Comparative Histological Study of Lacrimal Gland in Ruminants

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ABSTRACT

The present study was carried out to observe the histological differences of the lacrimal gland in ruminants. Lacrimal gland is a tubuloalveolar or tubuloacinar gland located in dorsolateral aspect of the eyeball, responsible for production and secretion of tears which clean and nourish the cornea. Tear film acts as the medium through which protective cells, immunoglobulins and oxygen can reach the cornea. In the present study, lacrimal gland was covered by a capsule which consisted of loose connective tissue and smooth muscle fibers. It was tubuloacinar type and serous in nature. The secretory units were lined by pyramidal cells. Myoepithelial cells were observed at the base of the secretory cells. The plasma cells, macrophages, multinucleated giant cells and lymphocytes were observed in the interstitial tissue. The lacrimal gland consisted of intercalated, intralobular and interlobular ducts. Goblet cells were observed in interlobular ducts of sheep and goat.

Keywords: Histology, Lacrimal gland, Ruminants, Myoepithelial cells

Lacrimal gland is a capsulated gland situated at the dorsolateral aspect of the eye ball. The connective tissue septa radiate from the capsule and divide the gland into lobules. The septa contain blood vessels, nerves and interlobular ducts. The gland is serous type in all domestic mammals other than the pig in which it is mucoid (Konig and Liebich, 2009). Lacrimal gland is responsible for production and secretion of tears which clean and nourish cornea and helps to maintain its health. The plasma cells are found in the interstitial spaces of the gland and migrate into it from lymphoid organs such as the gut-associated lymphoid tissue (GALT). These plasma cells secrete immunoglobin A (IgA) which is important in protecting the ocular surface from infection (Walcott, 1998). The dysfunction of lacrimal gland causes keratitis sicca. The present study conducted to correlate the histological variations in lacrimal gland of ruminants.

MATERIALS AND METHODS

Six samples of lacrimal gland were collected from each species of adult cattle, buffalo, sheep and goat. The glands were collected from the local slaughter house in Bidar and were fixed in different fixatives like 10% neutral buffered formalin, Zenker's fluid and Bouin's fluid and were processed by isopropyl alcohol-xylene sequence and embedded in paraffin by routine method. The sections of 5-6µ thickness were taken and were stained by haematoxylin & eosin method (Luna, 1968), Van Geison's stain for collagen fibres (Bancroft *et al.*, 2008), Gomori's method for reticular fibres (Luna, 1968), Aldehyde fuschin's method for elastic fibres (Bancroft *et al.*, 2008) and Toludine blue method for mast cells (Singh and Sulochana, 1996).

RESULTS AND DISCUSSION

The lacrimal gland of ruminants was covered by a connective tissue capsule which consisted of collagen, elastic, reticular and smooth muscle fibers. This finding was similar to the report of Singh *et al.* (1975) in buffalo whereas, Maala and De Ocampo (2007) stated that the capsule of the water buffalo mainly consisted of collagen fibers. Septa from the capsule divided the gland into different lobules and it consisted of collagen, elastic, reticular fibers,

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interlobular ducts, arteries, veins and nerve fibers in all the animals under study. This finding was similar to the earlier reports of Singh *et al.* (1975) in buffalo, Pinard *et al.* (2003) in bison and cattle, Maala and De Ocampo (2007) in water buffaloes, Getty (2012) in ruminants. However, in the present study the elastic fibers were more in the septa of cattle and goat compared to that of buffalo and sheep, which may be due to the species variation.

The lacrimal gland was tubulo acinar type and serous in nature in all animals under the study.This finding is similar to the earlier reports of Banks (1993), Konig and Liebich (2009) and Trautmann and Fiebiger (2002). However, it differs from the earlier reports of Abbasi *et al.* (2014) in sheep, Daryuos and Ahmed (2012) in sheep and goat and Kleaekowska *et al.* (2012) in Roe deer where the gland was mixed in nature and contained both serous and mucous acini. The secretory cells were pyramidal with basal nuclei. The nuclei were spherical to oval in shape in cattle, buffalo, sheep and goat similar to the earlier reports of Singh *et al.* (1975) in buffalo and Garguilo *et al.* (1999) in sheep. The cytoplasm of the secretory cells was granular and acidophilic in cattle and sheep as reported by Pinard *et al.* (2003) in cattle and Abbasi *et al.* (2014) in sheep. The cytoplasm was acidophilic at the base and basophilic towards the apical portion of the secretory cells in buffalo. In goat some cells were acidophilic and granular, others were basophilic and granular.

Myoepthelial cells were observed at the base of the secretory cells in cattle, buffalo, sheep and goat which is in agreement with the reports of Kleaekowska *et al.* (2012) in Roe deer, Eurell and Frappier (2006) in ungulates and Daryuos and Ahmed (2012) in sheep and goat. These cells help in the expulsion of the secretion out of the secretory cells. The secretory units were surrounded by thin layer of collagen fibers and reticular fibres in goat.

Plasma cells, macrophages, multinucleated giant cells and mast cells were observed in the interstitial tissue but the number of plasma cells were more in buffalo. Aggregation of lymphoid cells was observed in the parenchyma of the gland in buffalo indicating its role in defence mechanism protecting the ocular surface from infection as reported by Walcott (1998).

Duct system of the gland was made up of intercalated, intralobular and interlobular ducts. Intercalated duct was lined by simple cuboidal epithelium in cattle, buffalo and sheep; it was bilayered cuboidal in case of goat.The intralobular ducts were lined by simple columnar epithelium in cattle and buffalo, which is similar to the earlier reports of Singh *et al.* (1975) in buffalo; it was bilayerd columnar in sheep and goat which differs with earlier reports of Abbasi *et al.* (2014) in sheep where it was lined by simple cuboidal epithelium. The interlobular ducts were lined by bilayered columnar epithelium in cattle and buffalo which is in contrast to the report of Shadkhast and Bigham (2010) in buffaloes where the interlobular ducts were lined by pseudostratified epithelium. The duct was lined by stratified columnar epithelium with goblet cells in sheep and goat.This finding is similar

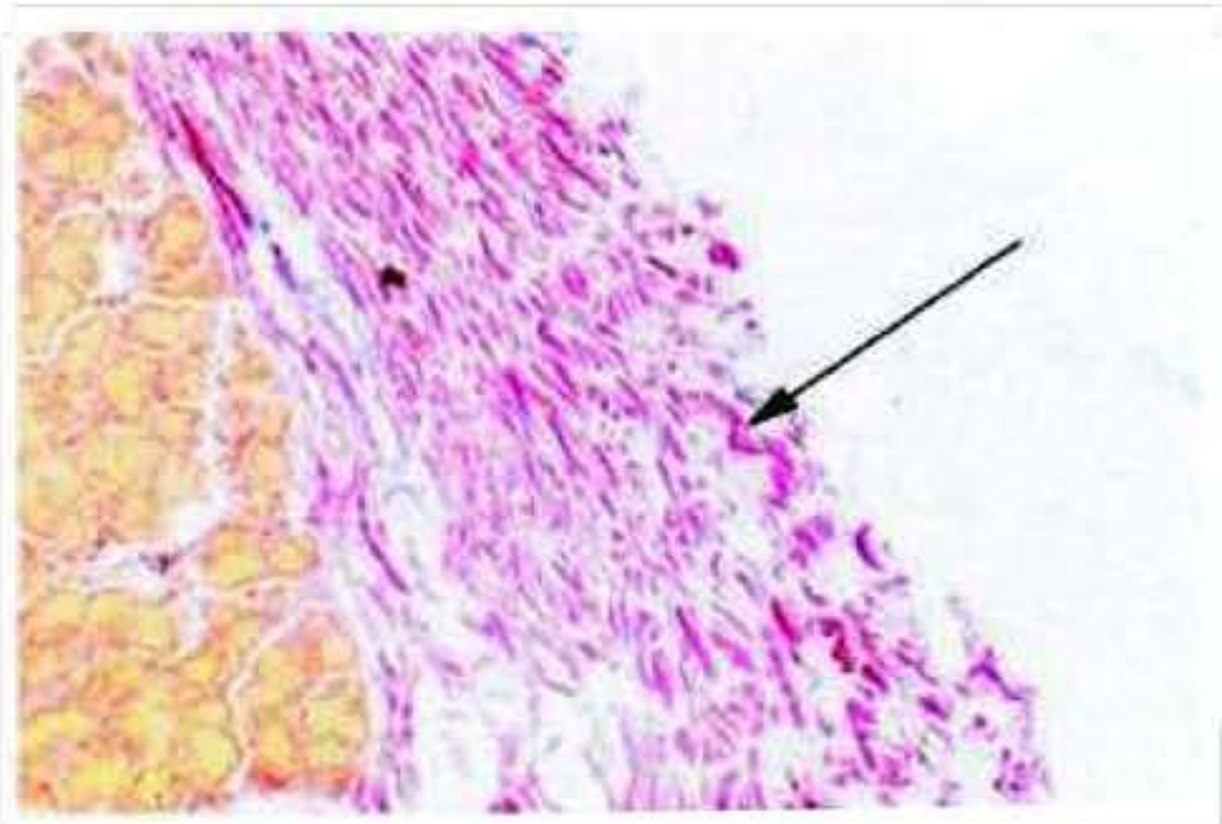

Fig. 1: Photomicrograph of lacrimal gland of goat showing collagen fibers (arrow) in the capsule (Van Gieson's stain x 10)

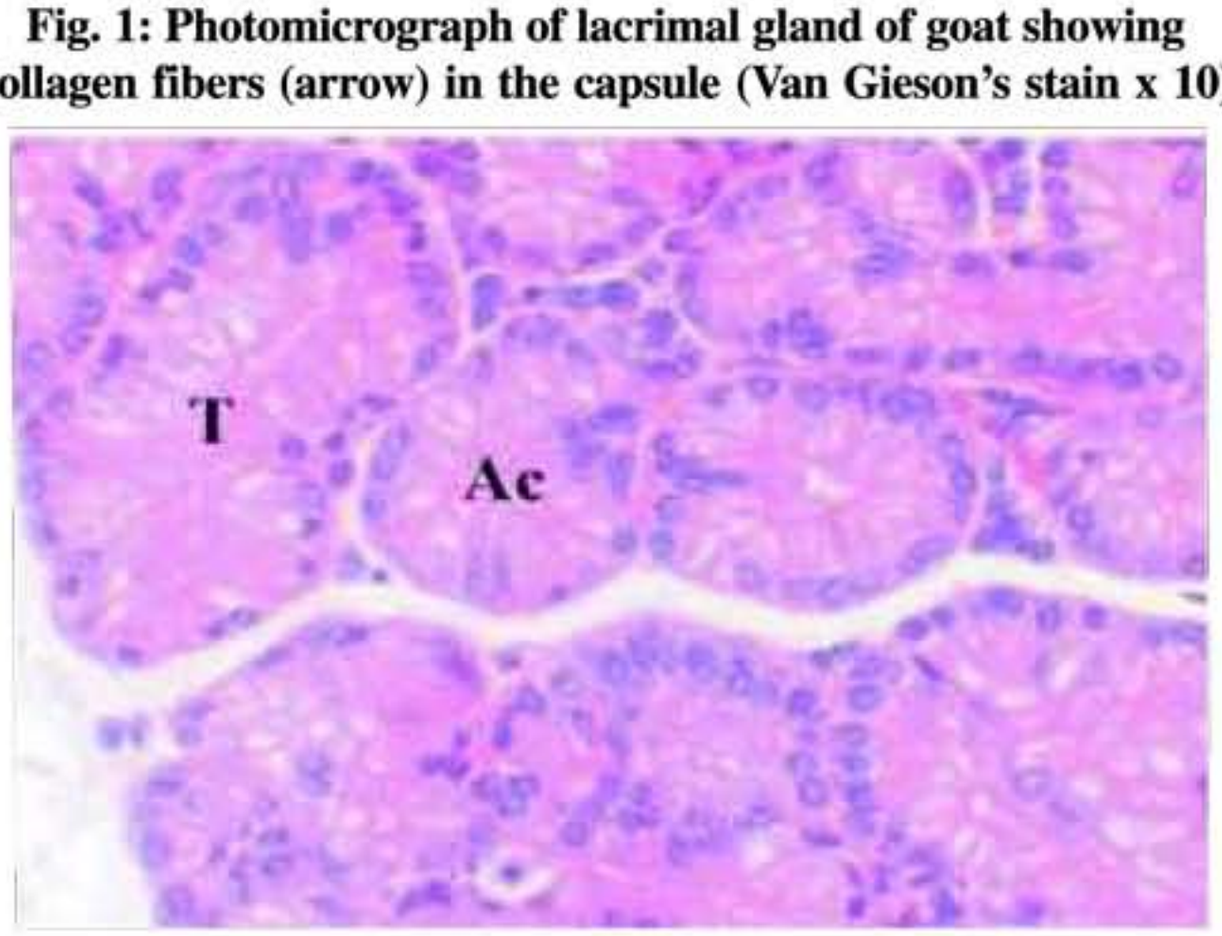

Fig. 2: Photomicrograph of parenchyma of lacrimal gland of buffalo showing tubular (T) and acinar (Ac) serous secretory units in buffalo (H&E x 40)

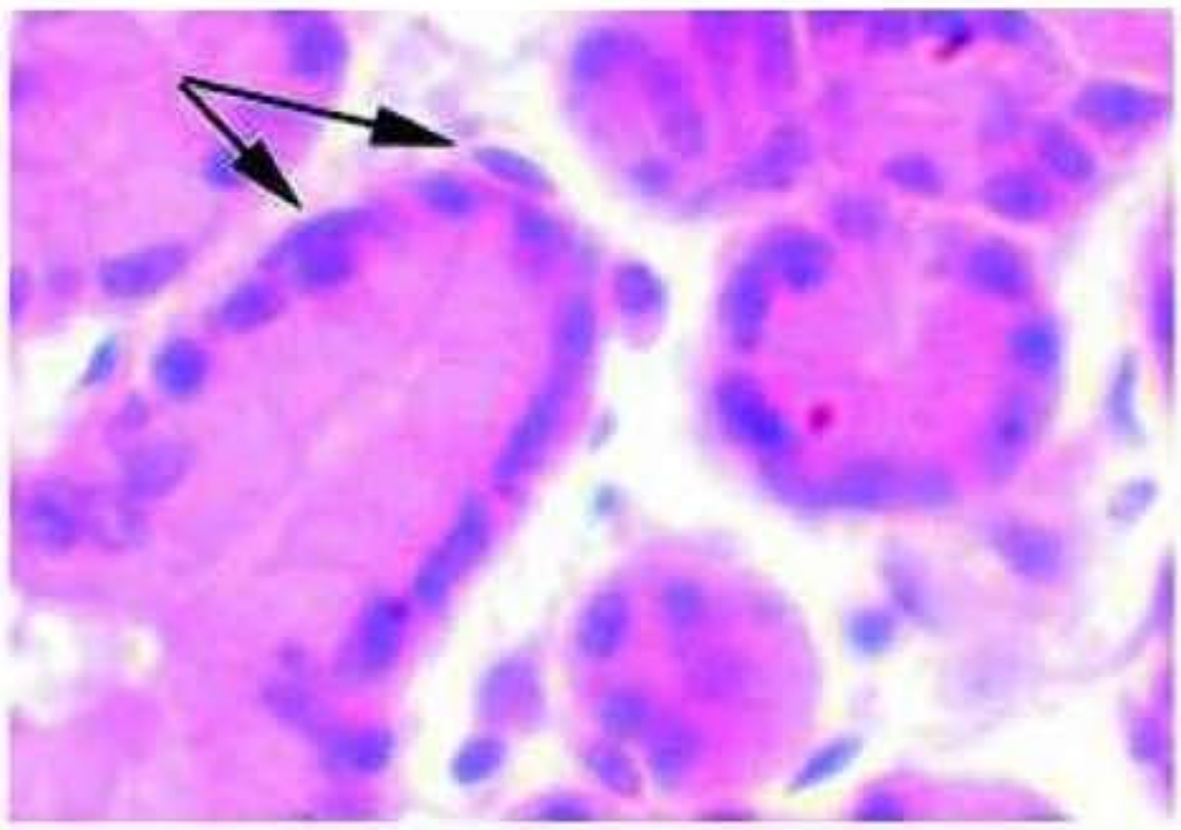

Fig. 3: Photomicrograph of the lacrimal gland of cattle showing myoepithelial cell (arrow) at the base of secretory cells (H&E x 100)

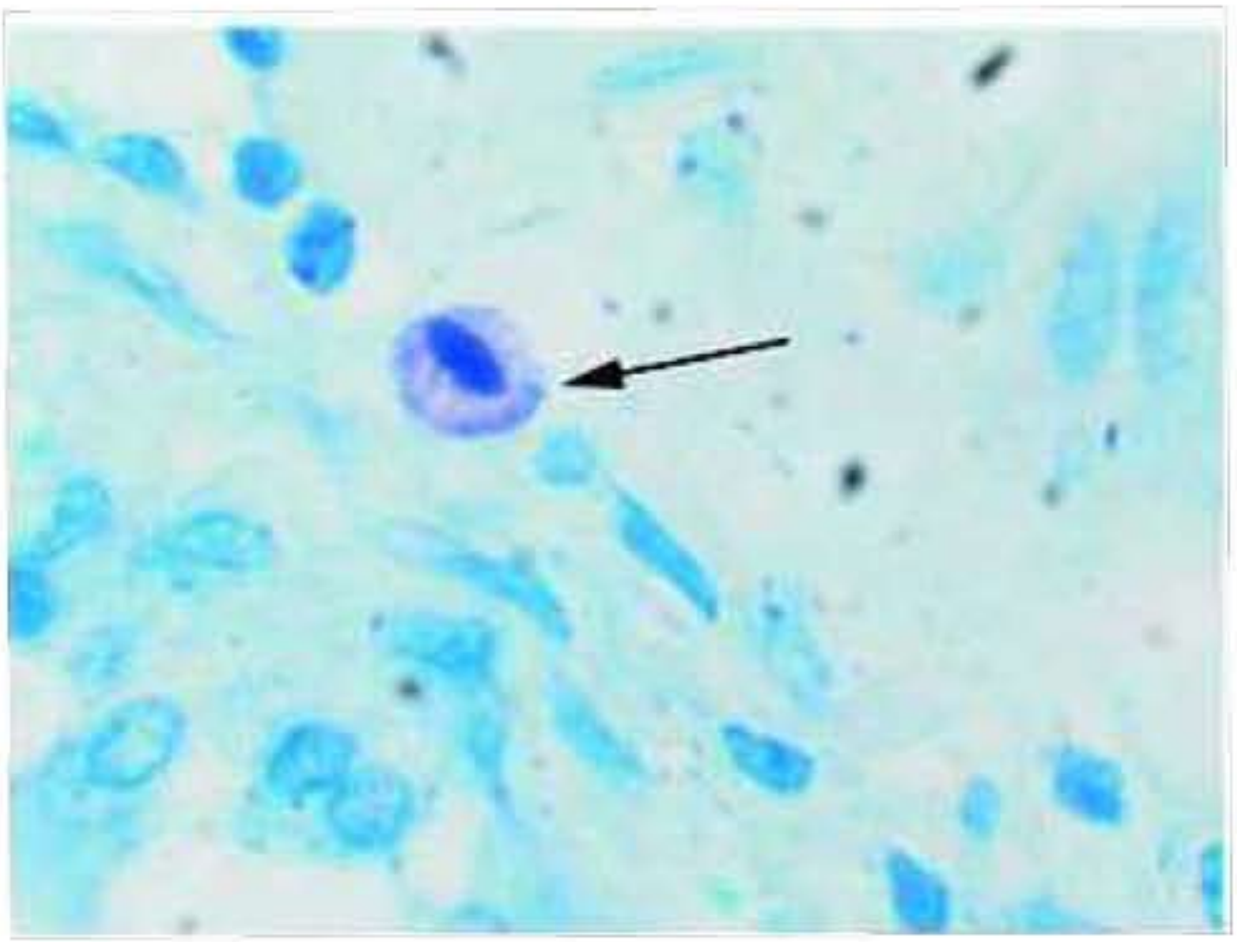

Fig. 4: Photomicrograph of lacrimal gland of buffalo showing mast cell (arrow) in the septa (Toludinie blue stain x 100)

Fig. 5: Photomicrograph of lacrimal gland of sheep showing binucleated cell (arrow) in the interstitial tissue (H&E x 100)

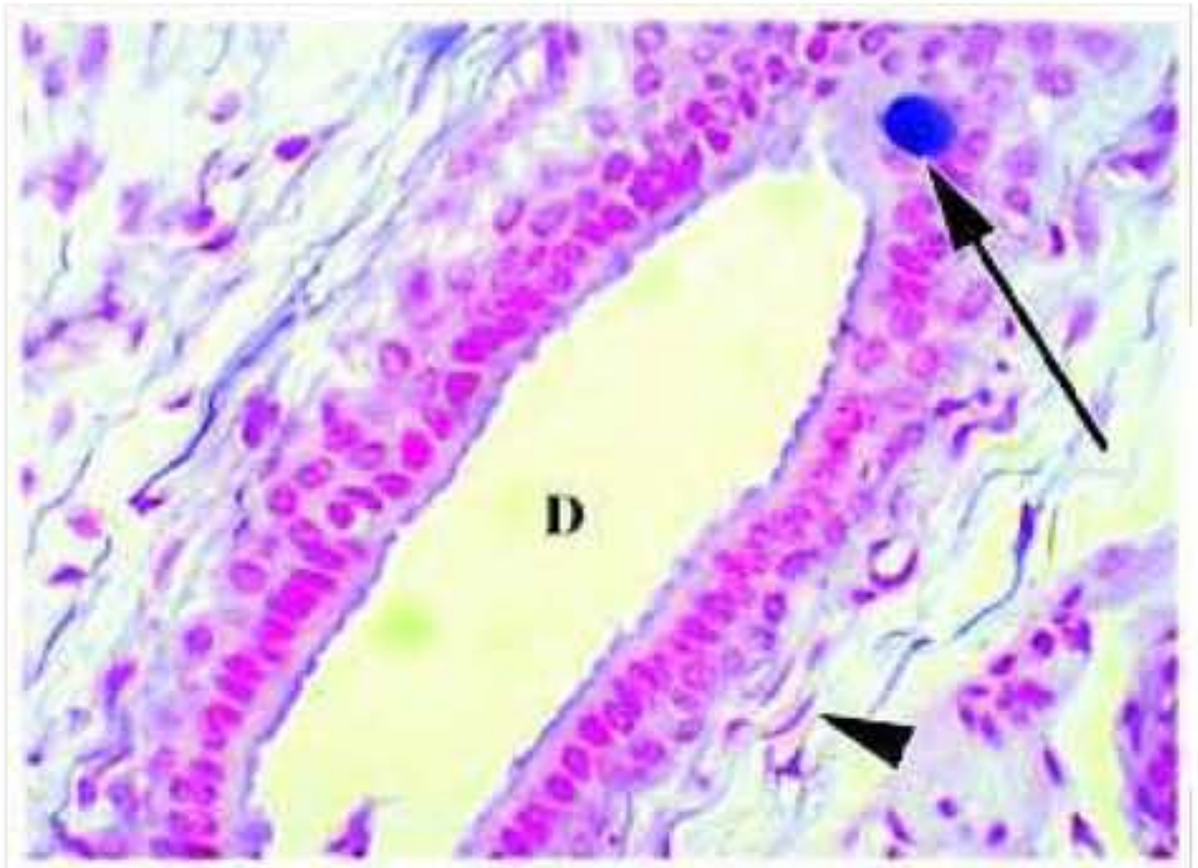

Fig. 6: Photomicrograph of lacrimal gland of goat showing goblet cells (arrow) in the epithelium and smooth muscle fibers (arrow head) around the interlobular duct (D) (Aldehyde fuschin x 40)

to the earlier reports of Daryuos and Ahmed (2012) in sheep and goat. All the ducts were surrounded by smooth muscle fibers to aid the expulsion of sercretion.

CONCLUSION

The lacrimal gland in ruminants was covered by a connective tissue capsule and was tubulo acinar type and serous in nature in cattle, buffalo, sheep and goat. The acini and tubules were lined by pyramidal cells with granular cytoplasm. The acini and tubules were larger in buffalo. The plasma cells and lymphocytes were more in case of buffalo. The duct system of lacrimal gland consisted of intercalated duct, intralobular duct and interlobular duct. Intralobular ducts were more in cattle.The goblet cells were present in the epithelium of interlobular ducts in sheep and goat.

