value in rats of Group-IV, but alterations in R_{max} values were not statistically significant. Following withdrawal of chromium exposure, spontaneous myometrial activity data of the uteri of rats of Group-V, VI and VII revealed that compared to Group-III and IV; absolute tension (g) and mean integral tension (g) were significantly decreased in rats of Group-VI and VII, respectively. Compared to the control, the E_{max} values of CaCl_2 , KDS, Oxytocin, $\text{PGF}_{2\alpha}$ and

BAY-K8644 in uteri of rats of Group-II, III and IV and rats of Group-V, VI and VII were found to be significantly reduced. The dose response curves of CaCl₂, Oxytocin, PGF_{2α} and BAY-K8644 were found to be significantly shifted towards right in all the chromium withdrawal Groups V, VI and VII as compared to the DRC of oxytocin in control group. *In vivo* studies suggested that chromium significantly decreased the contractile effect of CaCl₂, KDS, PGF_{2α}, oxytocin, BAY- K8644 at low doses while higher doses increased it. Possibility of chromium inhibiting the Ca²⁺ channels and/or intracellular signaling pathways involved in mediating contractile effects of oxytocin and PGF_{2α} cannot be ruled out. The results of the present *in vitro* study suggest that chromium decreases the influx of Ca²⁺ through L-type Ca²⁺-channels, stimulates K_{ATP} and K_v channels and also stimulates β₂ and β₃-adrenergic receptors to produce inhibitory effect on rat myometrium.

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DEPARTMENT OF VETERINARY PHYSIOLOGY

S. No	Title	Author/ Student	Guide	Year	Page No.
M.V.Sc. Degree Programme					
1	A study on effect of Sericin supplementation on expression profile of heat shock protein genes in spermatozoa before and after cryopreservation of buck semen	A. Vidya Sagar Reddy	Dr. Brijesh Yadav	2017	180
2	Effect of Quercetin on sperm characters, capacitation like changes and seminal antioxidative status in cryopreserved Barbari buck semen	Richa Sharma	Dr. Mukul Anand	2019	180
Ph.D. Degree Programme					
1	Studies on protective effect of different avian species egg yolk, LDL concentration and antioxidants supplementation on quality of cryopreserved Barbari buck semen	Mukul Anand	Dr. Sarvajeet Yadav	2016	182

M.V.Sc. Degree Programme

1. **A study on effect of Sericin supplementation on expression profile of heat shock protein genes in spermatozoa before and after cryopreservation of buck semen**

A. VidyaSagar Reddy and Brijesh Yadav

The present experiment was designed to study the effect of Sericin supplementation on semen quality, semen biochemical indices and expression profile of HSPs in Barbari buck. Study was conducted in five fertile, healthy adult Barbari bucks of similar age and body weight. Thirty ejaculates were collected in total by employing artificial vagina. Each ejaculate was divided into three aliquots. Sericin was supplemented at 0 %, 0.25 % and 0.5%. The progressive motility (%), sperm livability (%), HOST (%), total sperm abnormal morphology (%), acrosomal integrity (%) and kinematic parameters were assessed at different stages of cryopreservation. qRT-PCR was done to evaluate the relative expression of HSP70 and HSP90 and immunoblotting of HSP70 protein was also carried out. Assessment of antioxidative status, enzyme leakage, cryocapacitative damages and apoptotic changes were recorded in post-thaw samples. The expression of HSP70 and HSP90 gene in spermatozoa was downregulated both after equilibration and post thaw as compared to extended semen, however the decrease in expression was more prominent after post thaw. All the semen quality parameters deteriorated during the cryopreservation. Progressively motility, HOST positivity and acrosomal integrity of spermatozoa were significantly ($p < 0.05$) in all the post-thaw samples. A significant ($p < 0.01$) reduction of abnormal spermatozoa was observed in Sericin supplemented samples. Sericin supplementation improved the antioxidative status (SOD, GST, CAT) besides reducing lipid peroxidation. The enzyme leakage (ALT, LDH), cryocapacitative and apoptotic changes (Capacitation like changes, tyrosine phosphorylation and mitochondrial membrane potential) were also minimized after sericine supplementation. Sericin supplementation had a beneficial effect on HSPs/HSP mRNA expression either by induction or by protection of HSPs/HSP mRNA which is evident from the gene expression and immunoblotting studies. A positive correlation was found between expression of HSPs and semen quality parameters and a negative correlation was found between expression of HSPs and cryocapacitation and apoptotic changes. It can be concluded that sericin supplementation improved the semen quality of goat by improving the antioxidative status, increasing HSPs/HSP mRNA expression and decreasing the enzyme leakage, cryocapacitative and apoptotic changes in post thaw spermatozoa.

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2. **Effect of Quercetin on sperm characters, capacitation like changes and seminal antioxidative status in cryopreserved Barbari buck semen**

Richa Sharma and Mukul Anand

A study was designed to evaluate the cryoprotective effect quercetin during freezing and thawing process in Barbari buck semen. Six healthy Barbari bucks of similar age (1.5-2 years) and weight (30-35 kg) were selected as semen donor during the experiment. The semen was collected twice a week from each buck using artificial vagina. A total of 48 ejaculates were collected (eight from each buck) during each experiment. The collected semen was pooled. Pooled semen was divided into five aliquots. Each aliquot was diluted with extender

containing different concentration of quercetin viz. 50 μ M, 75 μ M, 100 μ M and 125 μ M while no Quercetin was added in control. The seminal attributes and sperm characters viz. volume (ml), mass activity (0-5 scale), sperm concentration (million/ ml), progressive motility (%), sperm livability (%), HOST (%), total sperm abnormal morphology (%) acrosomal integrity (%) and kinematic parameters, membrane fluidity, DNA fragmentation, intracellular Ca level and antioxidative enzyme level were evaluated in the frozen thaw semen. The result of the study showed that Quercetin supplementation @ 75 μ M improved the percent viable spermatozoa, membrane integrity as evaluated through HOST. Quercetin improve the path velocity (VCL, μ m/sec; VAP, μ m/sec and VSL, μ m/sec) of sperm but do not affect the motion characters of sperm except BCF and ALH. Quercetin @ 75 μ M supplement reduces the ROS evident through higher values recorded for antioxidative enzyme status (SOD, GST, CAT, GPX). Quercetin maintains the acrosomal integrity, DNA compaction and prevents cells from cryocapacitation and apoptotic changes. Quercetin did not show any significant difference in intracellular Ca ion concentration in post thaw semen.

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Ph.D. Degree Programme

1. **Studies on protective effect of different avian species egg yolk, LDL concentration and antioxidants supplementation on quality of cryopreserved Barbari buck semen**

Mukul Anand and Saravjeet Yadav

A study was designed to evaluate the cryoprotective effect of egg yolk from different avian species, low density lipoproteins and antioxidant supplementation in semen extender during freezing and thawing process in Barbari buck semen. Four healthy Barbari bucks of similar age and weight were selected as semen donor during the experiment. The semen was collected twice a week from each buck using artificial vagina. A total of 24 ejaculates were collected (six from each buck) during each experiment. The study was divided into six phases. Semen collected from each buck was initially evaluated before the start of each phase and samples with more than 85% live spermatozoa were selected and later pooled. Pooled semen sample was divided into equal parts. Each part was diluted separately with a TRIS-based extender containing 6% glycerol. The non-penetrating cryoprotectant was replaced with different concentration of egg yolk; completely replace with LDL or supplemented with LDL and antioxidants. Dilution was made to have final sperm concentration to of 200 million spermatozoa per ml, as standardized in phase I. The diluted samples were later subjected to freezing and thawing process. Different seminal attributes and antioxidative enzyme level were evaluated after equilibration and thawing. Kinematic characteristic exhibited by spermatozoa were evaluated after thawing during different phases. During phase II, it was observed that 15% Hen egg yolk, 10% Turkey egg yolk, 10% Quail egg yolk and 15% Duck egg yolk in semen extender recorded significantly ($P < 0.5$ or $P < 0.01$) higher values of different seminal attributes and kinematic characteristic of spermatozoa. On comparison of egg yolk from different avian species it was observed that Hen egg yolk@15% and Quail egg yolk @10% gives significantly ($P < 0.5$ or $P < 0.01$) higher values. In phase III, significantly ($P < 0.5$ or $P < 0.01$) higher values of different seminal attributes and kinematic characteristic of spermatozoa was observed at 8% LDL replaced with whole egg yolk in semen extender. In phase IV, it was observed that the semen extender containing 15% Hen egg yolk when supplemented with 3% LDL give better result. On comparison of the groups with best result in phase II, III and IV, group IV diluted with extender containing 15% Hen egg yolk and 3% LDL recorded better post thaw semen quality. Supplementation of different antioxidant to the extender that gave best results in phase V, with Glutamine @5mM recorded a better semen quality as compared to Bovine serum albumin @ 10mg/ml and Hypotaurine @ 5mM. So, it may be concluded that the extender containing 15% Hen egg yolk and supplemented with 3% LDL and 5 mM of glutamine gives better protection during freezing and thawing process and recommended for semen dilution in Barbari Buck.

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DEPARTMENT OF VETERINARY PUBLIC HEALTH

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1	Quality analysis of ground water and recreational water sources in Brij region with special reference to <i>E. coli</i> 0157:H7	Waquar Ahmed	Dr. Udit jain	2014	185
2	Prevalence of Verocytotoxic <i>E. coli</i> in milk and milk products in certain areas of Agra District	Manjula Devi	Dr. Basanti Bist	2014	186
3	Studies on prevalence of verocytotoxic <i>E. coli</i> (VTEC) including 0157:H7 in domestic and wild ruminants with its public health significance	Raghavendra Prasad Mishra	Dr. Udit jain	2015	187
4	Study on evaluation of microbial quality and <i>E.coli</i> (VTEC) contamination in milk and milk products of Brij region with special reference to public health	Subhasmita Behera	Dr. Udit jain	2016	187
5	Prevalence and antimicrobial resistance of VTEC in cattle farms (indigenous and exotic), goats and their environmental sources in Brij region	Ravneet Singh	Dr. Udit jain	2017	188
6	Prevalence and antimicrobial resistance of <i>Escherichia coli</i> (VTEC) in pets (dog and cat) and its public health significance in Brij region	Usha Bais	Dr. Udit jain	2017	189
7	Molecular characterization and antimicrobial resistance of pathogenic strains of <i>E. coli</i> in poultry, poultry products and its environmental sources with public health significance	Neha Saini	Dr. Udit jain	2018	190
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Ph.D. Degree Programme					
1	Studies on virulence associated factors and drug resistance profile of verocytotoxin producing <i>Escherichia coli</i> (VTEC) in cattle	Parul	Dr. Basanti Bist	2014	192
2	Quality analysis (physico-chemical and microbiological) of drinking and Yamuna water from different areas of Mathura and Agra regions with special reference to <i>E.coli</i> (VTEC) and <i>Campylobacter</i> spp	Barkha Sharma	Dr. Basanti Bist	2016	193
3	Prevalence and risk factors analysis associated with bovine brucellosis and its public health significance especially in peri-urban areas of brij region of U.P.	Raghavendra Prasad Mishra	Dr. Udit Jain	2019	194

M.V.Sc. Degree Programme

1. Quality analysis of ground water and recreational water sources in Brij region with special reference to *E. coli* 0157:H7

Waquar Ahmed and Udit Jain

The present study was emphasized to access the physico-chemical and microbiological quality including the prevalence of *E. coli* 0157:H7 (Enteropathogen) in ground water and recreational water sources in Brij region. A total of 200 water samples comprising of 130 ground water samples from 5 blocks (Baldeo, Farah, Maant, Mathura and Raya) and 70 samples of recreational water including 32 samples from four swimming pools (primary contact recreation sources) and 38 samples from Yamuna river ghats & Ponds (secondary contact recreation sources) were analyzed.

Ground water quality of Maant block were found good as all ground water samples of Maant block were sweet in taste whereas in Baldeo, Farah and Raya block most of the samples were found sweet and few were brackish in taste. In Mathura block most of the sample were sweet whereas some salty and few were brackish. All ground water samples were odourless except few samples of Mathura block contained oily odour. Physical parameter viz. pH and temp were found within the prescribed limit whereas total dissolved solids, total hardness, sulphate, nitrate were found beyond the permissible limit. Fluoride values were found beyond the permissible limit in most samples of the Mathura block. Ground water samples were almost free from microbiological contamination as only 9.23% samples contained *E. coli*. None of the ground water samples were found positive for VTEC.

In swimming pools, pH and temp were found within the limit whereas the range of dissolved oxygen was 17.5-34.2 mg/l and for residual chlorine, 0.1-0.6 mg/l. In swimming pools 100% of the samples had faecal coliforms >1MPN/100ml whereas 53.13% of samples of swimming pools had SPC content >200/ml and were found unacceptable. Percentage positivity of *E. coli* was observed in 68.75% samples. No VTEC strains could be detected in the water samples of all four swimming pools. In water samples of Yamuna river ghats and ponds overall range of pH and temp were found as 8.50 and 12.40.0-30.0°C respectively. The range of dissolved oxygen was reported as 0.2-0.4 mg/l. The overall range of total hardness in Yamuna river ghats & ponds were found as 116-198 mg/l. The total coliform range was recorded as 275-1800 MPN /100 ml. In Yamuna river ghats and ponds, 100% of water samples were found positive for *E. coli*. In Yamuna river ghats 43.33% samples were found positive for VTEC whereas no VTEC isolates could be detected from ponds. 43.33% samples of Yamuna river ghats (100% VTEC positive samples) contain stx1 and stx2 genes whereas 10% samples of Yamuna river ghats (23.07% VTEC positive samples) contain hlyA gene. Out of 13VTEC strains isolated from Yamuna river ghats, only 3 were found positive for 0157:H7 on MUG-Sorbitol agar.

In the present study 23.08% VTEC strains were found to be phenotypically positive for a-Haemolysin & Enterohaemolysin detection on washed sheep blood agar. Antibigram studies of isolates (13) revealed that 62 % VTEC strains were resistant to Amoxycylav whereas 88 % isolates were sensitive to Cefotaxime/Clavulanic acid.

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2. Prevalence of Verocytotoxic *E. coli* in milk and milk products in certain areas of Agra District

Manjula Devi and Basanti Bist

The present study was undertaken to find the prevalence of Verocytotoxic *E. coli* in milk and milk products from certain areas of Agra district. During the present study a total of 350 samples comprising of 200 milk samples (150 raw milk and 50 pasteurized milk) and 150 milk product samples (30 of each paneer, curd, peda, burfi and milk powder) were processed for detection of *E. coli*. The overall prevalence of *E. coli* in milk samples was found to be 26.5% (53/200). The prevalence in raw and pasteurized milk was found to be 20.67% (31/150) and 44% (22/50), respectively. The prevalence was higher in cow's milk i.e. 23.63% (26 /110 samples) in comparison to buffalo milk 12.5% (5/40).

In milk products, the overall prevalence of *E. coli* was found to be 18 % (27/150) with highest prevalence reported in curd 33.33% (10 / 30) followed by paneer 26.67% (8/30), burfi 16.67%(5/30) and peda 13.33%(4/30) whereas no *E. coli* was found in milk powder.

The overall prevalence of VTEC in milk samples was found to be 6.5% (13/ 200). The presence of VTEC in raw milk suggests that milk was apparently not produced under hygienic conditions and thus could have been subjected to faecal contamination. The prevalence of VTEC in raw milk samples was 8.0% (12/150) which was higher in cow milk 10% (11/110) in comparison to buffalo milk 2.5% (1/40). In pasteurized milk samples only 2.0% (1/50) samples were found positive for VTEC Among the milk products, one out of 30 samples each of paneer and curd (3.33 %) were found positive for VTEC. No VTEC was found in other milk products like peda, burfi and milk powder.

Out of 350 samples, 80 *E. coli* (53 milk, 27 milk products) isolates were screened by PCR to detect the presence of *Stx*₁, *Stx*₂, *eae* and *hlyA* genes. A total of 15 VTEC strains were detected from milk and milk products samples harboring different gene combinations. These VTEC isolates were obtained from various sources (raw milk - 12, pasteurized milk-1, paneer-1, curd-1). 6 strains of VTEC (40%) from raw milk and milk products samples harbored *Stx*₁ gene, one VTEC (6.66%) was positive for *Stx*₂ gene, 4 strains of VTEC (26.66%) carried both *Stx*₁ and *Stx*₂ genes whereas 2 strains of VTEC (13.33%) were found to be positive for all four genes (*Stx*₁, *Stx*₂, *eae* and *hlyA*) and 2 VTEC strains (13.33%) detected one from curd and another from pasteurized milk samples carried *Stx*₁ and *hlyA* genes.

In present study, the overall haemolytic activity and congo red dye binding ability was found to be exhibited by 46.67% and 86.67% VTEC respectively. The antibiogram assay of verocytotoxic *E. coli* revealed that the strains were highly sensitive for Ciprofloxacin (100%) followed by Ofloxacin (93.33%), Cefotaxime (86.66%), Ceftriaxone (80.0%), Gentamicin (80.0%), Chloramphenicol (73.33%), Cefoperazone (73.33%). In the present study, highest resistance was observed against antibiotics like Co-trimoxazole (80%), followed by Levofloxacin (40.0%), Ampicillin (33.33%) and Amoxyclav (26.0%).

The antibiotic sensitivity pattern of the isolates of verocytotoxic *E. coli* revealed that resistance to antibiotics is on increase. The multiple drug resistance is of alarming nature, development of resistant pathogenic strains of *E. coli* impose considerable threat to public health.

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3. Studies on prevalence of verocytotoxic *E. coli* (VTEC) including O157:H7 in domestic and wild ruminants with its public health significance

Raghavendra Prasad Mishra and Udit Jain

In present study, a total 420 samples comprising of faecal (n=240), soil (n=60), water (n=60) and 60 human stool samples were processed for VTEC. Out of 420 samples, 291 *E. coli* isolates were obtained. All *E. coli* isolates were subjected to multiplex polymerase chain reaction assay. Of these, only 37 *E. coli* isolates were found positive for VTEC. These isolates were obtained from various sources (faeces 27, soil 6 and water 4). 2 isolate from faecal sample of cattle was found to be positive for *stx*₁ gene (180 bp) and 6 isolates was found to be positive for *stx*₁ and *stx*₂ (180 & 255 bp). In buffalo 3 sample found positive for *stx*₁ gene (180 bp) and 5 isolates was found to be positive for *stx*₁ and *stx*₂ (180 bp & 255 bp), in sheep out of 4 VTEC, 1 found to be positive for *stx*₁ gene (180 bp) and 3 isolates was found to be positive for *stx*₁ and *stx*₂ for cattle (180bp & 255 bp) and in deer out of 7 VTEC, 1 found to be positive for *stx*₁ gene (180 bp), 1 isolates was found to be positive for *stx*₂, (255 bp) 4 eae (384 bp) and one *stx*₁, with eae (180 bp & 384 bp). In present study the prevalence of VTEC in soil was 10 %. In the 60 sample, 1 isolate from soil was found to be positive for *stx*₁ gene (180 bp), 1 isolates was found to be positive for *stx*₂ (255 bp), 2 positive for *stx*₁ and *stx*₂ (180bp & 255 bp) and 2 have eae gene (384bp). In 60 sample of water, one isolate was found to be positive for *stx*₂ (255 bp), 1 isolates was found to be positive for hlyA gene (534 bp), 2 positive for *stx*₁, *stx*₂ and *hlyA* (180 bp, 255 bp and 534 bp).

In present study, the overall hemolytic activity and Congo red dye binding ability of 37 VTEC was found to be 56.75% and 83.7% respectively.

All VTEC strains carried out for antibiotic sensitivity test against 16 commonly used antibiotic discs. Of these, Ciprofloxacin and Imipenem was found to be hundred percent sensitive followed by Cefotaxime/clavulanic acid (86.48%), Gentamicin (83.78%), Amikacin (83.78%), Chloramphenicol (81.09%) and Norfloxacin (78.37%) were found highly sensitive against VTEC strains. Enrofloxacin (81.09%) showed highest resistance followed by Cefixime (62.16%), Erythromycin (59.4%) and Tetracycline (54.05%).

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4. Study on evaluation of microbial quality and *E. coli* (VTEC) contamination in milk and milk products of Brij region with special reference to public health

Subhasmita Behera and Udit Jain

A total of 380 samples comprising of 150 milk samples (90 raw milk, 30 boiled milk, 30 pasteurized milk), 140 milk products (20 paneer, 20 Khoa, 20 Curd, 20 Burfi, 20 Peda, 20 Rasmalai, 20 Ice cream) and 90 samples from environmental sources (30 hand swabs of milkers and vendors, 20 utensils swabs, 20 udder swabs, 20 water samples) were screened for total microbial load, total coliform count and *E. coli* with verotoxigenic potential in Brij region.

Total viable counts (TVC) for milk, milk products and environmental samples in wet period were log 6.99±0.318, log 5.73±0.168 and log 3.87±0.162 respectively. Total viable counts (TVC) for milk, milk products and environmental samples in dry period were log 5.76±0.188, log 5.23±0.111 and log 3.99±0.147 respectively. Mean coliform count for milk, milk products and environmental samples in wet period were log 4.39±0.306, log 4.51±0.201 and log 1.90±0.253

respectively. Mean Coliform count for milk, milk products and environmental samples in dry period were $\log 4.74 \pm 0.199$, $\log 3.91 \pm 0.166$ and $\log 3.29 \pm 0.223$ respectively.

Out of 380 samples, 120 *E.coli* isolates were obtained, out of which 68 and 52 *E.coli* isolates were obtained in wet and dry season from 190 samples in each season, respectively. The overall percent of *E.coli* from milk, milk product and environmental samples were found to be 43.33 %, 22.14 % and 26.66%, respectively. A total no. of 30 VTEC were obtained, which is 25% of the total *E.coli* and 7% of the total sample collected. The overall percent of VTEC from milk, milk product and environmental samples were found to be 16.66%, 2.14% and 2.22% respectively. Out of 30 VTEC, 22 samples were found positive for *stx1*, 1 for *stx2*, 1 for both *stx1* and *eaeA*, 3 for both *stx1* and *hlyA* and 3 for both *stx1* and *stx2*. Out of total *E.coli*, 3.33% samples were found positive for *rfb O157* and 0 for *rfb O111*. 23.33% of VTEC were positive for SHV genes, 23.33% for CTX genes and 40% for both SHV and CTX genes. All the VTEC were subjected to antibiotic drug sensitivity test against 15 antibiotics. Imipenam (96%) showed highest sensitivity followed by Chloramphenicol (86.66%), Trimethoprim (86.66%), Tetracycline (83.33%), Amikacin (73.33%) and antibiotics like Cefoperazone (100%) and Cefixime (100%) showed highest resistance followed by Ofloxacin (90%), Erythromycin (90%), Enrofloxacin (86.66%), Amoxy Sulbactam (80%), Gentamicin (76.66%), Norfloxacin (66.66%) and Cotrimazole (50%).

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5. Prevalence and antimicrobial resistance of VTEC in cattle farms (indigenous and exotic), goats and their environmental sources in Brij region

Ravneet Singh and Udit Jain

The study was conducted to provide the information on prevalence and antimicrobial pattern of VTEC in Cattle and Goats feces as well as from their environments. Out of 375 fecal samples taken, 18.93% were found positive for virulent genes; 20.94% were from Cattle and 14.75% were from Goats fecal samples. Among Cattle, 21.28% were from Indigenous Cattle and 20% of them were from Exotic Cattle. In environmental samples, 7.14% samples were found positive for VTEC and the positivity among them was 5%, 10%, 5%, 5%, 20%, and 5% from soil, manure, effluent, water, hand swabs, and flies respectively. In different organized farms taken in this study, the highest percentage (27.5%) was found in Kamdehnu dairy, Karnaval, Mathura.

Molecular characterization of isolates through mPCR revealed 53.08% isolates positive for the *stx1* gene alone while all the other isolates were found carrying two or more VTEC genes. Also, the combinations found were *stx1* & *stx2*, *stx1* & *hlyA*, *stx1* & *eae*, and *stx1*, *stx2* & *hlyA* with a percentage of 11.11%, 13.58%, 3.70% and 18.51% respectively. The *eae* gene was found among 3 isolates only while the *Saa* gene was found in 21 isolates with overall percentage of 29.92%. The virulent genes combinations were found among Cattle isolates however *stx1*, *stx2* and *stx* & *eae* combinations was not found among any of the VTEC isolates of Goats. All the VTEC isolates were also screened for O157 by mPCR but no positive results was found.

The antibiotic resistant genes, ESBL genes viz TEM, CTX, and SHV through mPCR screened from VTEC positive strains and found 42 isolates carrying the ESBL genes either singly or in combinations with TEM being the most prevalent of all. The overall percentage of ESBLs genes from the VTEC isolates came as 54.43%, out of which more percentage found in the indigenous breeds with a total percentage of about 67.85%. The positive of ESBL in VTEC isolates of Cattle and Goats were 60.37% and 50% respectively.

The VTEC positive isolates were also screened phenotypically by Combination disk test (CDT) and Ezy MIC™ Strip test which revealed 6 isolates as ESBL positive by CDT (Cefotaxime) method while the Ezy MIC™ Strip method revealed 7 as MBL+ESBL positive while 39 as MBL positive. Also, by mPCR the screening of ESBL genes (TEM, SHV& CTX) showed 54.43% positive VTEC strains carrying these genes alone and in combinations. The overall percentage of ESBL in the indigenous breeds, exotic breeds of cattle as well as in goats was found 67.85%, 52% and 50% respectively. Most of the isolates found positive phenotypically also revealed ESBL genes taken in this study, using mPCR. Also, the antibiogram testing has been done on all positive VTEC isolates using 16 antibiotics. Out of which Imipenam/EDTA (96.62%) showed highest sensitivity followed by Imipenam (76.50%). While antibiotics like Ampicillin (100%), Cefixime (94.39%) and Cefaperzone (92.14%) showed highest resistance towards the positive VTEC isolates.

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6. Prevalence and antimicrobial resistance of *Escherichia coli* (VTEC) in pets (dog and cat) and its public health significance in Brij region

Usha Bais and Udit Jain

A total no. of 310 samples comprising of 180 dog faeces (60 healthy, 60 diarrhoeic and 60 diseased), 30 cat faeces (25 healthy and 5 diarrhoeic) and 100 samples from environmental sources (10 hand swabs of dog owner, 10 hand swabs of veterinarian, 10 commercial dog food, 10 home-made food, 20 dog drinking water, 20 surface swabs of dog kennel and veterinary hospital, 20 flies around dog kennel and veterinary hospital) were processed to screen *E.coli* with verotoxigenic potential in Brij region. Out of total 310 samples, 142 *E.coli* isolates were obtained. The overall percent of *E.coli* from dogs, cats and environmental samples were found 61.66 %, 60.00 %, 13.00%, respectively. A total no. of 69 VTEC were obtained, which is 48.59% of the total *E.coli* and 22.25% of the total sample collected. The overall percent of VTEC from dogs, cats and environmental samples were found 32.22% (26.66% in healthy, 40.00% in diarrhoeic and 30.00% in diseased), 16.66% (16.00% in healthy and 20.00% in diarrhoeic) and 6.00% (15.00% in fly, 10.00% in surface swabs and 10.00% in hand swabs of dog owner), respectively. Out of 69 VTEC, total 44 samples were found positive for *stx1* (38 from dogs, 3 from cats, and 3 from environmental samples), 8 for both *stx1* and *stx2* (6 from dogs, 1 from cats and 1 from environmental samples), 3 for both *stx1* and *hlyA* (2 from dogs, 1 from cats), 12 for both *stx1* and *eaeA* (11 from dogs, 1 from environmental samples), 2 for *eaeA*, *stx1* and *hlyA* (1 from dogs and 1 from environmental samples). Among virulence markers of VTEC Congo red dye binding ability played an important role in identifying the pathogenicity of bacteria. In present study, Congo red dye binding ability was found 86.95%. Epidemiological risk factors associated with prevalence of VTEC in dogs are breed, age, sex, coprophagic habit and cohabitation with other dogs. Prevalence of VTEC was higher in non-descript breed, 0-3 month age group of pups, female, coprophagic dog and dog having co-habitation with other dogs in comparison to global breed, higher age group (more than 3 month), male, non-coprophagic dogs and dogs not having co-habitation with other dogs, respectively. All the VTEC were subjected to antibiotic drug sensitivity test against 17 antibiotics. Imipenam EDTA (92.75%) showed highest sensitivity followed by Chloramphenicol (68.11%). Antibiotics like Ampicillin/Sulbactam (100%), Erythromycin (100%), Ofloxacin (100%), Cefotaxime/ Clavulanic acid (100%), Clindamycin (100%) showed highest resistance.

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7. Molecular characterization and antimicrobial resistance of pathogenic strains of *E. coli* in poultry, poultry products and its environmental sources with public health significance

Neha Saini and Udit Jain

The purpose of study was to determine molecular characteristics and antimicrobial pattern of VTEC in poultry, poultry products and its environment. Out of total 350 samples (200 cloacal swabs, 90 environmental samples & 60 from poultry products), 202 *E. coli* isolates were obtained. Out of 202 *E. coli* isolates from various sources, 27 pathogenic *E. coli* (only VTEC, no EPEC) were obtained, which was 13.37% of the total *E. coli* and 7.71% of the total samples collected. Percentage of pathogenic *E. coli* (VTEC) from cloacal swab, environmental samples & poultry product were 11.5%, 2.22% & 3.33%, respectively. From cloacal swab % pathogenic *E. coli* (VTEC) in Turkey, Quail, Chabro and broiler species were 14%, 12%, 12% & 8%, respectively. From environmental samples 6.67% positivity for pathogenic *E. coli* (VTEC) was found in utensil swab and litter sample, each. No pathogenic *E. coli* was found in hand swab, surface swab, feed and water. Among poultry products, 10% raw meat samples were positive for pathogenic *E. coli* (VTEC). Egg and ready to eat products were negative for VTEC. Molecular characterization of isolates through mPCR revealed 25.93% isolates positive for the *stx1* gene alone and 7.4% for *stx2* alone while all the other isolates were found carrying two or more VTEC genes. Also, the combinations found were *stx1* & *stx2*, *stx1* & *hlyA*, *stx2* & *hlyA* with a percentage of 3.7%, 18.5% and 44.4%, respectively. In 27 pathogenic *E. coli* (VTEC) positives, 7 *stx1* (4, 2 and 1 from cloacal swabs, environmental samples and poultry products, respectively), 2 *stx2*, 1 *stx1* & *stx2* and 5 *stx1* & *hlyA* (all from cloacal swabs only) and 12 *stx2* & *hlyA* (11 from cloacal swabs and 1 from poultry products) were found. No samples were found positive for *eaeA*, *hlyA* (single), *saa*, *rfb O111*, *rfb O157* and *fliC H7* gene and variants of *stx2* (C and D). All pathogenic *E. coli* (VTEC) positive isolates were also screened phenotypically by Combination disc test (CDT), Modified double disc synergy test (MDDST) and Ezy MIC™ Strip test. All pathogenic *E. coli* (VTEC) isolates were negative for ESBL production. Congo red dye binding ability was found 37.03% (10 out of 27). Out of which the percent positivity in cloacal swab, environmental sources and poultry product was 30.43%, 50% and 100%, respectively. Also, the antibiogram testing has been done on all positive pathogenic *E. coli* (VTEC) isolates using 15 antibiotics. Result revealed that Imipenem and Chloramphenicol (100%) showed highest sensitivity followed by Amikacin (96.3%), Kanamycin (92.6%), while antibiotics like Clindamycin (100%), Enrofloxacin (100%), Erythromycin (88.89%) showed highest resistance towards the positive VTEC isolates.

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8. Studies on seroprevalence of brucellosis and molecular detection of organisms causing reproductive disorders in ruminants

Gourab Basak and Udit Jain

A study was conducted to know the prevalence of brucellosis in ruminants and human of Brij region, Mathura, India, using different serological tests viz. RBPT, STAT and i-ELISA. A total of 493 serum samples were collected comprising Cattle (90), Buffalo (125), Sheep (135), Goat (113) and Human (50). On analysis total 463 animal samples, RBPT recorded 3.46% (16/463) in animals [6.05% (13/215) in Large Ruminants; 1.21% (3/248) in Small Ruminants] whereas 0% (0/50) in human. STAT showed 10.37% (48/463) in animals, 10.23%

(22/215) in Large Ruminants; 10.48% (26/248) in Small Ruminants and 2% (1/50) in human. Similarly, i-ELISA revealed 6.05% (28/463) in animals, 6.51% (14/215) in Large Ruminants; 5.65% (14/248) in Small Ruminants and 4% (2/50) in human. Distribution of the antibodies against *Brucella* antigens did not vary significantly ($P < 0.05$) in large ruminants but it varies in small ruminants. On the basis of i-ELISA, percent positivity of brucellosis in goat (10.62%) was found the highest among all the species of animals (10%, 4%, 1.48% in cattle, buffalo, sheep respectively) and human (4%). Females were found more prone to brucellosis.

Large ruminants of more than 4 years of age and small ruminants of 2 years to 4 years in sheep and goat respectively found more sensitive towards *Brucella organisms*. In comparison to i-ELISA among total animals, sensitivity of RBPT and STAT were 53.57% and 42.86% respectively with substantial agreement whereas specificity of RBPT and STAT were 99.77% and 91.72% respectively with fair agreement. PCR detected amplicons of 223bp in 28 sera samples employed whereas only 1 sample was detected with *B. abortus* by AMOS PCR at 498bp. Multiplex PCR was standardized for simultaneous detection of *Brucella spp.*, *Mycoplasma spp.*, *Listeria spp.* and *Leptospira spp.* at amplification bands at 223bp, 270bp, 456bp and 331bp respectively.

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Ph.D. Degree Programme

1. Studies on virulence associated factors and drug resistance profile of verocytotoxin producing *Escherichia coli* (VTEC) in cattle

Parul and Basanti Bist

The present research work was conducted to investigate the prevalence, virulence associated factor and antibiogram of Verotoxic *Escherichia coli* (VTEC) in cattle faeces, raw milk and soil. The VTEC were detected by Verocell line assay (VCA) and virulent genes of VTEC strains characterized by mPCR and gene sequencing. Other virulence factors enterohaemolysin and invasiveness were studied through hemolysis assay and Congo red dye assay. The results showed that out of 372 *E. coli* isolates, 60 (16.12%) strains were positive for cell line assay (VCA and HeLa cell line assay). Out of these 60 VTEC isolates, 54 strains were belonged to cattle faeces (10 diarrhoeic calves, 22 healthy calves, 18 health dairy cattle and 4 from cattle bull). Out of the 60 VTEC isolates, 4 VTEC strains were obtained from raw cow's milk and 2 VTEC strains from farm soil. The prevalence of VTEC was reported highest at Bareilly (16.11%) followed by Agra (10.55%) Mathura farm (6.66%). The VTEC were found more prevalent in healthy calves at all three farms. Overall prevalence of VTEC in cattle faeces was found to be 14.28%, comprising of 9.25%, 20.37 %, 16.66% and 7.40% in diarrhoeic calves, healthy calves, healthy dairy cows and cattle bull, respectively. The prevalence of VTEC was revealed to be 3.70% in both raw cow milk and farm soil. Among the three seasons, highest prevalence of VTEC was found in summer (17.77%) followed by rainy (10.00%) and winter (5.55%). In the summer season, highest isolation rate of VTEC was recorded from healthy calves (30.55%) followed by dairy cows (27.77%). The serotyping of 60 VTEC strains isolated from different sources showed that all strains were typable and belonged to 18 different 'O' serogroups. The different VTEC serotypes obtained in this study were (O9, O10, O11, O17, O26, O34, O52, O56, O74, O81, O83, O84, O91, O121, O125, O134 and O156). Serotypes O9, O34, O91 and O156 were obtained from dairy cow faeces as well as from raw cow milk. The serogroups O11 and O91 revealed from soil of different farms also reported from cattle faeces. The entire 60 VTEC strains positive on VCA were subjected to multiplex PCR for the detection of virulent genes (*vt1*, *vt2*, *eaeA* and *hlyA*). Out of 54 VTEC isolates from cattle faeces 18 strains (33.33%) were shown to harboured *vt1* gene, 25 (46.29%) strains carried *vt2* gene and 11 (20.37%) strains possessed both *vt1* & *vt2* genes. Out of these 54 VTEC, 9 strains (16.66%) harbored *eaeA* gene and 17 strains (31.48%) were positive for *hlyA* gene. Results showed that out of 54 VTEC of cattle faeces, 5 different gene combinations were obtained. These 5 gene combinations belonged to 24 different VTEC serotypes of cattle faeces. Out of 4 VTEC isolated from cow milk, 1 strain (25%) were positive for *vt1* gene, 1 strain (25%) harbored *vt2* gene, 2 strains (50%) revealed both *vt1* & *vt2* while in other virulent genes, 2 strains (50%) carried *eaeA* gene and 2 strains (50%) bearing *hlyA* gene. The three combinations were present in the VTEC of cow milk as (*vt1*, *vt2*, *eaeA* & *hlyA*) as 25.00%, (*vt1*, *vt2* & *hlyA*) as 25.00% and (*vt1* & *eaeA*) as 25%. The 2 VTEC strains were isolated from farm soil samples, both the strains (100%) were carried *vt1* gene while one strain also had *eaeA* and another possessed *hlyA* gene. Thus the gene combination obtained was (*vt1* and *hlyA*) and (*vt1* and *eaeA*) as 50% each. A total of 51 (85%) isolates were found positive for enterohaemolysin production and showed the turbid haemolysis on sheep blood agar while remaining 9 (15%) isolates showed alpha haemolysis. A total of 60 VTEC strains were screened to observe the invasiveness of strains through Congo red dye assay, out of which 59 (98.33%) strains were found positive with CR assay. *In vitro*, antibiogram of all the 60 VTEC strains was performed

against the 15 commonly used antimicrobial drugs. Strains were shown to be highly sensitive to Co-trimoxazole (98.33%) followed by Ceftriaxone (90%), Ciprofloxacin (81.66%), Cefuroxime and Ceftriaxone (80%) while high resistance was observed against Ampicillin (91.66%), Streptomycin and Tetracycline (80%).

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2. Quality analysis (physico-chemical and microbiological) of drinking and Yamuna water from different areas of Mathura and Agra regions with special reference to *E.coli* (VTEC) and *Campylobacter* spp

Barkha Sharma and Basanti Bist

The present study was conducted to assess the physico-chemical and microbiological quality of water from different sources in Mathura and Agra along with the prevalence of *E.coli* (VTEC) and *Campylobacter* spp in these water samples. A total of 480 water samples (drinking water n=180, underground water n=60, surface water (Yamuna and pond water (n=120 each)) were collected during Dec 2013-15. The overall ranges of pH, turbidity, TDS, TH, chloride, fluoride, nitrate and iron were between 6.5-8.9, 0- 36.2 NTU, 20-2000 ppm, 117-1500mg/, 100-3250 mg/l, 0.12-2.5 mg/l, 0-100.2 mg/l and 0-0.5 mg/l, respectively. In surface water, pH, chloride, TH, fluoride, nitrates and iron were within the allowable limits in surface water. The pH and TDS were higher during post monsoon season than premonsoon. The DO and EC in surface water ranged between 0.20-0.40 mg/l and 727-1250 μ S/cm, respectively. The SPC and MPN were within 0-6x10⁷ cfu/ml and 0->1800 coliforms/100ml. All surface water samples were positive for coliforms whereas 53.89% drinking water samples had coliforms. No coliforms were detected in packaged water of national brands. Sachet and locally packaged water was more contaminated. A total of 162 *E.coli* (33.75%) were isolated from 480 water samples. Surface water had 52.92 % *E.coli*, followed by underground water 26.67% (16/60) and drinking water, 10.56% (19/180). None of the packaged water samples had *E.coli*. The prevalence of VTEC was 4.8% (23/480) with maximum 6.11% (11/180) in drinking water, followed by ground water samples 5% (3/60) and surface water 3.75% (9/240). There prevalence of *E.coli* in surface water during premonsoon (65%) was more than post monsoon (40.83%). VTEC in premonsoon and post monsoon water was 3.33% (4/120) and 4.17% (5/120), respectively. 35 *E.coli* (21.6%) had pathogenic genes. The EHEC *hlyA* gene was the most common virulent gene with prevalence of 74.29%, followed by *stx*₁, (62.58%) and *stx*₂ gene (8.58%). Gene combination of *stx*_t+*hlyA* was predominant. 33 isolates (94.28%) were positive on Congo red medium indicating pathogenic nature. No *E.coli* 0157 and *E.coli* 0111 serotypes could be isolated from water sources in this study. A total of 36 ESBL producer *E.coli* (22.22%) were detected among 162 *E.coli*. Out of these, 19 (52.8%) were VTEC. Maximum prevalence (66.7%) of ESBL bacteria was in surface waters. 6/a-SHV(69.4%) occurred more than Wa-CTX-M (58.3%).The serotypes obtained in this study were 0140, 0120, 083, 086 and O118 with one VTEC untypable. The isolates were highly sensitive to cefotaxime-clavulanic acid (77.14%), followed by chloramphenicol (74.28%) while highest resistance was shown against amoxicillin (80%). Intermediate sensitivity was shown against gentamicin (80%). 23 (65.71%) isolates were multi drug resistant with an MDRI >20%.No *Campylobacter* spp could be isolated from surface waters of this region in this study.

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3. Prevalence and risk factors analysis associated with bovine brucellosis and its public health significance especially in peri-urban areas of Brij region of Uttar Pradesh

Raghavendra Prasad Mishra and Udit Jain

During present study an attempt was made to find seroprevalence of brucellosis in bovine milk, serum, aborted fetal tissue and human serum sample from different peri urban places of four districts of Brij region which include Mathura, Agra, Hathras and Kasganj. A total of 1860 samples comprising of 700 bovine milk samples, 567 bovine serum samples, 84 aborted fetal tissue samples and 509 human serum samples were processed. Out of 1860 samples, in 700 milk sample species wise prevalence of brucellosis in 450 cattle milk sample was found to be 05.78 % (26/450) and 08.45% (38/450) positive by MRT and I- ELISA respectively. On the other hand in buffaloes milk the prevalence of brucellosis in 250 milk sample was found to be 07.20% (18/250) and 09.02% (23/250) positive by MRT and I- ELISA respectively. In 567 animal serum sample the prevalence of brucellosis in 391 cattle was found to be 07.93% (31/391), 08.69% (34/391) and 10.74% (42/391) shows positive by RBPT, STAT and I- ELISA respectively and in 176 buffaloes tested serum sample the seroprevalence was found to be 09.66% (17/176), 10.79% (19/176) and 12.5% (22/176) positive by RBPT, STAT and I- ELISA respectively. Out of 509 tested human serum sample (male 394 and female 115), the seroprevalence of human brucellosis was found to be 02.75% (14/509), 04.31% (21/509) and 03.73% (19/509) shows positive by RBPT, STAT and I- ELISA respectively. A total of 228 samples, 144 ELISA positive samples (61bovine milk, 64 bovine serum and 19 human serum samples) and 84 tissue samples were screened by PCR to detect the presence of *Brucella* genus specific gene (*bcp31*, *omp2* and *16S rRNA*) and *Brucella abortus* species specific *IS711* genes. A total 49 samples were found positive for *Brucella* genus specific genes.