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Isolation, Biochemical Characterization and Antibiogram of *Salmonella* spp. from Backyard Poultry

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ABSTRACT

Salmonella infection remains a global issue in the poultry industry. Major sources of *Salmonella* infection in poultry include contaminated feed, water and environmental reservoirs. Food animals are important reservoirs of infectious pathogens and hence it is essential to understand role of *Salmonella* spp. in food born infection.The continuous use of antimicrobial drugs in food animals are major source of selection of drug resistant pathogens.The present study was undertaken to evaluate the antimicrobial susceptibility of *Salmonella* to various common antibiotics. A total of 45 samples (Cloacal swabs) were collected for isolation of bacteria and were inoculated to different media Identification of *Salmonella* spp. was performed based on the morphological, cultural and biochemical properties. Out of 45 samples, 7 isolates were identified as *Salmonella* spp. (15%).Anti biogram study revealed that *Salmonella* spp. were highly sensitive to Enrofloxacilin group of antibiotics and resistance to different antibiotics like Cefepime, Spectinomycin, Amoxycillin / clavulanic acid, Tetracycline, Ampicillin / Sulbactum, Ampicillin and Cloxacillin.

Keywords: *Salmonella*, poultry, backyard poultry, antibiogram

Salmonellosis is one of the most prevalent diseases in birds caused by a vast range of *Salmonella* serotypes. The non–motile serotypes (*S. pullorum* & *S. gallinarum*) are adapted mostly for chickens and turkeys, being less prevalent in the world (Shivaprasad, 2003). These *Salmonella* spp. cause heavy economic loss interms ofmortality and reduced production in poultry industry (Talha *et al.*, 2001). Avian Salmonellosis infection may occur in poultry either acute or chronic form by one or more member of genus *Salmonella,* under the family Enterobacteriaceae (Hofstad *et al.*, 1992). *Salmonella* species have been considered as one of the most important food borne pathogens all around the world (Gillespie *et al.*, 2003). Meat and poultry products are recognized as the major sources for transmitting *Salmonella* species to human with 40 % of the clinical cases attributed to the consumption of egg and poultry product (Ruban *et al.*, 2010). Backyard chickens can also be infected through contact with wild animals, domestic mammals and commercial poultry that are carriers of *Salmonella* and consequently may play a role in the transmission of the organism to other animals and humans. The wide spread use of antibiotics in human veterinary medicine has lead to an increase in the number of resistant *Salmonella* strain isolated from poultry and poultry environment. (Anthony *et al.*, 2001; Fey *et al.*, 2000).

MATERIALS AND METHODS

A total of 45 samples *i.e.* cloacal swabs were collected from backyard poultry in Bidar district, Kuttabad village.Inoculated in selenite broth and incubated at 37° C for 24hrs. One loopful of sample from salenite broth was streaked onto *Salmonella-Shigella* agar, Brilliant Green agar and MacConky agar and incubated at 37°C for 24hrs. The plates were examined for the presence of typical colonies of *Salmonella* spp. (Rajeshwari, 2013) and then subculture was performed on XLD to get pure culture. Suspected colonies were confirmed by conventional biochemical methods (Parmesh, 2016).

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Antibiotic sensitivity test was conducted using antibiotic discs (Oxoid, UK) (Table 2) according to Kirby-Bauer antibiotic disc diffusion techniques. Briefly described, Mueller-Hinton agar was prepared in petri-dishes. Pure colonies of the *Salmonella* isolates emulsified in normal saline and the turbidity matched against McFarland No. 0.5 turbidity standard were used in ABST.

Fig. 1: Selenite broth

RESULTS AND DISCUSSION

In the present study, colony morphology and biochemical tests were typical for *Salmonella* spp. Out of 45 cloacal swabs collected, 7 were *Salmonella* positive. After 24 h of culturing in selenite broth change in colour was observed from colorless to turbid orange indicating growth of *Salmonella* spp. (Fig.1).

Cultural and Morphological characterization: On Mac Conkey agar plates, all the *Salmonella* colonies appeared lactose non fermenter, colourless and transparent. On brilliant green phenol red lactose sucrose agar, the colonies of *Salmonella* test isolates were pinkish to red coloured and transparent. On *Salmonella-Shigella* agar black color colonies due to production of H_2S, on xylose lysine deoxycholate agar, black color colonies due to production of H_2S, were observed.On Gram's staining, the morphology of the isolated bacteria was assessed. The organisms were small rod shape, Gram negative, non-motile, single or paired in arrangement (Fig.2). Based on the morphological, cultural and biochemical characterization indicate that all isolates were confirmed to be *Salmonella* spp.

Biochemical characterization: All suspected colonies of *Salmonella* on the basis of cultural and

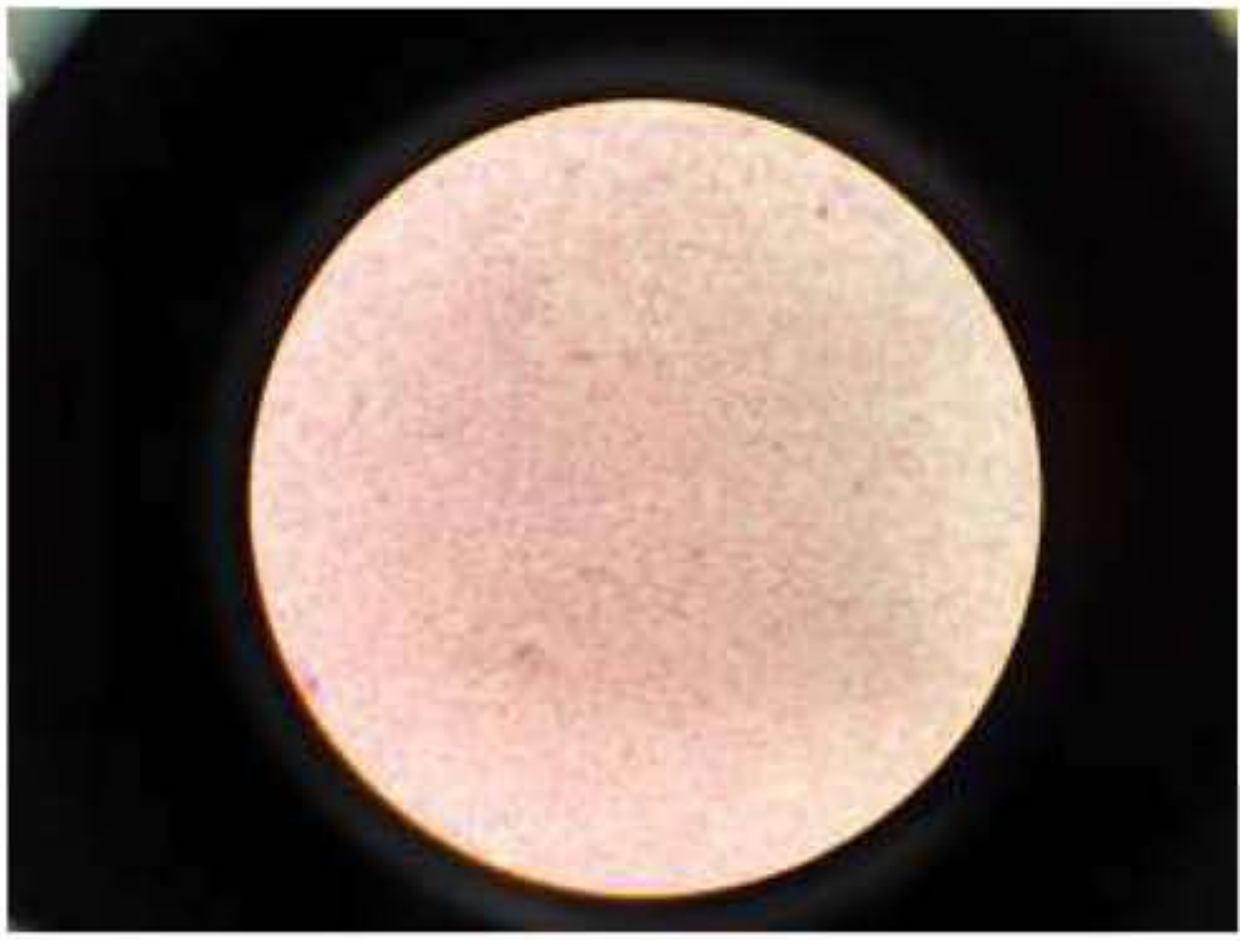

Fig. 2: On Gram's staining, the morphology of the isolated bacteria was small rod shape,Gram negative, single or paired in arrangement.

C134 579 10 10ZZZ 10101

Fig. 3: Indole formation test

10 97 5 431C

Fig. 4: Methyl red test

Fig. 5: Voges-Proskauer reaction

Fig. 6: Citrate utilization test

morphological properties were subjected to biochemical tests *viz.* Indole formation (Fig.3), Methyl red (Fig.4) and Voges-Proskauer reaction (Fig.5), Citrate utilization (Fig.6), Urea hydrolysis Triple sugar iron agar (Fig. 7) and ornithin decarboxylase. Out of the 7 suspected

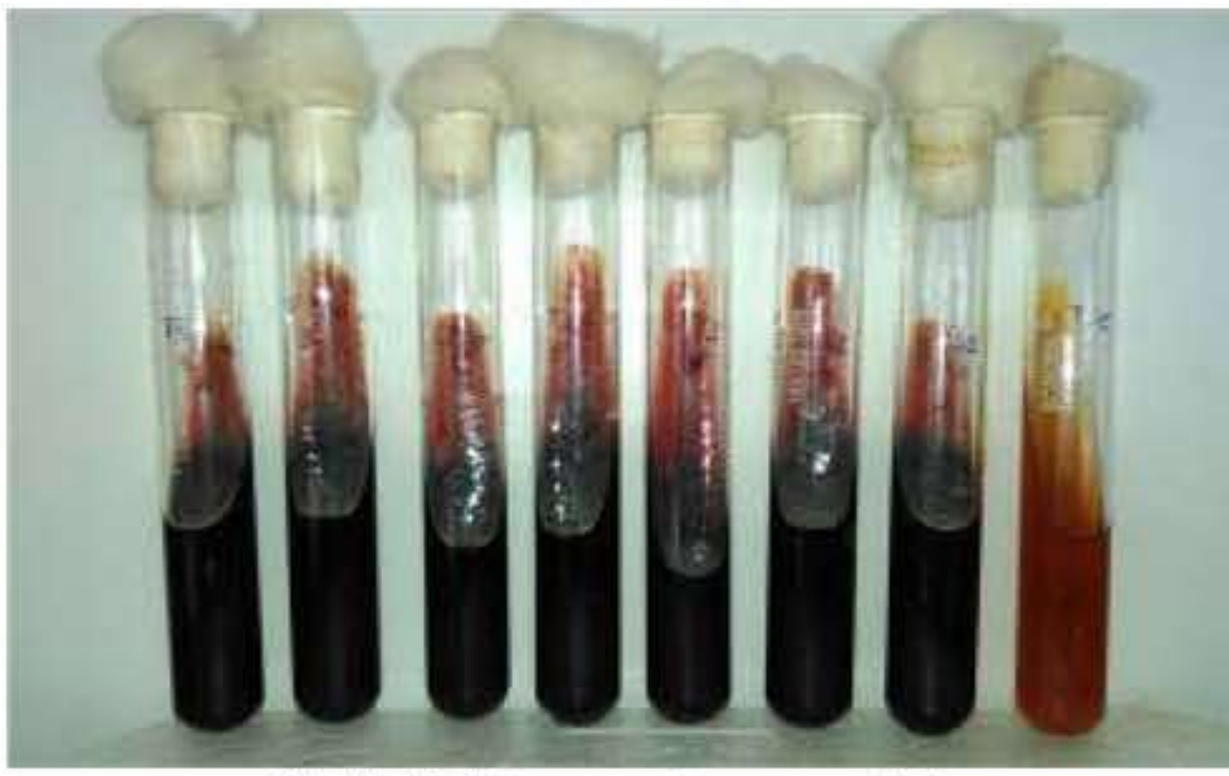

Fig. 7: Triple sugar iron agar test

Fig. 8: Plate showing the Anti biogram sensitivity pattern of *Salmonella* isolate

colonies, 7 isolates were confirmed to be *Salmonella* species after the biochemical results. On the basis of cultural characteristics out of total 45 samples 7 were

Table 1: Summary of the biochemical test results of *Salmonella* isolates

Sl.No.	Biochemical test	S1	S2	S3	S4	S5	S6	S7
1	Indole *Formation	+	+	+	+	+	+	+
2	Methyl red test	+	+	+	+	+	+	+
3	Voges-Proskauer Reaction	-	-	-	-	-	-	-
4	Citrate Utilization	+	+	+	+	+	+	+
5	Urea hydrolysis	-	-	-	-	-	-	-
6	TSI H_2S Production	+	+	+	+	+	+	+

(+) Positive after 48h of incubation
(S) Isolates *Salmonella*
Indole *formation test: V - (Bisping, W and Amtsberg, G. 1988)

Table 2: List of antimicrobial susceptibility discs used in antibiogram study

Sl. No	Antimicrobial Agent	Symbol Disc content	Results in mm						
			S1	S3	S4	S5	S7	S9	S10
1	Cefepime	CPM30	10	10	10	10	10	10	10
2	Spectinomycin	SE100	10	10	10	10	10	10	10
3	Norfloxacin	NX10	**23**	**22**	**24**	**28**	**22**	**21**	**22**
4	Tetracycline	TE30	10	10	10	10	10	10	10
5	Amoxycillin/clavulanic acid	AMC30	10	10	10	10	10	10	10
6	Ampicillin/Sulbactum	A/S10/10	10	10	10	10	10	10	10
7	Ampicillin	AMP2	10	10	10	10	10	10	10
8	Co-Trimoxazole	COT25	**16**	**16**	**17**	**17**	**16**	**17**	**17**
9	Cloxacillin	CX30	10	10	10	10	10	10	10
10	Enrofloxacin	EX10	**24**	**26**	**27**	**27**	**26**	**27**	**26**

confirmed to be *Salmonella* by biochemical characteristics (Table 1).

Antibiogram: Serotypic variation in drug sensitivity has been shown by the isolates in the present study. In addition to the quinolone group of antibiotics, all seven isolates *Salmonella* spp. were sensitive to Enrofloxacin (100%), Norfloxacin (85%) and Co-Trimoxazole (100%) in the present study. Seven isolates *Salmonella* spp. Showed 100% resistance against Cefepime, Spectinomycin, Amoxycillin/Clavulanic acid, Tetracycline, Ampicillin/ Sulbactum, Ampicillin and Cloxacillin. Antibiotic resistance has been reported to be more common in *Salmonella* spp. than the other serovars. Sensitive to some of the aminoglycosides such as Enrofloxacin (Fig.8) is great concern since it has been a major drug of choice in the treatment of enteric infections.

CONCLUSION

The present study indicated that the prevalence of salmonella infection in the backyard chicken resistant to most anti-bacterials used in the therapy, posing a risk to industrial poultry farms, and public health. Therefore, any prophylactic programme in controlling *Salmonella* infections must also take into account the infection in backyard chicken.

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Occurrence of Nitrofuran (AOZ) Antibacterial Residues in Chicken Eggs

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ABSTRACT

Like in human, antibacterials are used in animals for treating infectious diseases caused by bacteria. However, they are also used for non-therapeutic purposes like growth promotion in intensive farming of food-producing animals such as chicken, pigs and fish. Nitrofuran group of antibiotics, employed for the treatment of bacterial diseases in livestock production, were banned from use in the European Union (EU) in 1995 due to concerns about the carcinogenicity and mutagenicity of their residues in edible animal products. In the present study 150 commercial chicken eggs were analysed for estimation of 3-amino-2-oxazolidinone (AOZ), a metabolite of furazolidone by ELISA method using commercially available Ridascreen® Nitrofuran (AOZ) ELISA kit. In 145 samples AOZ values were found to be within MRPL (1000 ng/kg) and in 5 samples AOZ values were more than the MRPL. Regulatory authorities and producers are required to identify and eliminate the contamination source to ensure the chemical safety of foods available to the consumer.

Keywords: Nitrofuran, ELISA, 3-amino-2-oxazolidinone (AOZ), MRPL

In response to the growing global demand of animal proteins, antibacterial/antibiotic growth promoters (AGPs) have become an integral part of commercial farm practices. Growth promotion refers to increase rate of weight gain and/or feed utilisation by other thanpurely nutritional means. This definition applies to food-producing animals which are reared for meat production. Antibiotics as AGPs are extensively used across the world in an uncontrolled way except in certain countries that have started regulating such use over the last decade. In view of the antibiotic resistance and its strong association with antibiotic use, the World Health Organisation (WHO) came up with a list of critical antibiotics that need to be preserved for human use (World Health Organisation, 2011). In India, unlike the countries with stringent regulations, several such antibiotics are used in food-producing animals for non-therapeutic reasons. Almost all AGPs used in the Indian poultry industry are banned in countries of European Union (EU).

Nitrofurans are synthetsic broad-spectrum antibiotics, which are frequently employed in animal production for their excellent antibacterial and pharmacokinetic properties. They had been also used as growth promoters in shrimps, poultry and pig production. Nitrofurans were prohibited within the EU for use in food-producing animals (European Commission, 1995) because of their potentially carcinogenic and mutagenic effects (Van Koten-Vermeulen *et al.*, 1993). The EU has established a minimum required performance limit (MRPL) of 1000 ng/kg, for edible tissues of animal origin (Commission Decision, 2003).

The analysis of residues of nitrofuran drugs needs to be based on the detection of the tissue bound metabolites of the nitrofuran parent drugs (Hoogenboom *et al.*, 1991; Cooper *et al.*, 2005). Since the parent drugs are very rapidly metabolized, they are not detectable shortly after treatment. The tissue bound nitrofuran metabolites are detectable for a long time after administration and therefore they are used for the detection of the abuse of nitrofurans. AOZ (3-amino-2-oxazolidinone) is a nitrofuran metabolite found after administration of Furazolidone.

MATERIALS AND METHODS

Commercially available 150 chicken eggs were collected from retail shops in different areas of Bangalore, Karnataka and stored at 4°C in refrigerator. These chicken eggs were subjected to quantitative

analysis of AOZ using commercially available Ridascreen® Nitrofuran (AOZ) ELISA kit from R-Biopharm AG, Germany.

Test principle: The basis of the test is the antigen-antibody reaction. The microtitre wells are coated with capture antibodies directed against anti-AOZ antibodies. AOZ standards or sample solution, AOZ enzyme conjugate and anti-AOZ antibodies are added. Free AOZ and AOZ enzyme conjugate compete for the antibody binding sites. At the same time, anti-AOZ antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. After adding substrate/chromogen, bound enzyme conjugate converts the chromogen into a blue product. The addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm. The absorption is inversely proportional to the AOZ concentration in the sample.

Sample extraction: The sample extraction procedure was followed as per the instructions given in the kit. 3.9 ml H_2O, 0.5 ml 1 M HCl and 200 ml 10 mM 2-nitrobenzoic aldehyde (in DMSO) was added to a 1 g sample of homogenized each chicken eggs. After thorough shaking, the tubes containing the samples were incubated at 50°C for 3 hours; 5 ml 0.1 M K_2PO_4, 0.4 ml 1 M NaOH and 10 ml ethyl acetate were then added and the tubes shaken vigorously for 30s then centrifuged 3000 rpm for 10 min at room temperature. A 2.5 ml portion of ethyl acetate (upper layer) was then transferred into a new vial and evaporated, after which the residue was dissolved in 1 ml n-hexane and mixed thoroughly with 1 ml sample buffer. The vials were centrifuged again, and then 50 µl of the aqueous layer was used.

Test procedure: The test procedure was followed as per the instructions given in the kit. All standards and samples (50 µl of) were added in duplicates to wells in the microwell plaste and the positions of the standards and samples were recorded. 50 µl of the enzyme conjugate was placed in the bottom of each well, and 50 µl of the antibody was added to each well, followed by gentle mixing, and then incubated for 1 hr at room temperature in the dark. After incubation all the liquids were poured out of the wells and microwell plate was tapped upside down against absorbent paper. 250 µl of washing buffer was added and discarded the liquid again. A 100 µl of substrate/chromagen was then added, followed by gentle mixing and then incubated for 1 hr at room temperature. 100 µl of the stop solution was then added to each well and the solutions gently mixed by manually. The absorbance at 450 nm was then measured against an air blank and the results were read within 30 min.

RIDA® SOFT Win (Art No. Z9999) software was used to evaluate the enzyme immunoassay.

RESULTS AND DISCUSSION

All the samples were analysed for estimation of AOZ. Standard curve for nitrofuran (AOZ) standards is shown in fig. 1. The calculated optical density, percentage of absorbance and calculated AOZ in ng/kg is given in table 1. Number of samples of chicken eggs in different range of nitrofuran (AOZ) values is given in table 2. All the calculated AOZ values were

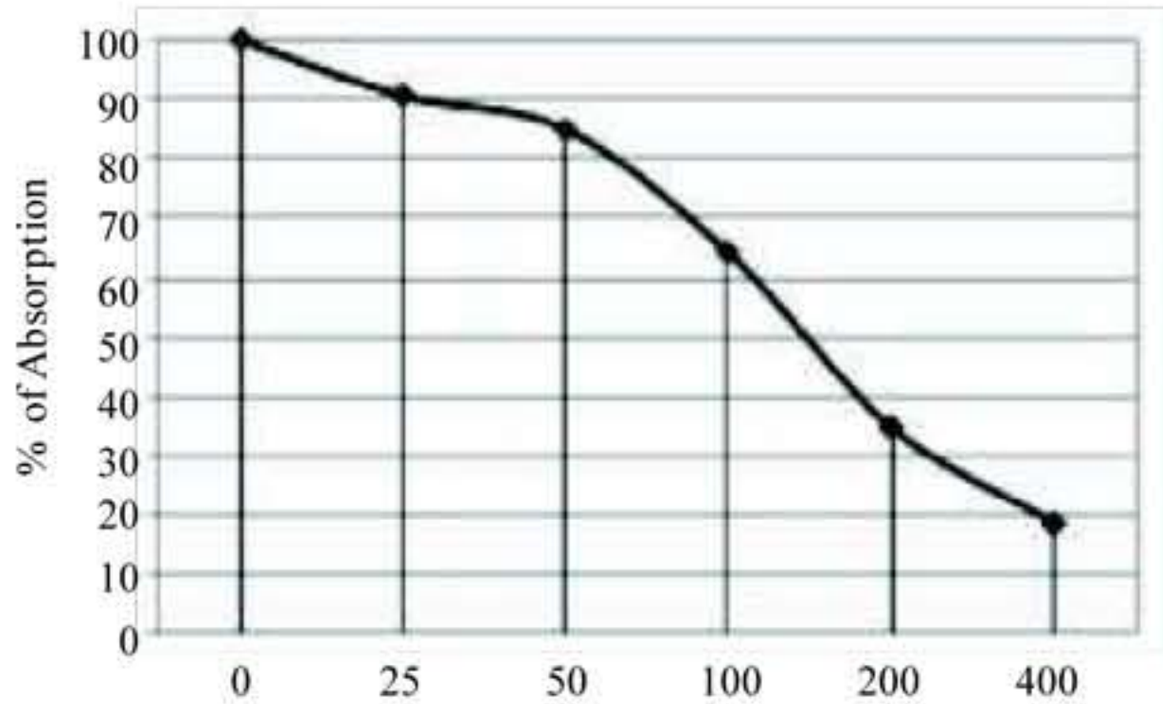

Fig. 1: Standard curve for nitrofuran (AOZ) standards

Table 1: Nitrofuran (AOZ) enzyme immunoassay results

Parameter	Mean values	Range
Optical Density	2.09554	0128-2.70
Percentage of absorbance	87.09	5.3-98.6
AOZ in ng/kg	119.154	4362-1489.71

Table 2: Number of samples in different range of nitrofuran (AOZ) values

Nitrofuran AOZ in ng/kg	No. of samples
Â100 ng/kg	142
100-500 ng/kg	0
500-1000 ng/kg	3
Ã1000 ng/kg	5

found to be within MRPL *i.e.*, 1000 ng/kg., except in five samples with AOZ values of 1485.69, 1323.55, 1489.71, 1348.61 and 1228.14 ng/kg which were found to be more than the MRPL. Present European legislation does not permit any confirmed concentration of nitrofuran residues in food commodities, although an MRPL of 1000 ng/kg has been laid down by the European Commission for nitrofuran metabolites in edible tissues of animal origin

CONCLUSION

The presence of nitrofuran residues in chicken eggs has been well documented in recent years by the European "Rapid Alert System for Food and Feed". Sampling procedures and monitoring plans for regulatory laboratories are necessary to ensure consumers safety. Present European legislation does not permit any confirmed concentration of nitrofuran residues in food commodities, although an MRPL of 1000 ng/kg has been laid down by the European Commission for nitrofuran metabolites in edible tissues of animal origin. Detection of a parent nitrofuran or its metabolite below the concentration of 1 µg/kg requires enforcement action (product withdrawal, issue of alert notifications by the RASFF etc.). Regulatory authorities and producers are required to identify and eliminate the contamination source to ensure the chemical safety of foods available to the consumer.