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M.V.Sc. Degree Programme

1. Studies on laparoscopic and conventional ovariectomy for canine birth control programme

Mritunjay Kumar Chaurasia and R.P. Pandey

Randomized surgery was done on twelve healthy female dogs of 5-12 month age and 5-10 kg body weight presented for owners' consented elective reproductive sterilization (spaying) by ovariectomy by three different methods to include four dogs each of Group A (Laparoscopic ovariectomy using intracorporeal haemostatic technique with use of CO₂ for laparoflation), Group B (Laparoscope assisted ovariectomy using extracorporeal haemostatic techniques with use of filtered air for laparoflation) and Group C (Conventional mid-line ovariectomy) to select the most suitable technique for ABC programme. Procedures were done under intravenous anaesthesia with a combination of glycopyrrolate-midazolam-xylazine-ketamine or glycopyrrolate-midazolam-propofol-ketamin. Pelvis down lateral recumbency position was found good in group A & B while in group C dorsal recumbency position was used. In group A and B surgery was done at 12 mm Hg IAP and 2 liter/min flow rate. Two port site (both median port 5 mm) of Group A and four port site (two median ports 5 mm, two flank incision ports 5-10 mm) in Group B were adequate to visualize, access and remove the ovaries. 5-8 cm midline incision (considering it as a single port) was adequate to visualize access and remove the ovaries in group C. The operative time ranged from 57 to 67 minutes, 44 to 56 minutes and 45 to 53 minutes in group A, B and C respectively. Mean port making time was 7.5 ± 0.29 , 10.25 ± 0.63 and 9.5 ± 0.65 minutes; mean left OVE time was 20 ± 0.71 , 13.25 ± 1.03 and 11.75 ± 0.85 minutes; mean right OVE time was 24.5 ± 1.19 , 16.25 ± 0.48 and 15.25 ± 0.63 minutes; mean port closure time was 10 ± 3.03 , 10 ± 0.41 and 12.75 ± 0.63 minutes; and mean operative time was 62 ± 2.08 , 49.75 ± 2.46 and 49.25 ± 1.75 minutes in group A, group B and group C respectively. Mean perioperative time which included preparation time, operative time and upto post operative recovery time was 100.8 ± 4.2 , 108.5 ± 14.4 and 97.5 ± 10.1 minutes for group A, B and C respectively. Mean incision length was 1.63 ± 0.13 , 3.13 ± 0.13 and 6.25 ± 0.6 cm in group A, B and C respectively. Ease and intraoperative maneuverability was poor in group A, excellent in group B and good in group C. Intraabdominal organ visualization ease and intra abdominal clarity of vision was excellent in group A and B while in case of group C, it was poor. The ease of port site delivery of ovary after Ovariectomy was poor in group A and excellent in group B and C. Subcutaneous emphysema was not observed in group A but in one case of group B, it was observed at flank port. In one case only, the liver got penetrated with Veress needle but it was of little consequence. No instance of electrical injury or accidental burn during use of diathermy occurred. Failure of haemostatic technique was observed in one case in group A and this had to be converted in exploratory laparotomy. Changes in mean heart rate, respiration rate and rectal temperature in group A, B and C at different time intervals and between the groups were non significant. In group A and B the mean values of SpO₂ just before first skin incision and after left OVE were significantly lower in comparison to the base line value. Comparison among different groups revealed that there was non significant difference in mean SpO₂ at different intervals. The mean SAP, DAP and MAP values in group A and B did not differ significantly from the base value but in group C, there was a significant increase just after completion of surgery. IInd lead tracing ECG in all the animals did not register any conduction abnormality except in one instance likely to be due to anaesthetic agents. The changes registered in mean Haemoglobin and Packed cell volume values, TLC and differential cell count in group A, B and C showed largely non significant differences and ranged within the normal physiological limits. Post operative

inflammation was of low grade in group A and B and of medium grade in group C. In group A, no change in behavioural manifestation was found and group B but mild alteration in behaviour was reported in animals of group C. On the basis of tolerance to abdominal palpation, there was no resentment in group A and mild resentment in group B and C. Mean volume of CO₂ utilized was 106±3.24 grams. Mean total cost of procedure was calculated on the basis of cost of antiseptics, anaesthetics, surgical consumables and post operative expenses. It varied with weight of animal and minimum cost of procedure (for 5 kg female dog in each group) was 306, 280, 422 rupees respectively in group A, group B and group C

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2. B-Mode ultrasonographic evaluation of teat and udder in buffaloes

Neeraj Awasthi and Sanjay Purohit

The aim of the present study was to determine the ultrasonographic normo-echotexture of teat and udder of healthy and lactating buffaloes. On the basis of these the clinical cases presented for treatment of teat and udder affections were evaluated ultrasonographically. The study was carried out in two phases. In phase I the normo-echo texture of the teat and udder of the healthy and lactating buffaloes were standardized (N=6) using gel application method and in phase II the teat and udder affections were sonographically evaluated by gel application method in buffaloes (N=50). Ultrasonography was performed using convex 8 MHz and 5MHz transducer of mindray device. Ultrasonographic examination of teat was done by immersing the teat in plastic transparent glass filled with water and the transducer being in contact with the glass wall from outside. During the scan, the transducer was positioned along the length of the teat designated vertical position outside the plastic glass with application gel. The udder were examined by gel application method by placing the transducer longitudinally/sagittally at the udder after applying a coupling gel.

Eight types of teat and udder affections were diagnosed sonographically. Total sixty six affections were diagnosed in these animals. In few cases more than one affection was diagnosed like mastitis and fibrosis together, mastitis and abscess together etc. The incidence of abscess, fibrosis, haematoma, mastitis, oedema, polyp, teat obstruction, udder tumour was 4.55, 42.42, 7.58, 19.70, 21.21, 1.52, 1.52, and 1.52 percent, respectively.

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3. Clinical studies on the maintenance of anaesthesia by constant rate infusion in bitches presented for oophorectomy

Hasanin Ansari and Sanjay Purohit

Two anaesthetic protocols were evaluated in two groups (A and B) of animals presented for conventional and laparoscopic oophorectomy. Each group was subdivided in to 2 subgroup (A₁, A₂ and B₁, B₂) each contain 5 animals. These five animals in each group were used to evaluate two different anaesthetic protocols. All the animals of four groups were receive glycopyrrolate (0.005 mg/kg) and xylazine (0.5 mg/kg) intramuscularly as preanaesthetic medication taking the time interval of 10 minutes between glycopyrrolate and xylazine administration. After 10 minutes, of the administration of preanaesthetics, induction of anaesthesia was achieved by administering midazolam (0.2 mg/kg) and ketamine (5.0 mg/kg) i.v., till effect. After 5 minute of induction, the maintenance of anaesthesia was started with midazolam (0.4 mg/kg/hr)-ketamine (20 mg/kg/hr)-xylazine (1.0 mg/kg/hr) in animals of group A | and B, while propofol (3.6 mg/kg/hr)-ketamine (5.4 mg/kg/hr) was

used in the animals of groups A₂ and B₂ as constant rate infusion (CRI) with definitive rate using an infusion pump.

The effects of these anaesthetic combinations were evaluated on the basis of alteration in clinicophysiological, haemodynamic, haematological and biochemical parameters. These parameters were recorded at base line, maximum depth of sedation, 5 min after induction, middle of maintenance and at complete recovery of drug administration.

Xylazine produced mild to moderate sedation in animals of all groups. Degree of analgesia and muscle relaxation was mild to moderate at maximum depth of sedation, moderate to excellent at 5 min after induction and excellent at middle of maintenance. Palpabral reflex and pedal reflex was weak to very light at maximum depth of sedation, very light to abolished at 5 min after induction and abolished at middle of maintenance. Response to intubation was varied from allowed deeper entry but coughed to easy intubation without coughing in animals of all groups. Sternal recombency time (SRT) and Complete recovery time (CRT) were significant higher in animals of A₁ and B₁.

Haemodynamic (ECG, SpO₂ and MAP), haematological and biochemical parameters in animals of all groups altered within physiological limit and nearly normalised at complete recovery, indicating non significant alteration in body systems. Total cost of anaesthesia (Rs/kg b. wt.) in A₁ B₁ groups was significantly (P<0.05) higher than A₂, B₂ groups.

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4. Evaluation of propofol as constant rate infusion for maintenance of anaesthesia in horses premedicated with xylazine acepromazine butorphanol and diazepam in different combinations

Ashish Kumar Kasoudhan and Bharat Singh

The study was conducted on three clinically healthy experimental female horses as well as on clinical cases. All horses were utilized in a latin square design using four different anaesthetic combinations in four different groups viz. A, B, C and D. Horses were premedicated with administration of acepromazine(0.02mg/kg)+ xylazine(0.5mg/kg) mixed in same syringe intravenously in A group, xylazine(0.5mg/kg) immediately followed by diazepam(0.1 mg/kg) intravenously in B group, acepromazine(0.02mg/kg) + butorphanol(0.02mg/kg) + xylazine(0.5 mg/kg) mixed in same syringe intravenously in C group and with butorphanol(0.02mg/kg) + xylazine(0.5 mg/kg) mixed in same syringe immediately followed by diazepam(0.1 mg/kg) intravenously in D group of animals. Induction of anaesthesia was achieved by i.v. administration of combination of Xylazine and Ketamine in ratio of 3:5 by weight, till effect in all four groups. Maintenance of anaesthesia was done by constant rate infusion (CRI) of propofol (1%) for 30 minutes. The anaesthetic groups were compared on the basis of anaesthetic, physiological, haematological and biochemical parameters.

Xylazine-diazepam combination (B group) was found better preanaesthetic in comparison to other three combinations as this produced strong sedation quality with shorter time of occurrence of maximum sedation with reduction in doses of induction and maintenance agents to a significant level. This combination was also produced less depression of cardiopulmonary system with good to excellent quality of recovery from anaesthesia. Significantly lesser doses of xylazine and ketamine for induction of anaesthesia was observed (0.74±0.024 and 1.23±0.039 mg/kg, respectively) in B group in comparison to A, C and D groups (1.48±0.015 mg/kg and 2.46±0.025 mg/kg , 0.86±0.051 and 1.43±0.085 mg/kg,

0.75±0.059 and 1.25±0.098 mg/kg, respectively). The required infusion rates of propofol to maintain the adequate depth of anaesthesia in A, B, C and D groups were 0.159±0.014, 145±0.026, 0.112±0.016 and 0.073±0.007 mg/kg/min, respectively. All the drug combinations produced adequate surgical anaesthesia. Cardiopulmonary functions were well preserved during whole period of anaesthesia. Results on present study on the various anaesthetic, physiological (HR, RR, RT, MAP, SpO₂, ECG), haematological (PCV, Hb, TLC, TC, DLC) and biochemical (serum glucose, creatinine, SUN, ALT, AST, chloride, sodium, potassium, cortisol) parameters did not reveal any deleterious effects on any vital function and organ in the body and these drug regimens can safely be used in routine clinical cases of surgery in field conditions without any risk.

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5. Clinical studies on the use of acepromazine xylazine and butorphanol in different combinations for standing sedation in horses

Abhishek Rajpoot and Vivek Malik

The aim of this study was to investigate the sedative, clinicophysiological, hematological and biochemical effects of acepromazine, xylazine and butorphanol in different combinations in clinical cases of horses. Study was conducted on 24 adult horses of either sex weighing 200 to 4000 kg presented for various surgical procedures. The animals were divided into 4 groups viz. A, B, C and D comprising six animals in each group. The animals of group A received an intravenous bolus of mixture of acepromazine (0.04mg/kg) + butorphanol (0.02mg/kg). Animals of group B received xylazine (0.5mg/kg) + butorphanol (0.02mg/kg) as an intravenous bolus. Animals of group C received acepromazine (0.04mg/kg) + xylazine (0.5mg/kg) intravenously. Animals of group D were administered with a mixture of acepromazine (0.03mg/kg) + xylazine (0.5mg/kg) and butorphanol (0.02mg/kg) intravenously. After medication with proposed drugs in A, B, C and D groups of animals, time of occurrence of first signs of sedation was 8.00±0.912, 3.50±0.645, 3.50±0.288 and 3.25±0.629. In animals of group A significantly (P<0.05) higher time for start of sedation was noticed when compared to animals of B and C and D groups. Values of start of maximum sedation time recorded in different groups were 13.50± 1.322, 6.00±0.408, 6.25±0.478 and 5.75±1.18 min respectively. Comparison among different groups revealed that the animals of group A took significantly (P<0.05) more time to reach the stage of maximum sedation than that of B, C and group D. Mean values of start of recovery time observed in different groups were 35.75±2.174, 31.50±2.533, 36.75±2.688 and 38.25± 1.652. Animals of D group took non significant (P>0.05) longer time to recover from sedation in comparison to A, B and C groups. Mean values of complete recovery time observed in different groups were 83.75±4.732, 73.75±4.269, 89.50±2.101 and 95.75±6.169 respectively. Animals of group D took significantly (P<0.05) longer time for complete recovery in comparison to A, B and C groups. Comparison among different groups revealed that the animals of group D have significantly (P<0.05) longer duration of maximum sedation than that of C, B and A groups. Among the four groups highest sedation score was observed in group D. Excellent sedation was observed in animals of this group with characteristic signs of sedation. None of the animals among in these four groups showed any sign of excitement during the observation period. All the animals of A, B, C and D groups do not exhibit any signs of ataxia and animals remained in standing position, without any incoordination during the period of observation.

The animals were evaluated for the changes in different physiological parameters, which includes heart rate, respiration rate, mean arterial pressure, rectal temperature and SpO₂,

before administration of any drug (baseline) and at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 90 minutes after administration of drug. Electrocardiography (ECG) done before administration of any drug at time 0 (baseline), and at 15, 30, 45 and 60 min after administration of drug. Haematological and biochemical studies included measurement of Hb, PCV, TLC, DLC, glucose, creatinine, ALT and AST, respectively at time 0 (base line) and at 15, 30, 45, 60 and 90 minutes after administration of sedative agents. In all the groups xylazine and acepromazine induced significant decrease in heart rate, respiratory rate, rectal temperature and SpO₂. Haemoglobin (Hb), packed cell volume (PCV), and total leukocyte count (TLC) decreased in all the groups. Changes in physiological and Haematological parameters were transient in nature. Xylazine and acepromazine produced an increase in glucose, creatinine, and AST and ALT levels. However these parameters fluctuate within normal limits and returned to normal during recovery ruling out any renal or hepatic toxicity. The study indicates that None of the sedative combination used in the present study for standing sedation produce any serious deleterious effect on various clinico-physiological and haemato-biochemical parameters indicating their safety on various vital organ functions hence all of these sedative drug regimens can safely be used in routine clinical cases of surgery of short duration under field conditions. Drug combination of Acepromazine (0.02 mg/kg) + xylazine (0.5 mg/kg)+ butorphanol (0.02 mg/kg) used for standing sedation in the animals of group D was found best in terms of the duration and quality of sedation among the four combinations used in the present study. On the basis of the results of the present study it is recommended to conduct further investigations using higher dose rates of different drugs in different combinations to produce sedation of higher quality and of more duration.

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6. Clinical studies on the effect of glycopyrrolate xylazine acepromazine dexmedetomidine and butorphanol in different combinations on propofol halothane anaesthesia in dogs

Saurabh Yadav and Vivek Malik

Three anaesthetic protocols were evaluated in three groups (I, II and III) of animals presented for various surgical procedures. Each group consisted of 6 animals. As preanaesthetics combination a mixture of glycopyrrolate (0.01 mg/kg), xylazine (0.5mg/kg) butorphanol (0.2 mg/kg) was administered intramuscularly in the animals of group I. In the animals of group II, a mixture of glycopyrrolate (0.01 mg/kg), dexmedetomidine (0.005mg/kg) and Butorphanol (0.2 mg/kg) was administered intramuscularly and in the animals of group III, a mixture of glycopyrrolate (0.01 mg/kg), acepromazine (0.05 mg/kg) and butorphanol (0.2 mg/kg) was administered intramuscularly. In all anaesthetic protocols, 15 min after administration of preanaesthetic, anaesthesia was induced with propofol (10 mg/ml) given slow intravenously, to effect using small boluses (0.5 mg/kg, every 30 sec) until a plane of anaesthesia suitable for endotracheal intubation was achieved. Soon after the desired level of anaesthesia was achieved, endotracheal intubation was performed and maintenance of anaesthesia in all groups was started with halothane in 100% oxygen using semiclosed rebreathing system of anaesthesia with a oxygen flow rate of 1 lit/min. The vapourizer was set at 1% initially and then increased or decreased in increments as per the need to maintain an adequate level of anaesthesia throughout the surgical procedure. Anaesthesia was maintained for at least 45 minutes or until the surgical procedure was completed.

The effects of these anaesthetic combinations were evaluated on the basis of alteration in clinicophysiological, haemodynamic, haematological and biochemical parameters. These

parameters were recorded at base line, fixed time intervals and at complete recovery of drug administration. All the three preanaesthetics- xylazine, dexmedetomidine and acepromazine produced mild to moderate sedation in the animals. Premedication in all the three groups decreased the requirement of propofol for anaesthetic induction. However, group III premedicated with acepromazine, required the highest dose of halothane for maintenance followed by group I premedicated with xylazine and group i. premedicated with dexmedetomidine. Palpebral reflex and pedal reflex was light to abolished during the different time intervals in the course of observation in all the groups. Complete recovery time was the highest in group II administered with dexmedetomidine.

Haemodynamic (ECG, SpO₂ and MAP), haematological (Hb, PCV, TLC and DLC) and biochemical parameters (serum urea nitrogen, serum glucose, serum creatinine, ALT and AST) in animals of all groups altered within physiological limit and nearly normalised at complete recovery, indicating non significant alteration in body systems.

Preanaesthetic combination of glycopyrrolate (0.01 mg/kg), butorphanol (0.2 mg/kg) and dexmedetomidine (5 mcg/kg) was found best among three combination in terms of the quality of sedation, early onset of sedation and dose sparing effect on induction and maintenance agents. However, the recovery time was highest in this group.

All the three preanaesthetic combinations were comparable in terms of the cardio-respiratory and haemodynamic stability and did not produce any serious allegations on these parameters and hence recommended for surgical procedures of about 60 min of duration. None of the anaesthetic combinations imposed any deleterious effects on any vital organ function as evidenced by the haemato- biochemical analysis and hence can safely be used in routine clinical cases of surgery.

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7. B-Mode ultrasonographic biometry of intraocular structures in Murrah buffalo (*Bubalus bubalis*)

Sudhir Singh and Sanjay Purohit

Ultrasonography is a relatively easy, safe and non-invasive examination method which can be used in diagnosis of ocular disorders as complementary to routine ophthalmic examinations. As there has been no collated study undertaken on the normal echomorphometric measurements of ocular structures in live murrah buffalo (*Bubalus bubalis*), obtaining these measurements could be a benchmark to diagnose some of the diseases and eye problems of this breed. Transcorneal ultrasonographic scanning of left and right eyes in male and female of calf as well as adult healthy buffaloes (n=6) was performed. Qualitative echo-biometric findings of the eyes were described and measurements of the ocular structures were obtained. In present transcorneal intraocular echo-biometric studies five parameters were measured i.e. aqueous chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), axial globe length (AGL) and scleroretinal rim thickness (SRRT) by 6- 8 MHz convex transducer at 6-9 cm scanning depth with suitable gain. Non-significant (P<0.05) difference was observed in all parameters when compared left and right eye of male as well as female calf and male and female calf. The same finding was observed in case of adults except LT which was significantly (P<0.05) higher in female than male and VCD was non-significantly (P<0.05) lower in female than male. The result of the present study revealed that parameters of the eye such as the aqueous chamber depth, lens thickness, vitreous chamber depth and axial globe length were significantly (P<0.05) increased with age. A prospective study of ocular affections in murrah buffaloes was carried out. Five types of ocular affection

were diagnosed in 15 buffalos and evaluated on echo- morphometric finding, out of which 3 (20%) were male and 12 (80%) were female buffaloes. Left eye (46.66%) had more affections than right eye (33.33%) and both eyes (20%). Traumatic lesions (39.98%) showed higher percentage of affection followed by tumour/dermoids (26.66%), blepharitis/conjunctivitis (20%), corneal opacity (6.66%) and ectropion (6.66%). Therefore, it can be documented that the obtained values as the standard parameters of eye in buffaloes. The present study provides an inside echo-morphometric view of the inner ocular structures in healthy as well as in certain eye affections where ophthalmoscopic examination was not possible.

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8. Clinical studies on the effect of glycopyrrolate, dexmedetomidine, fentanyl and butorphanol in different combinations on propofol-isoflurane anaesthesia in dogs of different age groups

Rohan Kumar Vijay and Vivek Malik

Two anaesthetic protocols were evaluated in four groups (A1, A2, B1 and B2) of animals of two different age groups (A1 and A2: less than 8 years; B1 and B2: more than 8 years) presented for various surgical procedures. Each group consisted of 6 animals. As preanaesthetics combination a mixture of glycopyrrolate (0.01mg/kg), dexmedetomidine (5µg/kg) and butorphanol (0.1 mg/kg) was administered intramuscularly in the animals of group A1 and B1. In the animals of group A2 and B2, a mixture of glycopyrrolate (0.01mg/kg), dexmedetomidine (5µg/kg) and fentanyl (4µg/kg) was administered, intramuscularly. In all anaesthetic protocols, 15 min after administration of preanaesthetics, anaesthesia was induced with propofol (10 mg/ml) given slow intravenously, to effect using small boluses until a plane of anaesthesia suitable for endotracheal intubation was achieved. Soon after the desired level of anaesthesia was achieved, endotracheal intubation was performed and maintenance of anaesthesia in all groups was started with isoflurane using semiclosed rebreathing system of anaesthesia with a oxygen flow rate of 30 ml/kg/min. The vapourizer was set at 2% initially and then increased or decreased in increments as per the need to maintain an adequate level of anaesthesia throughout the surgical procedure. Anaesthesia was maintained for at least 60 minutes or until the surgical procedure was completed. The effects of these anaesthetic combinations were evaluated on the basis of alteration in clinicophysiological, haemodynamic, haematological and biochemical parameters. These parameters were recorded at base line and at fixed time intervals till complete recovery of drug administration. Preanaesthetics combinations used in group A1 and B1 produced better sedation than A2 and B2 groups. Premedication in all four groups decreased the requirement of propofol for anaesthetic induction. However, group A2 and B2 premedicated with glycopyrrolate, dexmedetomidine and fentanyl, required the higher dose of isoflurane for maintenance than group A1 and B1 premedicated with glycopyrrolate, dexmedetomidine and butorphanol. Palpebral reflex and pedal reflex were completely abolished during post induction and maintenance period in all the groups. Complete recovery time was the higher in animals of group B2 followed by group B1, A2 and A1. Haemodynamic (SBP, DBP, MAP and SpO₂), haematological (Hb, PCV, TLC and DLC) and biochemical parameters (serum urea nitrogen, serum glucose and serum creatinine) in animals of all groups altered within physiological limit. Preanaesthetic combination of glycopyrrolate, butorphanol and dexmedetomidine was found better in comparison to combination of glycopyrrolate, dexmedetomidine, fentanyl in terms of the sedation quality, recovery time, dose sparing action on the induction and maintenance agents used and better maintenance of the cardiopulmonary and

haemodynamics. All the four preanaesthetic combinations were comparable in terms of the cardio-respiratory and haemodynamic stability and did not produce any serious allegations on these parameters and hence recommended for surgical procedures of about 60 min of duration. None of the anaesthetic combinations imposed any deleterious effects on any vital organ function as evidenced by the haematobiochemical analysis and hence can safely be used in routine clinical cases of surgery.