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Influence of BCS on Conception Rate and Frequency of Multiple Births in Estrus Synchronized NARI Suwarna Ewes with Different Systems of Feeding*

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ABSTRACT

The present study was under taken to assess the effect of BCS (Body Condition Score) on conception rate and the frequency of multiple births in estrus synchronized NARI Suwarna ewes maintained under different systems of feeding. Sixty adult NARI Suwarna ewes of same age and BCS of ≤ 2.5, 2.5-3.0 and 3.0-3.5 were divided randomly in to two groups. Ewes in both the groups were subjected for estrus synchronization protocol for 12 days by using intravaginal progesterone sponges with PGF2α and PMSG. Ewes were inseminated with freshly collected diluted semen and pregnancy was diagnosed with Ultrasonography 30 days of post AI. The mean conception recorded in BCS 2.5-3.0 was 83.33 and 50%. Similarly, the mean conception recorded in BCS 3.0-3.5 was 90.1 and 71.4% under scientific feeding and pasture grazing respectively. The frequency of multiple births recorded was 80 and 60% with BCS 2.5-3.0 and 100 and 80% with BCS 3.0-3.5 under scientific feeding and pasture grazed ewes respectively. In conclusion, significantly higher conception and higher multiple births were observed in ewes with BCS of 2.5-3.0 and 3.0-3.5 under scientific feeding than pasture grazing.

Keywords: Ewes, BCS, conception, frequency of multiple births, scientific feeding and pasture grazing

Body Condition Score (BCS) has been proved to be one of the most important management tools, for assessing the nutritional status of ewes. Body condition directly affect hypothalamic activity and GnRH secretion, but not pituitary sensitivity to GnRH, and these effects on reproductive performance are also mediated through changes in ovarian hormones or in hypothalamo-pituitary sensitivity to ovarian hormones (Rhind *et al.*, 1989b).The effect of body condition on the ovulation rate of ewes has been extensively reported (Gunn and Doney, 1979; Gonzalez *et al.*, 1997) and high body condition score has been associated with an increase of ovulation (Xu *et al.*, 1989). Husein & Ababneh (2008) and Contreras-Solis *et al.* (2009) recommend a BCS of 2.5 to 3.0 either for natural or artificially breeding in sheep. Aliyari *et al.* (2012) reported that conception rate was 86, 82 and 48 per cent at BCS of 3.5, 3.0 and 2.5 respectively, during first estrus of Afshari breed of ewe.

The absolute effects of BCS and live weight than their variations have greater impact on sheep reproduction efficiency, which suggest the importance of breed and interactions with nutritional and physiological conditions and its impact on reproduction efficiency (Contreras-Solis *et al.,* 2009; Koycegiz *et al.*, 2009).

Improving the BCS by flushing before breeding season is necessary, because it activates the most important reproductive characters such as fertility, livability and prolificacy (Xu *et al.,* 1989). Increasing prolificacy in sheep is a cost effective approach to increase the efficiency of meat production in a sustainable manner. Advances in genetic selection and breeding have significantly increased the proportion of multiple-pregnancies. However, competition between twin fetuses in mid-late pregnancy leads to restricted fetal growth, organ and tissue development, lower birth weight and increased mortality compared to their singleton counterparts (Wu *et al.*, 2006)

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Insufficient energy results in decreased growth, delays in reaching puberty and a decrease in reproductive performance (Schillo *et al.,* 1992). The adoption of flushing before and during the mating period results in a significant increase in ovulation rates (Molle *et al.*, 1997), decrease of follicular atresia (Schillo *et al.,* 1992), better body condition at mating and higher incidences of twin births (Mukasa-Mugerwa and Lahlou-Kassi, 1995). Therefore, the present study was conducted to investigate the effect of Body Condition Score on conception rate and the frequency of multiple births in estrus synchronized NARI Suwarna ewes with different systems of feeding.

MATERIALS AND METHODS

Sixty adult Nari Suwarna ewes of same age group were randomly divided in to 2 groups. All the ewes were vaccinated against Foot and Mouth, Enterotoxemia, Peste des petits ruminants and Black Quarter diseases and also dewormed once in 3 months using broad spectrum anthelmintics. Ewes in group I (n=30) received approximately 250g of balanced sheep feed, comprising of yellow maize 45%, soya bean meal 15%, wheat bran 35.5%, salt 2% and mineral mixture 2% daily as per the recommendations described by Brown, (1994). In addition to the concentrate feed, ewes were fed with *adlib* ragi straw and water. Ewes in group II (n=30) did not receive any concentrate feed or mineral mixture and were allowed to graze in the field for a period of 10 hr daily and these ewes were maintained under pasture grazing throughout the period of study. Body Condition Scoring (BCS) of all ewes were determined as per the procedure described by Aliyari *et al.* (2012) as ≤ 2.5, 2.5-3.0 and 3.0-3.5 by a team of three scorers and the average was taken in to consideration to group the ewes. Further, ewes in both the groups were subjected for estrus synchronization protocol for 12 days by using intravaginal progesterone sponges with PGF2α and PMSG. Further, ewes in both the groups identified to be in estrus were artificially inseminated (AI) using freshly collected and diluted ram semen of the same breed. All the inseminations were carried out at approximately 12 hr after the identification of estrus and ewes were artificially inseminated only once. Those ewes not returning to estrus were subjected to pregnancy diagnosis between 30-35 days of post AI using ultra sound scanner. Following lambing, single/ multiple births in ewes were recorded. Statistical analysis was carried out by using student 't' test.

RESULTS AND DISCUSSION

The conception rate was 83.33% in balanced feed group with BCS of 2.5-3.0 while only 50% of the sheep conceived with a similar BCS in pasture grazing group. A similar trend was observed in sheep with a BCS of 3.0-3.5 in which the conception rate was 90.1 and 71.4% in balanced feed and pasture grazing groups respectively (Table 1).

The multiple births were recorded as 80% when BCS was between 2.5-3.0 in group of ewes maintained

Table 1: Influence of BCS on conception rate and frequency of multiple births in estrus synchronized NARI Suwarna ewes maintained under two different systems of feeding.

BCS	Scientific feeding (n=30)			Pasture grazing (n=30)		
	Conception rate	Single	Multiples	Conception rate	Single	Multiples
≤2.5	0	0	0	1 (33.33%)	1 100%	–
2.5 - 3.0	10 (83.33%)[a]	2[a] 10%	8[b] 80%	05 (50%)[b]	2 40%	3 60%
3.0 - 3.5	10 (90.1%)	0	10[b] 100%	05 (71.4%)	1[b] 20%	4[a] 80%

The values with different superscript in a row differ significantly at p ≤ 0.05.

on a balanced feed and 20% higher than the incidence recorded in pasture grazed ewes with a similar BCS. A similar trend was observed in animals with a BCS of 3.0-3.5, in which the frequency of multiple births in ewes maintained with a balanced feed and pasture grazing being recorded as 100% and 80% respectively (Table 1).

Flushing refers to the practice of having ewes on a raising plane of nutrition for some defined period to improve ovulation rates prior to the introduction of the ram as noted by Rhind *et al.* (1989b). The practice of flushing is an excellent example of the interactions that exist between nutrition and reproduction. The body fat content (body condition) directly affects hypothalamic activity and GnRH secretion and those effects on reproductive performance are mediated by way of changes in ovarian hormones or in hypothalamic- pituitary sensitivity to ovarian hormones (Rhind *et al.*, 1989a).

The ewes with a high BCS had a higher number of gonadotrophin dependent follicles than ewes in low body condition (Xu *et al.*, 1989). Low BCS in ewes also related with prevention of estrus and fertility (Rhind *et al.*, 1985). It is therefore, appears that the BCS at the time of mating directly or indirectly influence the hypothalamo-pitutary ovarian axis and has a definite role to play in reproduction.

Therefore, it is concluded that, the BCS between 2.5- 3.5 in ewes on a balanced feed proved to be beneficial in terms of good conception as well as obtaining more multiple births.

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Effect of *Azadirachta indica* on Body Weight Gain in Diabetes Induced Rats

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ABSTRACT

Neem (*Azadirachta indica*) is an evergreen tree that grows throughout India. Aqueous extract is known to produce antihyperglycemic and hypoglycemic activity in diabetic dogs. The present study was performed to evaluate effect of *Azadirachta indica* on body weight gain in diabetes induced rats. Diabetes was induced by streptozotocin @ 40 mg/kg body weight. Rats were administered *Azadirachta indica,* gilbenclamide and a combination of both. A significant reduction in the mean body weight was observed in the diabetic control animal. An improvement was observed in the body weight of rats administered with *Azadirachta indica* alone. Diabetic rats treated with Glibenclamide @ 600 µg/kg b.w. dose showed significant improvement in their body weight when compared to that of diabetic control. *Azadirachta indica* @ 500 mg/kg body weight dose showed superior ameliorative effect than @ 250 mg/kg bodyweight in combination with 300 µg/kg body weight glibenclamide. Overall study revealed satisfactory effect on body weight gain in diabetic rats treated with Azadirachtaindica.

Keywords: *Azadirachta indica*, body weight, diabetic rat and glibenclamide

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins. India had highest number of diabetic people in 2000, *i.e.*, 31.7 million and expected to rise to 79.4 million by 2030. Therefore, India is being termed as "diabetes capital of the world".

Hypoinsulinism causes catabolism of carbohydrates, proteins and fats leading to loss of weight (Warkins, 2003). Inability to utilize glucose is reflected in the clinical signs of diabetes, loss of weight, polyuria, polydipsia and in advanced stages, ketoacidosis. In no other disease is an understanding of metabolic alteration so important in diagnosis and proper treatment (Kaneko, 2008).

Neem is known to produce antihyperglycemic and hypoglycemic activity in diabetic dogs (Satyanarayana *et al.,* 1978). The present study was carried out to evaluate efficacy of *Azadirachta indica* extract on body weight gain in diabetes induced rats and to compare it with an oral hypoglycemic agent glibenclamide.

MATERIALS AND METHODS

Streptozocin: To induce diabetes in rats, streptozotocin (M/s Sigma Chemicals. St. Louis, USA) was dissolved in ice-cold citrate buffer (O.1M, pH 4.5) (Babu and Prince, 2004).

Plant extract: Alcoholic extract of Neem used in the present study was procured from M/s Himalaya Drug Company, Bangalore.

Design: Female Wistar albino rats weighing 180-200 g obtained from a breeder were used in the present study. They were maintained under standard laboratory conditions with free access to standard feed (Vetcare Feeds, Bangalore) and drinking water. The animals were acclimatized for 2 weeks. The study was carried out with prior approval from the institutional Animal Ethics Committee.

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Experimental design: All the experimental animals were divided into 5 groups with 12 animals in each group as follows:

Group I : Non diabetic control/normal rats, administered NSS p.o.

Group II : Diabetic control, administered ST2 I/P p.o.

Group III : Diabetic rats supplemented with *Azadirachta indica* extract p.o. at the dose rate of 500 mg/kg body weight in distilled water for 60 days.

Group IV : Diabetic rats supplemented with glibenclamide p.o. at the dose of 600 µg/kg body weight for 60 days.

Group V : Diabetic rats supplemented with 50% of the dose of glibenclamide (300 µg/kg) and *Azadirachta indica* (250 mg/kg) extract p.o.for 60 days.

Experimental induction of diabetes: The animal were fasted overnight and diabetes was induced in animals of Group II to V by a single intraperitoneal injection of freshly prepared solution of streptozotocin at the dose rate of 40 mg/kg b.w. in 0.0 M cold citrate buffer of pH 4.5 (Babu and Prince, 2004). Control group (G-1) animal received citrate buffer alone. The blood glucose levels were estimated 72 hours post STZ injection using Glucochek glucometer (Aspen Diagnostic Pvt. Ltd. Delhi, India). The glucometer has been designed to measure the blood glucose level by GOD-POD method (Glucose oxidase-peroxidase method). The animals with blood glucose levels above 200 mg/dl were considered as diabetic. After confirmation of diabetic status, all the groups received their respective treatments daily for 60 days. Rats of all the groups were observed for feed and water intake, general behavior, alertness, urine output, diarrhea and any other clinical symptoms. The rats were weighed on the day of commencement of experiment and on 15th, 30th, 45th and 60th day of the experiment to evaluate the effects of various treatments on body weight.

Statistical analysis: Statistical analysis was performed using the statistical software Graph Pad Prism, Trial version 5 for windows, Graph Pad Software (San Diego Califormia UAS). Mean values and standard error were calculated and all values were expressed as mean (± SE). The data were analyzed by two way analysis of variance (Anova) for all parameters.

RESULTS AND DISCUSSION

In the present study, the normal control rats (Group I) remained healthy throughout the experimental period. All the rats from the Group II to V became diabetic on 3rd day and the mean glucose values ranged from 322 mg/dl to 680 mg/dl against 86 mg/dl of control rats of Group I.

The mean (± SE) body weights of different groups are presented in Table 1 and Fig. 1. All animals in the normal control group remained healthy at different time period of the study. All the body weight values were within the normal range and indicated their healthy status.

A significant reduction in the mean body weight was observed throughout the study in the diabetic control animals. The decrease was statistically significant ($P \leq 0.001$) from 15th day post STZ injection. The decrease in the weight could be attributed to hypoinsulinism that occurs in diabetes. Similar findings were also noticed by earlier workers (Pillion *et al.*, 1988/ Kakkar *et al.,* 1998; Virdi *et al.*, 2003; Dhanush, 2009 and Mallikarjuna, 2009).

As insulin is an anabolic hormone, its deficiency causes catabolism of carbohydrates, proteins and fats leading to loss of body weight. The other factors contributing for the reduced weight include decreased protein synthesis in the absence of insulin, partly because of diminished transport of amino acids to the muscle (Warkins, 2003), loss of fluids leading to dehydration through glycosuric polyuria and altered uptake of glucose and glycogenesis by target cell (Hakim *et al.,* 1997 and Rubin and Strayer, 2008).

An improvement in body weight was observed in the groups treated with *Azadirachta indica* extract

Table 1: The mean (±SE) animal body weight (g) values of normal control, diabetic and diabetic treatment groups at different intervals of time

Groups	Days-Post Treatment				
	3	15	30	45	60
Group I (NC)	184.64±4.16^{a}	192.15±4.19^{a}	201.57±4.23^{a}	222.80±2.70^{a}	247.07±2.65^{a}
Group II (DC)	188.44±4.41^{ba}	175.32±3.06^{b}	165.42±3.88^{b}	153.15±3.76^{b}	132.67±2.97^{b}
Group III (AI)	185.92±3.75^{cabe}	202.4±3.94^{cae}	214.42±3.37^{cae}	229.42±2.34^{cae}	247.07±2.65^{cae}
Group IV (GC)	178.55±2.63^{dabe}	186.35±2.91^{dac}	198.30±1.97^{dac}	206.27±2.58^{dac}	224.79±2.40^{dac}
Group V (AI+GC)	190±4.34^{eabd}	197.54±4.88^{eab}	207.27±4.74^{ea}	216.17±4.56^{e}	235.35±4.62

The means with at least one common superscript within the rows are not significantly different.
Values are statistically significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$

(both Group-III and Group V) as compared to diabetic rats (Group II). These findings are in accordance with the observations of earlier workers (Bopanna *et al.*, 1997; Rasheed *et al., 2008;* Das *et al.*, 2010; Bhat *et al.*, 2011; Akpan *et al.*, 2012 and Akter *et al.*, 2014). The increased body weight could be due to some constituents of the neem extract which might have mimicked or stimulated the actions of growth factors hence its ability to enhance the repair and regeneration of damaged pancreatic tissue.

The major active constituents of the tree are nimbin, nimbidin and nimbinene. The leaves yield quercetin (flavonoid) and nimbosterol (â-sitosteriol) as well as a number of liminoids. The trunk bark contains nimbin (0.004%), nimbinene (0.001%) and tannins (6), while, stem bark contains tannins (12-16%) and non-tannins (8-11%). The oil extracted from the seeds contains nimbosterol and flavonoids (Biswas *et al.*, 2002).

There was a gradual decrease in severity of clinical signs such as polyuria, polydipsia, polyphagia and weight loss in diabetic animals treated with the *Azadirachta indica* (Group III and IV) treated rats. The improvement in the body condition could be attributed to the hypoglycemic, hypolipidemic, hepatoprotective, insulinomimetic and insulin secretagogue effects of the plant extract in diabetic subjects.

Based on all these results it can be construed that *Azadirachta indica* showed a superior ameliorative effect with the higher dose @ 500 mg/kg body weight than *Azadirachta indica* @ 250 mg/kg body weight in combination with 300 µg/kg body weight glibenclamide.

Glibenclamide is a potent second generation sulphonylurea drug which is used as a cardinal drug in the treatment of type-II diabetes mellitus. Glibenclamide improves glucose control by acting both by acting both on insulin secretion and insulin action.

Group-IV diabetic rats were treated with Glibenclamide @ 600 µg/kg b.w. from 3rd day to 60th day. The animals showed significant improvement in their body weight when compared to that diabetic control rats. Similar findings were reported by Virdi *et al. (*2003), Dhanush (2009), Mallikarjuna (2009), Pragathi (2011), Nazreen (2012) and Manjunath (2013).

There was a drastic decrease in the body weight of diabetic control group throughout the study. The animals in the treatment groups showed improvement by gaining the body weight. The body weight gains of Group III (diabetic rats treated with *Azadirachta indica* extract @ 500 mg/kg b w) and Group IV (diabetic rats treated with glibenclamide) was comparable with normal control group.

The above findings reveal that alcoholic extract of *Azadirachta indica* @ 500 mg/kg bw) has satisfactory effect on improvement of body weight gain in diabetic rats. *Azadirachta indica* would facilitate reduction in dose of glibenclamide to 300 µg/kg body weight when administered in combination. Further studies should focus on effect of *Azadirachta-indica* on biochemical, enzyme and pathomorpho logical changes in diabetic animal.

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Host Preferences of Dominant *Culicoides* (Diptera: Ceratopogonidae) Species Near Domestic Animals

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ABSTRACT

Culicoides midges are the smallest haematophagus insects involved in the transmission of Bluetongue. The possibility of disease transmission mainly depends on the possible species of vector in that area and the effective host-vector contact. The present study discusses the host preferences of *Culicoides imicola* and *C. oxystoma*, species on different domestic animal species in Bidar, Karnataka. The midges were collected from the body of different domestic species using electrically operated insect aspirator during dawn, dusk and nearing to the midnight. Based on the number of midges collected from the body it was found that *Culicoides imicola* prefer goats followed by sheep, cattle and buffaloes in decreasing order. Host preference of *C. oxystoma* based on aspirations revealed its highest preference for buffaloes followed by cattle. Its preferences for sheep and goats were low when compared to cattle and buffaloes. Since, *C. imicola* is a proven vector for transmission of bluetongue and its preference to sheep and goats indicated that this species may be probably a candidate vector for bluetongue in this region.

Keywords: *Culicoides*, insect aspirator, bluetongue

Species of *Culicoides* have been found in almost all parts of the world. About 1,400 species are known to live in a wide variety of habitats (Mellor *et al.*, 2000). Despite their small size, ranging from 1 to 3 mm, members of this genus are associated with the transmission of several different viruses (n = 66), protozoa (n =15) and filarial nematodes (n = 26) to a diversity of hosts (Meiswinkel *et al.*, 2004; Borkent, 2005). At least three orbiviruses, African horse sickness (AHSV), bluetongue (BTV) and epizootic haemorrhagic disease virus (EHDV) cause viral diseases of such international significance that they have been classified as notifiable diseases to the Office International des Epizooties (OIE).

The host-seeking and feeding behaviours of the midges are poorly described partially because of the difficulty in collecting these small insects directly on animals. The host range of biting midges is not completely known. Most *Culicoides* species are mammalophilic or ornithophilic, although some of them feed on reptiles and frogs. The majority of biting midges are exophagous feeders and do not attack their hosts in enclosed places (Borkent, 2005). This explains the importance of keeping cows inside stables in the most active hours of the biting midges, around dusk and dawn to curtail the transmission of bluetongue virus (Baylis *et al.*, 2010). However, endophagy has been demonstrated in some mammalophilic biting midge species, the females of which feed on animals inside barns (Barnard, 1997).

Culicoides imicola and *C. oxystoma* were identified as dominant midges in Bidar, Karnataka based on light trap collection (Satheesha *et al.*, 2014). In the present study an attempt has been made to collect these dominant *Culicoides* midges from the body of different domestic animal species.The trapping of large

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numbers of certain *Culicoides* near specific host animals is generally used as an indicator of host preference (Meiswinkel *et al.*, 2004). Host preference will influence the biting rate of a species and is therefore one of the critical factors that will influence the vector capacity of a *Culicoides* species (Mullens *et al.*, 2004). In turn, this may help in understanding the species of midge involved in transmission of bluetongue disease.

MATERIALS AND METHODS

Culicoides midges were aspirated from the body of different host species *viz.*, sheep, goats, cattle and buffaloes using an electrically operated insect aspirator (Plate) fabricated using a 230V, 20W instrument cooling fan. The method of aspiration was followed as per Saenz *et al.* (1994). The aspiration procedure was performed during sun set, nearing to midnight and early morning. The insect aspirator was moved close to the body of the animal over the neck, back and sides and underneath the abdomen for 5 minutes. Such 5 to 6 aspirations were made each at 15 minutes intervals. The insects collected at each run were transferred to the plastic container having 70 percent ethyl alcohol. Identification of dominant *Culicoide s*species was done based on the wing pattern of *Culicoides* species following the method of Wirth and Hubert (1989).

RESULTS

The aspiration of insects using insect aspirator near cattle, buffaloes, sheep and goats at Veterinary College, Bidar, yielded only two *Culicoides* species *viz., C. imicola* and *C. oxystoma*. The overall collections near all the species of animals revealed higher numbers of *C. imicola* compared to *C. oxystoma*. The details of aspiration of midges near all the species of animals were presented in the Table 1.

A total of 36 aspirations were made from the body of cattle which yielded a total of 139 midges. The percentage of abundance of *C. oxystoma* was higher compared to *C. imicola*. The total number of midges collected nearing to the midnight was higher compared to aspirations made during sunrise and immediately after sunset near all the species of animals. In case of buffaloes the aspiration of *C. oxystoma* was significantly higher compared to *C. imicola*. The aspirations made near sheep and goats yielded significantly higher percentages of *C. imicola* than *C. oxystoma.*

DISCUSSION

The method of aspiration of midges made near the animals in the present study was similar to that of Saenz *et al.* (1994) who used modified hand held electric vaccum cleaner. The abundance of each species of these *Culicoides* midges in the catches made during the study period in the sheds of each host species were compared to find the order of preference for host species by the two dominant individual species of midges. Both aspirations and light trap collections of *Culicoides* midges were considered for host preference study by Greiner *et al.* (1990).

The circadian rhythm of both *C. imicola* and *C. oxystoma* species was studied, which revealed activity of these midges throughout the night. Fall *et al.* (2015) reported that the *Culicoides* midges are crepuscular or nocturnal and the continuous activity of *C. oxystoma* throughout the diel with peaks in numbers collected after sunrise and sunset, while the activity of *C.imicola* was mostly nocturnal with peak after sunset.

Based on the number of midges aspirated on different species of animals it was considered that *Culicoides imicola* was found to prefer goats followed by sheep, cattle and buffaloes in decreasing order and the difference in the preferences. *Culicoides imicola* was reported to prefer sheep (Mellor *et al.*, 1985) and cattle (Braverman *et al.*, 2003). Randal (1982) collected large number of *C. imicola* and *C. oxystoma* using hand net near sheep and cattle in Natal, Pietermaritzburg. Since the serological evidence of BT in both cattle and sheep are well documented, the association of *C. imicola* with these host species in the present study may have an epidemiological significance for bluetongue disease. Since, *C. imicola* which is already proven as vector of BT in South Africa (DuToit, 1944) and Middle East (Boorman and Wilkinson, 1983) and was found to have preference for goats and sheep in the present study and it stands first as a candidate species as BTV vector based on

Table 1: Host preferences of *Culicoides* species collected over the body of animals using insect aspirator at different times of the day during the period of study

Species of animals	Time of collection	Number of collections	No. of midges collected		Total midges collected
			C. imicola	*C. oxystoma*	
Cattle	Around sunrise	11	13	15	28
	After sunset	11	09	16	25
	nearing to midnight	14	38	48	86
	TOTAL	36	60(43.17)	79(56.83)	139
Buffaloes	Around sunrise	10	04	21	26
	After sunset	8	02	15	17
	nearing to midnight	11	10	24	34
	TOTAL	29	16(21.05)	60(78.95)	76
Sheep	Around sunrise	11	12	03	15
	After sunset	07	08	07	15
	nearing to midnight	11	15	06	21
	TOTAL	29	35(68.63)	16(31.37)	51
Goats	Around sun rise	10	14	5	19
	After sunset	7	09	2	11
	nearing to midnight	10	17	5	22
	TOTAL	27	40(76.92)	12(23.08)	52

Fig. 1: Electrically operated insect aspirator

the presence of vector capacity traits such as host preference and abundance.