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9. Clinical Studies on Comparative evaluation of halothane isoflurane and sevoflurane anaesthesia on glycopyrrolate xylazine premedicated and ketamine diazepam induced anaesthesia in canine

Hari Pal Singh Gangwar and Sanjay Purohit

Three anaesthetic protocols were evaluated in three groups (A, B and C) of animals presented for various surgical procedures. Each group consisted of 6 animals. All the animals of three groups were received glycopyrrolate (0.01mg/kg) + xylazine (0.5 mg/kg) intramuscularly as preanaesthetic medication taking the time interval of 10 minutes between glycopyrrolate and xylazine administration. After 10 minutes of the administration of preanaesthetics, induction of anaesthesia was achieved by administering diazepam (0.25mg/kg) and ketamine (5.0mg/kg) IV, till effect (the administration of induction agent was continued over a period of 60 seconds till the absence of response of animal to noxious stimulus, pedal reflex, relaxation of jaw muscles and absence of resistance to pulling out the tongue). After complete induction, endotracheal tube intubation was performed and maintained on inhalation anaesthesia. For maintenance in animal of group A used halothane, for animal of group B used isoflurane and for animal of group C used sevoflurane in 100% oxygen using semiclosed rebreathing system of anaesthesia machine. The vapourizer was set at 1% initially and then increased or decreased in increments as per the need to maintain an adequate level of anaesthesia throughout the surgical procedure. The anaesthesia was maintained for at least 60 minutes or until the surgical procedure is completed. The effects of these anaesthetic combinations were evaluated on the basis of alteration in clinico-physiological, haemodynamic, haematological, biochemical observations and cost of anaesthesia. These observations were recorded at base line and other fixed time intervals during anaesthesia. Preanaesthetics, glycopyrrolate and xylazine, produced mild to moderate sedation in the animals of all the three groups. Premedication in all the animals of three groups decreased the requirement of ketamine and diazepam for sufficient anaesthetic induction. All the animals of group A, B and C were maintained by halothane, isoflurane and sevoflurane respectively. Palpebral reflex and pedal reflex was light to abolished during the different time intervals in the course of observation in all the three groups. Sternal recumbency time (SRT) and complete recovery time (CRT) were lowest in group C maintained with sevoflurane and were highest in group A maintained with halothane. While SRT and CRT in group B maintained with isoflurane was slightly higher than group C maintained with sevoflurane anaesthesia. Haemodynamic (SpO₂ and MAP), haematological (Hb, PCV, TLC and DLC) and biochemical parameters (serum glucose, serum urea nitrogen, serum creatinine, ALT and AST) in animals of all the three groups altered within physiological limit and nearly normalised at complete recovery, indicating non significant alteration in body systems. Faster induction and recovery were recorded in animals of sevoflurane group in comparison to isoflurane and halothane groups. All the three anaesthetic protocols were comparable in terms of the cardio-respiratory and haemodynamic stability and did not produce any serious

allegations on these parameters and hence recommended for surgical procedures of about 60 min of duration. None of the anaesthetic combinations imposed any deleterious effects on any vital organ function as evidenced by the haematobiochemical analysis and hence can safely be used in routine clinical cases of surgery. In all the animals of three groups, induction cost (Rs/kg) was similar in all the groups while maintenance cost (Rs/kg/ min) was minimum in halothane group in comparison to isoflurane and sevoflurane group.

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10. Studies on ORO-dental conditions buffaloes (*Bubalus bubalis*)

Ravindra Kumar Singh and R.P. Pandey

Present study was carried out on the cadaver specimens of adult buffalo head and selected clinical cases with oral/dental affections as well as normal healthy buffaloes presented during study period at TVCC and ILFC, DUVASU, Mathura. A total number of 10 cadaver specimens of adult buffalo heads were collected from abattoir or from hospital casualty cases. The morphometric and radiographic measurements for the crown and root lengths, total tooth length, oral cavity width, occlusal surface dimension (MD& BL) and diastema were measured by using a Vernier calipers to the nearest 0.5 mm, needle point divider and scale. The radiographic measurements were done using image work 10.09 software of Konica regius 110 CR system. Morphometric and radiographic study of the dental arch of Buffalo revealed deeply and firmly anchored 8 incisors in lower jaw. The length of root was 0.8 times that of the crown in the incisor. The morphometric measurement were non significantly higher than the radiographic measurement except second premolar of lower jaw and first premolar of upper jaw which was significantly differ at ($P \leq 0.05$). The crown length, root length and total tooth length of the maxillary teeth was constantly more than that of the mandibular cheek tooth. The oral cavity width ranged from 5.24 to 9.23 cm. Oral cavity was 'V' shaped on lower jaw and oval on upper jaw and it was maximum at the level of M1. The comparison of morphometric and radiographic measurements as well as morphometric comprision between the upper and lower jaw was also done. In this study it was found that there is no significant difference in mesiodistal measurement of upper and lower jaw premolar and molar teeth but when the buccolingual measurements of same teeth were compared, the difference was highly significant and the mean values (0.95 ± 0.05) obtained for lower jaw were markedly less than those obtained for the upper jaw (1.35 ± 0.07). The oral cavity examination were performed in 10 healthy buffaloes and 07 clinical cases reported at TVCC during study period. In healthy animal the Saliva pH 8.5 in 30% and 9 in 70% cases, Color of mucus membrane pink in 60% and pale in 40 % cases, Plaque Index grade 0 in 50% , grade 1 in 30% and grade 2 in 20% cases, Gingivitis Index grade 0 in 80% and grade 1 in 20% cases, Dental caries and supernumerary teeth in 10% cases, Calculus, Tooth mobility ,sharp molar, odontoma, facial/ mandible swelling were not observed in any case. In clinical cases the Saliva pH 8.5 in 28.5% (2) and 9 in 71.5% (5) cases, Color of mucus membrane pink in 71.5% (5) and pale in 28.5% (2) cases, Plaque Index grade 0 in 28.5% (2) , grade 1 in 57.1% (4) and grade 2 in 14.2% (1) cases, Gingivitis Index grade 0 in 57.1% (4) and grade 1 in 42.8% (3) cases, Sharp molar and odontoma were observed in 28.5% (2) cases, Facial/ mandible swelling was observed in 42.8% (3) cases, abnormal eruption of teeth in 14.2%(1) Dental caries, Calculus, Tooth mobility and supernumerary teeth were not observed in any cases.

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11. EXCRETORY urographic and ultrasonographic Studies of urinary system in canines (*Canis familiaris*)

Achintya Gautham and Sanjay Purohit

Present study was conducted on 8 healthy dogs of either sex to evaluate the urinary system by excretory urography, ultrasonography, urine analysis, hematology, and serum biochemistry. Nine radiographic biometric parameters were measured i.e. kidney length, width, length of second lumbar vertebral body, ratio of kidney length to the length of second lumbar vertebrae, ratio of kidney width to the length of second lumbar vertebrae (at 5 min interval), ureter luminal diameter, ureter length (at 15 min interval) and ratios of ureteral length to the length of second lumbar vertebrae using the in-built calipers in the CR system. VD and right lateral radiographic exposures were made 0, 5, 15, 30 and 45 min intervals after iohexol contrast agent infusion @ 800 mg I/kg body weight. The excretory urograms were evaluated based on the visibility of iohexol in the urinary organs in both the views wherein, the best visualization of the kidneys, ureters and urinary bladder were observed at 0 min to 5 min intervals, 15 min interval, and 15 to 45 min intervals, respectively. Seven ultrasonographic biometric parameters were measured i.e. kidney length, width, height, volume, cortex thickness taken length-wise and width-wise, and urinary bladder wall thickness by 3.5-7.5 MHz micro-convex transducer with suitable gain. Urine analysis, haematological and biochemical parameters were measured by using standard techniques. Non-significant ($P \leq 0.05$) difference was observed in all radiographic, ultrasonographic, urine, hematological and biochemical parameters when compared male and female dogs. Significant ($P \leq 0.05$) difference was observed when the ultrasonographic measurement of cortex thickness taken length wise was compared with that taken width-wise, irrespective of side of the kidney and sex of the animal. A prospective study of affection of urinary system was carried out. Six types of urinary system affection were diagnosed in 17 dogs wherein excretory urographic, ultrasonographic, urine analysis, hematological and serum biochemical parameters were evaluated, out of which 9 (52.94%) were male and 8 (47.06%) were female dogs, aged between 2 to 11 years of various breeds. Urolithiasis (52.94 %), chronic renal failure (11.76 %), urethritis (11.76 %), vaginal tumour (11.76 %), urinary bladder tumour (5.88 %) and pyelonephritis, prostatitis and testicular tumour in monorchid dog (5.88%) were the six type of cases encountered. It was concluded that combination of diagnostic procedures are required for accurate evaluation of anatomy and physiological function of the urinary system.

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12. Clinical studies on ultrasonographic and the eye and ocular affection in dog, horse, cattle and buffalo

Deepak Mani Tripathi and Vivek Malik

The purpose of this study was to describe the ultrasonographic appearance, to measure different intraocular echo-biometric indices in normal adult dogs, horses, cattle and buffaloes and to study hospital incidence of different ocular affections and their management during May 2016 to June, 2017. As there has been no such study undertaken on the measurement on the normal intraocular echobiometric parameter in these animals, obtaining these measurements could be a benchmark to diagnose some of the disease and eye problems in these animals. B-mode transcorneal ultrasonographic scanning of left and right eyes of six adult healthy animals of each species viz. horse, cattle, and buffalo were performed. Additionally, six healthy adult dogs from three different breeds viz. German shepherd, Labrador retriever and Indian mongrels were also selected for the same purpose. Qualitative

echo-biometric findings of the eyes were described and measurements of the intraocular structures were obtained. In present transcorneal intraocular echo-biometric study six parameter were measured i.e. aqueous chamber depth (ACD), lens depth (LDe), lens diameter (LDi), vitreous depth (VD), scleroretinal rim thickness (SRT), and globe axial length (GAL) by using high end ultrasound machine (Mylab30vet), with 2.5-7.5 MHz microconvex transducer and the depth of scanning was set at 5-9 cm with suitable gain without administration of any general/local anesthetic. Non-significant difference ($P \leq 0.05$) was observed in all parameters when compared between left and right eye of different breeds and species of animals used in the study. The average values of LDi and GAL of both eye of German shepherd dog were significantly differ from Labrador retriever and Indian mongrel dogs. The average value of SRT of both eye of German shepherd and Labrador were significantly higher than that of Indian Mongrel dogs. The average value of ACD of both eye of horse showed significant difference from buffalo. The average value of LDe of both eyes of horse were significantly different from cattle. The average value of LDi of both eyes of buffaloes and horses were significantly different from cattle. The average value of VD of both eyes of horse showed significant difference from cattle and buffaloes. The average value of SRT of both eyes of horse show significant difference with cattle. The average values of GAL in both eyes were significantly different in all three species. The study was conducted to evaluate the hospital incidence of ocular affections. Total 65 cases were presented with complaints of ocular affections during the study period. The study revealed that overall incidence of ocular affections in canine contributed (57%) of the ophthalmic cases, followed by buffalo (22%), bovine (12%) and equine (9%). Ocular affection recorded during this study period were corneal opacity (17 %), cataract (14%), neoplasm (9 %), dermoid (9 %), cherry eye (8%), trauma (8%), exophthalmos (5%), eye worm (5%). The incidence of each affection viz. corneal ulcer, descemetocoele, chemosis, traumatic lens expulsion and prolapse of iris was (3%) whereas, the incidence percentage of blindness, glaucoma, pigmentary keratopathy, vitreous hemorrhage, anterior uveitis and anophthalmia was 2% for each affections. In all the cases, where possible, ultrasonography was performed and cases were managed by medicinal, surgical or medico-surgical interventions as per the requirement.

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13. Excretory urographic and ultrasonographic studies of urinary system in goats (*Capra hircus*)

Manoj Kumar Verma and Sanjay Purohit

Present study was conducted on 8 healthy goats of either sex to evaluate the urinary system by excretory urography, ultrasonography, urine analysis, hematology, and serum biochemistry. Four radiographic biometric parameters were measured i.e. kidney length, width, ratio of kidney length to the length of second lumbar vertebrae, ratio of kidney width to the length of second lumbar vertebrae (at 5 min interval) using the in-built calipers in the CR system. VD and right lateral radiographic exposures were made at 0, 5, 15, 30 and 45 min intervals after iohexol contrast agent infusion @ 700 mg I/kg body weight. The excretory urograms were evaluated based on the visibility of iohexol in the urinary organs in both the views wherein, the best visualization of the kidneys, ureters and urinary bladder were observed at 0 min to 15 min intervals, 0 min interval, and 15 to 45 min intervals, respectively. The kidneys and ureters were visualized in the right lateral view than the VD view. Seven ultrasonographic biometric parameters were measured i.e. kidney length, width, height, volume, cortex thickness taken length-wise and width-wise, and urinary bladder wall thickness by 3.5-7.5 MHz microconvex transducer with suitable gain. Urine analysis,

haematological and biochemical parameter were measured by using standard techniques. Non-significant ($P \leq 0.05$) difference was observed in almost all radiographic, urine, hematological and biochemical parameters except some ultrasonographic parameters, when compared male and female goat. However Significant ($P \leq 0.05$) difference was observed in the radiographic biometry of right kidney length and left kidney length; the ultrasonographic biometry of right kidney length, width and volume and left kidney; left kidney length width, height, volume, length of cortex thickness taken width-wise; urinary bladder thickness and total protein level in the serum. A prospective study of urinary tract affection was carried out. Two types of urinary tract affection were diagnosed in 6 goats wherein excretory urographic, ultrasonographic, urine analysis, hematological and serum biochemical parameters were evaluated, out of which all were males (100%) goats, aged between 1 month to 2 years of various breeds. Urolithiasis (83.33%), and urethral diverticulum (16.67%) were the two type of cases encountered. It was concluded that combination of diagnostic procedures are required for accurate evaluation of anatomy and physiological function of the urinary system.

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14. Echocardiographic studies in healthy mongrel dogs

Prashant Raj Singh and Vivek Malik

Twelve healthy mongrel dogs which included seven males and five female dogs, were selected for the present study to perform echocardiographic examinations. The animals were confirmed as healthy based on their clinico - physiological, radiographical, haematological and electrocardiographical examinations. Animals found with any deviation in the values of pre - echocardiographic evaluations were excluded from the study. 2D mode, M-mode and Doppler mode examinations were performed for each animal in order to fulfil the aim of this study which was to generate the reference values of echocardiographic parameters in mongrel dogs. The mean age of male and female animals included in the study was 2.92 ± 0.27 (mean \pm S.E.) years and 2.70 ± 0.29 (mean \pm S.E.) years, respectively. The selected male and female animals were with their mean body weight of 19.35 ± 1.19 (mean \pm S.E.) kg and 14.10 ± 1.02 (mean \pm S.E.) kg, respectively. 2D mode echocardiographic examinations of animals showed normal structural conformations of various cardiac structures. On M-mode echocardiographic examinations in present study, most of the parameters were found to be non - significantly different between male and female animals, except posterior wall thickness percentage (PW%) value was found significantly higher in male animals than female animals. The values of right ventricular dimension in diastole (RVDd) of all animals, aortic root diameter (Ao) of female animals, ejection fraction (EF %) and cardiac output of male animals were found to be correlated negatively with body weight and body surface area of the animals, in M - mode echocardiography. Remaining all the parameters of M - mode echocardiographic examinations of the animals showed positive correlations with body weight and body surface area of the animals. In Doppler mode echocardiography, significantly higher values were recorded in the values of the A wave peak of mitral and tricuspid valves and aortic flow velocity in female animals than male animals. In male animals, all the Doppler mode measurements were found to be correlated negatively with body weight and body surface area. However, in female animals A peak of mitral valve (MVApeak) and aortic flow velocity (AV) showed positive correlation and remaining Doppler mode parameters correlated negatively with body weight and body surface area.

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15. Clinical studies on upper gastrointestinal endoscopy in dogs

Ajeet Kumar Singh and Vivek Malik

Endoscopic evaluation of oesophagus, stomach and duodenum was conducted in twelve dogs of either sex, belonging to different breeds and age groups presented to the Teaching Veterinary Clinical Complex (TVCC), Kothari Veterinary Hospital, College of Veterinary Science and A.H, U.P. Pandit Deen Dayal Upadhyay PashuChikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura (U.P.) with the history of reduction in food intake, regurgitation or chronic vomiting and not responding to symptomatic treatment. Diagnosis was made on the basis of clinical symptoms, haematobiochemical, radiographic, ultrasonographic, endoscopic and histopathologic evaluation and appropriate treatment was done as per need. The breeds of dogs reported with different gastro-intestinal problems which were subjected to endoscopy were Labrador Retriever (8), German Shepherd (2), Rottweiler (1) and Lhasa apso (1). There were seven males and five females among the animals investigated. The age of the dogs ranged from five months to ten years with an average value of 3.95 ± 0.87 (mean \pm S.E.) years. The body weight of dogs ranged from 10 kg to 35 kg with average values of 20.65 ± 2.56 (mean \pm S.E.) kg. The prominent clinical signs observed were chronic vomiting, regurgitation and anorexia. Glycopyrrolate (0.01 mg/kg) + xylazine (0.5 mg/kg) + butorphanol (0.2 mg/kg) combination was used as preanesthetics. Induction of anesthesia was achieved with propofol (2-4 mg/kg) till effect to intubate the animals. A surgical plane of anesthesia was maintained throughout the procedure with isoflurane (1.5-2.5%) in oxygen using a semiclosed system of inhalation anaesthesia. Left lateral recumbency was found appropriate for performing upper gastro-intestinal endoscopy in all animals under study. Upper gastro-intestinal endoscopy was performed using a multipurpose video endoscope with a working length of 140 cm, outer diameter of 7.9 mm and a channel diameter of 2.8 mm in twelve dogs. The different conditions diagnosed on endoscopy included gastric foreign body obstruction (2), oesophagitis (3), megaesophagus (2), oesophageal diverticulum (1), extramural mass and ulcer (1), haemorrhagic gastritis (2) and gastric adenocarcinoma (1). Radiographic (plain and contrast), ultrasonographic finding complemented the endoscopic examination. Foreign bodies were retrieved by endoscopy in two cases and management of clinical cases was carried out to achieve optimum success. Out of 12 clinical cases that have undergone endoscopic procedure, 10 cases showed significant clinical progress, however two cases could not survive. Endoscopy was found to be minimally invasive and efficient diagnostic tool to visualize precise location of the lesion and facilitated surgical manoeuvres to be undertaken in dogs.

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16. Studies on ultrasonography of adrenal glands of dogs

Akash and Gulshan Kumar

The present study was undertaken in dogs to standardize ultrasonographic examination protocol and generation of reference images. This study was conducted in two parts. Part I of the study was conducted on 18 apparently healthy dogs divided into three groups of six animals each namely, Group I, II and III. Part II comprised of patients reporting to the TVCC with symptoms like lethargy, anorexia vomiting, weight loss, bradycardia, weak femoral pulse and abdominal pain, or polydipsia, polyuria hyper-glycaemia etc. Ultrasonographic examination was done in lateral recumbency in all the dogs without using any sedative or anaesthetic agent. Ultrasonographic images of adrenal glands were recorded only in sagittal plane because adrenal glands could not be differentiated from the surrounding structures in

transverse plane. The left adrenal was best visualised by keeping transducer at left paralumbar fossa just behind the last rib. The right adrenal was best visualised by keeping transducer at right paralumbar fossa just behind the last rib. The length of left adrenal gland was 1.61 ± 0.17 cm in Group I, 2.2 ± 0.177 cm in Group II and 2.44 ± 0.30 cm in Group III. The cranial pole diameter of left adrenal gland was 0.32 ± 0.033 cm, 0.49 ± 0.048 cm and 0.54 ± 0.08 cm in Group I, II, III, respectively. The caudal pole diameter of left adrenal was 0.35 ± 0.037 cm, 0.54 ± 0.029 cm and 0.52 ± 0.08 cm in Groups I, II, III, respectively. The left adrenal gland was at a distance of 1.1 ± 0.107 cm from the skin in Group I, 2.02 ± 0.43 cm in Group II and 2.05 ± 0.25 cm in Group III. The volume of gland was 0.60 ± 0.155 ml, 1.85 ± 0.29 ml and 2.32 ± 0.52 ml in group I, II, III respectively. The length of right adrenal gland was 1.57 ± 0.12 cm, 2.1 ± 0.10 cm and 2.20 ± 0.27 cm in Groups I, II and III, respectively. The cranial pole diameter of right adrenal gland was 0.36 ± 0.03 cm, 0.48 ± 0.03 cm and 0.61 ± 0.07 cm in Groups I, II and III, respectively. The caudal pole diameter of right adrenal gland was 0.39 ± 0.04 cm, 0.54 ± 0.05 cm and 0.56 ± 0.08 cm in Groups I, II and III, respectively. The right adrenal gland was at a distance of 1.1 ± 0.13 cm from the skin in Group I, 1.56 ± 0.27 cm in Group II and 1.86 ± 0.21 cm in Group III. The volume of gland was 0.63 ± 0.14 ml, 1.68 ± 0.24 ml and 2.06 ± 0.44 ml in Groups I, II and III, respectively. The left adrenal gland appeared as a peanut shaped hypoechoic area, and was homogenous in all the groups. The outline of the left adrenal was clear. The difference in the echotexture of cortex and medulla were not discernible. The cranial and caudal poles of the left adrenal were easily discernible. The left adrenal gland was the first structure to appear in ultrasonogram beneath the skin, aorta appeared as an anechoic pulsating oblong structure below the left adrenal gland. The right adrenal gland was almost oval shaped hypoechoic structure as compared to the surrounding tissue, and was homogenous in all groups. The outline of the adrenal was smooth but the cortex could not be differentiated from the medulla as in case of the left adrenal. The right adrenal gland appeared either dorsal to the caudal vena cava or at the level of caudal vena cava. In Part II of the study, which comprised of clinical cases, the adrenal measurements and the echotexture of both the adrenal glands were well within the normal ranges and comparable to those of the Part I of the study. However, in one animal, the ultrasonographic examination of left adrenal revealed enlargement of caudal pole thickness (2.09 cm) and the echotexture of the gland was slightly heterogenous. So it was suspected for the adrenal carcinoma. Ultrasonography of adrenal glands in dogs does not require anaesthesia. Subcostal approach is the best approach to scan left and right adrenals, the scanning of adrenal can be easily done using a 7.5 MHz Linear transducer, in lateral recumbency by placing the probe caudal to the last rib and ventral to the lumbar process. The dimensions and the echotexture of the adrenal glands may assist in the diagnosis of pathologies of the adrenals or other organs.