Host preference of *C. oxystoma* based on aspirations over the body of these animals revealed its highest preference for buffaloes followed by cattle. Its preferences for sheep and goats were low when compared to cattle and buffaloes. In a study for host preferences conducted at Marathwada region of Maharashtra, by Narladkar *et al.* (1993) using insect aspirator, and light trap (Narladkar and Shivpuje, 2014) it was reported that *C. schultzei* showed preferences for feeding on cattle, buffaloes, sheep and goats. Dadawala *et al.* (2012) isolated BTV serotype 1 from *C. oxystoma*in India. The preference of *C. oxystoma* in buffaloes and cattle and to an extent sheep and goats as revealed in the present study may get epidemiological significance in transmission of bluetongue. Further attempts on host preferences of species of *Culicoides* for different host species using various other techniques like bait traps and blood meal identification in vectors may yield better clarity on their rolein the transmission of arboviral diseases like bluetongue.

Since *C. imicola* and *C. oxystoma* are the potential species. The higher abundance of *C. imicola* is higher on the body of goats followed by sheep, cattle and buffaloes respectively. *Culicoides oxystoma* abundance was higher on buffaloes followed by cattle, sheep and goats respectively. The preferences of these potential midges for sheep and goats supports the

possibility of their role in the transmission of Arboviral diseases like bluetongue in this region.

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Serum Creatinine, Cholesterol and Triglyceride Levels in Ochratoxicosis and its Amelioration Using DAE and Vitamin E in Broiler Chickens

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ABSTRACT

The present work was conducted to study the important serum biochemical alterations in ochratoxicosis and the amelioration effects of DAE and Vitamin E in broiler chickens. The toxigenic strains of *Aspergillus, A. parasiticus*, NRRL 2999 and *A. ochraceus* NRRL 3174 cultures were used for the present study. A total number of 320 birds were divided into 40 each in the Group I to VIII, Group I being the control. Number of treatments were eight and selection of birds used for base line data were six and the duration of the experiment was 35 days. Powdered ochratoxin was incorporated into the feed at the rate of 1 ppm, vitamin-E was fed to the birds at the rate of 80 mg/kg feed and DAE at the rate of 2000 mg/kg feed. Birds from each group were sacrificed on day 7, 14, 21, 28 and 35 and the sera samples were analysed for biochemical parameters like serum creatinine, cholesterol and triglycerides. Administration of ochratoxin to broiler chicken resulted in significant increase in serum creatinine and significant decrease in cholesterol and significant decrease in triglycerides only during first two weeks of experiment. Supplementation of DAE and Vit E significantly reduced the creatinine and cholesterol levels in OA fed group birds and indicated their ameliorative effects on ochratoxin toxicity.

Keywords: DAE, Ocrotoxin A, *Aspergillus fumigatous, A. ochraceus*

Ochratoxins are nephrotoxic, hepatotoxic, carcinogenic, immunotoxic and teratogenic mycotoxins. The exposure to low concentration of ochratoxin causes structural and functional changes in kidney and liver of birds, thus altering the biochemical parameters. The present study has been attempted to assess the efficacy of DAE, a toxin binder and Vitamin E, an anti-oxidant to ameliorate the toxic effects of ochratoxin.

MATERIALS AND METHODS

The present research work was carried out at the Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru to study the ameliorating effect of diatomaceous earth (DAE) and Vitamin E in the experimentally induced ochratoxicosis in broilers.

Fungal Cultures

Source of Fungus

The toxigenic strain of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture maintained at the Department of Veterinary Pathology, Veterinary College, KVAFSU Bengaluru were used in the study.

Production of Mytotoxins

Ochratoxin

Ochratoxin was produced on the broken wheat using *Aspergillus ochraceus* NRRL 3174 as outlined by Trenk *et al.* (1971) with minor modifications. Overnight soaked wheat (50 G + 25ml Tap water) was autoclaved at 121^{0}C at 15 psi for 20 minutes and inoculated with fungal spore suspension. The inoculum was incubated for 12 days at room temperature in dark

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place with vigorous shaking once a day to break the white mycelial masses. The fermented wheat was autoclaved to kill the spores and dried at 80^{0}C in hot air oven, over night. The dried material was powdered and stored in the dark place for further use.

Mycotoxin Quantification

Ochratoxin was quantified using Thin Layer Chromatography (TLC) at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institution (TANUVAS), Namakkal-367 002.

Experimental Information

a) Number of birds : 320
b) Number of treatments : 08
c) Number of birds per treatment : 40
d) Number of birds used as base line data : 06
e) Type of management : Battery Cages
f) Duration of experiment : 05 Weeks

Experimental birds and rearing system

A total of 320 day old commercial broiler Venkobb chicks were procured from a reputed commercial Sri Kadakeshwar hatchery, Bengaluru. The chicks were weighed individually and reared in battery brooder fitted with electrical bulbs on raised wire mesh floor under optimum condition of brooding and management.

Experimental Feed

Commercial broiler starter (0-3 weeks) and finisher feed (4-5 weeks) were procured from Department of Poultry Science, KVAFSU, Bengaluru. The feed samples were screened for mycotoxin content prior to use.

Ochratoxin Feed

Powdered ochratoxin culture material was incorporated into the feed at the rate of 1 ppm. The yield of OA was 25 ppm.

Vitamin E

Vitamin E Tocopherol Acetate adsorbed on precipitated silicon dioxide from Merck Pvt. Ltd., Goa) was mixed with the feed at the rate of 80 mg/kg feed.

Mycotoxin Ameliorating Agent

Diatomaceous Earth (DAE) obtained from M/s Agripower, Australia, was incorporated in feed at the rate of 2000 mg / kg feed).

Biochemical Reagents

Reagent Kits

Biochemical reagent kits of creatinine, cholesterol and triglycerides, were procured from Swemed Diagnostics, Bengaluru.

Methodology

Experimental Design

Table 1: Experimental Design

Treatments	Ochratoxin (ppm)	DAE (mg/ kg feed)	Vitamin E (mg/kg feed)
Group I (Control)	0	0	0
Group II	0	2000	0
Group III	0	0	80
Group IV	0	2000	80
Group V	1	0	0
Group VI	1	2000	0
Group VII	1	0	80
Group VIII	1	2000	80

A total of 320, day old commercial broiler chicks were procured and randomly divided into eight groups of 40 chicks each on day one. Six chicks were examined for biochemical parameters to establish the base lisne data for the experiment. The control and experimental diets were fed from 0 to 35 days to different dietary treatment groups as detailed below:

Sequential Pathology

Six experimental birds form each group were sacrificed on Day 7, 14, 21, 28 and 35 to study the biochemical parameters.

Biochemical Parameters

During each screening 5 ml of blood was collected from six birds of each group at weekly interval in a day test tube and serum was separated. Sera samples were analysed for creatinine, cholesterol and triglycerides using the Lab Met clinical analyzer as per the procedure described in the manual with the reagent kits supplied by Swemed Diagnostics Ltd., Bengaluru.

Statistical Analysis

The data generated from different parameters of the experimental study was subjected to one way analysis of variance (ANOVA) test using graph pad prism soft ware.

RESULTS AND DISCUSSION

Serum biochemistry

Serum creatinine

The weekly mean serum creatinine (mg/dL) and triglyceride (mg/dL) values of birds fed with different dietary treatments have been summarized in Table No. 2, 3 and 4 respectively.

The mean serum creatinine values showed no significant alterations in Group II, III and IV while, it was significant in Group V when compared to control (Group I) throughout the experimental period.

Supplementation of diatomaceous earth, Vitamin E and both (Group VI to VIII) showed significant ($P < 0.05$) decrease as compared to toxin alone fed birds (Group V) till the end of experiment.

The levels of serum creatinine were increased in birds fed with ochratoxin as compared to control (Group I). Similar findings were also reported by Huff *et al.* (1998), Kubena *et al.* (1998) and Bailey *et al.* (1989). The increase in serum creatinine concentration in the Group V birds could be due to damage of glomeruli and PCT by ochratoxin (Krough *et al.*, 1976).

Supplementation of either DAE or Vit E alone or in combination significantly decreased the creatinine level in the serum of birds in Group VI to VIII respectively and indicated the ameliorative effect. The findings of the present study are in agreement with those of Denli *et al.* (2009) and Anand (2015) who reported an amelioration effect by supplementation of DAE to the AFB1 fed birds.

The mean serum cholesterol values of birds of Group II and III did not alter significantly on all the days of observation. When compared to control group (Group-I) a significant ($P<0.05$) decrease was noticed in Group IV and V birds as compared with control (Group I) birds, from second week of observations. A significant ($P<0.05$) effect was observed from 2nd week onwards in Group VII and only during 2nd and 5th weeks in Group-VII as compared to toxin alone fed group (Group V) birds.

Table 2: Mean ± SE values of serum creatinine (mg/dL) in broiler chicks fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/Weeks	1	2	3	4	5
I	0.14±0.01^{a}	0.15±0.01^{a}	0.13±0.01^{ac}	0.14±0.01^{a}	0.15±0.01^{a}
II	0.12±0.01^{a}	0.14±0.01^{a}	0.14±0.01^{a}	0.16±0.01^{a}	0.15±0.18^{a}
III	0.12±0.01^{a}	0.18±0.01^{ab}	0.14±0.03^{ac}	100.15±0.0^{a}	0.14±0.01^{a}
IV	0.16±0.01^{ac}	0.17±0.01^{ab}	0.13±0.01^{a}	0.14±0.01^{a}	0.14±0.16^{a}
V	0.28±0.20^{b}	0.28±0.01^{b}	0.29±0.01^{b}	0.31±0.01^{b}	0.30±0.01^{d}
VI	0.21±0.04^{d}	0.16±0.01^{ac}	0.17±0.01^{c}	0.18±0.01^{a}	0.20±0.01^{b}
VII	0.21±0.02^{d}	0.21±0.01^{c}	0.16±0.01^{b}	0.22±0.01^{a}	0.25±0.01^{c}
VIII	0.20±0.01^{cd}	0.15±0.01^{a}	0.15±0.01^{a}	0.17±0.01^{a}	0.18±0.01^{b}

Mean values bearing common superscript within columns did not differ significantly ($P<0.05$)

Table 3: Mean ±SE values of serum cholesterol (mg/dL) in broiler chicks fed with ochratoxin (OA), diatomaceous earth (DAE), vitamin E and their combination

Groups/Weeks	1	2	3	s4	5
I	42.06±10.69^{a}	64.61±1.56^{c}	150.66±1.22^{de}	142.96±6.19^{e}	142.89±1.91^{e}
II	47.91±10.11^{ab}	61.88±1.39^{bc}	149.32±9.97^{de}	138.53±1.92^{e}	144.18±1.28^{e}
III	59.29±4.91^{ab}	61.49±0.74^{bc}	159.96±2.72^{e}	152.32±8.88^{e}	140.12±0.86^{de}
IV	97.77±12.51^{ab}	44.87±1.21^{ab}	93.14±3.12^{a}	72.67±2.15^{a}	90.74±4.18^{a}
V	84.77±22.09^{ab}	41.37±0.18^{a}	112.37±5.5^{b}	87.67±5.18^{ab}	102.44±1.3^{b}
VI	71.67±15.72^{ab}	44.9±4.92^{ab}	122.1±1.51^{b}	96.67±4.63^{bc}	110.5±2.96^{b}
VII	50.6±9.19^{ab}	76.84±4.71^{c}	137.94±3.68^{d}	112.4±4.85^{cd}	120.47±3.99^{c}
VIII	71±20.33^{ab}	101.54±10.05^{d}	122.2±5.76^{b}	94.57±2.02^{b}	120.3±5.11^{c}

Mean values bearing common superscript within columns did not differ significantly (P<0.05)

Table 4: Mean ±SE values of serum TGL (mg/dL) in broiler chicks fed with ochratoxin (OA), Diatomaceous earth (DAE) Vitamin E and their combination.

Groups/Weeks	1	2	3	4	5
I	108.80±6.81^{d}	121.64±5.33^{cd}	123.74±5.82^{abc}	117.47±4.97^{ab}	120.97±6.30^{b}
II	108.10±11.67^{d}	123.34±5.01^{cd}	130.30±7.51^{c}	118.10±3.85^{ab}	116.84±5.52^{b}
III	108.67±6.66^{d}	126.80±8.17^{d}	125.17±5.29^{bc}	124.50±12.32^{b}	117.87±8.03^{b}
IV	56.64±1.77^{a}	99.57±3.18^{a}	105.77±3.90^{a}	105.70±3.36^{a}	100.84±0.72^{a}
V	70.37±8.97^{ab}	102.20±4.96^{ab}	112.24±4.65^{ab}	113.30±4.62^{ab}	107.20±1.56^{ab}
VI	84.77±1.17^{bc}	109.20±3.26^{abc}	111.74±3.25^{ab}	108.27±2.81^{ab}	115.24±1.30^{ab}
VII	96.27±0.64^{cd}	117.24±4.31^{bcd}	116.70±2.18^{abc}	119.14±1.15^{ab}	114.74±1.81^{ab}
VIII	72.67±7.24^{ab}	107.17±6.26^{abc}	109.54±3.19^{ab}	112.40±0.95^{ab}	113.64±3.38^{ab}

Mean values bearing at least one common superscript within columns did not differ significantly (P ≥ 0.05)

However, in Group VIII numerical increase in serum cholesterol value was seen as compared to Group V from second week onwards but significant increase was seen on Day 14 and 35 of observation.

Serum cholesterol decreased significantly in birds of Group IV and V from second week onwards as compared to control (Group I). Similar observations were made by Kalorey *et al.* (2005), Sakhare *et al.* (2007), Srikanth *et al.* (2013). The reduction in the serum cholesterol levels during ochratoxicosis reflects impaired liver metabolism, leading to reduced synthesis of cholesterol. However, the significant improvement in the serum cholesterol levels in birds supplemented with DAE and Vit-E along with toxin (Group VII and VIII) as compared to toxin alone fed group (Group-V) is indicative of their protective effect. However, birds supplemented with DAE and Vit-E along with toxin (Group VII and VIII) as compared to the toxin fed birds (Group V), is indicative of their protective effect.

No significant differences were seen in mean serum triglyceride level of Group II and III birds throughout the period of the experiment and significant decrease seen in group IV birds except during 3rd week when compared to control group. However, a significant decrease was noticed in toxin fed groups (Group V) on Day 7 and 14 of the observation. Significant differences were not observed in triglyceride levels of Group VI, VII and VIII when compared to toxin alone fed group (Group V) except the significant increase ($P<0.05$) during 1st week in Group VIII birds.

The decrease in serum triglycerides level in birds fed with toxin (Group V) as compared to control (Group I) observed in this study was in accordance with that of earlier reports by Srikanth *et al.* (2013), Sawarkar *et al.* (2011) and Sakhare *et al.* (2007). Reduction in triglycerides reflects impaired liver metabolism leading to reduced synthesis in triglycerides. However, birds supplemented with DAE, Vit E and both (Group VI, VII and VIII) showed non significant increase in levels of triglycerides in comparison to toxin fed birds (Group V) which indicates the protective role of DAE in binding of ochratoxin.

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Studies on the Prevalence of Gastrointestinal Helminth Infection in Captive Wild Birds in Karnataka State*

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ABSTRACT

A total 177 faecal samples of captive birds in different zoological parks of Karnataka were screened for parasitic ova by sedimentation and flotation technique. Out of 177 samples 45 (25.42%) samples were found positive for helminth infection. With regard to prevalence in various zoological parks of Karnataka state, 37.83% faecal samples from Bannerghatta Biological Park, Bangalore, 18.18% of the samples from Sri Chamarajendra Zoological Garden, Mysore, 40% of samples from Tyavarekoppa Lion and Tiger Safari and 33.3% from WRRC, Bannerghatta were found positive for gastrointestinal helminths. Positive samples were subjected to Mc Master's egg counting technique for estimation of worms load. The eggs of *Ascaridia* spp., *Capillaria* spp., *Heterakis* spp., *Echinostoma* spp. and tape worm eggs were detected.

Keywords: Gastrointestinal helminth, captive wild birds

Birds are the most populous life forms on the planet. They are the colourful attractive creatures being displayed in the zoos primarily to entertain the visitors and to educate as well. Parasitic infection causes considerable loss to wildlife. In zoological parks, the birds are under constant stress and are prone to parasitic infection. Particularly helminthic infections can frequently be a major problem causing even mortality in captive birds.

Inadequate information on parasites of zoo birds is a major limiting factor in many zoos. The present work was undertaken to make a systematic study on the prevalence of GI parasites among captive birds in various zoological gardens in Karnataka.

MATERIALS AND METHODS

Birds from Bannerghatta Biological Park, Bengaluru, Sri Chamarajendra Zoological Garden, Mysore, Tyavarekoppa Lion and Tiger Safari, Shimoga and WRRC, Bannerghatta, Bengaluru were utilised for the study. Individual faecal dropping from large birds and droppings from smaller birds in cages were collected and pooled. Fresh faecal sample approximately one-two grams were collected into a clean polythene cover and transported to laboratory in cold chain. The macroscopic examination of the faecal sample were carried out to note the characteristic features like consistency, colour, presence of mucus, blood, helminth parasites, segments and their stages.

The samples were subjected for microscopic examination for detection of parasitic ova using centrifugal sedimentation and floatation techniques as per the procedure described by Soulsby (1982). Positive samples were subjected to McMaster's egg counting technique. The results were statistically analyzed as per the standards given by Snedecor and Cochren (1994).

RESULTS AND DISCUSSION

Macroscopic examination of the faecal sample

In captive birds, the colour of the faecal samples ranged from brown, green, white, black, orange, slit red or mixed colour. The consistency of the faecal samples varied from semisolid, watery, pasty, hard and mucus mixed. Only one faecal sample of dove from Bannerghatta Biological Park revealed adult ascarid

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worm and no blood was observed in faecal sample of captive birds.

Microscopic examination

In the present study, a total of 177 faecal samples of captive wild birds were collected for detection of parasitic ova. Overall 45 (25.42%) faecal samples were found positive for helminth eggs. Fourteen (37.83%) out of 37 faecal samples in Bannerghatta Biological Park, 20 (18.18%) out of 110 faecal samples, in Sri Chamarajendra Zoological Garden Mysore, 6 (40%) out of 15 faecal samples in Tyavarekoppa Lion and Tiger Safari, Shimoga and 5 (33.3%) out of 15 faecal samples from WRRC, Bannerghatta, Bengaluru were positive for parasitic eggs (Table 1 and Figure 1). Statistically there was a significant ($P<0.0416$) difference in prevalence of gastrointestinal parasites of captive birds among various zoological parks.

The present finding of overall 25.42% occurrence is in agreement with the finding of Shanmugasundaram (2012) who reported 27.50% prevalence of gastrointestinal helminth infection at Arignar Anna Zoological Park, Vandalur. At the same time, Shanmugasundaram (2012) reported 20% prevalence of gastrointestinal parasitic infection at Guindy Children's Park, Chennai. Further, higher prevalence of gastrointestinal parasitic infection was reported by Patel *et al.* (2000) at Kamala Nehru Zoo, Ahmedabad and Sayajibaug Zoo, Vadodara (48.11 %). Similarly, Parsani *et al.* (2001) recorded 71.43% prevalence of gastrointestinal parasitic infection at Sakkarbagh Zoo, Junagadh, Gujarat. Interestingly, Borghare *et al.* (2009) reported 100% prevalence in Maharajbagh Zoo, Nagpur and Thanveer *et al.* (2011) reported 68.6% prevalence of gastrointestinal nematode parasites in domestic pigeons in Lahore, Pakistan.

This variation in prevalence of gastrointestinal parasitic infections by different workers may be attributed to difference in geographical area, season and managemental practices adopted at different zoos. Birds in captivity are susceptible to stress due to factors such as captivity, proximity to different types of birds, more number of birds in individual cages and unsatisfactory hygienic conditions due to restricted movement space provided to birds (Varghese, 1987).

Table 1: Prevalence of gastrointestinal helminth infection in captive wild birds

Sl.No	Location	No. of faecal samples	No. of positive samples	Per cent positive
1.	Bannerghatta Biological Park, Bengaluru	37	14	37.83
2.	Sri Chamarajendra Zoological Garden, Mysore	110	20	18.18
3.	Tyavarekoppa, Lion and Tiger Safari, Shimoga	15	6	40
4.	WRRC, Bannerghatta, Bengaluru	15	5	33.3
	Total	177	45	25.42

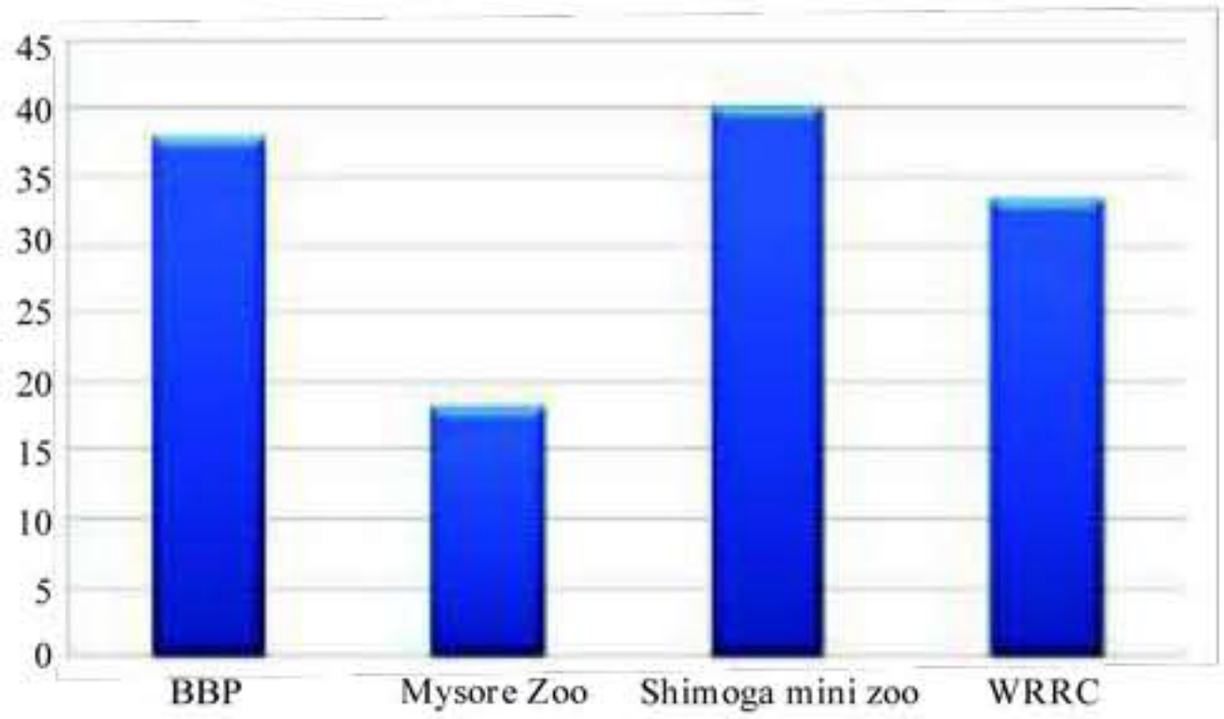

Fig. 1: Prevalence of gastrointestinal helminth infection in captive wild birds

Thus produced physiological stress make captive birds more prone for various diseases including parasitic infection as rightly opined by Parsani *et al.* (2001). This may be possible reason for observation of 25.42% occurrence on gastrointestinal parasitic infection in the present study.

When compared with the occurrence of gastrointestinal parasites in the four zoos in Karnataka, Sri Chamarajendra Zoological Garden had lowest (18.18%) occurrence. It might be due to regular deworming, better hygienic management and low

density of birds in each enclosure in Sri Chamarajendra Zoological Garden, Mysore. Further, only water birds (Anserifomis, Pelecaniformis) were positive for nematodes, cestodes and trematodes in Sri Chamarajendra Zoological Garden, Mysore. Many trematodes and cestodes have indirect life cycle. Various species of crustaceans, insect larvae, annelids (earthworm), mollusks (snail) and fishes serve as intermediate hosts. It is difficult to control these intermediate hosts. This might be the reason for water birds being positive for nematode, cestode and trematodes as rightly opined by Atkinson *et al.* (2008).

Faecal eggs per gram of different species of captive birds

The egg counts of positive faecal samples obtained in different species of captive birds are presented in Table 2. The egg counts ranged from 400 to 5500 EPG.

Bannerghatta Biological park, egg counts ranged from 700 to 5500 EPG with lowest egg count of 700 EPG in pelican and the highest egg count of 5500 EPG in white dove.

The egg count in captive wild birds ranged from 400 to 5500 EPG. In Sri Chamarajendra Zoological Garden, painted stork had lowest egg count of 400 EPG where as faecal sample from white dove from Bannerghatta Biological Park revealed an egg count of 5500 per gram. Nematodes of *Ascaridia* spp., *Heterakis* spp., *Capillaria* spp. and mixed infection were recorded in captive birds at different zoological gardens.

Low level of parasitic load may not cause severe lesion in these birds. However, birds with higher parasitic load can cause severe lesion. Further,

Table 2: Faecal eggs per gram in different species of captive wild birds in different locations

Location	Species	EPG
WRRC, Bannerghatta Biological Park, Bengaluru	Dove	2700
	Pea fowl	1300
Sri Chamarajendra Zoological Garden, Mysore	Pink pelican	600
	Painted stork	400
	Black swan	1400
	Bar headed goose	1000
Tyavarekoppa, Lion and Tiger Safari, Shimoga	Red jungle fowl	1400
	Lady amherst pheasant	1000
Bannerghatta Biological Park, Bengaluru.	Pond heron	1100
	White dove	5500
	Silver pheasant	900
	Pelican	700

In WRRC, Bannerghatta, egg counts ranged from 1300 to 2700 EPG with lowest egg count of 1300 EPG in pea fowl and the highest egg count of 2700 EPG in dove. In Sri Chamarajendra Zoological Garden, egg counts ranged from 400 to 1400 EPG with lowest egg count of 400 EPG in painted stork and the highest egg count of 1400 EPG in black swan. Further, Tyavarekoppa Lion and Tiger Safari, egg counts ranged from 1000 to 1400 EPG with lowest egg count of 1000 EPG in lady amherst pheasant and the highest egg count of 1400 EPG in red jungle fowl. Similarly, in

pathogenicity varies from one type of worm to another type. There is paucity of information on pathogenic burden of egg count of different parasites of wild birds. Further work need to be carried out in this area.

Parasitic eggs identified in captive wild birds

The different types of parasitic eggs observed in captive wild birds in different study sites are presented in Table 3. Among 37 faecal samples examined at Bannerghatta Biological Park, 14 (37.83%) revealed *Ascaridia* spp., 5 (13.51%) revealed

Table 3: Different types of gastrointestinal helminth infections prevalent in captive birds in different locations

Parasitic ova	Bannerghatta Biological Park (N=37)	Mysore Zoo (N=110)	Tyavarekoppa Lion and Tiger Safari (N=15)	WRRC, Bannerghatta (N=15)	Total N=177
Ascaridia sp.	14 (37.83%)	9 (8.18%)	4 (26.66%)	4 (26.66 %)	31 (17%)
Capillaria sp.	5 (13.51%)	5 (4.54%)	2 (13.33%)	1 (6.66%)	13 (7.35%)
Heterakis sp.	6 (16.21 %)	3 (2.72%)	2 (13.33%)	2 (13.33%)	13 (7.35%)
Tape worm	–	3 (2.72%)	–	–	3 (1.70%)
Echinostoma sp.	2 (5.40 %)	1 (0.90%)	–	–	3 (1.70%

Capillaria spp., 6 (16.21%) were positive for *Heterakis* spp., and 2 (5.40%) for *Echinostoma* spp., eggs. Among 110 faecal samples examined at Sri Chamarajendra Zoological Garden, 9 (8.18%) revealed *Ascaridia* spp., 5 (4.54 %) revealed *Capillari* spp., 3 (2.72 %) showed *Heterakis* spp., 3 (2.72 %) revealed tape worm and 1 (0.90%) was positive for *Echinostoma* spp., eggs.

Among 15 faecal samples examined at Tyavarekoppa, Lion and Tiger Safari, 4 (26.66 %) revealed *Ascaridia* spp. 2 (13.33 %) revealed *Capillaria* spp. and 2 (13.33 %) were positive for *Heterakis* spp. eggs. Among 15 faecal samples examined at WRRC, Bannerghatta, 4 (26.66 %) revealed *Ascaridia* spp., 1 (6.66 %) revealed *Capillaria* spp., and 2 (13.33 %) showed *Heterakis* spp., eggs.

In captive birds, overall occurrence of *Ascaridia* spp., *Capillaria* spp., *Heterakis* spp., tapeworms and *Echinostoma* spp., was found to be 17.5%, 7.35%, 7.35%, 1.70% and 1.70% respectively indicating highest occurrence of *Ascaridia* spp., and least occurrence of tapeworm and *Echinostoma* spp.

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Effect of *Ocimum sanctum* Extract on Haematological and Biochemical Changes in Streptozotocin Induced Diabetes Mellitus in Rats

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ABSTRACT

The present study was undertaken to observe the haematological and biochemical changes noted in streptozotocin induced diabetes mellitus in rats and to evaluate the hypoglycemic effect of *Ocimum sanctum* extract for a period of 60 days. Alcoholic *Ocimum sanctum* extract was gavaged at the dose of 150 mg/kg b.w. and 300 mg/kg b.w. to the rats daily for 60 days and compared with a standard hypoglycemic drug glibenclamide at the dose of 600 µg/kg b.w. There was significant difference in mean values of parameters such as serum glucose, triglyceride, cholesterol, ALT, AST, RBC, hemoglobin, PCV, platelets and WBC in diabetic rats when compared to rats of control groups. *Ocimum sanctum* extract at the dose of 300 mg/kg showed good improvement in haematological and biochemical profile. Glibenclamide and *Ocimum sanctum* extract at a dose of 300 mg/kg b.w. were almost at par in several biochemical parameters. There was no synergistic or additive effect seen between *Ocimum sanctum* extract and glibenclamide at half the dose (300 µg/kg b.w.). There was no toxic effect in the rats gavaged with *Ocimum sanctum* extract at the dose of 300 mg/kg b.w. daily for 60 days.

Keywords: Hypoglycemic, *Ocimum sanctum,* streptozotocin, glibenclamide, haematological

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. In animals, particularly in canines and felines incidence of diabetes mellitus is increasing. Rare cases of diabetes are reported in horses, cattle and sheep (Anderson and Low, 1990). According to the world health organization, more than 70 percent of the world's population use traditional medicine to satisfy their principal heath needs. A great number of medicinal plants used in the control of the diabetes mellitus have been reported (Bailey and day, 1989). This interest is due to reasons such as ease of access, better culture acceptability and compatibility, cost effectiveness and also the bid to "Go Natural" (Gita *et al.,* 2014). Tulsi *i.e. Ocimum sanctum* is a plant with enormous properties for curing and preventing diseases. Tulsi is known as "Queen of plants" and "The mother medicine of nature". Leaves of *Ocimum sanctum* have been shown to possess hypoglycaemic effects in experimental animals. Decoction prepared with various parts of plant lowers the blood sugar level.

The study was undertaken to evaluate the hypoglycemic effect of *Ocimum sanctum* in STZ induced diabetic rats and to compare its efficacy with glibenclamide, a reference hypoglycemic drug.

MATERIALS AND METHODS

Eighty four female, healthy, Wistar albino rats weighing 180-250g were procured from areputed breeder in Bengaluru. They were divided into 7 groups each comprising of 12 rats. The groups included; Group I (Norman control), Group II (Diabetic control), Group III (*Ocimum sanctum* @ 150 mg/kg b.w.), Group IV (*Ocimum sanctum* @ 300 mg/kg b.w.), Group V (Glibenclamide @ 600 µg/kg b.w.), Group VI (*Ocimum sanctum* @ 150 mg/kg + glibenclamide @ 300 µg/kg b.w.) and Group VII (*Ocimum sanctum* @ 300 mg/kg b.w.) for non-diabetic rats.

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The experimental rats were maintained under standard laboratory conditions and offered *ad libitum* standard commercial rat feed (Vet Care Rodent Feeds, Bangalore) and clean drinking water. The animals were kept for acclimatization in the experimental lab house for 2 weeks. Necessary permission from IAEC was obtained for conduct of the experimental study.

Diabetes was induced in rats of Group II-VI using streptozotocin @ mg/kg b.w. administered intraperitonially for once. Fasting blood glucose level was estimated 3 days after STZ injection and rats blood glucose level above 200 mg/dl were considered diabetic. For the next 60 days the rats of all groups received their respective treatments. At regular intervals of time, the rats were subjected to evaluate various haematological and biochemical parameters such as hemoglobin, RBC, WBC, PCV, MCV, MCH, MCHC, platelets, serum glucose, serum cholesterol, serum triglyceride, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) using haematology auto analyzer (BC-2800 vet. Mindray) and semi-automatic biochemical analyzer (CPC diagnostics pvt ltd., India) respectively.

RESULTS AND DISCUSSION

The rats of Group I and Group VII remained healthy throughout the experiment as evaluated by various haematological and biochemical parameters.

Significant improvement was noticed in diabetic rats of Groups III, IV,V,VI and VII in Hb, TEC, PCV and platelet levels and reduction in WBC level during treatment. The improvement was significant ($P \leq 0.01$) in Group V animals closely followed by Group IV, Group III and Group VI (Table 1). Anemia could be attributed to the destruction of RBCs and reduced rate of its release from the bone marrow to blood (Helal, 2000 and Rabbani *et al.*, 2010). Leukocytosis observed may be a response to stressful condition (corticosteroid release) after induction of DM by STZ injection (Ramadan *et al.*, 2009; Papazafiropoulou, 2010 and E1-Baky, 2013). In the present study, there was significant improvement in the levels of circulating

Table 1: Heamatological values on Day 60 of the experiment in *Ocimum sanctum* treated diabetic rats compared to normal and diabetic control rats

Groups	Values on Day 60 of the experiment				
	Total Erythrocy tecount (TEC) (X 10^6/µl)	Heamo-globin (g/dl)	Packed cell volume (PCV) (%)	Total leucocyte count (TLC) (X 103/µl)	Platelet count (x 10^3/µl)
Group I					
Normal Control	8.38±0.1	14.95±0.13	44.00±1.31	17.48±0.38	655.50±20.37
Group II					
Diabetic Control	4.98±0.1	8.72±0.09	32.50±0.76	27.62±0.67	269.83±24.35
Group III					
Ocimum sanctum @ 150 mg/kg b.w. in distilled water	7.17±0.10	13.28±0.14	42.20±0.52	19.02±0.10	450.83±24.05
Group IV					
Ocimum sanctum @ 300 mg/kg b.w. in distilled water	8.10±0.05	14.67±0.05	43.25±0.51	18.68±0.08	449.83±27.57
Group V					
Glibenclamide Control	8.50± 0.24	15.67 ± 0.18	44.25± 0.77	17.83±0.05	562.83 ± 50.06
Group VI					
Glibenclamide @ 300 µg/kg + *Ocimum sanctum* @ 150 mg/kg b.w.	7.68±0.40	13.00±0.07	41.67±0.41	19.32±0.10	562.00±24.37
Group VII					
Ocimum sanctum @ 300 mg/kg b.w. in distilled water for non-diabetic rats	8.2±0.10	15.32±0.27	41.37± 0.53	18.18±0.04	562.00± 24.37

platelets in blood in Group III, IV and VI when compared with diabetic control. This could be attributed to inhibitory activity of *O. sanctum* on platelet aggregation.

The diabetic control rats showed significant ($P<0.05$) increase in the serum glucose values on 3rd day of the study which progressively increased till the end of the study. In all the treatment groups improvement in the serum glucose was observed. In Groups III, IV and Group VI, improvement was comparable to that of glibenclamide treated rats. In general Group IV showed better improvement than Group III and VI (Table 1). *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused rat pancreas, isolated rat islets and a clonal rat â-cell line in a concentration dependent manner (Gholap *et al.*, 2004). Similar blood glucose lowering effect of *O. sanctum* was observed by many workers (Joglekar *et al.*, 1959; Dhar *et al.*, 1968; Chattopadhyay, 1993; Rai *et al.*, 1997; Agrawal *et al.*, 1996; Vats *et al.*, 2002).

There was significant increase in the mean serum cholesterol and triglyceride levels in diabetic control rats compared to normal rats. The treatment used in the present study was effective in decreasing serum cholesterol and triglyceride levels. The levels of Group IV reached to the levels of normal control by the end of study and improvement was comparable to glibenclamide group (Table 2). The change observed in lipid profile in the present study is in accordance with previous clinical trials (Sarkar *et al.*, 1994; Geetha and Vasudevan, 2004; Khan *et al.*, 2010). Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant

Table 2: Biochemical values on Day 60 of the experiment in *Ocimum sanctum* treated diabetic rats compared to normal and diabetic control rats

Groups	Values on day 60 of the experiment				
	Serum glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)	ALT(IU/l)	AST (IU/l)
Group I					
Normal Control	102.33±36	97.08±8.23	73.87±2.53	64.52±1.98	85.33±0.81
Group II					
Diabetic Control	565.00±7.37	182.58±8.22	226.50±4.88	238.38±5.12	241.82±2.40
Group III					
Ocimum sanctum @ 150 mg/kg b.w. in distilled water	169.03±8.74	108.32±0.72	117.03±2.52	101.60±5.68	114.72±2.73
Group IV					
Ocimum sanctum @ 300 mg/kg b.w. in distilled water	143.00±9.12	103.78±1.48	94.87±1.90	87.37±1.34	95.08±1.77
Group V					
Glibenclamide Control	187.67±2.70^{ab}	84.00±1.21^{b}	82.00± 1.68^{b}	76.97± 1.50^{ab}	93.50± 2.27^{ab}
Group VI					
Glibenclamide @ 300 µg/kg + *Ocimum sanctum* @ 150 mg/kg b.w.	163.33±5.67	104.53±1.28	115.33±0.76	92.92±2.05	95.73±1.72
Group VII					
Ocimum sanctum @ 300 mg/kg b.w. in distilled water for non-diabetic rats	107.00±7.53^{bc}	82.50± 5.45^{b}	57.43± 9.11^{bc}	59.67± 2.38^{bc}	79.00± 2.40^{bc}

Statistical significance at $P<0.05$

activity (Afanas'ev *et al.*, 1995). *O. sanctum* partitionates showed the presence of flavonoids and related phenolic compounds (Khan *et al.*, 2010).

A significant increase in serum AST and ALT levels were observed in the diabetic control rats when compared to normal rats. The Groups III, IV and VI have shown decrease in serum ALT and AST levels but they were still higher when compared to normal control (Table 2). The animals of Group IV showed improvement when compared to Group III and Group VI. The improvement was comparable with glibenclamide group. Eugenol, a major essential oil in *Ocimum sanctum* may contribute to this action since it has been shown that oral administration of eugenol reduced iron-induced hepatic damage (Reddy and Lokesh, 1996).

Ocimum sanctum had no significant effect on serum glucose on non-diabetic rats. The results were in accordance with those of Thamolwan and Thanapat (2005).

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Prevalence of Gastrointestinal Helminth Infection in Sheep and Goats in and around Hassan, Karnataka State, India*

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ABSTRACT

Prevalence of gastrointestinal parasites was studied in sheep and goats in and around Hassan. A total of 287 faecal samples collected from four different farms and examined by direct smear, floatation and sedimentation techniques. Quantitative examination was done by McMaster's technique. Out of 287 faecal samples processed 241 samples were found positive for gastrointestinal parasites. The overall prevalence was 83.97 %. The most common gastrointestinal parasites were Haemonchus, Trichostrongyle, Cooperia, Oesophagostomum, Trichuris, Monezia and Amphistomes. The results of the present study suggest that Haemonchus is the main gastrointestinal parasite of sheep and goats in Hassan region. Necessary steps should be taken in timely manner to improve the productivity from these animals.

Keywords: Gastrointestinal parasites, helminths, sheep and goats, Hassan, prevalence

Gastrointestinal nematode parasitism is well recognized as a major production-limiting disease. Gastrointestinal parasitic infections in sheep and goats are of much economic importance because small ruminants' rearing has been a major source of income especially to the marginal farmers of the country (Pathak and Pal, 2008). Gastrointestinal parasites cause heavy economic losses in meat and wool production (Gordan, 1974). Recurring losses in productivity due to widely prevalent parasitic infection is an important and common problem for small ruminant production in most parts of the world (Gall, 1981).

Commonly occurring gastrointestinal parasitic diseases in goats and sheep are Haemonchosis, Ostertagiosis, Strongyloidosis, Oesophagostomiosis. Among the parasites *Haemonchus contortus* is the most important as it feed on blood and causes anaemia. The degree of infestation may be sub clinical or clinical depending on level of parasitic load. Sub clinical infections remain dominant and as such are not recognized by the clinicians and owners. Thus the sub clinical and clinical infection should be tackled timely for better economic return. Prevalence of gastrointestinal helminths has been reported ranging from 0.72 to 84.1% in domestic animals from various parts of the world including India. (Regassa *et al.*, 2006; Mamata *et al.*, 2007; Khan *et al.*, 2010). For successful formulation and implementation of an efficient and effective strategic helminth control regime, a periodic surveillance of the prevalence of gastrointestinal helminthiosis within given environment and associated risk factors that influence their transmission is required.

MATERIALS AND METHODS

In the present study faecal samples of 272 sheep and goat from ILFC farm, Veterinary College, Hassan, Chittanahalli, Veerapura, Kammarage, Channarayapatanna goat farms around Hassan were examined to know the prevalence of gastrointestinal parasites in these animal during the study period 2013-2014 (Table-1). Faecal samples were collected from the rectum of each animal. Gross examination was done for colour, consistency and presence of any adult worms. The faecal sample were processed and screened by direct smear method, flotation, sedimentation techniques. The ova of parasite were identified from their morphological features (Soulsby, 1982). Quantitative examination of faeces was conducted to know the intensity of parasitic infestation by Egg Per Gram (EPG) by McMaster's

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technique. Animals detected positive by faecal examination were classified in to sub clinical (EPG<1600) and clinical (EPG>1600) (Arora *et al.*, 2003 and Sing *et al.*, 2013) (Table 2). Further classification was made by type of parasites eggs present and as pure and mixed infections (Table 3). Faecal samples from each region were pooled and cultured separately for identifying the third stage infective larvae (Zajac and Conboy, 2012).

Table 1: Animal population studied

Species	Place	No of animals studied
Sheep	ILFC farm, Veterinary College Hassan.	50
Sheep	Sheep farm, Chittanahalli, Hassan	65
Sheep	Sheep farm, Veerapura. Hassan	73
Goat	Goat farm, Kammarage, Hassan	46
Goat	Goat Farm near Channarayanapatna, Hassan	53
	TOTAL	**287**

Table 2: Range of EPG values in sub clinical and clinical gastrointestinal helminth parasites in goats and sheep at different places of Hassan district

Place	Animal	Range of EPG value		Average EPG value	
		Subclinical	Clinical	Subclinical	Clinical
ILFC farm, Veterinary College Hassan	Sheep (50)	320-1080	1660-4200	700	2930
Chittanahalli, Hassan	Sheep(65)	560-1480	1780-3860	1020	2820
Veerapura, Hassan	Sheep(73)	380-1520	1720-4600	950	3160
Kammarage, Hassan	Goat (46)	360-1380	1640-3200	870	4842
Channarayanapatna, Hassan	Goat (53)	460-1540	1700-2840	1000	2270

RESULTS AND DISCUSSION

In ILFC farm of Veterinary College, Hassan, 46 (92%) out of 50 sheep were found to be positive for gastrointestinal helminth parasites. In Chittanahalli farm, out of 65 faecal samples of sheep 51 (78.46%) were found positive where as in Veerapura farm 69 (94.52%) out of 73 faecal samples of sheep were found positive for gastrointestinal helminth parasite. In Kammarage goat farm, out of 46 faecal samples 35 (76.08%) were found positive for gastrointestinal helminth parasites where as in Channarayanapatna goat farm, 40 (75.47%) out of 53 sample were found positive. By EPG values for parasitic infected sheep, the prevalence of subclinical infection (<1600) at ILFC farm Veterinary College, was 39.13%, while the clinical infection (>1600) was 60.86% (Table 4). The prevalence of subclinical infection (<1600) and clinical infection at Chittanahalli sheep farm was 29.41% and 70.58%, at Veerapura farm was 33.33% and 66.66%, at Kammarage goat farm 28.57% and 71.42% and at Channarayapatna goat farm was 24.52% and 50.94% respectively (Table 4). Culturing of faecal sample showed the presence of third stage larvae of *Haemonchus* spp., *Trichostrongyle* spp., *Cooperia* spp. and *Oesophgostomum* spp. (Fig. 1-4).

The infection of gastrointestinal parasites was recorded (94.52%) the highest in Veerapura farm, followed by 92% at ILFC farm, Veterinary College,

Table 3: Prevalence of gastrointestinal parasites in goats and sheep at different places of Hassan district

Place	Animal	Single species infection					Mixed infection										Total
		S	M	A	T	F	S+M	S+A	S+T	S+F	M+T	S+M+A	S+M+T	S+A+F	S+A+T	S+A+T+F	
ILFC farm, Veterinary College Hassan	Sheep (50)	13	2	1	1	3	9	7	3	2	1	1	1	2	-	-	46(92.00%)
Chittanahalli, Hassan	Sheep(65)	22	2	3	-	3	4	6	2	4	1	2	1	1	-	-	51(78.46%)
Veerapura, Hassan	Sheep(73)	25	4	8	3	4	6	4	2	-	1	5	2	3	-	2	69 (94.52%)
Kammarage,Hassan	Goat (46)	19	-	2	1	2	4	2	1	-	-	-	-	2	2	-	35(76.08%)
Channarayanapatna, Hassan	Goat (53)	16	1	6	1	3	1	3	-	2	-	2	2	-	2	1	40(75.47%)

S- Strongyle, M-Moniezia, A-Amphistome, T-Trichuris, F-Fasiola

Table 4: Prevalence of clinical and sub clinical gastrointestinal parasites in goats and sheep at different places of Hassan district

Place	Animal	Prevalence		Overall prevalence
		Subclinical	Clinical	
ILFC farm, Veterinary College Hassan	Sheep (50)	18 (39.13%)	28 (60.86%)	46 (92.00%)
Chittanahalli, Hassan	Sheep(65)	15 (29.41%)	36 (70.58 %)	51 (78.46%)
Veerapura, Hassan	Sheep(73)	23(33.33%)	46(66.66%)	69 (94.52%)
Kammarage, Hassan	Goat (46)	10 (28.57%)	25 (71.42%)	35(76.08%)
Channarayanapatna, Hassan	Goat (53)	13(24.52%)	27(50.94%)	40(75.47%)

Plates showing third stage larvae of *Haemonchus* spp., *Trichostrongyle* spp, *Cooperia* spp., *and Oesophagostomum* spp.,

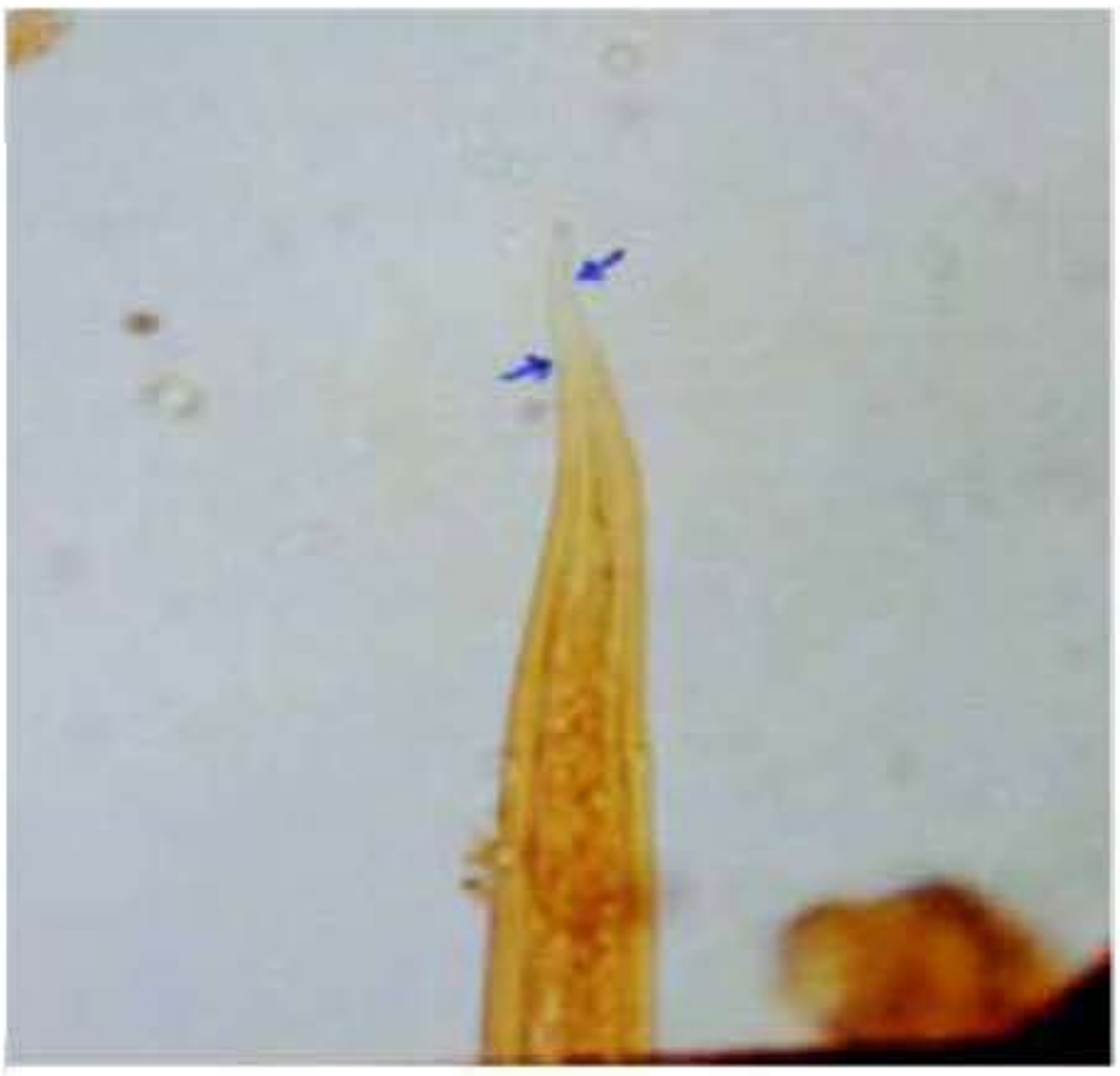

Fig. 1: Third stage larvae of *Haemonchus* species with a kink in the tail sheath 40x.