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17. Canine oculoopathies- A clinical study on incidence, diagnosis and surgico-therapeutic management

Rajesh Kumar Pathak and R.P. Pandey

The study was done on the eyes of 18 normal dogs three age group i.e. 5 year (group III) and 74 clinical cases of the same age group presented at TVCC, Duvasu, Mathura from May 2017 to March 2018. Ophthalmic examination including B-mode sonography (except in normal dogs) was carried out. Hospital incidences of ocular affections were recorded and managed by therapeutic or surgical procedures as per need of the conditions. Total 20 ocular affections in 74 dogs (148 eyes) belonging to 16 different breeds were diagnosed. The mean value of STT for both eyes in group I, II, and III in normal dogs were 19.16 ± 0.60 , 17.08 ± 0.83 ,

17.83 ± 0.81 mm/min, respectively. The average mean value of IOP for both eyes in group I, II, and III in normal dogs were 15.57 ± 0.25, 15.63 ± 0.85, 16.82 ± 1.13 mm Hg, respectively. STT and IOP were significantly low in KCS and high in glaucoma cases respectively. Breed wise maximum hospital incidences of ocular affection was found in Labrador followed by Pug, Pomeranian, Rottweiler and GSD. Age wise distribution of cases revealed maximum incidence of affected eyes in the age group of 1 to 5 years (28 cases, 37.8%) followed by 5 year (21 cases, 28.3%). Sex wise distribution indicated that more males (48, 65%) were affected than females (26, 35%). Ophthalmological conditions were 4.34 % of total surgical cases, 1.40 % of total canine cases, and 0.6% of total hospital cases. Incidence of cherry eye was maximum (30 eyes, 20%), followed by cataract (25 eyes, 16.8%), KCS (22 eyes, 14.8%), corneal opacity (15 eyes, 10.1%), corneal ulcers (10 eyes, 6.75%), pigmentary keratopathy (12 eyes, 8.1%), glaucoma (4 eyes, 2.70%), traumatic injury (8 eyes, 5.40%), retinal detachment (4 eyes, 2.70%), vitreal degeneration (5 eyes, 3.33%), conjunctivitis (7 eyes, 4.72%), Blepharitis (4 eyes, 2.7%) and enophthalmos (3 eyes, 2.0%). One case each of proptosis, eye dermoid, aphakia, lens luxations, vitreous hemorrhage was also recorded. Therapeutic management protocol adopted was successful for corneal opacity, corneal ulcer, KCS, pigmentary keratopathy, conjunctivitis, glaucoma and blepharitis only. For cherry eye, Morgan's pocket technique was found better than excision. ECCE was attempted in 4 cases under adrenaline mydriasis and vision restoration could be achieved in only one. 15 dogs had more than one oculopathy and cases of diabetes and canine ehrlichiosis were also encountered.

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18. Radiographic, electrocardiographic and echocardiographic studies in goats

Vimlesh Kumar Sanjay Purohit

Present study was conducted on twelve apparently healthy goat free from cordiothoracic diseases. The animals were divided into two groups each containing 6 animals to evaluate various the cardiothoracic parameters. Mean ± S.E values of body weight and age were measured 11.83 ± 0.70 (range 10 - 15) kg, 3.75 ± 0.31 (range 3 - 5) month and 25.67 ± 1.73 (range 16-30) kg, 8.58 ± 0.95 (range 6.5 - 12) month in animals of the group-I and II, respectively. The purpose of this study was to establish the standard values (range) for parameters of the heart and thorax. Most of the parameters were found to be non - significantly different between group-I and group-II animals, except VHS- (Buchanan and Buchler (1995) and Ljubica et al. (2007) method); Cardiosternal contact, Cardiac height/T3- T5, Cardiac height + cardiac width /T3-T5 and right side castophrenic angle. Significant positive correlation with age and body weight were observed in 2TD/3, while it was significant negative in cardiac width / T3-T5. Positive correlation with age and body weight were observed in tracheal angle, cardiac width /thoracic height, cardiac height / R3-R5, cardiac height + cardiac width/R3-R5, CVC/AO, CVC/T4, CVC/R4, AO/R4 and castophrenic angle. While with body weight Cardiac width/R3-R5 and AO/T4 while other parameters show negative correlation with age and body weight were observed. In electrocardiographic studies, the mean ± S.E. values of the heart rate were 153.83 ± 5.85 (range 133 - 166) bpm and 142.17 ± 8.99 (113 - 166) bpm in the goats of the group I and II, respectively. Significant (p < 0.05) negative correlation was found in the value of heart rate with body weight and age of the goats. No significant difference was recorded in the mean values of electrographic amplitude and duration. Amplitude of P wave showed positive correlation with both age and body weight, R and T wave with only body weight while R wave was also showed the significant positive correlation with age while other parameter of amplitude are negative correlation with both age and body weight of the animal.

Significant positive correlation with both age and body weight were observed in duration in QRS complex, R-R interval while PR and S-T interval showed only with age while other parameter of duration are negative correlation with both age and body weight of the animal. In echocardiographic studies, B- mode, M-mode and Doppler mode examinations were performed to generate the reference values of echocardiographic parameters in goats. In Bmode echocardiographic examinations of animals showed normal structural conformations of various cardiac structures. On M-mode echocardiography, most of the parameters were found to be non-significantly different between group-I and II of the animals, except RVDd, cardiac output, and mitral velocity of A peak (MVA) in M - mode and doppler echocardiography, respectively. Positive correlation with age and body weight were observed in M - mode echocardiographic measurements of EPSS, RVDd, LVDd, PWD, IVSs, LVDs, EF%, S%, LVM, LA/AO, EDV, ESV, and cardiac output (CO) while with age stroke volume and body weight with PWs. Significant positive correlation with body weight were observed in stroke volume while other parameter was negative correlation with age and body weight of the animals. Significant positive correlation with age were observed in of pulse wave doppler echocardiographic measurements of tricuspid E peak and tricuspid E/A ratio. Positive correlation with age and body weight were observed in of pulse wave doppler echocardiographic measurements of peak mitral velocity (E peak, A peak) and aortic velocity. While with age ME/A, tricuspid A peak and with body weight tricuspid E peak, TE/A. while other parameter was negative correlation with age and body weight of the animals.

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19. Echocardiography of anaesthetised dogs undergoing surgical procedure

Amolak Sharma and Gulshan Kumar

This study was conducted on twelve dogs of different breeds and age (8 males and 4 females) reporting for various surgical procedures. A baseline (T0) echocardiography was performed before administration of any preanaesthetic or anaesthetic agent. The animals were then administered. Atropine and Xylazine and induced with ketamine and maintained with isoflurane. They were then subjected to echocardiography immediately after induction, 10 minutes into anaesthesia/surgery (T10) and at recovery. M-mode parameters were recorded. The baseline RVDd mean value was 0.82 ± 0.04 cm, whereas, it was 0.90 ± 0.04 cm immediately after induction (at 0 minute), 0.82 ± 0.03 cm at 10 minute and 0.78 ± 0.02 cm at recovery. The mean values of LVDd were 2.84 ± 0.16 cm at baseline, 2.57 ± 0.15 cm at 0 minutes, 2.43 ± 0.25 cm at 10 minutes and 2.24 ± 0.26 cm at recovery. The mean values of LVDs were 1.39 ± 0.11 cm at baseline, 1.57 ± 0.11 cm at 0 min, 1.75 ± 0.11 cm at 10 minute, 1.61 ± 0.11 cm at recovery phase. The mean values of IVSd were 0.77 ± 0.03 cm at baseline, 0.85 ± 0.03 cm at 0 minute, 0.80 ± 0.03 cm at 10 minute, and 0.77 ± 0.03 cm during recovery. None of these values varied significantly over different points of time. The mean values of IVSs were 0.99 ± 0.03 cm at baseline, 0.90 ± 0.03 cm at 0 minute, 0.82 ± 0.03 cm at 10 minutes and 0.78 ± 0.03 cm at recovery phase. The decrease in thickness of IVSs was significant ($P < 0.05$) at 0 minute, 10 minute and recovery as compared to the baseline values. However, variation was not significant variation between 10 and recovery phase, but there was significant decrease in thickness from 0 min. to 10 min. and recovery phase. The mean values of PWd were 0.93 ± 0.04 cm at baseline, 1.07 ± 0.04 cm at 0 minute, 0.93 ± 0.04 cm at 10 minute and 1.00 ± 0.05 cm at recovery phase with no significant variation in thickness at different time interval. The mean values of PWs were 1.35 ± 0.05 cm at baseline, 1.22 ± 0.06 cm at 0 minute, 1.10 ± 0.05 cm at 10 minute and 1.07 ± 0.05 cm at recovery. There was significant ($P < 0.05$). The mean values of FS were 44.83 ± 0.76 % at

baseline, 35.17 ± 0.94 % at 0 min, 33.13 ± 1.08 % at 10 min. and 28.83 ± 0.98 % at recovery phase. The decrease in the fractional parameters FS was observed at 0 min, 10 min and recovery phase; that at 0 min in comparison to baseline value and that at 10 min. and recovery phase in comparison to 0 min were significant ($P < 0.05$). The mean values of EF were 77.75 ± 0.72 % at baseline, 65.50 ± 0.81 % at 0 min, 59.00 ± 1.13 % at 10 min and 56.75 ± 0.79 % at recovery phase. The decrease in EF values observed at T0, T10 and recovery phase as compared to baseline value and that from 0 min. to 10 min. and recovery phase, were significant ($P < 0.05$). The mean values of LVM were 63.25 ± 7.25 gm at baseline, 62.20 ± 7.71 gm at 0 min, 60.58 ± 7.78 gm. at 10 minute and 60.67 ± 7.65 gm. These variations were non significant ($p > 0.05$). Perioperative echocardiography can be done in dogs when the site of the proposed surgical procedure is away from the site for echocardiography avoiding any risk of breach of asepsis and can also be used for monitoring the heart function during the surgical procedure.

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20. Echocardiography and cardiac biometry in Muzaffarnagari sheep

Anil Singh and R.P. Pandey

The present study was carried out on 12 apparently healthy Muzaffarnagari sheep maintained at ILFC, DUVASU, Mathura. The animals were divided into two groups each containing 6 animals to evaluate various Vertebral Heart Score (VHS) and Echocardiographic parameters. Mean \pm S.E values of body weight and age were measured (17.83 ± 0.70 , range 10 - 20) kg, (4.67 ± 0.33 , range 3 - 6) months and (37.58 ± 0.66 , range 25 - 40) kg, (13.83 ± 0.40 , range 12 -15) months in animals of the group-I and II, respectively. The purpose of this study was to establish the standard values for parameters studied. Most of the parameters were found to be non-significantly different between group I and group II animals, except VHS. The mean LA, SA and VHS in group I was 10.83 ± 0.23 cm, 6.53 ± 0.14 cm and 8.40 ± 0.14 v respectively, whereas in group II these mean values were 12.47 ± 0.23 cm, 7.55 ± 0.30 cm and 8.23 ± 0.21 v respectively. The mean LA and SA values showed positive correlation with age and body weight in both the groups, however, mean VHS value showed negative correlation. In echocardiographic studies, B-mode, M-mode and Doppler mode examinations were performed to generate the reference values of the parameters in Muzaffarnagari sheep. B-mode echocardiographic examinations of animals showed normal structural conformations of various cardiac structures. On M-mode echocardiography, The mean EPSS (mm), RVDd (mm), IVSd (mm), LVDd (mm), PWd (mm), IVSs (mm), LVDs (mm), LVDs (mm), PWs (mm), EF%, FS%, S%, LVM (gms), PW% of group I was 2.92 ± 0.52 , 10.98 ± 0.87 , 8.27 ± 0.31 , 24.00 ± 1.50 , 8.05 ± 0.52 , 11.00 ± 0.41 , 12.50 ± 1.02 , 10.98 ± 1.06 , 81.17 ± 1.99 , 48.17 ± 1.99 , 33.00 ± 6.08 , 40.33 ± 4.01 , 38.00 ± 10.97 respectively, whereas in group II these mean values were 2.03 ± 0.31 , 11.15 ± 0.91 , 7.87 ± 0.75 , 26.47 ± 0.91 , 7.90 ± 0.58 , 10.00 ± 1.06 , 13.28 ± 0.96 , 11.08 ± 0.47 , 82.67 ± 2.30 , 49.83 ± 2.41 , 46.17 ± 10.63 , 46.33 ± 6.21 , 45.00 ± 4.58 respectively. Most of the parameters were found to be non-significantly different between group I and II of the animals, except RVDd. Positive correlation with age and body weight were observed in M-mode echocardiographic measurements of RVDd, LVDd, IVSs, LVDs, PWs, EF%, FS%, S%, LVM, PW%, while other parameter was negative correlation with age and body weight of the animals. The mean left atrium diameter (LA), aorta diameter (AO) and LA/Ao in group I was 1.495 ± 0.49 , 1.462 ± 0.21 , 0.77 ± 0.03 respectively, whereas in group II these mean values were 1.462 ± 0.21 , 1.778 ± 0.81 and 0.83 ± 0.03 respectively. The left atrium diameter (LA), aorta diameter (AO) showed negative correlation with age and body weight in both the groups, however, LA/AO value showed positive correlation. The mean E peak, A peak, and E/A

value for peak mitral velocity and peak tricuspid velocity of group I was 0.51 ± 0.02 , 0.32 ± 0.01 , 1.59 ± 0.04 , 0.58 ± 0.03 , 0.35 ± 0.02 , 1.66 ± 0.08 respectively whereas in group II it was 0.51 ± 0.03 , 0.31 ± 0.03 , 1.66 ± 0.07 , 0.51 ± 0.02 , 0.32 ± 0.02 , 1.62 ± 0.06 respectively. The E peak and A peak for both peak mitral velocity and peak tricuspid velocity showed negative correlation with age and body weight, while the E/A for peak mitral velocity showed positive correlation with age and body weight whereas E/A for peak tricuspid velocity showed negative correlation with age and body weight. The mean pulmonary velocity and aortic velocity of group I was 0.69 ± 0.03 , 0.70 ± 0.02 where as in group II it was 0.64 ± 0.03 , 0.68 ± 0.01 respectively, however both pulmonary velocity and aorta velocity showed negative correlation with age and body weight. Color doppler study of the flow pattern through mitral, aortic, pulmonary and tricuspid valves revealed unidirectional flow through the valves was seen with absence of mosaic pattern signifying absence of regurgitation.

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21. Studies on thoracic radiography, electrocardiography and echocardiography in canine

Mamta Singh and Sanjay Purohit

The present study was conducted in two groups to establish the reference values of various cardiothoracic parameters. In group-I, total eight apparently healthy German shepherd dogs aged 36 - 72 months and body weight 22 - 33.2 kg free from cardio-thoracic diseases were selected, group-II contained three German shepherd dogs suffering with cardiothoracic diseases. Highly significant ($p \leq 0.05$) negative correlation of tracheal diameter/ T4 with age was observed. Significant positive ($p \leq 0.01$) correlation of cardiac inclination angle with body weight, significant negative ($p \leq 0.01$) correlation was seen in cardiac height and tracheal diameter with age and body weight while tracheal diameter/ T4 was found significantly negatively correlated with body weight. Positive correlation with age and body weight were observed in cardiosternal contact, cardiophrenic contact, tracheal angle, cardiac width/ T3-T5, cardiophrenic contact /cardiac height, CVC/ T4, CVC/ R4, AO/ T4, AO/ R4, 2TD/3, cardiac distance and right costophrenic angle. Cardiac width/ R3-R5, cardiac height + cardiac width / T3-T5 were positively correlated with bodyweight. Negative correlation with age and body weight were observed in VHS, cardiac height/thoracic height, cardiac width/thoracic height, cardiac height/ T3-T5, cardiac height/ R3-R5, cardiac height + cardiac width / R3-R5, cardiac height + cardiac width / thoracic height, CVC/AO, cardiac index, cardiothoracic ratio, leftside costophrenic angle and CdLA/R9. Cardiac width/ R3-R5, cardiac height + cardiac width / T3-T5 were negatively correlated with age. Heart rate showed negative correlation with age and body weight. Positive correlation of amplitude of S and T wave, QRS complex, duration of T wave, P-R interval and R-R interval with age and body weight; amplitude of Q wave, duration of P wave and Q-T interval with body weight was observed. Positive correlation of amplitude of S and T wave, QRS complex, duration of T wave, P-R interval and R-R interval with age and body weight; amplitude of Q wave, duration of P wave and Q-T interval with body weight was observed. Negative correlation of amplitude of P and R wave, S-T interval with age and body weight; amplitude of Q wave, duration of P wave and Q-T interval with age was observed. In B-mode examinations of animals showed normal structural conformations of various cardiac structures. On M-mode echocardiography, positive correlation with age and body weight was observed in Mmode echocardiographic measurements of EPSS, RDVd, PWd, PWs and LVM. IVSd was found positively correlated with age and stroke volume index (SVI), LA/Ao with body weight. Negative correlation with age and body weight was observed in M-mode echocardiographic measurements of LVDd,

IVSs, LVDs, EF %, FS %, S %, PW %, EDV, ESV, stroke volume, cardiac output and cardiac index. IVSd was found negatively correlated with body weight and stroke volume index (SVI), LA/Ao with age. Positive correlation with age and body weight was observed in Doppler echocardiographic measurements of Peak tricuspid velocity (A peak) while age was positively correlated with Peak mitral velocity (ME/A). Negative correlation with age and body weight was observed in Doppler echocardiographic measurements of Peak mitral velocity (A peak, P peak), Peak tricuspid velocity (E peak, TE/A), pulmonary velocity and aortic velocity while body weight was negatively correlated with Peak mitral velocity (ME/A).

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22. Studies on ultrasonography of the pancreas of dogs

Pradeep Kumar and Gulshan Kumar

The present study was undertaken to standardize ultrasonographic examination protocol in dogs and generation of reference images. This study was conducted in two parts. Part I of the study was conducted on 18 apparently healthy dogs, divided into three groups of six animals each namely, Group I comprising of animals of 01 to 09 months of age, Group II comprising of animals of more than 09 months to 06 years of age and Group III comprising of animals above six years of age. Part II comprised of patients reporting to the VCC with symptoms like lack of appetite, weight loss, acute vomiting, diarrhoea, dehydration, abdominal pain, tucked-up belly were enrolled to assess echoarchitecture and size. Ultrasonographic examination was done in lateral recumbency in all the dogs, but in some cases ultrasonography was also done in dorsal recumbency without using any anaesthesia or sedatives. In all the dogs (except one) not all the regions of the pancreas were visualized by ultrasound, only the thickest portion of the pancreas was identified by ultrasound and was measured by in-built caliper of the ultrasound machine. Ultrasonographic images of pancreas were recorded in sagittal plane and transverse plane. The right limb of pancreas was visualized in a transverse view in right cranial abdomen by placing the transducer just behind the last rib. Direct visualization of the pancreas was not possible. The right limb of the pancreas could be seen dorsomedial to the duodenum, ventral to the right kidney and lateral to portal vein. The left limb of pancreas could be identified in a sagittal plane in left cranial abdomen by keeping transducer midway between the xiphisternum and last rib beneath the subcostal arch. The left pancreatic lobe was visualized in a triangular region cranial to the left kidney, caudal to stomach, and medial to the spleen in left cranial abdomen. The pancreas appeared to be hypoechoic to isoechoic as compared to the surrounding structures in all the groups. The echoarchitecture of both limbs of pancreas were homogeneous in the animals of all the groups and lacked capsular marginations. The mean width of right limb of pancreas was 1.2 ± 0.03 cm in Group I, 1.31 ± 0.06 cm in Group II and 1.5 ± 0.06 cm in Group III, being highest in Group III and least in Group in I. The mean width of left limb of pancreas was 1.47 ± 0.16 cm in Group I, 1.77 ± 0.10 cm in Group II and 1.92 ± 0.15 cm in Group III, mean thickness of left limb of pancreas was 1.17 ± 0.06 cm in Group I, 1.35 ± 0.09 cm in Group II and 1.5 ± 0.10 in Group III. The mean width of left limb of pancreas was maximum in Group III and minimum in Group I. In the second part of the study, the echoarchitecture of both limbs of pancreas were different from those of part I of the study. In part II the pancreas were larger, with irregular demarcation and appeared hypoechoic whereas; the mesentery adjacent to it was hyperechoic. In one animal the width and thickness were 3.22 cm and 2.31 cm respectively, which were considerably higher than group II (mean width 1.77 ± 0.1 cm, mean thickness 1.35 ± 0.09 cm) and in another case the width and thickness were 3.57 cm and 2.62 cm respectively, which were considerably higher than the values of animals in group III (mean

width 1.92 ± 0.15 cm, mean thickness 1.5 ± 0.1 cm). These ultrasonographic changes corroborated the clinical findings and suggested pancreatic inflammation. On the basis of this study it can be concluded that pancreas in dogs can be scanned without sedation and anaesthesia. Subcostal approach is the best approach to scan right and left limb of pancreas, using a 7.5 MHz curvilinear transducer in lateral recumbency by placing the probe caudal to the last rib and ventral to the lumbar process. The dimensions and the echoarchitecture of the pancreas may assist in the diagnosis of pathologies of the pancreas or other organs.

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Ph.D. Degree Programme

1. Endoscopic, ultrasonographic and urinalytic studies on female bubaline urinary system

Gulshan Kumar and R.P. Pandey

This study was undertaken in female buffaloes to standardize ultrasonographic and urethroscoposcopic examination protocol and generation of reference images and their compilation, urinalysis for physical and chemical attributes in normal buffaloes and identification, diagnosis and classification of affections of urinary system on the basis of urinalysis, urethroscopy and ultrasonography in clinical cases. Therefore, this study was conducted in two parts. Part I of the study was conducted on 30 adult female farm buffaloes divided into three groups of ten animals each namely, Groups I, II and III. Part II comprised of females buffaloes possibly reporting for urinary tract affections. The left kidney and urinary bladder could be examined per-rectum, while the right kidney could be scanned transcutaneously through the right lumbar and paralumbar region. The urinary bladder could also be scanned transcutaneously through the ischio-rectal fossa. In the left kidney, the vertical diameter of the kidney was 7.4 ± 0.435 cm, 7.9 ± 0.437 cm and 6.9 ± 0.32 cm in Group I, II and III, respectively. The horizontal diameter of the kidney was 8.9 ± 0.732 cm, 8.6 ± 0.433 cm and 8.6 ± 0.45 cm in Group I, II and III, respectively. The left kidney was therefore, more wide than thick. The vertical diameter of the sinus was 3.7 ± 0.279 cm, 3.7 ± 0.23 cm and 3.7 ± 0.37 cm in Group I, II and III, respectively. The horizontal diameter of the sinus was 5.4 ± 0.719 cm, 4.3 ± 0.312 cm and 4.3 ± 0.36 cm in Group I, II and III, respectively. The dorsal dimension of the corticomedullary tissue was 1.8 ± 0.12 cm, 2.0 ± 0.162 cm and 1.9 ± 0.14 cm in Group I, II and III, respectively and the lateral dimension of the corticomedullary tissue was 2.1 ± 0.134 cm, 2.1 ± 0.125 cm and 2.3 ± 0.25 cm in Group I, II and III, respectively. In the right kidney, the vertical diameter of the kidney was 6.4 ± 0.41 cm, 7.0 ± 0.211 cm and 7.2 ± 0.407 cm in Group I, II and III, respectively. The horizontal diameter of the kidney was 7.9 ± 0.53 cm, 8.3 ± 0.571 cm and 9.0 ± 0.44 cm in Group I, II and III, respectively. The vertical diameter of the sinus was 3.4 ± 0.298 cm, 3.8 ± 0.292 cm and 3.4 ± 0.294 cm in Group I, II and III, respectively. The horizontal diameter of the sinus was 5.0 ± 0.547 cm, 4.6 ± 0.401 cm and 4.3 ± 0.36 cm in Group I, II and III, respectively. The dorsal dimension of the corticomedullary tissue was 1.8 ± 0.106 cm, 2.0 ± 0.087 cm and 2.0 ± 0.145 cm in Group I, II and III, respectively and the lateral dimension of the corticomedullary tissue was 2.0 ± 0.221 cm, 2.3 ± 0.154 cm and 2.1 ± 0.137 cm in Group I, II and III, respectively. The bladder wall measured between 0.3 and 2.1 cm. The echogenicity of the various structures of the kidney was variable. The renal outline was clear but the cortex could not be differentiated from the medulla with the 3.5 MHz transducer. The corticomedullary tissue appeared homogenously hypoechoic as compared to the surrounding tissue as well as the sinus of the kidney. The sinus was hyperechoic and irregularly shaped. The medullary pyramids appeared as round to triangular structures in the renal parenchyma and were hypoechoic to anechoic as compared to the corticomedullary tissue. The renal calyces were not seen. In some frames hypoechoic structures were visible within the sinus and were interpreted as vasculature or collecting tubules which could not be differentiated. The kidney appeared to be enveloped by hyperechoic perirenal fat surrounding the capsule. The renal cortex and medulla could be differentiated and had better resolution with a 5-10 MHz transducer. However, the penetration was greatly limited. The urinary bladder appeared as an anechoic almost circular area on an ultrasonogram in transverse scan and pear shaped in longitudinal scan. The tissue area beyond the bladder appeared hyper echoic due to acoustic enhancement resulting from the sound waves travelling through fluid contained in the

bladder. The bladder wall was distinctly hyperechoic with uniform thickness. On endoscopic examination the mucosal folds of the bladder were visualised in case of evacuated bladders. The urethral mucosa appeared glistening, smooth, off-white to yellowish in colour. The vessels traversing the mucosa could also be visualised upon closing in. A cicatrized remnant of the opening of the urachus could be seen immediately in the fundus region. In full bladders, the mucosal folds were less marked with a smooth pale pink mucosa. The mucosa of the urethra was glistening, smooth, uniformly pink and appeared to be collapsing over what appeared like longitudinal mucosal folds while the endoscope was withdrawn. The specific gravity of the urine varied between 1.000 and 1.020 whereas, the pH values varied between 7.0 and 8.5. urobilinogen and protein were present in all buffaloes. In clinical cases, one case was diagnosed as having haemorrhagic cystitis, and post-partum vaginal trauma in addition to having retained placenta; two cases were diagnosed as having urinary bladder tumour, one case was diagnosed as having hydronephrosis and a cyst beneath the rectum, and two cases were diagnosed as having purulent cystitis and urethritis with renal cysts, on the basis of the findings of urinalysis, ultrasonography and urethroscopy. Ultrasonography does not require any chemical restraint whereas endoscopy requires epidural anaesthesia. The urinalysis, ultrasonographic and endoscopic examination of the urinary tract in female buffaloes can help in early diagnosis of possible diseases of kidney urethra and urinary bladder in buffaloes.