Fig. 3: Head of *Cooperia* third stage larva showing two refractile bodies at anterior end of oesophagus 40x

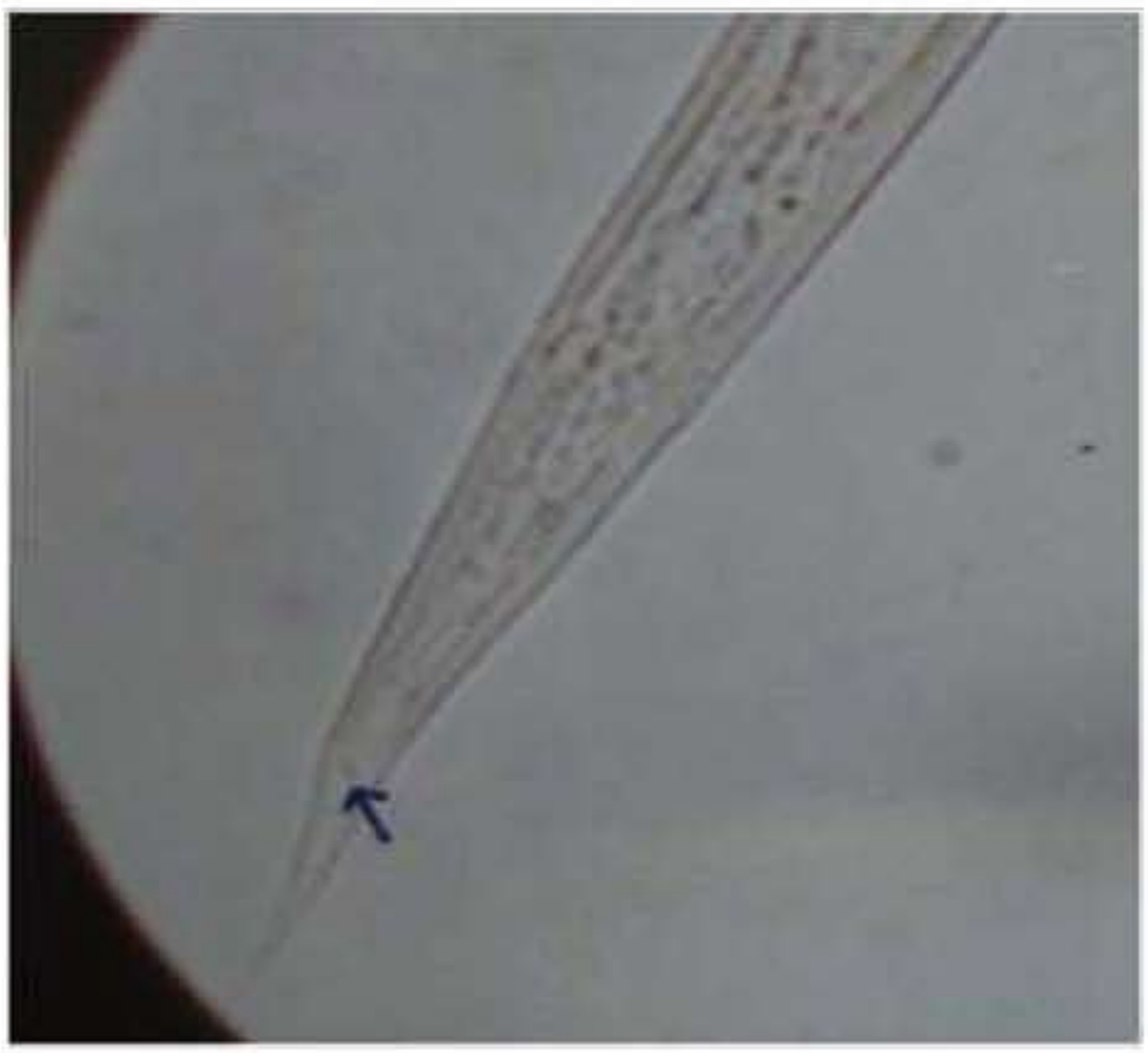

Fig. 2: *Trichostrongylus* species with tail sheath is having one or two tuberosities or indistinctly rounded tail 40 x

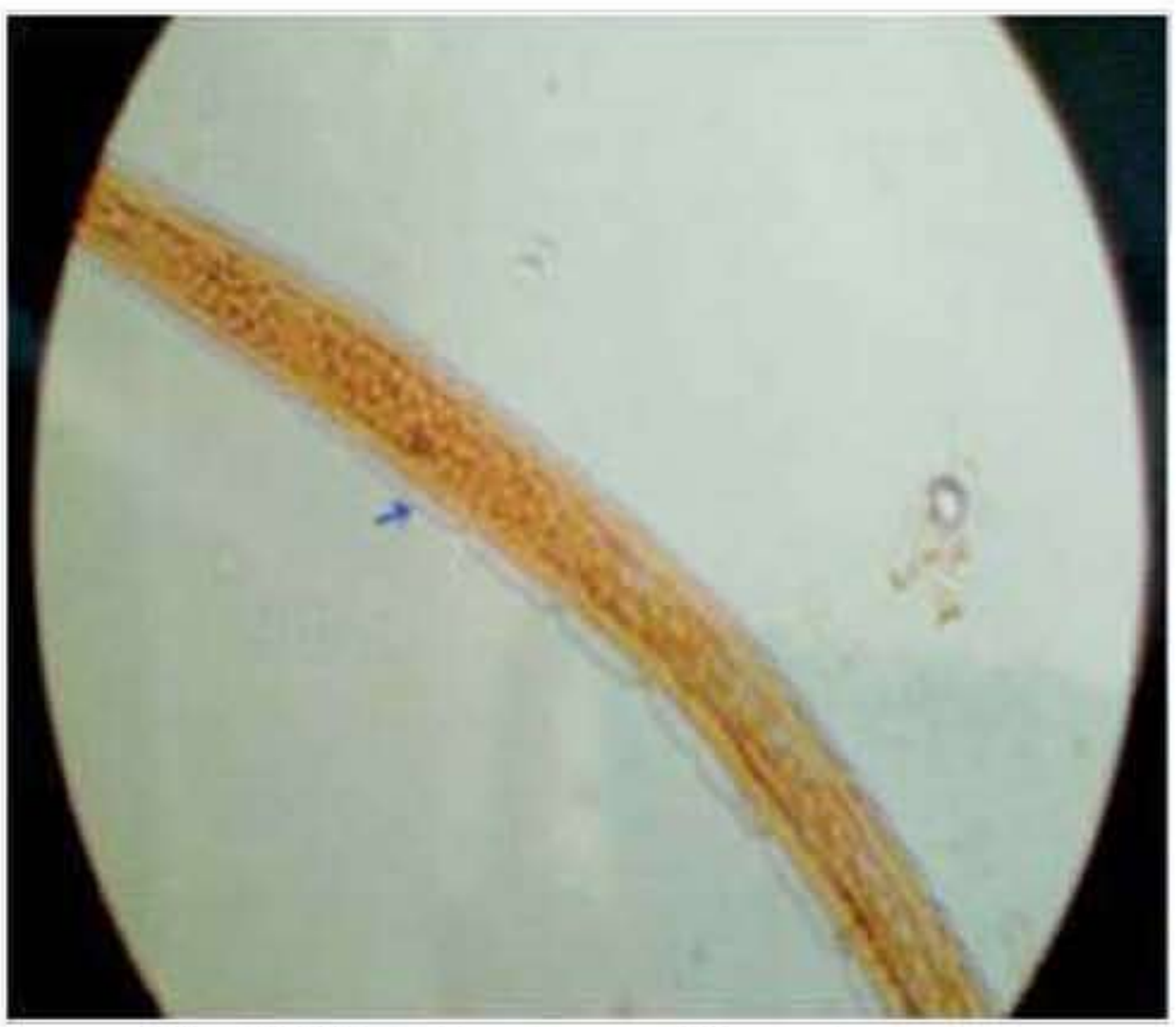

Fig. 4: Third stage larva of *Oesophagostomum* species with wrinkled sheath 40x.

Hassan. Out of 46 parasitic infected sheep at ILFC farm of Veterinary College, 20 (43.47%) were found infected with a single species of helminth, whereas 17 (36.95%) animals had mixed infection. Among the 65 sheep screened at Chittanahalli farm 51(78.46%) were found to be positive for gastrointestinal helminth parasites. A total of 30 (58.82%) animals had single species infection and 21 (41.17%) had mixed infection

(Table 3). Various studies have been conducted on prevalence of gastrointestinal parasites in sheep and goats in this country and abroad (Pathak and Pal, 2008; Pandey *et al.*, 1994; Pant *et al.*, 2009). Prevalence of gastrointestinal helminths has been reported ranging from 0.72 to 84.1% in domestic animals from various parts of the world (Regassa *et al.*, 2006; Khan *et al.*, 2010). This variation in prevalence of parasitic

infestation depends upon difference in agroclimatic condition and availability of susceptible host (Radostits *et al.*, 2000).

Kammarage goat farm 35(76.08%) animals were found to be positive for gastrointestinal helminth parasitic infection 24 (68.57%) had single species infection and 11(31.42%) had mixed infection. Channarayapatna goat farm 40 (75.47%) animals were found to be positive for gastrointestinal helminth parasitic infection with 27(67.5%) had single species infection and 13 (32.5%) had mixed infection (Table 3).

The percentage infection levels at different sheep and goat farms are mentioned in Table 4, among five farms studied the prevalence rate was more in Veerapura farm followed by Chittanahalli, ILFC, Channarayapatna and Kammarage farm. Dhanalakshmi *et al.* (2001) reported prevalence rate of 82.3% with predominant infection of strongyles in sheep in few farms of Karnataka state belonging to Bangalore, Tumkur, Kolar and Mandya districts. Mamata and placid (2007) reported overall prevalence rate of gastro intestinal parasites rate as 91.33 and 78.66 per cent in sheep and goat respectively from 7 districts of Karnataka. Variation may be due to change in management practices of different flocks and opportunity of grazing in the infected field. There are many associated risk factors influencing the prevalence of gastrointestinal helminthes including age, sex, weather condition and husbandry or management practices (Miller *et al.*, 1998; Khan *et al.*, 2009).

The range of EPG value in sheep at ILFC farm Veterinary College, Hassan was from 320-1080 and 1660-4200, at Chittanahalli farm from 560-1480 and 1780-3860, at Veerapura farm from 380-1520 and 1720-4600 in subclinical and clinical infection respectively. EPG values in goat at Kammaragi farm ranged from 360-1380 and 1640-3200, at Channarayapatna farm ranged from 460-1540 and 1700-2840 in subclinical and clinical infection respectively. Mamatha and Placid (2007) reported the average mean EPG count of Strongyle as 1567 ± 144.7 with a range from 300-42000 in sheep. Palampalle *et al.* (2002) reported 5525 ± 359 EPG from Maharashtra. Dhanalakshmi *et al.* (2001) recorded low to moderate counts from some sheep farms in Karnataka. But the threshold limits of EPG are not well defined for various nematode parasites. Regarding the levels of EPG to be considered as pathogenic, many workers have expressed different levels.

Faecal culture revealed the highest prevalence of *Haemonchus* spp., followed by *Trichostrongylus* spp., *Cooperia* spp.and *Oesophgostomum* spp., larvae. Palampalle *et al.* (2002) from Maharashtra reported *Haemonchus, Bunostomum* along with *Trichostrongylus* spp., in sheep and goats. Jeya Thilakan and Sathhianesan (1997) reported highest incidence of *H. contortus* with lowest incidence of *Bunostomum* spp. in goats from Kerala. Yadav *et al.* (2003) encountered predominant infection of *Haemonchus, Trichostrongylus* spp. and *Oesophgostomum* in sheep and goats from North West India. Mamata and Placid (2007) reported highest incidence of *Bunostomum* spp. followed by *Oesophgostomum, Haemonchous, Trichostrongylus* spp., in sheep and goat.

CONCLUSION

The results of the present study suggest that, the overall prevalence of gastrointestinal parasites in and around Hassan was 83.97%. *Haemonchus* spp., is the main gastrointestinal parasite of sheep and goats in Hassan region. Necessary steps should be taken in timely manner to improve the productivity from these animals. A periodic surveillance of the prevalence of gastrointestinal helminthiasis helps in successful formulation and implementation of an efficient and effective strategic helminth control regime. The present finding of high rate of infection is alarming and therefore emphasis should be laid on managemental practices with regular dosing of anthelminthics.

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Effects of Ochratoxin on Serum Total Protein, Albumin and Globulin levels and its Amelioration with DAE and Vitamin-E in Broiler Chickens

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ABSTRACT

The present work was conducted to study the important serum biochemical alterations in ochratoxicosis and the amelioration effect of DAE and Vitamin-E in broiler chickens. The toxigenic strains of *Aspergillus parasiticus*, NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture were used for the present study. A total number of 320 birds were divided in to 40 each in the Group I to VIII, Group I being the control. The numbers of treatments were 8 and selection of birds used for base line data were 06 and the duration of the experiment was 35 days. Powdered ochratoxin was incorporated in to the feed at the rate of 1.0 ppm, the vitamin-E was fed to the birds at the rate of 80 mg / kg feed and DAE at the rate of 2000 mg / kg feed. Six birds from each group were sacrificed on day 7, 14, 21, 28 and 35, sera samples were obtained by collecting 5 ml of blood from each bird and alterations in serum total protein, albumin and globulin were studied. A significant decrease in total protein, albumin and globulin levels was noticed in toxin fed group, while supplementation of DAE, Vitamsin E, and both significantly increased the total protein, albumin and globulin in toxin fed group and suggested the ameliorating effect of ochratoxin.

Keywords: Total Protein, Albumin, Globulin, DAE, Ochratoxin A, Vitamin E

Ochratoxins are nephrotoxic, hepatotoxic, carcinogenic, immunotoxic and teratogenic mycotoxins. Exposure to low concentration of ochrotoxin cause structural and functional changes in kidney and liver of birds thus alters the levels of serum, total protein, serum albumin and serum globulin. The present study has been attempted to access the efficacy of DAE, a toxin binder to ameliorate the toxic effect of ochratoxin and Vitamin E an anti oxidant.

MATERIALS AND METHODS

The present research work was carried out at the Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru to study the ameliorating effect of diatomaceous earth (DAE) of Vitamin E in the experimentally induced ochratoxicosis in broilers.

Fungal Culture

Source of Fungus

The toxigenic strain of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture maintained at the Department of Veterinary Pathology, Veterinary College, KVAFSU Bengaluru were used in the study.

Production of Mycotoxin

Ochratoxin

Ochratoxin was produced on the broken wheat using *Aspergillus ochraceus* NRRL 3174 and NRRL 2999 as outlined by Trenk *et al.* (1971) with minor modifications such as over night soaked wheat (50 G + 25ml Tap water) was autoclaved at 121^{0}C 15 psi for 20 minutes & inoculated with fungal spore suspension. The inoculum was incubated for 12 days at room temperature in dark place with vigorous shaking once a day to break the white mycelial masses. The fermented wheat was autoclaved to kills the spores

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and dried at 80^{0}C in hot air oven, over night. The dried materials were powdered and stored in the dark place for further use.

Mycotoxin Quantification

Ochratoxin was quantified using Thin Layer Chromatography (TLC) at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institution (TANUVAS), Namakkal-367002 which yielded 25 ppm of toxin.

Experimental Information

a)	Number of Birds	:	320
b)	Number of Treatments	:	08
c)	Number of birds per treatment	:	40
d)	Number of birds used as base line data	:	06
e)	Type of management	:	Battery Cages
f)	Duration of Experiment	:	05 Weeks

Experimental Birds and Rearing System

A total of 320 days old commercial Venkobb broiler chicks were procured from a reputed commercial hatchery named Shri Khadkeshwara Hatchery in Bangalore. The chicks were weighed individually and reared in battery brooder fitted with electrical bulbs on raised wire mesh floor under optimum condition of brooding and management.

Experimental Feed

Commercial broiler starter (0-3 weeks) and finisher feed (4-5 weeks) were procured from Department of Poultry Science, KVAFSU, Bengaluru. The feed samples were screened for mycotoxin content prior to use in feed.

Ochratoxin Feed

Powdered ochratoxin culture material was incorporated into the feed at the rate of 1 ppm and the toxin yield was 25 ppm.

Vitamin E

Vitamin E (Tocopherol Acetate adsorbed on precipitated silicon dioxide from Merck Pvt. Ltd., Goa) was mixed with the feed at the rate of 80 mg/kg feed.

Mycotoxin ameliorating agent

Diatomaceous Earth (DAE) obtained from M/s Agripower, Australia was incorporated in feed at the rate of 2000 mg / kg feed).

Biochemical reagents

Reagent Kits

Biochemical reagent kits of serum, total protein, serum albumin and serum globulin were procured from Swemed Diagnostics, Bengaluru.

Methodology

Experimental Design

A total of 320 day-old commercial broiler chicks were procured and randomly divided into eight groups of 40 chicks each on day one. Six chicks were examined for biochemical parameters to establish the base line data for the experiment. The control and experimental diets were fed from 0 to 35 days to different dietary treatment groups as detailed below:

Sequential Pathology

Six experimental birds form each group were sacrificed on Day 7, 14, 21, 28 and 35 to study the serum biochemical parameters.

Table 1: Experimental Design

Treatments	Ochratoxin (ppm)	DAE (mg/ kg feeed)	Vitamin E (mg/kg feed)
Group I (Control)	0	0	0
Group II	0	2000	0
Group III	0	0	80
Group IV	0	2000	80
Group V	1	0	0
Group VI	1	2000	0
Group VII	1	0	80
Group VIII	1	2000	80

Biochemical Parameters

During each screening 5 ml of blood was collected from six birds of each group at weekly interval in a dray test tube and serum was separated. They

were analyzed for serum total protein, albumin and globulin using Lab Met clinical analyzer as per the procedure described in the manual by using reagent kits supplied by Swemed Diagnostics Ltd. Bangaluru.

Statistical Analysis

The data generated from different parameters of the experimental study was subjected to one way analysis of variance (ANOVA) test using graph pad prism soft ware.

RESULTS AND DISCUSSION

Total Serum Protein

The weekly mean serum total protein levels in different groups have been shown in Table 2.

It was observed that the mean serum total protein of Group II to IV did not alter significantly throughout the period of experiment when compared to control group (Group-I). The mean serum total protein of toxin fed group (Group V) decreased significantly ($P < 0.05$) when compared with control (Group I) from 2nd week till the end of the experiment. However, supplementations of diatomaceous earth, Vitamin E and both to the toxin treated birds of Group VI, VII and VIII respectively showed significant

Table 2: Mean (± SE) values of serum total protein (g/dL) in broiler chicken fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/Weeks	1	2	3	4	5
I	2.33±0.22^{a}	2.68±0.18^{ac}	2.48±0.12^{ac}	2.56±0.13^{ac}	2.55±0.15^{ab}
II	2.23±0.12^{a}	2.37±0.09^{abc}	2.53±0.14^{ac}	2.15±0.11^{ac}	2.57±0.07^{ab}
III	2.56±0.78^{a}	2.99±0.27^{a}	2.33±0.13^{a}	2.71±0.02^{ac}	3.05±0.23^{a}
IV	2.45±0.29^{a}	2.51±0.23^{ac}	2.79±0.05^{c}	2.91±0.33^{c}	3.12±0.27^{a}
V	2.72±0.28^{a}	1.81±0.05^{bd}	1.71±0.02^{b}	1.83±0.09^{b}	1.70±0.06^{c}
VI	2.27±0.02^{a}	2.29±0.09^{cd}	2.23±0.05^{a}	2.30±0.06^{abc}	2.28±0.06^{bc}
VII	2.21±0.13^{a}	2.36±0.06^{ad}	2.14±0.03^{a}	2.24±0.08^{ab}	2.00±0.04^{bc}
VIII	2.32±0.16^{a}	2.12±0.04^{cd}	2.47±0.08^{ac}	2.54±0.11^{ac}	2.30±0.05^{bc}

Mean values bearing common superscript within columns did not differ significantly ($P<0.05$)

Table 3: Mean (± SE) values of serum albumin (g/dL) in broiler chicken fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/Weeks	1	2	3	4	5
I	1.33±0.11^{ad}	1.50±0.02^{a}	1.65±0.04^{a}	1.50±0.02^{a}	1.80±0.05^{a}
II	1.38±0.06^{a}	1.44±0.04^{ac}	1.48±0.04^{ad}	1.41±0.02^{acd}	1.70±0.07^{ad}
III	1.34±0.04^{ad}	1.32±0.04^{bcd}	1.38±0.02^{bd}	1.47±0.02^{ac}	1.72±0.02^{ad}
IV	1.41±0.02^{a}	1.39±0.02^{ab}	1.44±0.08^{b}	1.44±0.04^{acd}	1.69±0.06^{ad}
V	0.94±0.02^{b}	0.99±0.04^{ac}	1.04±0.04^{c}	1.01±0.02^{b}	1.10±0.04^{b}
VI	1.10±0.03^{bd}	1.18±0.06^{df}	1.28±0.01^{be}	1.26±0.04^{cd}	1.45±0.03^{c}
VII	1.03±0.03^{bc}	1.02±0.03^{cf}	1.14±0.03^{ce}	1.24±0.10^{d}	1.37±0.08^{c}
VIII	1.19±0.02^{ab}	1.27±0.04^{bf}	0.41±0.01^{bd}	1.46±0.05^{a}	1.51±0.04^{cd}

Mean values bearing common superscript within columns did not differ significantly ($P<0.05$)

Table 4: Mean (± SE) values of total serum globulin (g/dl) in broiler chicks fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/Weeks	1	2	3	4	5
I	0.99±0.01^{ae}	1.18±0.01^{abc}	0.84±0.03^{a}	1.05±0.01^{a}	1.80±0.05^{a}
II	0.85±0.02^{bd}	0.94±0.04^{bc}	1.05±0.01^{b}	1.34±0.01^{bd}	1.70±0.07^{a}
III	1.22±0.05^{c}	1.66±0.07^{a}	0.92±0.06^{a}	1.24±0.02^{b}	1.72±0.02^{b}
IV	1.04±0.02^{de}	1.12±0.02^{ac}	1.35±0.01^{c}	1.47±0.08^{d}	1.69±0.06^{ad}
V	1.78±0.02^{b}	0.83±0.01^{c}	0.66±0.01^{d}	0.82±0.04^{c}	1.10±0.04^{a}
VI	1.17±0.02^{a}	1.16±0.06^{ac}	0.95±0.01^{ad}	1.04±0.01^{a}	1.45±0.03^{a}
VII	1.18±0.03^{a}	1.34±0.03^{ac}	0.83±0.02^{a}	0.71±0.02^{ac}	1.37±0.08^{a}
VIII	1.13±0.02^{b}	1.35±0.04^{bc}	1.05±0.02^{b}	1.05±0.01^{a}	1.51±0.04^{a}

Mean values bearing common superscript within columns did not differ significantly ($P \geq 0.05$)

increase ($P<0.05$) in the serum protein as compared to only toxin treated birds from 2nd week onwards.

Serum Albumin

The weekly mean serum albumin values in the birds of Group I to VIII were presented in Table 3.

The serum albumin values did not show significant ($P < 0.05$) differences in birds of Group II to IV when compared to Group-I during the entire period of experiment. The mean serum albumin value of Group V (Toxin fed group) was decreased significantly ($P<0.05$) when compared with that of the control group throughout the period of study. While the birds of Group VI to VIII showed a significant ($P<0.05$) increase from 1st to 5th week of age as compared to only toxin fed birds (Group V).

Serum globulin

The weekly mean serum globulin values in birds of Group I to VIII are presented in Table 4.

The mean serum globulin values of Group II to IV did not show significant ($P < 0.05$) differences, but showed significant ($P < 0.05$) decreased in birds fed with toxin as compared to control birds. However, birds of Groups VIII showed significant ($P < 0.05$) increase in serum globulin values as compared to only toxin fed birds during 3rd and 4th week of the experimental study.

In the present study, the mean serum total protein, albumin and globulin values were significantly decreased in toxin fed birds as compared to control birds (Group I). A similar feature was reported by Singh *et al.* (1990); Prakash (2001); Rajeev (2001) and Gupta *et al.* (2005).

Decrease in the serum total protein albumin and globulin values in these birds could be due to inhibition of hepatic protein synthesis which occurred at the post transcription level by competitive inhibition of phenylalanine –tRNA, and in turn stoppage of amino-acylation and peptide elongation (Creppy *et al.*, 1979). Further, Kamagani (1985) reported that one of the primary effects of albumin binding on OA was to retard its elimination by limiting the transfer of OA from the blood strain to hepatic and renal cells contributing to its long half-life.

Supplementation of DAE to the birds fed with toxins slightly improved the serum protein concentration indicating the protective effect of DAE against ochratoxin. The results of present study were in agreement with the Shi *et al.* (2006), who reported that serum biochemical changes associated with AFB1 contamination would be ameliorated by the supplementation of a modified montmorillonite nanocomposite (a compound comprising of DAE) at dose of 3g/Kg. Similarly, Bailey *et al.* (2006) reported that montmorillonite clay (5 g/kg) in broiler diets provided protection in serum biochemistry and the relative organ weights from over 4 mg of AFB1/kg

diets. In the light of the above it can be concluded that the reduction of OA toxicity might be similar to that of AFB1.

The higher values of serum total protein, albumin and globulin in Vitamin-E treated group in contrast to only toxin fed group indicate the restorative role of herbal preparations as for as protein synthesis is concerned. Further, supplementation of toxin binder DAE to the birds fed with toxins slightly improved the serum protein concentration indicating the protective effect of toxin binder against ochratoxin. The results of present study were in agreement with those of Anita (2001) and Bhanuprakash (2002). However, supplementation of Vitamin-E, toxin binder and both together with toxin increased the protein, albumin and globulin levels in birds of Group VIII and suggest the ameliorative effects on alterations induced by the ochratoxicosis.

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Clinical, Haematobiochemical, Venous Blood Gas and Ruminal Fluid Changes Before and After Therapy in Acute Ruminal Acidotic Goat*

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ABSTRACT

A two and half year old pregnant goat was presented to TVCC with a history of accidental ingestion of rice. Evaluation of clinical, haematobiochemical, ruminal fluid and venous blood gas parameters were undertaken in the affected goat for confirmation of ruminal acidosis. The ruminal fluid evaluation showed pH - 5.0, whitish colour with sour odour and absence of microflora. There was significant increase in heart rate, respiration rate, Hb, PVC, blood glucose and SGOT and decrease in VpH and $VHCO^{-}_{3}$ were observed. The case was treated with isotonic sodium biocarbonate and Bufzone® as oral ruminal buffering agent. All parameters returned to normalcy within 72h to 120h after the treatment and goat recovered uneventfully.

Keywords: Goat, Acidosis, Blood gas, Ruminal, Bufzone

In goats ruminal acidosis is a metabolic disorder caused by ingestion of highly fermentative carbohydrates like rice, jawar, wheat, ragi, grams, fruits, vegetable wastes, and leftover kitchen and ceremonial wastes. Acute ruminal acidosis is the most remarkable forms of ruminal microbial fermentative disorders (Radostits *et al.*, 2007). The decrease in ruminal pH to less than 5.00 in most cases is observed due to speedy fermentation of carbohydrates that results in proliferation of acid resistant bacteria (*Lactobacillus and Streptococcus bovis*). Sodium bicarbonate is an important buffer of ruminal pH (Ding and Xu, 2006). Its additives or probiotics along with ruminal buffers are administered more commonly in the ruminants as therapeutic regimen to cure the metabolic lactic acidosis. The present paper reports mainly the blood gas changes in ruminal acidotic goat where not many reports were available.

HISTORY AND CLINICAL SIGNS

A two and half year-old pregnant goat was presented with a history of accidental ingestion of rice. After 12 hours of ingestion it stopped taking the food and water. The chief compliant was distention of abdomen, fluid splashing sound and passing of loose semisolid feaces. The case was tentatively diagnosed as ruminal acidosis based on history and clinical signs.

CLINICAL EXAMINATION AND TREATMENT

The detailed clinical examination was performed to arrive at confirmative diagnosis. The physiological parameters like temperature, heart rate and respiration rates, ruminal fluid (pH, odor, consistency, and concentration of protozoa), haematological (TLC, PCV and Hb), venous blood (pO_2, pCO_2, HCO^{-}_{3}, H^{+} and base deficit) and the biochemical (SGOT, SGPT and blood glucose) were evaluated to know the status of animal before treatment 0h, 12h, 24h 72h and 120h after treatment at different intervals with details in Table 1. Before treatment the ruminal fluid of 20-30ml was collected from the rumen by extraction pump and on immediate evaluation showed, whitish colour with

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Table 1: Showing the clinical, ruminal fluid, haematobiochemical and venous blood gas changes in acidotic goat

Sl No.	Parameters	Before treatment	After treatment			
		0 h	12 h	24h	72h	120h
1	Temperature °F	101.8	101.2	101.6	101.8	102.2
2	Heart rate / min	120	112	105	84	68
3	Respiratory rate / min	54	46	42	40	38
4	pH	5.0	5.6	5.8	6.2	6.3
5	Colour	Milky white	Milky white	Milky white	Light green	Greenish
6	Consistency	Watery	Watery	Watery	Viscous	Viscous
7	Odour	Sour	Sour	Sour	Aromatic	Aromatic
8	Concentration of protozoa	Nil	Nil	Nil	Present	Present
9	Hb g/dL	11.3	11.5	12.0	10.7	10.0
10	PCV %	42	37	30	31	32
11	TLC $x10^3\mu L$	10.3	9.82	11.12	12	11.36
12	Blood Glucose mg/dL	114.42	95.52	92.23	54.44	52.67
13	SGOT IU/L	125.68	66.03	68.87	52.63	24.23
14	SGPT IU/L	17.75	12.78	3.55	8.54	7.33
15	VpH	6.948	7.281	7.429	7.457	7.441
16	$VpCO_2$ mmHg	28.5	22.6	42.9	35.4	36.5
17	VpO_2 mmHg	70.9	51.4	40.7	48.0	45.0
18	$VHCO^-_3$ mmol/L	7.2	14.3	26.7	25.7	24.2
19	VH^+nmol/L	112.7	52.4	37.3	34.9	35.0

pungent odor, absence of microflora and the pH-5 were suggestive of acute ruminal acidosis.

The case was treated with isotonic sodium biocarbonate as alkalizing agent parenterally following to this Ringers lactate 100ml I/V Tribivet 3ml I/V, Chlorpheniramine maleate 2ml I/M, Streptopencillin 3ml intra ruminal and oral ruminal buffering agent Bufzone 50gm were given for five days as supportive medications.

RESULTS AND DISCUSSION

The chief clinical signs were distention of abdomen and passing of loose semi solid feaces. The signs of dullness, distension of abdomen and fluid splashing sound after abdominal ballottement were observed. High molecular weight lactic acid in rumen was responsible for increased osmolarity of ruminal epithelium in acute ruminal acidosis which is in turn causes accumulation of fluid, splashing sound and over distension of abdomen (Radostits *et al*., 2000).

Significant increase in heart rate and respiratory rate was observed which returned towards normally within physiological limit at 72 hr after treatment. Patra *et al*.(1996) and Tufani *et al*. (2013) were also observed similar findings. The ruminal fluid showed significant changes in odour, colour, consistency and concentration of protozoa before treatment suggestive of ruminal acidosis. The colour changed from milky white to greenish, consistency from watery to viscous, odour from sour to aromatic. Appearances of microflora were observed at 72 h after treatment. The above changes in the present case corroborate earlier

findings of Kasaralikar *et al.* (2007) and Tufani *et al.* (2013). Among the haematological values TLC has not showed significant changes however significant increase in Hb 11.3g % and PCV 42 % were observed and returned towards within physiologiscal limit at 72 hrs after treatment. This finding was in accordance with the Sharma *et al.* (2010) and Tufani *et al.* (2013) where dehydration was the cause for increasing the values and after fluid therapy the values come down to normalcy (Radostits *et al.*, 2000).

The blood glucose and SGOT were increased and returned towards within physiological limit at 72 h to 120 h after treatment.This finding was in accordance with the Kasaralikar *et al.* (2007) and Sharma *et al.* (2010). The elevated blood glucose and SGOT were attributed to glycogenolysis with decreased level of insulin and hepatocellular damage due endotoxins. The VpH and $VHCO^{-}_{3}$ mmol/L were decreased and returned towards within physiological limit at 24 hrs after treatment. Ullah *et al.* (2013) and Rahima *et al.* (2012) also observed the decline in blood pH and bicarbonates respectively. Increase in the production of volatile fatty acids and lactate and absorption of acids into the blood stream leads to systemic and metabolic acidosis (Radostits *et al.*, 2007). However, $VpCO_2$ mmHg, VpO_2 mmHg and VH^{+}nmol/L were increased and returned towards within physiological limit at 24 h to 120 h after treatment. This observation was similar to observations made by Rahima *et al.* (2012).

The case was treated with isotonic sodium biocarbonate alkalizing agent parentally. The similar treatment was also recommended by the many workers were Patra *et al.* (1996), Kasaralikar *et al.* (2007) and Ding and Xu (2006). Following to this supportive treatment oral ruminal buffering agent Bufzone helped in buffering the ruminal environment. In this study appearances of microflora were observed at 72 h after oral administration of Bufzone (rumen buffer enriched with metabolic boosters and yeast). Tufani *et al.* (2013) recommended use of prebiotic and proboitic products in ruminal acid indigestion cases for early rejuvenation of the ruminal microflora. The goat started taking little feed next day however the marked improvement in food and water intake was observed after the fourth day after treatment. It passed semi solid feces on third day and complete pelleted feces on fifth day (120 hrs) after treatment.

CONCLUSION

Acute ruminal acidosis is an emergency metabolic disorder caused by ingestion of highly fermentative carbohydrates. It is very essential to know the status of the animal. In this case the various diagnostic parameters especially the ruminal and blood gas changes evaluated for knowing the systemic status of the animal. The administration of sodium biocarbonate and Bufzone were really very much essential in speedy recovery of case without any complication.

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Effect of Incorporation of Hydrolysed Fascia on the Quality of Buffalo Meat Patties*

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ABSTRACT

Epimysial fascia was collected from buffalo carcasses under hygienic conditions from organised slaughter house, was cut into thin slices, defatted, further subjected to mild drying and finally powdered in a hammer mill. The powdered fascia was subjected to enzymatic hydrolysis by suspending them in mild 0.1 N hydrochloric acid solution containing 0.1% pepsin (w/w of fascia) of 1:10,000 strength for 8 hours at 37°C. Physico-chemical properties like proximate principles, collagen contentand *in-vitro* pepsin digestibility of hydrolysed fascia weredetermined. Buffalo meat patties were prepared by replacing lean meat at 0, 10, and 20%. Replacement of lean with hydrolysed fascia did not affect proximate composition of patties except proteins. Amongst processing qualities, though pH increased, the emulsion stability, shear force, cooking yield of patties showed a declining trend. Nutritional qualities like available lysine, collagen increased significantly whereas calculated PER decreased but remained well above universally accepted index of 2.5. Texture and sensory attributes were also significantly affected but remained good to very good upto a level of 20%. Results from this study indicated that meat could be replaced up to 20% with hydrolysed fascia in buffalo meat patties formulations without affecting sensory qualities with nutritional gain.

Keywords: Hydrolysed fascia,Utilization, Buffalo meat, Patties, Quality

Fascia is one of the under - utilized byproduct from slaughter animals and has good amount of protein. In most of the slaughter houses in our country, fascia (epimysial collagen) is discarded as waste. The major protein of fascia is collagen. Meat containing raw material added with collagen or its fractions could enhance its technological and rheological properties and solve the problem of waste disposal from abattoirs (Rao and Henrickson, 1983). Though novel meat processing techniques resulted in superior meat products, high cost of these products making it difficult for an average consumer to use these products regularly in their diet (Malav *et al.*, 2013). Reports are available on various uses of collagen in different food products including meat products (Ranganayaki, 1982; Rao and Henrickson, 1983; Charvez *et al.,* 1985). Hence, an attempt was made to utilize epimysial fascia in meat products.

MATERIALS AND METHODS

Collection of fascia: Epimysial fascia was collected from buffalo carcasses under hygienic conditions and stored in deep freeze (-18°C) till further use.

Preparation of fascia: Frozen fascia after thawing was cut into thin slices of 1-1.5 mm thickness in a slicer and defatted with ten volumes of ethanol with continuous stirring for 6 hours and subjected to mild drying and finally powdered in a hammer mill.

Hydrolysis of fascia: Based on degree of hydrolysis, the powdered fascia was subjected to enzymatic hydrolysis by suspending them in mild 0.1 N hydrochloric acid solution containing 0.1% pepsin

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(w/w of fascia) of 1:10,000 units strength for 8 hours at 37^{0}C in an incubator.

Degree of hydrolysis: Powdered fascia was subjected to three different concentrations of pepsin (1:10,000) *viz.* 0.1%, 0.5% and 1% and degree of hydrolysis was ascertained at every 2 hours. Degree of hydrolysis was evaluated according to percent of trichloroacetic acid ratio method as described by Hoyle and Merritt (1994) and Fonkwe and Singh (1995). At every 2 hours of hydrolysis, 20 ml of hydrolysate was added to 20 ml of 20% (w/v) trichloroaceticacid to produce 10% trichloroacetic acid (TCA) soluble material. The mixtures were left to stand for 30 min to allow precipitation followed by centrifugation at 8000 rpm for 15 minutes. The supernatant was analysed for soluble nitrogen by Kjeldhal method (A.O.A.C., 1995). Samples from the hydrolysate was also analysed for protein content. Degree of hydrolysis (DH) was calculated using the formula below:

$$\% \text{ DH} = \frac{\text{Soluble N in TCA10\%(W/V)}}{\text{Total N in the sample}} \times 100$$

where N=nitrogen.

Recipe of patties: Deboned buffalo meat was packed in clean polyethylene bags and frozen at -20^{0}C until use. The standardized recipe contained 85 parts buffalo meat with 15 parts of sun flower oil and green condiments 5%, table salt 2%, dry spices mix1%, sugar 1%, phosphate 0.5%, sodium nitrite 0.02% and ice water 12%. Buffalo meat patties for the present study prepared by incorporating hydrolysed fascia at 0, 10, 20 percent levels by replacing lean meat.

Preparation of patties: Meat emulsion was made utilizing above mentioned ingredients. Sixty grams of meat emulsion was moulded in aluminium circular mould and placed on perforated trays and cooked for 18 minutes in a preheated oven at180 ^{0}C to obtain an internal temperature of about 75^{0}C. Six such trials were conducted for each level of incorporation.

Analysis of sample: Proximate composition was determined according to AOAC (1995) methods.The *in vitro* pepsin digestibility of the hydrolysed fascia was performed as per the standard method A.O.A.C. (1995) with slight modifications as per ICONTEC (1994). The pH of raw emulsion as well as cooked patties was determined by the method of AOAC (1995) using pH meter. Emulsion stability and percent cooking yield were determined by the method of Baliga and Madiah (1970) with slight modifications. Amount of collagen in hydrolysed fascia and meat patties was calculated by estimating hydroxyproline content according to the procedure of Neuman and Logan (1950). Available lysine content of patties was determined by the method of Carpenter (1960). Calculated protein efficiency ratio (PER) of patties was calculated by adopting the procedure of Lee *et al.* (1978) who by using varying amounts of collagen in the meat product bioassay, computed the following linear regression equation to estimate the PER of meat product from collagen content.

PER = - 0.229 (Collagen %) +3.1528.

Objective texture/ shear force value of the patties was recorded using a Warner- Bratzler shear device. Each patty was made into small piece of 1.5 cm and the force required to shear the patties was recorded. The sensory attributes of the product were evaluated by six semi trained panellists, using and point Hedonic scale as per Keeton (1983). Data obtained were analysed statistically as per the method outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Degree of hydrolysis: An attempt was made to use hydrolysed fascia in meat product by subjecting fascia to a minimum concentration of pepsin over a period of time, so that the product quality and nutritive value is enhanced.

For enzymatic hydrolysis, pepsin was used as the proteolytic enzyme to breakdown milled fascia resulting in both soluble and insoluble fraction. From meat processing point of view, the soluble fraction contains the hydrolyzed proteins that can be converted and incorporated with other main components while processing meat products.The degree of hydrolysis (DH) was determined and the results were shown in Table 1. As expected that DH increased with increasing reaction times ($P<0.05$). The 8 hours of reaction time gave DH in the range of 25.17 -27.33 % yet there were no significant differences in DH among

Table 1: Degree of hydrolysis of fascia by pepsin at different concentration and time

Reaction time in h	Conc. of pepsin enzyme(1:10000)		
	0.1%	0.5%	1.0%
2	10.5 ± 0.619[a]	10.60 ± 0.447[a]	15.00 ± 0.447[b]
4	15.30 ± 0.667[a]	16.75 ± 0.833[a]	18.10 ± 0.447[b]
6	20.17 ± 0.543[a]	19.22 ± 0.422[a]	22.00 ± 1.633[a]
8	25.17 ± 1.108[a]	27.33 ± 1.109[a]	26.67 ± 0.843[a]

the enzyme concentrations. Hence, pepsin (1:10000) at lowest possible concentration of 0.1% is used for hydrolysing fascia.

Physico-chemical composition of hydrolysed fascia: The physico-chemical composition of hydrolysed fascia is presented in Table 2. The similar observations with respect to protein % and collagen content of hydrolysed fascia were made by the authors of the committee on health aspect of collagen as a food ingredient (FDA, 1981), particularly with native hide collagen from slaughtered cattle.

Table 2: Physico-chemical composition of hydrolysed fascia

Proximate Composition	Hydrolysed fascia	
	Wet basis	Dry basis
Moisture %	67.50 ± 0.33	–
Protein %	29.94 ± 0.24	89.77 ± 0.175
Fat%	0.61 ± 0.065	–
Ash%	0.70 ± 0.04	–
Collagen(mg/g)	89.58 ± 0.068	
pH	4.58 ± 0.085	
Nutritional quality		
In- vitro pepsin digestibility %	70.00 ± 16.56	

Values are Mean± SE of six replicates: Means with different superscripts (row-wise) differ significantly (P<0.05).

Table 3: Processing quality, proximate composition and nutritional characteristics of cooked buffalo meat patties incorporated with different levels of hydrolysed fascia

Processing quality	Levels of incorporation (%)		
	0	10	20
Emulsion stability (%)	93.66 ± 0.45[a]	91.61 ± 0.37[b]	89.33 ± 0.40[c]
pH	6.35 ± 0.010[c]	6.47 ± 0.005[b]	6.52 ± 0.006[a]
Shear force value (Kg)	0.76 ± 0.009[a]	0.62 ± 0.017[b]	0.41 ± 0.018[c]
Cooking Yield(%)	88.33 ± 0.211[a]	87.65 ± 0.464[b]	85.7 ± 0.052[c]
Proximate composition			
Moisture (%)	61.04 ± 0.246[b]	60.73 ± 0.147[b]	63.11 ± 0.101[a]
Protein (%)	17.5 ± 0.502[a]	18.4 ± 0.238[a]	14.08 ± 0.327[b]
Ether extract (%)	17.61 ± 0.218	17.73 ± 0.307	17.68 ± 0.246
Total ash (%)	2.89 ± 0.045	3.07 ± 0.071	3.09 ± 0.047
Nutritional characteristics			
Collagen (mg/g)	17.38 ± 0.462[c]	22.34 ± 0.437[b]	30.39 ± 0.2[a]
Protein efficiency ratio	3.11 ± 0.005[a]	3.1 ± 0.001[b]	3.07 ± 0.001[c]
Available lysine (%)	1.32 ± 0.013[c]	1.63 ± 0.01[b]	1.79 ± 0.008[a]

Values are Mean± SE of six replicates: Means with different superscripts (row-wise) differ significantly (P<0.05).

Processing quality characteristics of patties: The processing quality, proximate composition and nutritional characteristicsof patties are presented in Table 3.

Emulsion stability: The emulsion stability decreased significantly (P<0.05) with the incorporation of hydrolysed fascia in this study might be attributed to loss of binding characteristics due to enzymatic hydrolysis of the fascia. Enzymatic hydrolysis resulted in the formation of low molecular weight components and peptides which do not possess any emulsification properties as opined by Cronlund and Woychik (1987), Bailey and Light (1988) as evidenced by degree of hydrolysis.

pH: The pH of cooked patties increased significantly (P<0.05) with the increasing addition of hydrolysed fascia in buffalo meat patties. Similar increase in pH values in bologna incorporated with hide collagesn was reported by Rao and Herickson (1983). Collagen molecule constitute about 40% polar amino acid residues of which 11% are basic and 9% acidic and about 17% are hydroxyl amino acids as opined by Cassel and Mckenna (1953). This higher proportion of basic amino acids in collagen might be responsible for the observed increase in the pH of patties incorporated with the hydrolysed fascia.

Shear force value: Measuring the force in shearing is one of measure for tenderness as opined by Kramer (1957). In the present study significantly lower shear values of the patties with hydrolysed fascia might be attributed to the disintegration of structure of the native collagen after enzymatic hydrolysis and similar results are obtained by Reddy *et al.*(1998).

Cooking yield: The results indicated that the cooking yield decreased (P<0.05) significantly when patties were incorporated with hydrolysed fascia that might be due to the higher moisture content of hydrolysate as well as loss of functional properties by way of formation of low molecular weight compounds on hydrolysis.

Proximate composition of patties: Amongst all groups, the moisture content increased significantly (P<0.05) whereas the protein content of patties decreased significantly (P<0.05) in patties containing 20 % hydrolysed fascia.The crude fat content of patties did not vary with the incorporation of hydrolysed fascia. No change in moisture and protein contents of patties incorporated with hydrolysed fascia at 10 % level was observed when compared to control. It was in agreement with findings of Rao and Henrickson (1983) and Reddy *et al.* (1998). The decrease in protein content of patties with 20% hydrolysed fascia in the present study might be due to relatively higher moisture content contributed by 0.1 N Hydrochloric acid added for enzymatic hydrolysis during preparation of hydrolysed fascia.

Nutritional characteristics of patties incorporated with fascia:-

Collagen: It increased significantly (P<0.05) with increase in the incorporation of hydrolysed fascia. In the present study increased collagen content was observed in treatment groups compared to control was in agreement with the findings of Strange and Whiting (1990) and Reddy *et al. (*1998). Strange and Whiting (1990) reported 2.5% of collagen in the restructured beef steaks incorporated with epimysium at 10% level. In the present study also the collagen content was similar to that reported by the above workers.

Calculated protein efficiency ratio: It is evident that the calculated protein efficiency ratio (PER) of patties incorporated with different levels of fascia showed significant decrease (P<0.05) from control. However, the calculated PER of patties with 20 % hydrolysed fascia was significantly lower compared to the groups with 10 % hydrolysed fascia. Protein efficiency ratio values reflect both the level of essential amino acid in a protein or proteinaceous food as well as bioavailability of those amino acids. In USDA (1989), a minimum PER of 2.5 have been specified for most fabricated foods. Lee *et al.* (1978) reported that a PER of 2.5 correspond to 28.5% of collagen in the test product and increasing the collagen content of meat products from 10 to 30% reduced it from 3 to 2.5. Collagen alone is a poor protein but has supplementary nutritional value when added in meat products. Vaughn *et al.* (1979) reported a PER value of 0.6 for pigs ear (70% collagen) while Happich *et al.* (1975) reported a PER value less than zero for

cattle hide (80% collagen) increasing to a PER value of 1.1 when only 50% collagen was present. Erbersdobler *et al.* (1970) reported that collagen if used with balanced proteins and within limits, did not decrease the nutritive value of the diet. However, Kofranyi and Jekat (1969) reported improved biological value of meat containing 15-20% collagen.

In the present study the calculated PER values for all the groups of patties ranged from 3.07 to 3.11. These values are more than the minimum PER limit of 2.5 as specified by Lee *et al.* (1978). The collagen content of different patties ranged from 1.73% in control to 3.03 % in patties with 20% hydrolysed fascia. The patties with 10% fascia corresponded to 2.23% collagen while that with 20% hydrolysed fascia contained 3.03% collagen. These observations were almost in accordance with those of Strange and Whiting (1990) who reported 2.5% of collagen content in restructured beef steaks incorporated with 10% epimysium. The reason for higher PER values recorded in the present study might be attributed to lower contents of collagen of the product than the maximum level i.e. 28.5% collagen for a minimum PER of 2.5 reported by Lee *et al.* (1978). In this study, patties incorporated with hydrolysed fascia recorded lower PER values than the patties with control and might be correlated with the higher content of collagen. From the above discussion it is evident that incorporation of hydrolysed fascia upto 20% level did not have any adverse effect on PER, rather it was well above cut off value of 2.5 and had increased availability of lysine.