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2. Radiographic and ultrasonographic study of limbs with reference to diagnosis of lameness in buffaloes

Ranvir Singh and R. P. Pandey

In the present study, mean length of metacarpal of animals was 18.98 ± 0.21 cm, and in metatarsal 23.77 ± 0.14 cm. Amongst the medial and lateral phalanges of fore and hind limbs, it was observed that the length of the first lateral phalanx of hind limb was higher than the length of lateral phalanx of the fore limb, the values of the length of second lateral phalanx of fore limb was significantly higher than the second lateral phalanx of the hind limb. Mean \pm SE values of radiographic total bone diameter (D) (mm) of bones of distal fore and hind limb of adult buffalo ranged from 21.99 ± 0.58 to 37.92 ± 1.12 mm. Diameter of metacarpal was not significantly different from diameter of metatarsal. Statistical analysis between the different bones of fore limbs and hind limbs showed that the values of radiographic bone diameter of first medial phalanx and first lateral phalanx of fore limb was significantly higher than that of the first medial phalanx and first lateral phalanx of hind limbs. The diameter of third medial phalanx and third lateral phalanx of fore limbs was significantly higher than that of the third medial phalanx and third lateral phalanx of hind limb. It could be due the fact that fore limbs bear more body weight in comparison to hind limbs. Between the medial phalanges and lateral phalanges, the total bone diameter of the second medial phalanx of both fore and hind limb were significantly higher than that of the second lateral phalanx of the same limb. In third phalanx the third medial phalanx of fore limb as well as hind limb was significantly lesser than that of the third lateral phalanx bone of fore and hind limbs, respectively. Mean \pm SE values of radiographic width of cortex and medulla of distal bones of adult buffaloes ranged from 8.74 ± 0.32 to 15.16 ± 0.32 mm and 13.01 ± 0.16 to 22.93 ± 1.09 mm respectively. Cortex to medulla ratio of different bones studied ranged from 0.52 to 0.78. Comparison between cortex to medulla ratio of both limbs depicted that the values of first medial phalanx and first lateral phalanx of fore limbs were significantly lesser than that of hind limb. Additionally, comparison between the medial and lateral side of bones revealed significantly lesser values

of first lateral phalanx than of first medial phalanx of hind limbs. Similarly, the values of second lateral phalanx was significantly lesser than the mean CM ratio of second medial phalanx, when compared in fore limbs. Cortex to diameter ratio of different distal bones of fore and hind limbs in the range of 0.31 to 0.43. The cortex to diameter ratio of first medial phalanx of hind limb was significantly higher than cortex to diameter ratio of first lateral phalanx. Similarly, the cortex to diameter ratio of second medial phalanx of hind limb was significantly higher than cortex to diameter ratio of second lateral phalanx. Comparison amongst different bones of fore and hind limbs the cortex to diameter ratio of first medial phalanx and first lateral phalanx of hind limb were significantly higher than cortex to diameter ratio of first medial phalanx and first lateral phalanx of fore limb respectively. Mean values of joint space measurements may act as reference values as there is paucity of reference values of joint space in animals. Radiographic evaluation of each limb was performed aiming at the identification of possible lesions that affected the digital extensors and flexor structures, consequently causing lameness. The ultrasound examination of the musculotendinous and ligamentous structures of buffalo distal limbs was performed. The examination on carcass limbs, was difficult to be performed due to certain technical artifacts absence of lividity, vascularity and muscle tone. Soft tissue relaxation also contributed to this observed difficulty. This all put together, made echolocation and identification of various structures difficult when done on carcass limb. It was observed that the absence of blood flow led to the presence of other technical artifacts, where the formation of anechoic circular areas could be interpreted as fluid accumulation in the tissues or the presence of a blood vessel at the site examined. This occurrence was observed mainly in the ultrasound evaluations of the interosseous muscle and the accessory ligament of the deep digital flexor tendon. It is not possible to attribute to the observed image pattern the existence of musculo-tendinous lesions. As an abstract observation it can be said that in live animals, identification and examination of echotexture of various musculo tendinous structures is easy. The structures visualized on ultrasound examination of the distal portion of the bubaline limbs included the superficial and deep digital flexor tendons, the interosseous muscle, the accessory ligament of the deep digital flexor tendon. Ultrasonographic assessment of bovine limbs was done by dividing the distal limbs into five transverse and two sagittal zones, the proximal third, the middle third, the distal third of the metacarpals or metatarsals, the area proximal to the dew claws, and that between the dew claws and the heel transversely and dorsal and palmer/planter surface in sagittal plane. Since buffalo is not an athletic animal and there is little possibility of frequent strain or sprain to occur. Lack of awareness amongst farmers about the indirect enormous economic impact of lameness in buffaloes in the form of reduced fertility and reduced production also needs to be addressed.

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M.Sc. Degree Programme

1. Analysis of polymorphic and expression pattern of heat shock gene and heat shock transcription factor-1 in goat

Varun Kumar Singh and P.K. Rout

The present study was designed to investigate the polymorphic pattern of HSP70 gene in Jamunapari goats and HSF-1 gene in Jamunapari and Barbari goats. Expression pattern of HSP70 gene was analyzed in different tissues viz. spleen, heart, kidney and brain during heat stress. Blood samples from 30 Jamunapari goats were collected in 10 ml vacutainer tubes using EDTA and DNA was isolated to analyze the polymorphic pattern using HRM. RNA was extracted from the tissues of organs viz. spleen, heart, kidney and brain of Jamunapari goats and the expression pattern of HSP70 gene was analyzed by Real Time qPCR. Blood samples from Barbari and Jamunapari goats was used to isolate DNA and to analyze the polymorphic pattern of HSF-1 gene by PCR.

The results of the HRM analyses showed 5 different genotypes of HSP70 gene among the analyzed samples. Relative quantification by RT-PCR indicated that the Hsp70 gene expression was almost similar in brain and heart. Its expression was about 6 folds higher in kidney as compared to that in heart and brain. Hsp70 expression was found highest in spleen which was about 20 folds greater than that in heart and brain and about 3 folds higher than that in kidney. Genotyping of different goat breed was carried out to analyze polymorphism in HSF-1 gene by PCR. The size of amplified product of HSF-1 gene was observed as 220 bp. Sequencing of the product was carried out and no difference was observed in all the analyzed samples in present study.

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2. Studies on oxidative stress and its role in mastitis with special reference to *Staphylococcus aureus*

Lalita Sharma and Amit Kumar Verma

Mastitis is considered as one of the most common diseases in dairy cows, causing significant economic losses to the dairy industry. Oxidative stress is believed to be a primary factor in various cattle diseases including mastitis, but scanty literature is available on its role in mastitis. *Staphylococcus aureus* is the most common bacterial pathogen causing acute as well as chronic mastitis in both cattle and buffaloes. The present study was carried out to estimate the level of oxidative stress in clinical and subclinical mastitis cases in dairy animals (cattle and buffalo); to compare the level of oxidative stress with apparently healthy mastitis free lactating animals; to detect the prevalence of *Staphylococcus aureus* infection in mastitis cases; and to assess the bacterial antibiotic susceptibility pattern of *Staphylococcus aureus*. Milk and blood samples were collected from 10 normal cows and 20 cows each with clinical and subclinical mastitis from dairy cows in Mathura, India. This study examined urea, activities of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), lipid peroxidase (LPO) and glutathione peroxidase (GPx) in the serum samples, and activities of lipid peroxidase (LPO) and glutathione peroxidase (GPx) in milk of lactating dairy cows with clinical and subclinical mastitis. Milk samples were also processed for isolation and identification of *S. aureus* using biochemical tests. All the confirmed 5. *aureus* isolated under study were examined for their antibiotic resistance pattern by disc diffusion method using 38 antibiotic discs. *Staphylococcus aureus* were isolated from only 27 samples showing the overall

incidence of *Staphylococcus aureus* in clinical as well as sub clinical mastitis as 33.75%. The incidence of *Staphylococcus aureus* was higher (50.00%) in clinical mastitis in comparison to that of subclinical mastitis (17.50%). The results revealed that the incidences of *Staphylococcus aureus* in clinical as well as sub-clinical mastitis were higher in cattle in comparison to buffaloes. Drug sensitivity revealed 100% resistance against penicillin followed by vancomycin (88.89%), nalidixic acid (77.78%), cefixime, methicillin, novobiocin (66.67% each), amoxyclav, colistin, pipemidic acid (55.56% each), ofloxacin, streptomycin, sulphamethizole (44.44% each), ampicillin/sulbactam, cefalexin, cefazolin, cefaperazone, enrofloxacin, floxidin, meropenem (33.33% each), cefuroxim, ciprofloxacin, clindamycin, gentamicin, levofloxacin, norfloxacin, tetracycline (22.22% each). Eighteen isolates were found to be methicillin-resistant, while the remaining (09) were methicillin-susceptible. Similarly, twenty four *S. aureus* isolates were intermediate to vancomycin, while 03 were vancomycin susceptible. None of the isolate was resistant to vancomycin. The occurrence of lipid peroxidation as revealed by elevated blood and milk lipid peroxidase levels, in present study, indicated the involvement of oxidative stress and the possible oxidative damage in clinical and subclinical mastitis in dairy cows. From the present study, it may be concluded that supplementation of antioxidants such as vitamins and minerals may enhance the resistance against subclinical and clinical mastitis in the dairy cows. The higher prevalence of methicillin resistant bacteria clearly indicate increase in drug resistance. Thus, the findings of the study are useful for formulating specific control programs for bovine mastitis caused by *S. aureus* in this region.

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3. Genetic polymorphism study of DGAT1& ABCG2 gene in Sahiwal and Haryana cattle

Anita Sharma and Madhu Tiwari

Acy-CoA: diacylglycerol acyltransferases I(DGAT1) and ATP binding cassette sub family G member 2(ABCG2) are two strong candidate genes for milk production traits. Diacylglycerol acyltransferase (DGAT) plays a central role in formation of lipid in different tissues of biological body and metabolism of cellular glycerolipids. DGAT catalyzes the final step in triacylglycerol (TAG) biosynthesis by converting diacylglycerol (DAG) and fatty acyl-coenzyme A (CoA) into triacylglycerol. A quantitative trait loci (QTLs) for milk production traits were mapped to the centromeric region of the bovine chromosome 14 (BTA14). ATP-binding cassette transporter ABCG2 is a member of the ABC transporter superfamily that actively extrudes xenotoxins from cells and is a major determinant of the bioavailability of many compounds. ABCG2 QTL for milk production trait on BTA 6 was found by many workers in various populations of *Bos taurus*. There is strong evidence that a polymorphism of the ATP-binding cassette superfamily G member 2 transporter (ABCG2) gene located on BTA 6 is associated with effects on milk yield and composition in the Holstein cattle. The genomic DNA was isolated from 50 blood samples of Sahiwal & Haryana cattle bred and the purity of DNA was checked spectrophotometrically and by agarose gel electrophoresis. A 491 & 292 bp amplified PCR product was obtained by using optimum primers and RFLP was performed by *EaeI* & *PstI* restriction endonucleases. The *EaeI*/PCR-RFLP assay showed the monomorphic nature of DGAT1 gene due to the presence of only KK genotype in screened population. The genotypic frequency of KK genotype was found to be 1 and the allelic frequencies of K allele was also observed to be 1. The *PstI*/PCR-RFLP assay showed the monomorphic nature of ABCG2 gene due to the presence of only AA genotype in screened population. The genotypic frequency of AA genotype was found to be 1 and the allelic

frequencies of A allele was also observed to be 1. By the present study it can be concluded that the Sahiwal & Haryana cattle had only wild type genotype for DGAT1 and ABCG2 genes.

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4. Genetic polymorphism of Kappa-Casein and Beta-Lactoglobulin genes in Sahiwal, Haryana cattle and Murrah buffalo

Abhishek Pal and Madhu Tiwari

In the present study, identification of Kappa-Casein (K-Cs) and Beta- Lactoglobulin (B-Lg) gene polymorphism and its association with milk production traits was undertaken in 130 animal including Sahiwal (n=50), Haryana (n=50) cattle and Murrah (n=30) buffalo maintained at ILFC, DUVASU, Mathura by using PCR-RFLP technique. The amplified fragments of the K-Cs and B-LG genes revealed 350 and 247 bp, respectively. The K-Cs/Hinfi PCR-RFLP assay of K-Cs gene revealed three types of genotypes AA, BB and AB with genotypic frequencies 61.0%, 23.0% and 16.0%, respectively and allelic frequency of A and B alleles as 0.69 and 0.31, respectively. The screened buffalo population used in the present study was monomorphic in nature with only B allele (1.0). Similarly, B-Lg /HaeIII PCR-RFLP assay of B-Lg gene revealed three types of genotypes AA, AB and BB with genotypic frequencies 19.0%, 20.0% and 61.0%, respectively and allelic frequency as 0.29 (A) and 0.71 (B). The screened buffalo population was monomorphic for this gene also with both types of alleles A (0.50) and B (0.50). On χ^2 analysis, screened Sahiwal and Haryana cattle population was not found in Hardy- Weinberg equilibrium. Association studies of K-Cs/Hinfi genotypes with milk production traits showed that gestation period (GP), lactation period (LP), calving interval (CI), peak yield (PY) and days to reach peak yield (DRPY) had non-significant variation among all the three genotype over first and second lactation. However, a significant difference was found among three genotypes for total milk yield (TMY), dry period (DP) and milk yield in 300 days (MY300). The BB genotype showed higher milk yield value than AA and AB in first lactation. GP, LP, DP, CI, PY and DRPY showed non- significant variation among three genotypes over first and second lactation on association studies of B-Lg/HaeIII genotypes with milk production traits but significant difference ($P < 0.00$) were found among three genotype for TMY and MY300 with AA genotype showing higher milk yield value than BB and AB in first lactation.

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5. A comparative study on expression profile of HSP genes during different seasons in goat breeds

Devendra Kumar and Brijesh Yadav

Goats are most adapted species to all type agro-climatic conditions and play important role in rural economy. Livestock species experiences stress due to thermal challenges. Environmental stressors such as hypothermia or hyperthermia affect behavioral, physiological and molecular mechanisms. HSPs are highly conserved groups of proteins that expressed under various kinds of stresses and considered as potential indicator for animal adaptation. HSPs are group of well conserved proteins across the species that are expressed under various kinds of stresses and these are considered as potential indicator of animal adaptation. Present study was study was conducted in three different seasons viz. winter (January), Thermo-neutral (March) and summer (May) to examine comparative expression profile of HSP genes (HSP60, 70 and 90). Five animals from each breed of semi-arid region were selected for this study viz. Barbari, Sirohi and Jhakrana. All of the animals were regularly

monitored and similar management inputs were provided during the experimental period. The climatological data and physiological parameters during the experimental period were recorded. Recording of physiological parameters viz. respiratory rate, rectal temperature and collection of blood samples was done at 09:00 to 10:00 hours in all the seasons during experiment. During the experimental period the temperature humidity index (THI) in winter (January), thermo-neutral (March) and summer (May) was 59.63, 72.1, 81.63 respectively. Blood samples were collected and PBMCs were separated. Total RNA was isolated and reverse transcribed to cDNA. Real time PCR was applied to investigate the relative mRNA expression of HSP genes. The respiratory rate was found to be higher ($P<0.01$) during summer season as compared to winter and thermo-neutral season in all the goat breeds. The rectal temperature was found to be similar during all seasons. Expression data showed significant increase in mRNA expression of HSP genes during summer season as compared to winter and thermoneutral season. Fold change in relative mRNA expression of hsp60, HSP70 and HSP90 was significantly higher ($P<0.01$) in Sirohi breed as compared to Jhakrana and Barabri. It can be concluded that Barbari breed possessed better heat tolerance followed by Sirohi and Jhakrana.

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6. Molecular and functional characterization of voltage gated sodium channels in sperms of bulls

Dharmendra Singh Chauhan and Dilip Kumar Swain

In the current study, molecular and functional characterisation of Nav 1.8 and Nav 1.4 were carried out in spermatozoa of Haryana bulls. Forty ejaculates were collected from four bulls and were used for series of experiments. Immunoblotting and immunocytochemistry were employed for the molecular characterisation of Nav 1.8 and Nav 1.4. Immunoblotting reported a single band of 260kDa for Nav 1.8 and 220 kDa for Nav 1.4 confirming the presence of Nav 1.8 and Nav 1.4 in Haryana bull spermatozoa. Positive immunoreactivity was seen in head, neck, middle piece and tail parts of the spermatozoa for both Nav 1.8 and Nav 1.4. Selective blocking of NaV 1.8 by using the selective blocker A-803467 at 6 and 8 μM concentration significantly ($P<0.05$) decreased sperm motility in a time dependent manner, whereas, blocking with high concentration (10 and 15 μM) induced spermatozoa hypermotility after 2 hrs of incubation. Immunoblotting and immunocytochemistry confirmed the presence of tyrosine phosphorylated proteins along with B- pattern of spermatozoa in Chlortetracycline assay confirming the hypermotility was due to selective blocking of Nav 1.8 with high concentration. Treatment with veratridine showed concentration and time dependent increase in sperm motility which remained sustained up to 6 hrs. Veratridine at high concentration (10 and 15 μM) induced spermatozoa hypermotility after 2 hrs of incubation. Immunoblotting and immunocytochemistry confirmed the presence of tyrosine phosphorylated proteins along with B- pattern of spermatozoa in Chlortetracycline assay confirming the hypermotility was due to activation of Nav channels. The induction of hypermotility due to antagonist and agonist were mediated by different sets of tyrosine phosphorylated proteins. Both antagonist and agonist regulated high mitochondrial transmembrane potential in concentration and time dependent manner. At high concentrations of both antagonist and agonist (10 and 15 μM) induced bent neck condition in the spermatozoa along with lost membrane integrity (HOST negative). Treatment of spermatozoa with both antagonist and CPA significantly reduced progressive motility in a dose and time dependent manner. From the study, it was concluded that, voltage gated sodium channels are present in Haryana bull spermatozoa and are involved in regulation of

sperm motility along with spermatozoa function in terms of mitochondrial transmembrane potential. Further studies are required to have an insight in to the mechanism of action of NaV channels in regulation of Calcium fluxing, pH regulation and process of capacitation.

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7. Purification and characterization of urinary antimicrobial peptides of goat

Vaibhav Tomar and Rajesh Nigam

The present study was aimed for identifying urinary antimicrobial peptides in healthy Goats. In this study, urine samples were collected from 30 healthy non-pregnant goats and evaluated for physio-chemical parameters. The urine samples were pooled together and protease inhibitor was added. Thereafter filtered through 0.2 μ membrane filter and stored at 40C till further analysis. The samples were subjected to Dia-filtration using Amicon 10 kDa Fiter to concentrated proteins in urine and proteins were extracted by ion exchange chromatography and acetone precipitation. Cationic peptides were extracted from concentrated urine by using weak Cation exchange beads from the dia-filtered urine. Cationic and anionic peptides fractions obtained were estimated by different method for determining the recovery of protein. The results of protein estimation by different method revealed highest values of protein by BCA method compared to Lowry and Bradford method giving protein ranged between 0.029 μ g/ μ l to 6.03 μ g/ μ l. Further cationic peptides were confirmed by AU-PAGE revealed 5 bands and the anionic fraction by SDS PAGE revealed 12 bands of molecular weight ranged from 10.0 to 93 kDa proteins. Out of these one protein band from each were identified by MALDI-TOF MS analysis revealed the presence of significant proteins: Zinc Transporter 9 (ZnT9) in AU-PAGE HAUS augmin-like complex subunit 5 (HAUS5) in SDS-PAGE. The antimicrobial activity of both cationic and anionic fractions were examined by Radial Diffusion and microtiter broth dilution method. The cationic fractions revealed 23 and 26 mm zones of inhibition against *S. aureus*. and *E. coli*. respectively and MIC of cationic peptides observed was 0.039 μ g/ μ l and 0.0199 μ g/ μ l against *E. coli* and *S. aureus* respectively. It can be concluded from the study that the cationic peptides present in the goat urine may contribute in antimicrobial activity of goat urine.

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8. Genetic polymorphic studies of fecundity genes in Muzaffarnagari sheep

Parul Singh and Deepak Sharma

Three major genes whose mutations can increase ovulation rate have been discovered, and all related to the transforming growth factor β (TGF β) superfamily. Different mutations in the Growth differentiation factor (GDF9), Bone morphogenetic protein (BMP15) also known as growth differentiation factor 9B (GDF9B), and the mutant of FecB of Bone morphogenetic protein receptor 1B (BMPR1B) had an additive effect on ovulation rate in sheep. The aim of the current study was to determine the mutations in GDF9, BMP15, and BMPR1B genes & the possible polymorphism in the Muzaffarnagari sheep breeds. DNA was isolated from blood Samples were collected from the Muzaffarnagari sheep breeds (n=200) during 2017- 18 maintained at LFC of DUVASU, Mathura, U.P. The PCR products of 190bp of BMPR1B (part of exon-8), 141bp of BMP15 (part of exon-2) and, 139bp of GDF9 (part of exon-1) were amplified using reported primers. The PCR products of the genes were digested with *Av*II restriction enzymes for the BMPR1B (FecB) gene giving uncut of 190bp, *Hin*II for the BMP15

(FecX) gene producing 111bp and 141bp, and with DdeI for the GDF9 (FecG) gene giving 105 bp and 34 bp products. The results showed no difference in the band patterns of digested products as only the wild-type alleles (++) were detected in the loci of BMP15, BMPR1B genes & only mutant type in GDF9 gene, and were found monomorphic in nature and none of the sheep carried heterozygous genotype for FecB, FecX, and FecG variants in this breed. Our study revealed that Muzzafarnagari sheep population in the present study was homozygous and non-carrier of FecB, FecX, and FecG mutation. Further studied can be conducted with different regions of these genes to evaluate the relationship of different genotypes with litter size and ovulation rate.

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9. Heat shock protein 70 and redox status in fluid and spermatozoa in different segment of buck epididymis

Akhilesh Kumar and Brijesh Yadav

The present study was carried out to estimate antioxidative status and HSP70 level in spermatozoa and fluid of different segments of buck epididymis. The experiment was carried out on the testes collected from sexually mature and healthy buck aged 2-3 years. Within one hour after the slaughter the testes were brought to the laboratory in pre-chilled phosphate buffer saline. The entire epididymis was separated from testes which was demarcated and cut into caput (head), corpus (body) and cauda (tail). The epididymal fluid containing spermatozoa was diluted and used for further analysis. Tissue samples were stored in 10% formalin for histological examinations. After assessing the gross features of the spermatozoa, objective analysis was carried out using CASA and sperm viability was examined. The luminal fluid and spermatozoa were separated and used for further analysis. The kinematic pattern of spermatozoa in different segments of epididymis varied significantly ($p < 0.05$). Although the variation was less between caput and corpus but it was found to be more pronounced in cauda. Head to head adhesion was observed in the spermatozoa of corpus region as compared to caput and cauda region. The cytoplasmic droplets were found to be present at proximal, middle and distal part of the tail of the sperm in caput, corpus and cauda epididymis, respectively. The luminal epithelial lining was longer in corpus region as compared to caput and cauda region. A definite pattern of redox status was not observed either in the fluid or in the spermatozoa of different segments of epididymis. The HSP70 concentration in fluids was significantly ($p < 0.01$) higher in corpus epididymis as compared to caput and cauda epididymis. The HSP70 concentration in sperm lysate was found to be significantly ($p < 0.01$) higher in caput epididymis as compared to corpus and cauda epididymis. Similarly, relative expression of HSP70 mRNA decreased significantly ($p < 0.01$) in the spermatozoa of corpus and cauda epididymis as compared to caput epididymis. Immunoblot confirmed the presence of HSP70 in the sperm of all the three segments of epididymis. HSP70 was found to be localized on the surface of the acrosomal cap region of the spermatozoa in cauda epididymal only. The results confirmed that the antioxidative status and HSP 70 concentration in spermatozoa vary in the fluid and spermatozoa of different segments of epididymis.

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10. Isolation, identification and virulence typing of *Escherichia coli* from clinical cases (animals and humans) and their surroundings

Mini Kanchan and Udit Jain

The purpose of study was to determine molecular characteristics, biofilm production and antimicrobial pattern of *E. coli* in animals, humans and their environment. Out of total 200 samples [animals (n=70), humans (n=65) & environment samples (n=65), 35 *E. coli* isolates were obtained. Out of 35 *E. coli* isolates from various sources, 2 pathogenic *E. coli* (only VTEC, no EPEC) were obtained, which was 5.71% of the total *E. coli* and 1% of the total samples collected. Percentage of pathogenic *E. coli* (VTEC) from animal, human & environmental samples were 6.89%, 0% & 0%, respectively. From animal samples, % pathogenic *E. coli* (VTEC) in cattle faeces and urine were 10% & 9.09%, respectively. No pathogenic *E. coli* was found in human and environment samples. A total of 35 isolates of *E. coli* were screened by multiplex PCR for virulence to detect the presence of *stx1*, *stx2* (variants of *stx2*), *eaeA*, *hlyA* and *rfb O157* gene. Only one (4.45%) isolate was found carrying the *stx2* gene alone while the other isolate was found carrying two or more pathogenic genes (*stx1*, *stx2* and *hlyA*) in combinations. All samples were found negative for *eaeA* and *rfb O157* gene. The overall percent positivity by phenotypic detection of biofilm producing *E. coli* by CRA, TM & TCP method showed biofilm formation 62.85%, 62.85% and 74.28% respectively. Biofilm producing *E. coli* isolates showing the very high prevalence i.e. 45.71% (16/35) for the *luxS* gene which is confirmation of biofilm production Also, the Antibiogram testing has been done on all positive *E. coli* (VTEC) isolates using 18 antibiotics. After testing, result revealed that imipenem (100%) showed highest sensitivity. The sensitivity of all others was below 50%. While antibiotics like vancomycin (100%), linezolid (100%) ampicillin (97.14%) erythromycin (88.57%), and oxacillin (85.71%) showed highest resistance towards the *E. coli* isolates. The multi drug resistance of biofilm producing *E. coli* strains showing maximum to ampicillin (97.14%) followed by erythromycin (88.57%), gentamicin (88.57%) and co-trimoxazole (57.14%).

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11. Isolation and characterization of extended-spectrum β -lactamase producing organism from bovine female reproductive tract with special reference to *E. coli*

Samiksha Agarwal and Ajay Pratap Singh

Extended-spectrum β -lactamase (ESBL) producing Enterobacteriaceae, resistance against third- generation cephalosporin is a serious public health concern. The present study was conducted to obtain the prevalence of ESBL positive organism in bovine uterine infection. The study population included non-repetitive 70 cow and buffaloes from Mathura and adjoining region. Uterine samples were collected from animals with the history of clinical endometritis and processed in the laboratory for bacterial isolation. Bacterial isolates were characterized by biochemical test for genus and species determination. Antimicrobial susceptibility tests were performed for initial screening of ESBL organism. Phenotypic confirmation of ESBL suspected strains were done by combination disc method and double disc method. Multiplex PCR was carried out for ESBL genotyping using TEM, SHV and, CTX-M specific primers. During the study period, a total of 62 isolates were identified as ESBL producing Enterobacteriaceae, which includes *E. coli* (64.5%), *Klebsiella* Spp. (11.2%), *Citrobacter* (8.06%), *Serratia* (6.45%), *Enterobacter* (3.22%), and *Pseudomonas* (3.22%). Most of the isolate showed high rate (69%) of

resistance for three cephalosporin antibiotic (cefotaxime, ceftriaxone, and cefpodoxime) combined. A high proportion of isolates were found to have MIC value $\geq 32 \mu\text{g}$ for cefotaxime, amoxicillin, ampicillin, ceftazidime, and enrofloxacin. In the present study, bla CTXM was observed as a predominant beta-lactamase type with the preponderance of CTX-M group 1, whereas, 37.5% organisms were also found positive for bla TEM type ESBL. The study found carbapenemase prevalence of 6.45 % in the isolates using phenotypic tests. These CPE isolates were found to have co-localized bla VIM and bla IMP beta-lactamase. The phylogenetic grouping of the 40 *E. coli* strains was done using the Clermont multiplex PCR methods. Highest prevalence was observed for B1 (22.0 %) followed by A (20%), C (12.0%), D (12.0%), F (8.0%), E (3.0 %), Clad I (3.0 %). In vitro antimicrobial test using *Polyalthia longifolia* and *Eucalyptus robusta* extract showed minimum or no antimicrobial property against gram negative bacilli. In conclusion we have observed a very high prevalence of ESBL *E. coli* in bovine clinical endometritis.

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Ph.D. Degree Programme

1. A study on assessment of DNA integrity and protamination status of Barbari buck semen

Deepika Kritania and Saravjeet Yadav

Evidences regarding deprotamination, apoptosis like changes, DNA fragmentation and cryocapacitation during ultralow freezing of spermatozoa in different species of animals have been established, however literature regarding these alterations in buck spermatozoa is less known and hence the present study was proposed in buck spermatozoa to study these cryo-alterations after freezing and thawing. Six apparently healthy Barbari bucks and 8 ejaculates were collected from each buck. The study was designed with aim to evaluate the presence of Protamine I in buck spermatozoa and its variation at high and low temperature (if any), apoptosis and capacitation like changes in frozen thawed buck spermatozoa. Collected semen was frozen with 16% egg yolk and 6 % glycerol and analysis of various sperm attributes were done after 24 h and on different days (14 and 30) after freezing and thawing. Different sperm attributes like total progressive motile spermatozoa, per cent live spermatozoa, per cent spermatozoa having intact acrosome, intact membrane were evaluated using established and standard protocols of the department. Protamine I was evaluated using western blotting and immunolocalisation was carried out using indirect fluorescence. Early apoptosis like changes were evaluated by using Annexin V FITC assay and evaluation of mitochondrial membrane potential (MMP) using JC I staining. Late apoptotic like changes were evaluated by using TUNEL assay. Cryocapacitation like changes were studied by using chlortetracycline assay (CTC) and molecular insights in to capacitation like changes were evaluated using immunoblotting and immunofluorescence. Motion and kinematic analysis of spermatozoa was carried out using CASA. Immunoblot confirmed the presence of Protamine I in buck spermatozoa and immunofluorescence confirmed its nuclear organization. Results revealed significant reduction in per cent live spermatozoa, per cent total progressive motile spermatozoa, per cent spermatozoa having intact membrane, per cent spermatozoa having intact acrosome after all the days of freezing and thawing. Per cent spermatozoa showing early apoptotic like changes significantly increased in terms of Annexin V positive and spermatozoa showing low MMP after all the days of freezing and thawing. Per cent of spermatozoa showing deprotamination and DNA fragmentation significantly increased after all the days of freezing and thawing. Frozen thawed spermatozoa showed significant increase in B- and AR-pattern spermatozoa indicating rise in cryocapacitation and acrosome reaction like changes after all the days of freezing and thawing. It was evident that cryocapacitation like changes were mediated through phosphorylation of tyrosine containing proteins and in specific p54 exhibited highest phosphorylation. Deprotamination and DNA fragmentation were found positively correlated with B- and AR- pattern spermatozoa and all other parameters were found negatively correlated. Motion parameters as analyzed by CASA indicated reduction in total progressive motile spermatozoa, altered path velocities and hyperactivation in spermatozoa due to freezing and thawing. In conclusion- the study reported presence of Protamine I in buck spermatozoa and indicated that with freezing and thawing, cryocapacitation and apoptosis like changes occur in evidently in spermatozoa resulting in poor quality of spermatozoa after freezing and thawing.

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2. Insights into Toll-like receptors expression and Th1/Th2 cytokines regulation in peripheral blood of dogs with demodicosis

Priyambada Kumari and Rajesh Nigam

Canine demodicosis is a common but unwieldy noncontagious parasitic dermatosis caused by overpopulation of the host-specific follicular mites of various *Demodex* species. An over proliferation of *D. canis* mites is paramount factor for occurrence of clinical demodicosis and disease progression. Host immune response and mechanisms associated with *Demodex* mites population control requires extrapolation to unravel the host-parasite interface in demodicosis. There is limited knowledge related to clinical manifestation and/or over-proliferation of *Demodex* mites and TLRs transcription. Therefore, the present study was projected with objectives to insight the expression of TLRs in canine demodicosis; to divulge the association of TLRs with progression of canine demodicosis and to unveil the link between TLRs expression and Th1/Th2 cytokines levels for canine demodicosis. Client-owned dogs presented for clinical and dermatological examination was examined and diagnosis of demodicosis by detection of *D. canis*. The dogs diagnosed with localized (LD), with generalized (GD) and with generalized and concurrent pyoderma were grouped in to three respective groups. Another 12 apparently healthy dogs was kept as healthy controls. It was observed that *D. canis* mites might be utilizing cholinergic immunosuppressive pathways for their own perpetuation and induction of clinical demodicosis in dogs. Markedly elevated circulatory IL-10 and decreased TNF- α levels could be attributed to triggering and progression of canine demodicosis and thus shifting of Th1/Th2 cytokines balance towards Th2 side could be associated with induction of generalized demodicosis. An upregulation of TLR2 gene expression in peripheral blood of dogs might be accountable for *Demodex* induced clinical phenotypes in dogs, whereas down regulation of TLR4 & TLR6 genes expression could be the paramount strategy of mites to snag the innate immune response of dogs. Therefore, it is apparent that *D. canis* mites play with the both innate and adaptive immune system of the host and manage to evade the host immune response.

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3. Development of Omp28 protein based ELISA for diagnosis of bovine brucellosis

Deepti Singh and Sharad Kumar Yadav

Brucellosis, a zoonotic infection is recognized as an emerging public health disease that is endemic in most regions of the developing countries including India. It causes severe economic losses in the form of loss in productivity, abortion, repeat breeding in animals and loss of man days in human beings. In the absence of safe and effective isolation procedure, serological tests like complement fixation test (CFT), rose bengal plate test (RBT), standard tube agglutination test (STAT), milk ring test (MRT) and enzyme-linked immunosorbent assay (ELISA) are relied on for the clinical diagnosis of brucellosis. However, agglutination tests sometimes give false-positive results due to cross-reactions with other pathogenic organisms. There is need to have sensitive and specific diagnostic test. For this purpose the outer membrane proteins of *Brucella* spp. have been extensively studied for their immunogenicity and serodiagnostic applications. In the present study, cloning and expression of *B. abortus* Omp28 were accomplished by PCR amplification cloning into a prokaryotic expression system, and purification of a recombinant Omp28 protein. The immunogenicity of rOmp28 was confirmed by Western blot analysis with known *Brucella*- positive bovine serum.

On checkerboard titration, the optimum concentration of recombinant antigen which was able to differentiate both known positive and known negative serum was 100 ng per well and serum dilution was standardized at 1:100 dilution of serum for further analysis. Two hundred seventy six sera from cattle and buffalo collected from different parts of the Uttar Pradesh state were tested by rose bengal plate test and Indirect ELISA against the recombinant Omp28 antigen and commercially available ELISA kit. In case of bovine serum Omp28-ELISA showed (21.73%), while commercial-ELISA and RBPT showed (23.55%) and (21.38%), respectively. Concordance between Omp28-ELISA and commercial ELISA was slightly higher than concordance between Omp28-ELISA and RBPT. Kappa statistics between OMP28-ELISA and commercial ELISA showed almost perfect agreement, while RBPT and OMP28-ELISA showed substantial agreement. In conclusion, the recombinant Omp28 protein of *Brucella abortus* was successfully expressed in *E. coli* expression system and the yield of recombinant Omp28 protein was high. Relative sensitivity and relative specificity of Omp28-ELISA was 87.69% and 99.99%, respectively when compared with commercial ELISA. Thus, the developed in house test may be used by the local veterinary diagnostic laboratories for diagnosis of brucellosis and may have significant implications in control of brucellosis at least in Uttar Pradesh state.

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4. Molecular characterization and typing of methicillin-resistant *Staphylococcus aureus* (MRSA)

Jayshree and Sharad Kumar Yadav

The present study included 312 samples collected from 212 and 100 human and animal cases, respectively. The prevalence of *S. aureus* in human pyogenic and clinical cases of animals was comparable with 38.6% and 40%, respectively. The amplification of *coa* genes was not related with the ability to produce slide coagulase or bound coagulase test. The prevalence of MRSA in *S. aureus* isolates was significantly higher in animal isolates (50%). On drug sensitivity test, irrespective of group of drug, resistance was higher in human isolates in comparison to animal isolates. The drug resistance against β lactam antibiotics ranged up to 100%. Following to β lactam antibiotics, resistance was against cephalosporin and quinolones. All the animal isolates were sensitive to glycopeptide vancomycin, while only 80.4% human isolates were sensitive to vancomycin. Following the vancomycin, sensitivity was highest against clindamycin, followed by amino glycosides like amikacin and gentamicin. The drug resistance patterns suggested that the drugs like chloramphenicol and tetracycline, which are not being used in commonly used are regaining sensitivity against drug resistant bacteria. The antibiotype of all isolates revealed 51 antibiotypes including 30 human and 21 animal antibiotypes exclusively. None of the animal and human isolates shared antibiotype suggesting different pattern of exposure to antibacterials. The level of resistance in animal isolates was lesser than human isolates and majority of isolates showed resistance to less than four antibacterials and those are mainly β lactam antibiotics. The resistance of healthy human isolates against all the used antibacterials suggested harboring of drug resistant isolates as carrier. The AP-4 primer based RAPD typing revealed 5 and 6 patterns in human and animal MRSA with two common patterns. The presence of 9 different patterns suggested possible different phylogeny of isolates. AluI digestion of *mecA* amplicons revealed 2 and one patterns in animal and human MRSA. Similarity in human and animal MRSA, digestion pattern further suggested common ancestral relationship. The plasmid profiling clearly indicated difference in animal and human isolates. The presence of single plasmid in all human MRSA and multiple plasmid might be the reason of difference in drug resistance pattern of human

and animal isolates. The SDS-PAGE based typing of all MRSA revealed 23 polypeptides with 12 major polypeptides in all the human and animal MRSA in the range of 20 – 200 kDa. The major polypeptides were of 20kDa, 28 kDa, 33kDa, 39kDa, 43kDa, 59kDa, 64 kDa, 72 kDa, 86kDa, 97kDa, 121kDa, and 200kDa. To conclude, the prevalence of MRSA in *S. aureus* is alarming with high prevalence in human and their companion animals. The drug resistance pattern showed resistance against almost all antibacterials used in study. The presence or absence of pathogenicity related *coa* genes and methicillin resistance *mecA* genes were not related to major polypeptides and their immunoreactivity in *S.aureus*. The typing performed with limitation suggested, MRSA with common ancestral origin in human and animal with different phylogeny that can be further confirmed by sequencing of the PCR products and phylogenetic analysis.

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5. Development of omp31 Protein based ELISA for diagnosis of ovine and caprine brucellosis

Ajay Singh and Rajesh Nigam

In India goat population contributes a lot to the agrarian economy, particularly in regions where crop and dairy farming are not economical, thus play an important role in the livelihood of landless, small and marginal farmers. Brucellosis caused by different species of *Brucella* is considered as a major public health problem due to its zoonotic nature, worldwide distribution and the economic losses. Among various *Brucella* species, *B. melitensis* is most pathogenic and highly zoonotic and included as category B biothreat. The diagnosis of brucellosis in goat is very important for control of this disease as there is no vaccine available for human use. The present study was designed to develop the recombinant omp31 (rOmp31) protein antigen based in house ELISA for specific serodiagnosis of caprine brucellosis. Omp31 gene of *B. melitensis* strain was cloned and expressed in pET32b(+) expression system. The recombinant protein was purified under denaturing conditions using 8 M urea. The purified recombinant protein was confirmed by western blotting using known caprine *Brucella* positive and negative serum. The sero-reactivity of the recombinant protein was also checked by reacting with antisera of known *B. melitensis*. Serodiagnostic potential of recombinant antigen was tested against 92 clinical serum samples collected from goats by iELISA. Out of 92 samples tested, 18 (19.56%) were positive and 74 (80.43%) were negative by rOmp31 antigen based enzyme-linked immunosorbent assay (ELISA). In comparison to RBPT, the relative sensitivity and relative specificity of rOMP31-ELISA were found 92.85% and 93.59%, respectively. Positive predictive value of rOMP31-ELISA was found 72.22%, while negative predictive value was 98.64%. rOMP31-ELISA showed 93.40% in accuracy of prediction against RBPT. In comparison to commercial ELISA kit, the relative sensitivity and relative specificity of rOMP31-ELISA were found 94.11% and 97.33%, respectively. Thus, the test gave comparable results with the commercially available ELISA kit for diagnosis of brucellosis in goats. The rOMP31-ELISA showed 96.74% in accuracy of prediction against commercial ELISA kit in diagnosis of brucellosis in goats. Concordance was higher between rOMP31-ELISA and commercial ELISA, which was (96.74%) than RBPT and rOMP31-ELISA test with a concordance of (93.48%). Kappa statistics between rOMP31-ELISA and commercial ELISA showed almost perfect agreement as the value observed was 0.894, while RBPT and rOMP31-ELISA showed substantial agreement as their Kappa value was 0.774. From the results, it can be concluded that the developed in house test may be used by the local veterinary diagnostic laboratories for diagnosis of caprine brucellosis and may have significant implications in control of brucellosis at least in Uttar Pradesh state. However, the efficacy of serodiagnosis

also needs to be further evaluated using more number of sera samples from different geographic regions. Beside this, the comparative evaluation for rOmp31 i-ELISA with other reported recombinant Omps and also their combinational use can also be explored.

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6. Functional and molecular studies and associated signaling pathways of mercury-induced deleterious effects on spermatozoa of bucks

Bhawana Kushwaha and S.K. Garg

The present study was undertaken to evaluate the effect of different concentrations of mercuric chloride on functional attributes, redox status (both oxidative and antioxidative), tyrosine phosphorylation, immuno-localization, intracellular Ca^{++} release, levels of cAMP, DNA damage, Bax and Bcl-2 expressions in spermatozoa and, involvement of Ca^{++} signaling, and MAPK pathways in mediating mercury-induced effects on buck spermatozoa. Six, healthy adult fertile Barbari bucks of almost similar age and body weight were selected for this study. Total 625 ejaculates were collected from these bucks using artificial vagina. Each ejaculate was diluted (50×10^6 /ml) using PBS (pH 7.4) with 0.5% glucose. Diluted semen samples divided into five aliquots (12×10^6 /ml) in-vitro treated with control (PBS pH 7.4) and different concentrations of mercuric chloride (0.031, 0.125, 0.25 and 1.25 $\mu\text{g}/\text{ml}$) and observed for different motility and functional attributes parameters up to 3h and results compared with PBS control.

Compared to control, percentage of progressively motile, HOST positive spermatozoa, and acrosomal integrity, and mitochondrial transmembrane potential were significantly ($p < 0.05$) change in DNA damage, or relative mRNA expression of Bax gene, but significant ($p < 0.05$) reduced following exposure to higher concentrations of mercury (0.25 and 1.25 $\mu\text{g}/\text{ml}$) at 15 min and 3 h. The results of our study also showed that mercuric chloride even at the lowest used concentration of 0.031 $\mu\text{g}/\text{ml}$ significantly ($p < 0.05$) change in DNA damage, or relative mRNA expression of Bax gene, but significant ($p < 0.05$) increase in necrotic spermatozoa was observed and this effect was concentration and time dependent. Compared to no effect on Bax gene relative mRNA expression of Bcl-2 gene was found to be significant increased at higher concentration (0.25 and 1.25 $\mu\text{g}/\text{ml}$) of Hg. After 3 h exposure of semen samples, high intensity bands of 58 kDa, 80 kDa, 100 kDa and 105 kDa in PBS control and less intensity bands of tyrosine phosphorylation of 80 kDa and 100 kDa proteins were observed in 0.031 $\mu\text{g}/\text{ml}$ mercuric chloride-treated group but as the concentration of mercuric chloride increased from 0.125 to 1.25 $\mu\text{g}/\text{ml}$, no protein bands were observed. Compared to the PBS control (9.01 ± 0.42 %), only in lowest concentration (0.031 $\mu\text{g}/\text{ml}$) of mercury group immuno-reactivity in the head of spermatozoa (4.11 ± 0.02 %) was while observed at other concentrations (0.125, 0.25, and 1.25 $\mu\text{g}/\text{ml}$), mercuric chloride failed to exhibit any immuno-reactivity of tyrosine phosphorylated protein. Following exposure of semen samples to different concentration of mercury alone, no significant effect on total motility and other kinematic patterns of spermatozoa was observed at lower concentrations (0.031, 0.125, and 0.25 $\mu\text{g}/\text{ml}$) in up to 3 h. But higher concentration (1.25 $\mu\text{g}/\text{ml}$) significantly ($p < 0.05$) decreased the motility and other kinematic patterns of spermatozoa at 3 h compared to control. However, interestingly on concurrent treatment of semen samples even with the lowest used concentration of HgCl_2 (0.031 $\mu\text{g}/\text{ml}$) and Bay-K, Nifedipine, NNC and MEK inhibitor significant ($p < 0.05$) decrease in the total motility and other kinematic patterns of spermatozoa was observed and this effect was concentration and time-dependent. Thus, our findings suggest that mercury adversely affects the functional attributes of spermatozoa which in turn may be responsible for altering

the functionality and fertilizing capacity of buck spermatozoa. On in-vitro exposure, HgCl₂ even at 0.031 µg/ml concentration is toxic enough to induce necrosis and apparently it does not induce early apoptosis in buck-spermatozoa. Therefore, mercuric chloride at the used concentrations has not induced any DNA damage in spermatozoa. Motility of spermatozoa is not dependent on extracellular Ca⁺⁺; rather it is intracellular Ca⁺⁺-dependent. No direct effect of L-type and T-type channel blockers was observed and inhibitory effects of Hg in the presence of those blockers suggest that mercury seems to involve some other pathway(s) in mediating spermatozoa motility. MAPK/MEK pathway was found to be modulated in presence of Hg in reducing motility that indicating possible involvement of MEK pathway in presence of Hg. Mercury dose not seems to induce apoptosis in spermatozoa rather causes necrosis. Thus sperm toxicity of mercury seems to be mediated through ROS-dependent intracellular Ca⁺⁺ and/or cAMP mediated pathways that also modulating spermatozoa motility.

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7. Molecular mapping of biofilm related genes and accessory gene regulator (agr) typing in *Staphylococcus aureus*

Lalita Sharma and Sharad Kumar Yadav

Internalization of *S. aureus* is strain-dependent and internalized bacteria have been reported to over express adherence and biofilm-forming genes in comparison to those that remain in the supernatant particularly by expressing various biofilm producing genes. Strains yielding highest invasion percentages are mostly carry *icaA*, *icaD*, *bap*, *can*, *fnbA* and *clfA* genes irrespectively of the presence of other resistance genes. Further, Biofilm-embedded bacteria that gain resistance to immune defense and antibiotics by antibiotic degrading enzymes, efflux pumps, and certain gene products of which expression are changed by the quorum sensing cause chronic and recurrent infections such as indwelling device-associated infections. Moreover, most of the *S. aureus* strains of animal origin, reported from all over the world are multidrug-resistant and carry multiple virulence genes, posing a potential public-health risk. Therefore the present study was undertaken a) to determine ability of *S. aureus* to produce biofilm; b) to map the presence of biofilm related gene in biofilm forming *S. aureus* c) to determine accessory gene regulator (agr) typing in biofilm forming *S. aureus*. Out of 175 (100 human and 75 animals) samples, 86 (46 human and 40 animal) isolates were confirmed based on cultural, morphological, biochemical tests and by confirming the presence of species specific nuc genes. The overall prevalence of *S. aureus* was 49.14% (61.3% and 40.0% in human and animal). The confirmation of methicillin resistance *mecA* gene revealed its presence in 32 isolates (12 human and 20 animals) with 18.28% (16.0% and 20% in human and animal) overall prevalence of MRSA was in all the isolates. Congo red agar (CRA) method revealed 47 (23 human and 24 animal) biofilm producer isolates in 86 isolates with 54.65% (48.9% and 51.06% in human and animal) overall prevalence of biofilm producing *S. aureus*. The amplification of *icaA*, *icaD*, *bap*, *can*, *fnbA* and *clfA* biofilm forming genes showed highest presence of *IcaD* gene (41) followed by *clfA* (26), *fnbA* (24), *can* (18) and *bap* (8). None of isolate revealed the presence of *icaA* gene. The overall prevalence of *icaD*, *fnbA*, *clfA*, *bap* and *can* was 47.67%, 27.91%, 30.23%, 9.30% and 20.93% respectively. The agr typing has been recommended as an important tool for deciphering of important epidemiological information about *S. aureus* in clinical isolates. The global presence of these genes makes them an effective tool for the epidemiological studies, and also for investigating the genetic relatedness and heterogeneity of *S. aureus*. A majority of isolates belonged to agr Group II (51.16%), followed by agr Group I (32.55%) and agr Group III (16.27%). The agr typing of 86 isolates revealed 28 isolates of agr

type I (15 human and 13 animal), 44 isolates of agr type II (24 human and 20 animal) and 14 isolates of agr type III (7 human and 7 animal). None of the isolates was positive for agr type IV. 13 human isolates and 5 animal isolates revealed the amplification of more than one type of agr genes. However, 8 human and 12 animal isolates revealed no amplification of agr genes. The findings of study suggest that MRSA are adopting the environment and using multiple approaches to develop resistance. A single mechanism is not responsible for the methicillin resistance in *S. aureus*. *S. aureus* is very smartly using different mechanism to develop resistance. Thus, continuous monitoring is required to overcome the drug resistance in MRSA. The presence of multiple agr typed need further studies are required to establish these parentage and to link them with other MRSA.

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8. Studies on the toxicity of acrylamide and its amelioration in wistar rats

Pratishtha Sharma and Sharad Kumar Yadav

Present study was designed in Wistar rats in two phases to study acrylamide toxicity and its amelioration using Vitamin E, GSM and hot aqueous extract of *Ocimum sanctum* (HAE). In the first phase of study, acrylamide was fed @ 10, 20 and 30 mg/KgBW for 45 days and basing on neurophysiological as well as histopathological findings, the dose of acrylamide was finalised @ 30 mg/Kg BW and duration of experiment for 45 days. The Phase II study was the major study which was carried out for 45 days in eleven groups of rats along with a vehicle control group for corn oil. The different groups taken during the major study were- Group A: Acrylamide; Group B: Acrylamide + Vitamin E; Group C: Acrylamide + hot aqueous extract of *Ocimum sanctum* (HAE); Group D: Acrylamide + Reduced Glutathione (GSH); Group E: Acrylamide + Vit E + HAE; Group F: Acrylamide + Vit E + GSH; Group G: Acrylamide + HAE + GSH; Group H: Acrylamide + HAE + Vit E + GSH; Group I: Basal diet + GSH; Group J: Basal diet + Vitamin E (Antioxidant); and Group K: Negative control = Normal diet and Water. Corn oil group was taken as vehicle control. Three ameliorating agents were orally administered into rats namely- *Ocimum sanctum* hot aqueous extract @ 250mg/Kg BW; a-Tocopherol (Vit E) @ 5 IU/ Kg BW and reduced Glutathione @ 2mg/ Kg BW. After 45 days of experiment, erythrocyte oxidative stress markers, tissue oxidative stress markers, histopathological changes in various organs, plasma cytokine levels, blood biochemicals, cauda sperm attributes, testicular bioinjury markers and micronuclei formation in hepatocytes and spleenocytes were evaluated. Feeding acrylamide for 45 days resulted in significant increase in MDA, significant reduction in the activities of SOD, GST, GSH, and CA T in erythrocytes and all tissues indicating rise in oxidative stress and reduction in antioxidant defense due to acrylamide toxicity as compared to all other groups (B to I); however, concurrent feeding with Vitamin E (Group B) and combination of Vitamin E, GSH and HAE (Group H) rats showed significant improvement in antioxidant defense indicating ameliorative potential of these agents. All other ameliorating agents in alone improved the antioxidant status but not significant as compared to Group B and H. Histopathological findings indicated that acrylamide induced number of cellular, degenerative and inflammatory changes in liver, testes, lungs, spleen, heart, brain, and kidneys; however, concurrent feeding with Vitamin E (Group B) and combination of Vitamin E, GSH and HAE (Group H) rats showed significant protection of these organs from degenerative changes. Similar trend was observed for blood biochemical parameters and plasma cytokines which were decreased due to acrylamide toxicity. Cauda retrieved sperm cells showed significant reduction in functional attributes and spermatozoa showed decreased motility, viability,

membrane integrity and DNA fragmentation indicating toxic effects of acrylamide. Testicular bioinjury markers were significantly increased due to acrylamide toxicity along with higher formation of micronuclei in spleenocytes and hepatocytes. Concurrent feeding with Vitamin F (Group B) and combination of Vitamin E, GSH and HAE (Group H) rats showed significant amelioration from acrylamide toxicity'. Vitamin E feeding along with acrylamide (Group B) showed highest amelioration indicating the best agent to neutralize the toxic effects of acrylamide. From the findings of the study, it was concluded that acrylamide induces subtle changes in antioxidant system of host and it can be suitably ameliorated by using Vitamin E or GSH or HAE of *Ocimum sanctum* either alone or in combination. ACR toxicity can be minimized using antioxidants and therefore, it was concluded that ACR mostly acts by forming free radicals affecting oxidative status of the host.

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9. Development of multiplex PCR and hydrolysis probe based molecular assays for diagnosis of viral enteritis in neonatal goat kids

Sapna Prajapati and Ashok Kumar

In the present investigation, a total of diarrhoeal (254) and non-diarrhoeal (50) faecal samples of goat kids (232), lambs (22) and necropsied tissue sample (17) were collected from different outbreaks and farms of five states of and screened for Group A rotavirus, group B rotavirus and Bovine corona virus by a RT-PCR, real time RT-PCR. The prevalence of rotavirus small ruminants was recorded as 14.57% for GARV, 7.48% for BCoV and 1.18% for GBRV by conventional RT-PCR. The results of conventional RT-PCR and real time RT-PCR showed some significant differences. Season, age and gender-wise analysis revealed highest occurrence of rotavirus in winter period (November to February). All the positive samples were from kids under one month of age, and maximum numbers of cases were detected during months of October to March. The prevalence of group A rotavirus in lambs was recorded as 22.7%. All the cases were detected in the month of January and February. None of the non-diarrhoeal samples from any species was found positive for rotavirus, indicating strong association of Group A rotavirus with diarrhoea in small ruminants. It was observed that for maximizing diagnostic potential of rotavirus infection, conventional RT-PCR is relevant. Sequencing and phylogenetic analysis revealed two major branches, where CIRG F2 strain was closely associated with bovine and human GARV strains, indicating the relevance of genetic re-assortment and its zoonotic potential. Two more strains viz., CIRG 1873 and CIRG1841 were placed in a clade genetically close to the porcine GARV isolates. This shows the dynamic nature of the circulating strains. qRT-PCR was standardized and developed for GARV targeting two genes viz., VP6 and NSP4, and for BCoV targeting NC gene. Out of 94 diarrheic neonate fecal samples tested 5 were positive for both GARV VP6-qRT-PCR, and out of 74 tested by NSP4 qRT-PCR, 4 were positive. These samples were detected with very high sensitivity and specificity for GARV. While NC-qRT-PCR for detection of BCoV revealed 3 positive out of 94 samples tested. To conclude, the current study could reveal some important features of the enteric viral affections in small ruminants with respect to various criteria like age, season, gender etc.. The GARV were the most common enteric pathogen followed by BCoV and lastly GBRV. The GARV emerged as most important pathogen, that was associated with clinical diarrhoeic cases as evidenced by the conventional RT-PCR assay and the VP6 & NSP4 gene based qRT-PCR. This indicates the fact that GARV needs attention in field conditions leading to diarrhea, slower growth rate and mortality in goat kids, which can only be addressed by vaccination and hygiene measures. Although the GBRV incidence was less

than 2%, their importance with respect to clinical diarrhea and its combination with other bacterial pathogens like *Escherichia coli* or *Cryptosporidium* should be explored. The significance of enteric viruses in small ruminants was always not supported by ample data, which the current study could address in a way using the latest tools including qRT-PCR assays for their detection.

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10. Development of sodium polyacrylate nanoparticles based combined mastitis vaccine and assessment of its efficacy in mice model

Shalini Yadav and Sharad Kumar Yadav

The present study was conducted to develop a stable, safe and effective vaccine against mastitis. For the purpose, two most common mastitis causing bacterial pathogen *S. aureus* and *E. coli* were selected based on previous studies conducted locally and in different part of country. These were characterized by cultural, morphological, biochemical tests. Further, these were confirmed by the PCR based gene amplification. The amplified products were purified and sequenced. The sequences of PCR amplicons have been submitted to NCBI, GenBank to obtain Accession no. MH092071 and KY914488 for *S. aureus* and *E. coli*, respectively. Adjuvants always play critical role in the acceptance and success of any vaccine so well approved and recognized adjuvant Montanide™ GEL 1 was incorporated with virulent *S. aureus* (MH092071) and *E. coli* (KY914488) to develop formalized killed adjuvant combined mastitis vaccine PGV (Polymer gel based vaccine) to make it 8.9 X10⁸ and 1.85 X10⁸ CFU of formalin killed virulent *S. aureus* (MH092071) and *E. coli* (KY914488) in per shot (100µl) for mice. The PGV was tested for sterility and found sterile. Its safety was assessed in adult inbred albino female mice and it produced no untoward reaction. For stability testing PGV was stored at different temperature and PGV was found stable for 12 months at refrigeration temperature (4-8°C) and 9 months at room temperature. To assess efficacy of PGV, it was inoculated in 24 adult inbred albino female mice at the dose of 100µl {8.9 X10⁸ and 1.85 X10⁸ CFU of formalin killed virulent *S. aureus* (MH092071) and *E. coli* (KY914488)} through subcutaneous route. The control groups were inoculated equal volume of adjuvant and PBS (pH7.4) with similar route. All the mice were challenged on 28th day post vaccination with live virulent *S. aureus* (MH092071) and *E. coli* (KY914488) (10² CFU) through intra mammary route and sacrificed on 7 th day post challenge. During experiment, blood was collected at 7th, 14th, 21st, 28th and 35th days for serum as well as whole blood. The blood erythrocytes were used for the estimation of oxidative stress biomarker parameters, plasma for plasma cytokine level. Serum samples were used to know the status of serum antibodies by plate agglutination test (PAT) and in house indirect ELISA (iELISA). During the experiment all the mice were examined routinely for any gross pathological changes. On 35th day (7th day post challenge) all the mice were sacrificed to conduct postmortem examination. The vital organs were collected for live weight and estimation of oxidative stress biomarkers. Splenocytes were used for splenocytes proliferation assay. The spleen and mammary tissues were also used for live bacterial count and to study expression of oxidative stress biomarker, cytokines and TLR mRNA by Real time PCR. Histopathology was performed with the tissues of all the vital organs along with mammary tissues. Based on the findings of present study, it can be concluded that PGV is sterile, stable and safe for animal use. PGV is stable for the duration of 12 months under refrigeration temperature (4-8 °C). PGV is easy to administer. PGV confers good early serological as well as cell mediated immune response in mice mastitis model. PGV produced desirable oxidative stress for effective vaccination. Expression of mRNA for

oxidative stress biomarkers, cytokines and TLR revealed the basis of effective protection. None of the organ revealed pathological changes in vaccinated and challenged mice. Thus, Formalized killed combined mastitis vaccine can be used in animals safely and effectively for the prevention of mastitis. Further, PGV fulfill all sterility, safety, stability and efficacy parameters as desired in OIE guidelines and European pharmacopeia. Hence, it is recommended for field trial in homologous host.

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M.V.Sc. Biotechnology

1. Genetic polymorphism of TLR4, CD14 & DrB3 gene in Indian breeds of cattle and buffalo

Hitesh Lambha and Amit Kumar Verma

Toll-like receptors (TLRs) are a multi gene family play a central role in the initiation of inflammation response and subsequent adaptive immune system and CD14 gene is an important gene for immunomodulation, while BoLA-DRB3 class II genes trigger humoral immune response. Polymorphism study of BoLA-DRB3, TLR4 and CD14 gene in cattle and buffalo and its association with mastitis and production traits explore the possibilities of these genes being used as candidate marker gene. Keeping all these points in view the current study was proposed in Sahiwal & Haryana cattle breed and in Murrah buffalo breed. The study was undertaken in total 130 animals of Sahiwal, Haryana cattle and Murrah buffalo, maintained at ILFC, DUVASU, Mathura by using simple microscopic method for SCC and PCR-RFLP technique for gene study. The amplified fragments of the TLR4, CD14 and DRB3 genes revealed 493, 832 and 304 bp, respectively and the amplified products were digested with HaeIII, HinfI and HaeIII restriction endonuclease enzymes respectively. The TLR4/HaeIII PCR-RFLP assay revealed only one type of banding pattern (genotype); which was of 271 and 222 bp (BB genotype). This revealed that the Sahiwal, Haryana cattle and Murrah buffalo used in the present study were monomorphic in nature. The CD14/HinfI PCR-RFLP assay revealed three types of banding pattern (genotypes); 377, 272 and 183 bp (CC); 377, 225, 183 and 47 bp (DD) and 377, 272, 225, 183 and 47 bp (CD genotype) with frequencies 39.0%, 16.0% and 45.0% respectively. Allelic frequency of C & D alleles were 0.615 and 0.385, respectively. The DRB3/HaeIII PCR-RFLP assay revealed five types of banding pattern (genotypes); 170, 82, 52 bp (AA); 222, 170, 82 & 52 bp (AB), 222 & 82 bp (BB), 222, 193, 82 & 29 bp (BD), 170 & 134 bp bands (EE genotype) with frequencies 56.0%, 20.0%, 5.0%, 7.0% and 12.0%, respectively in screened cattle and 20.0%, 43.3%, 13.3%, 10.0% and 13.3%, respectively in screened Murrah buffaloes and allelic frequency of A, B, D and E were 0.660, 0.185, 0.035 and 0.120, respectively in screened cattles and 0.417, 0.183, 0.050 and 0.133, respectively in investigated Murrah buffaloes. In association studies of CD14/HinfI gene, D allele was responsible for higher TMY and lower DP. CD genotype had low SCC than DD and CC in screened cattle population. Association studies of HaeIII/DRB3 genotypes with production traits showed that BD genotype was significantly associated with lower CI and higher (TMY) and PY in both Haryana & Sahiwal cattle. Association studies of Hae III/DRB3, BD genotype had higher TMY and MY300 in first Lactation in total investigated Murrah buffalo population.

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2. Functional characterization of voltage-gated proton channel (Hv1) in bull spermatozoa

Abhishek Kumar Mishra and Dilip Kumar Swain

Acid extrusion and regulation of intracellular alkalinisation are the two key events during sperm capacitation which are mediated through proton gated channels (Hv1). Literature is meagre regarding the presence and involvement of Hv1 in bull spermatozoa. In the current study, molecular and functional characterisation of Hv1 was carried out in spermatozoa of Haryana bulls. Sixty four ejaculates were collected from four bulls and were used for series of experiments. Immunoblotting and immunocytochemistry were employed for the molecular

characterisation of Hv1. Immunoblotting identified a single band of 32 kDa corresponding to Hv1 in Hariana bull spermatozoa. Positive immunoreactivity was seen in principal piece of the spermatozoa for Hv1. Functional study was carried out using two Hv1 blockers namely 2-Guadinobenzimidazole (2GBI) @ 200 μ M and Zinc Chloride @ 1mM, whereas, one activator was used namely Anandamide (AEA) @ 0.3 μ M. In the study, three groups were used namely, control (100 μ L of sperm dilution medium (SDM) containing 1 \times 10⁶ cells), vehicle (3 μ L) and drug (2GBI/Zinc/AEA and their combinations). Different time of incubations was used depending on the experiments. Blocking of Hv1 resulted in significant ($P < 0.05$). Evaluation of 2-GBI, Zinc and AEA treated spermatozoa stained with CTC revealed significant ($P < 0.05$) increase in B-pattern of spermatozoa indicating induction of capacitation. Spermatozoa treated with different pH gradients showed significant ($P < 0.05$) reduction in motility as compared to control both with and without drugs modulating Hv1. Functions of Hv1 was found to be mediated through cAMP and PKA pathway in the induction of hypermotility in sperm cells as evident from inhibition of sAC and PKA. Both L- and T-type of calcium channels were found to be associated with Hv1 function as evident from their respective blocking and its effect on PSM. Blocking as well as activation of Hv1 showed significant ($P < 0.05$) reduction in sperm livability, per cent spermatozoa having intact membrane, per cent spermatozoa having intact acrosome, per cent spermatozoa showing high mitochondrial transmembrane potential indicating the involvement of Hv1 in the process of regulation of sperm functional dynamics. From the study- it was concluded that Hv1 channels are found in bull spermatozoa and are pH dependent. These channels mediate number of sperm functions like hyper motility, capacitation and acrosome reaction through complex interacting pathways through calcium and pH dependent mechanisms. Further studies are required to find out the possible relationship between Hv1 channels and other channels in regulating spermatozoa function and possible mechanisms associated with Hv1 activation as well as its role in sperm function regulation.

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