Available lysine: The available lysine content of patties incorporated with hydrolysed fascia at different levels were not only different from that of control but also differed significantly ($P<0.05$) from one another and increased with simultaneous increase in the amount of hydrolysed fascia incorporated. Lysine is one of essential amino acids and its amount in plasma proteins is taken as a criterion of the biological value of food. Lysine and threonine are the limiting amino acids in cereal foods. Collagen has lysine in fair quantity when compared to native muscle and other proteins of food animal. However, meat proteins containing 15 to 20% collagen have a considerable excess of these amino acids as supplementary effect of collagen is well appreciated (Rogov *et al.,* 1992). As the collagen is converted into gelatin on cooking it becomes more digestible and nutritionally more advantageous.In the present study patties incorporated with hydrolysed fascia showed increase in the amount of available lysine. This was in agreement with the findings of Dvorak and Vognarov (1965) who reported increase in the content of available lysine in canned meat due to gelatinization of collagen.

The amino acid lysine is present in the form of hydroxylysine residues are distributed along the peptide chains are known to be involved formation of intermolecular cross links. At cooking temperatures, collagen is solubilised and reduced to small fragments by heat as per Bailey and Light (1988). In the light of above explanation, the observed increase in the available lysine content of patties containing hydrolysed fascia might be attributed to the hydrolysis of fascia resulting in the release of bound lysine by cleavage of collagen cross links during cooking. FDA has already approved the use of collagen upto 15% in meat blocks and claims no disadvantages of using it to such an extent.

Sensory evaluation scores of buffalo meat patties with incorporation of hydrolysed fascia at different levels: The mean scores of sensory parameters of buffalo meat patties with different levels of fascia are presented in Table 4.

General appearance: There was a decrease in general appearance scores as compared to control, when hydrolysed fascia was incorporated in patties. The patties with 20% hydrolysed fascia scored significantly lower score amongst all groups. Inspite of significant differences in the general appearance scores, all the groups scored very good on subjective scale.

Flavour: The patties with 20% hydrolysed fascia scored significantly lower score amongst all groups. All the groups scored very good on subjective scale. Lower flavour scores observed for patties incorporated with higher levels of hydrolysed fascia were in agreement with the findings of Jones (1984) and

Table 4: Sensory evaluation scores of buffalo meat patties with incorporation of hydrolysed fascia at different levels

Processing quality	Levels of incorporation (%)		
	0	10	20
General appearance	6.88 ± 0.09[a]	6.78± 0.07[a]	6.23± 0.09[b]
Flavour	6.75 ± 0.09[a]	6.67± 0.06[a]	6.09± 0.04[b]
Juiciness	6.64± 0.06[c]	7.05± 0.07[b]	7.19± 0.07[a]
Texture	6.62 ± 0.09 [a]	6.39± 0.07[b]	5.82± 0.08[c]
Mouth coating	7.16± 0.10	7.08± 0.10	7.27± 0.06
Overall acceptability	6.50± 0.07[a]	6.39± 0.08[a]	6.15± 0.09[b]

Values are Mean± SE of thirty six replicates; means with different superscripts (row-wise) differ significantly (P<0.05).

Charvez *et al.*(1985) and might be attributed to the bland flavour of collagen as suggested by the above workers.

Juiciness: There was a gradual increase in juiciness scores compared to control which was significant statistically. Charvez *et al.* (1985) reported increase in juiciness scores as the collagen content increased as evidenced in this study.

Texture: The textural scores gradually decreased with simultaneous increase in the incorporation of fascia. The textural scores decreased as the incorporation levels of fascia increased which was in agreement with the findings of Jones (1984), Charvez *et al.* (1985), Strange and Whiting (1990) and Reddy *et al.* (1998).

Mouth coating: All the groups were scored more or less same.

Overall acceptability: There was a gradual decrease in overall acceptability scores when hydrolysedfascia was incorporated upto 20% level. However, all the groups scored very good on the subjective scale. The overall acceptability scores decreased as the fascia level increased which was in agreement with the findings of Jones (1984) and Charvez *et al.*(1985). It may be concluded that hydrolysed fascia may very well be used as a substitute of lean meat in buffalo patties with better processing and nutritional advantage.

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Detection of Factor XI Deficiency in Breeding Bulls

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ABSTRACT

Factor XI (*FXI*) is a plasma protein that participates in the blood coagulation process. Factor XI deficiency is autosomal recessive hereditary disorder with insertion of mutation into exon 12 in bovine chromosome 27 and may be associated with excess bleeding. Functional and physiological defects arising from Factor XI deficiency have negative impact on health and productivity of farm animals. Increased use of artificial insemination and worldwide use of service bull caused to widespread of this disorder through heterozygous carriers. Hence, it has a significant economic impact on farmers worldwide. The aim of this study was to optimise a diagnostic test for detection of this syndrome and to obtain a preliminary picture about their existence in breeding bull population. A total of 288 blood samples were collected from phenotypically normal bullsmaintained at different organized breeding stations. Genomic DNA was extracted from fresh blood samples. Amplicons of *FXI* exon 12 were obtained by Polymerase Chain Reaction and electrophoresed through 2% agarose gel stained with ethidium bromide. The result of this study showed that no mutation of Factor XI was observed in breeding bulls. Although we did not observe any carrier but, widespread screening programs for detection of factor XI deficiency is necessary to prevent the accidental transmission of genetic polymorphism in heterozygous form to a large number of offspring.

Keywords: Autosomal recessive disease, *FXI*D, Polymerase Chain Reaction

Factor XI is a plasma glycoprotein essential for blood coagulation and formation of stable fibrin clot. In cattle, the disorder was described in the Holstein bull (USA) as early as 1969 (Kociba *et al.*,1969) and later in British cattle (Brush *et al.*, 1987). The molecular basis of Factor XI deficiency is a 76 bp insertion of an imperfect poly-adenine tract occurring in exon 12 (Marron *et al.*, 2004). Animals that are *FXI* deficient can be asymptomatic or exhibit a number of indicators that may include prolonged bleeding after injection, production of bloody milk, anaemia and lower calving and survival rates and increased susceptibility to infectious diseases (Brush *et al.*, 1987). Screening of for autosomal recessive disorder is essential and mandatory as per Government of India prior to induction of bulls into breeding programs. To identify the animals with *FXI* genetic defect or carriers is the main the objective of the present study.

MATERIALS AND METHODS

A total of 288 fresh blood samples collected from phenotypically normal bulls from different organized bull farms (91 blood samples from Nandini Sperm Station,Hesaraghatta, Bangalore, 131 blood samples from Livestock breeding & Training Institute, Dharwad, 12 blood samples from State Livestock Breeding & Training Centre, Hesaraghatta, Bangalore and 51 blood samples from CFSP & TI, Hesaraghatta, Bangalore) for the routine screening of genetic defects. The isolation of genomic DNA from blood cells was performed by using QIAamp DNA Blood mini kit (Qiagen) and isolated DNA, stored at -20°C The DNA sample with 1.7 and 2.0 (OD260/OD280) was subjected to Polymerized Chain Reaction (PCR).

The PCR reaction mixture was prepared in a final volume of 25µl containing 1 X PCR buffer 12.5 µl, Red dye 2.5 µl, Nuclease free water 7.4 µl, Template DNA 1.0 µl, (~100ng), forward primer 0.8 µl

(8pmoles) (5'CCCACTGGCTAGGAATCGTT3') and reverse primer 0.8 (8pmoles) (5' CAAGGCAATGTCATATCCAC3') as suggested by Marron *et al.* (2004). Amplification was performed using initial denaturation of 10 minutes at 95°C followed by denaturation at 95°C for 30 seconds, annealing for 1 minute at 55°C, extension at 72°C for 30 seconds and final extension of 10 minutes at 72°C. Due to non-availability of positive control the mutant artificially

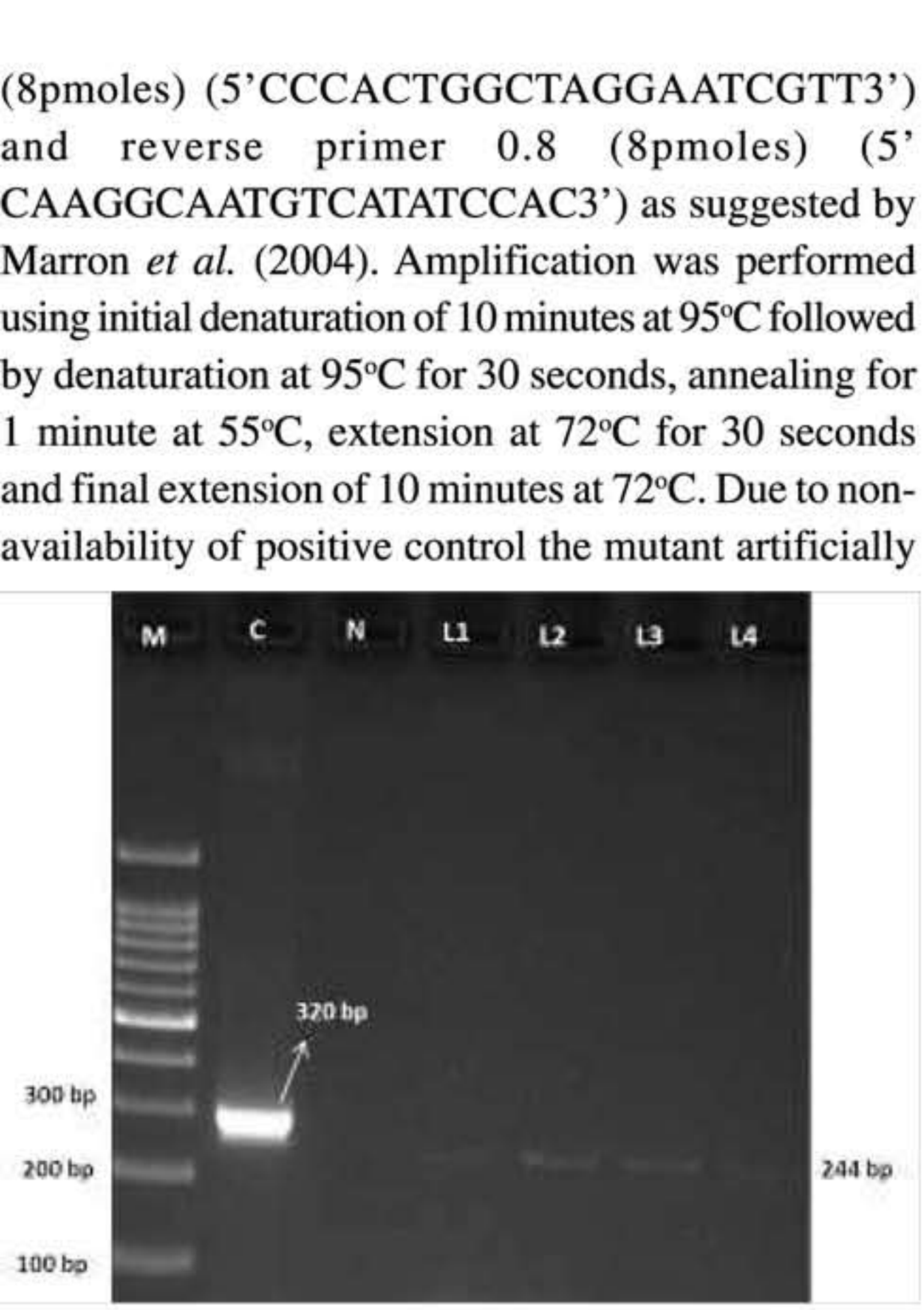

Fig. 1: Agarose gel electrophoresis of PCR product generated by amplification of genomic DNA using gene specific primer.

Lane M: 100 bp DNA ladder, L1 to L4: test sample with 244 bp fragment of normal animal, C: 320 bp fragment of synthetic DNA probe used as Positive control, N: Negative control (Without DNA).

synthesized DNA probe (purchased from Chromos biotech Ltd.) was used. Then the amplified products were analysed along with positive control by agarose gel electrophoresis in 1.5% agarose stained with ethidium bromide.

RESULT AND DISCUSSION

The PCR condition was optimized with respect to template and primer concentration and annealing temperature. Consistent results were obtained at an annealing temperature of 55 for amplification of *FXI* gene and results are in accordance with Patel *et al.* (2007) and Meydan *et al.* (2009). The PCR products of all 288 blood samples generated single 244 bp fragment, which is specific to *FXI*. The carriers of the *FXI* deficient animals exhibit two DNA fragments of size 320 bp and 244 bp, while homozygous recessive animal exhibits only one fragment of size 320 bp and normal animal exhibit only one fragment of size 244 bp (Fig.1 & Fig. 2). The present findings from 288 animals suggested that none of the animals were carrier for *FXI* deficiency. The present findings are in agreement with that of Mukhopadhyaya *et al.* (2006) who screened 307 HF cattle and 259 water buffaloes in India and Kade *et al.* (2014) who screened 50 HF crossbred animals. However, mutation for *FXI* deficiency has been reported by various investigators worldwide. Patel *et al.* (2007) from India reported two *FXI* carrier Holstein-Friesian bulls with frequency 0.6 per cent prevalence in India. Meydan *et al.* (2009) analysed of 225 Holstein cows in Turkey and reported the frequency of the mutant *FXI* allele and the prevalence of the carriers as 0.9% and 1.8% respectively.

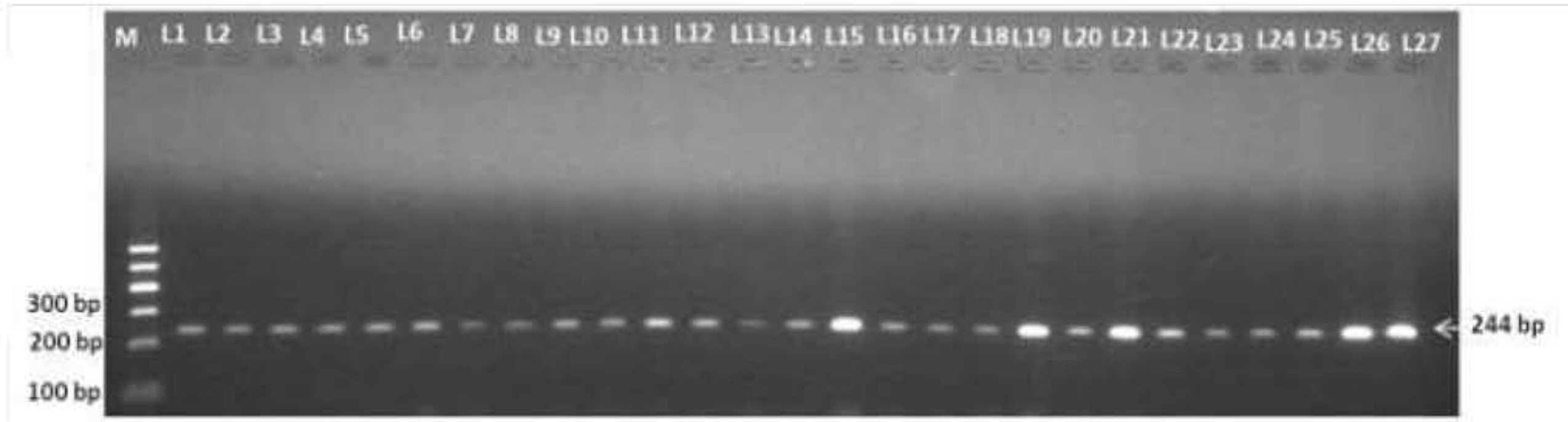

Fig. 2: Agarose gel electrophoresis of PCR product generated by amplification of genomic DNA using gene specific primer.

Lane M: 100 bp DNA ladder, L1 to L27: test sample with 244 bp fragment of normal animal.

CONCLUSION

Based on the results of the present study its can be concluded that, the DNA based diagnostic test described can detect *FXI* deficiency syndrome in breeding bull population. Although we did not observe any carrier but, in India semen from *Bos taurus* animals is extensively used for cross breeding programme with *Bos indicus* animals, it has become necessary to screen all breeding bulls to prevent the accidental transmission of genetic polymorphism in heterozygous form to a large number of offspring.

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Factors Affecting Age at First Calving in HF X Sahiwal (Frieswal) Crossbred Cattle

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ABSTRACT

Age at First Calving (AFC) is an important economic characteristic of dairy animals directly contributing to life time performance of a cow. The present study was carried out on Frieswal cattle of two military dairy farms. The influence of various genetic and non-genetic factors on AFC was studied by Harvey's least squares analysis of variance for unequsal non-orthogonal data. The overall mean Age at first calving obtained in the present study was 915.41±8.24 days. Genetic grade and period of birth were found to have significant effects on AFC in the present study. The highest mean AFC of 1021.13±23.97 days was observed in cows born during period (1992-1997) and was significantly higher compared to other periods. Mean AFC of cows born during period 2003-2007 was lowest (867.03±8.13 days) and differed significantly from other periods. Frieswal cattle with higher HF inheritance (≥ 75% HF, ≤ 25% Sahiwal) had lower (887.64±10.61 days) AFC than in genetic group ≤ 50% HF, ≥ 50% Sahiwal and genetic group 62.5% HF, 37.5% Sahiwal with respective mean values of 904.12±15.86 days and 954.18±6.97 days. Present investigation revealed that, AFC of Frieswal cows varied significantly with the level of HF inheritance. AFC of recently born (second, third and fourth periods) Frieswal cows had significantly lower AFC than the cows born during the first period.

Keywords: AFC, Frieswal, Least squares, Period, Season

Age at first calving (AFC) is one of the important early expressed traits directly contributing to the life time production since the milk production in dairy animals starts with first calving. It is an important economic characteristic of dairy animals directly contributing to life time performance of a cow (Katpatal *et al.*, 1978). Lower AFC reduces the cost of raising animals to productive life, and yields more number of calves and lactations. Lower AFC thus reduces the generation interval and provides opportunity to the breeders to raise more number of offspring, so as to increase the selection intensity and response per unit of time. This also facilitates for evaluation of breeding bulls.

MATERIALS AND METHODS

The data for the present investigation were collected from history sheets and daily milk yield records of Frieswal Crossbred Cattle maintained at Military Dairy Farms (MF) Belgaum and Secunderabad. The records on reproduction performance of Frieswal Crossbred Cattle at the two farms spread over a period of 20 years (1992-2011) were collected. HF x Sahiwal cows born during the period from 1992-2011 were only included in the present study. The effect of periods, was studied by classifying the data into 4 periods of approximately 5 consecutive years duration. The period was classified according to the year of birth as First (1992-1997), Second (1998-2002), Third (2003-2007) and Fourth Periods (2008-2011). Each year was subdivided into three seasons based on meteorological observations *viz*, summer (February to May), rainy (June to September) and winter (October to January) seasons to study the effect of season of birth. To study the effect of genetic grades the differences due to the data were classified into the three genetic groups *viz.*,

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G1 group (≤ 50% HF inheritance + ≥ 50% Sahiwal), G2 group (62.50% HF inheritance + 37.50% Sahiwal) and G3 group (≥ 75% HF inheritance + ≤ 25% Sahiwal), to analyze the differences due to genetic groups.

The influence of various genetic and non-genetic factors on AFC was studied by least squares analysis of variance for unequal non-orthogonal data using the technique described by Harvey (1966).

The statistical model assumed was

$$Y_{ijkmn} = \mu + S_i + P_j + F_k + Gm + e_{ijkmn}$$

Where,

Y_{ijkmn} = Dependent trait (AFC) of n^{th} cow born in i^{th} season, j^{th} period at k^{th} Farm and m^{th} genetic grade

μ = Over all mean

S_i = Effect of i^{th} season

P_j = Effect of j^{th} period

F_k = Effect of k^{th} farm

G_m = Effect of m^{th} genetic grade

e_{ijklmn} = Random error, assumed to be normally and independently distributed with mean zero and constant variance *i.e.* NID $(0,\sigma_e^2)$.

Duncan's multiple range test as modified by Kramer (1957) was used for testing differences among least squares means (using the inverse coefficient matrix).

RESULTS AND DISCUSSION

The computed overall least squares mean for age at first calving based on 1638 observations was 915.41±8.24 days (Table 1). The present estimate is comparable with report of 957.5±5 days by Reddy and Basu (1985) in HF x Sahiwal crosses. However, the present estimate is lower than the reports of Mukherjee (2005) and Anon (2014-15) who reported AFC estimates of 972.37±3.6 days and 973.25 days, respectively, in Frieswal cattle. It is also lower than the reports of Kaul *et al.* (1973), Bhat *et al.* (1978), Banerjee and Banerjee (2002) whose reports were in the range of 1097.402±11.40 days and 1905±31.2 days in HF x Sahiwal crossbreds.

Table 1: Least squares means of AFC in Frieswal crossbred Dairy Cattle

Factors	AFC	
	No.	Mean ± SE
Overall	1638	915.41±8.24
Farms		
Belgaum	575	918.62±9.68
Secunderabad	1063	912.21±8.68
Period of birth		
1992-1997 (P1)	44	1021.13±23.67[b]
1998-2002 (P2)	261	873.00±10.97[a]
2003-2007 (P3)	578	867.03±8.13[a]
2008-2011(P4)	755	900.49±7.20[a]
Season of birth		
Summer (S1)	653	920.56±9.28
Rainy (S2)	445	912.63±10.50
Winter (S3)	540	913.05±9.80
Genetic grade		
≤ 50%HF (G1)	115	904.12±15.86[b]
= 62.5%HF (G2)	1223	954.18±6.97[c]
≥ 75%HF (G3)	300	887.94±10.61[a]

LS means bearing common superscript within columns in sub groups do not differ significantly (P ≤ 0.05)

Table 2: Least squares analysis of variance (Mean squares only) for AFC in Frieswal Crossbred Cattle

Source of variation	AFC
Farm	14479.94(1)
Period	396739.26*(3)
Season	11563.77(2)
Genetic grade	588327.02*(2)
Error	23547.78
R^2- value (%)	6.00

Figures in parentheses indicate the degrees of freedom

* significant at 5% level (P ≤ 0.05)

Least square analysis of variance for age at first calving revealed significant ($P \leq 0.05$) influence of period of birth and genetic grades on AFC and non significant influence of season of birth and farm (Table 2).

Genetic grades had significant influence on AFC. The mean value for AFC of cows in G3 genetic group was lowest (887.94±10.61 days) followed by first genetic and secosnd genetic groups with respective mean values of 904.12±15.86 days and 954.18±6.97 days. This corroborates the earlier reports of Amble and Jain (1967), Kaul *et al.* (1973), Bhat *et al.* (1978), Dangi (1979), Jadhav (1990), Nair (1991), Hassani (2000), Akhter *et al.* (2003) and Mukherjee (2005) in HF x Sahiwal/ Frieswal cattle. However, Katpatal *et al.* (1978) did not observe any significant effect of genetic grades on AFC.

The highest mean AFC of 1021.13±23.67 days was observed in cows born during P-1 period (1992---1997) which was significantly higher compared to other periods. Mean AFC of cows born during P-3 period was lowest (867.03±8.13 days) and differed significantly from those born during P-1 period. The respective mean AFC of cows born during P-2 period and P-4 period were 873.00±10.97 days and 900.49±7.20 days but the differences among the three periods P-2, P-3 and P-4 were not significant. Similar to the present finding, AFC was reported to vary significantly with period of birth (Kaul *et al.* 1973; Bhat *et al.* 1978; Reddy and Basu, 1985; Jadhav, 1990; Hassani, 2000; Nair, 1991; Akhter *et al.,* 2003; Gaur, 2003; Mukherjee, 2005; Anon, 2014-15) in HF x Sahiwal crossbreds. However, Gaur *et al.* (2007) in HF x Sahiwal crosses reported non-significant effect of period of birth on AFC.

Farms had non-significant influence on AFC. Mean AFC of cows at MF, Belgaum was 918.62±9.68 days and 912.21±8.68 days at Secunderabad farm. This is similar to the earlier reports of Hassani (2000) in HF x Sahiwal crosses. However, significant effect of farm on AFC in Frieswal / HF x Sahiwal crosses was reported by several workers (Kaul *et al.,* 1973; Bhat *et al.,* 1978; Reddy and Basu, 1985; Raheja, 1997; Nair, 1991; Gaur, 2003; Mukherjee, 2005; Gaur *et al.,* 2007; Anon, 2014-15).

The mean age at first calving of 920.56±9.28 days, 912.63±10.50 days and 913.05±9.80 days were observed in cows born during summer, rainy and winter seasons, respectively. However, the differences in the AFC of cows born during the three seasons were non-significant. The non-significant effect of season of birth on AFC could be due to the endurance of Frieswal cattle to the varied climatic conditions with respect to AFC. It may also be a fact that the effect of season of birth gets diluted by the time the animal conceives. In agreement with the present finding of non-significant effect of season on AFC, Bhat *et al.* (1978), Katpatal *et al.* (1978), Reddy and Basu (1985), Mukherjee (2005), and Gaur *et al.* (2007) reported non-significant effect of season of birth on AFC in HF x Sahiwal crossbreds. Hsowever, several other workers reported significant affect season of birth on AFC (Kaul *et al.,* 1973; Jadhav, 1990; Hassani, 2000; Akhter *et al.,* 2003; Anon, 2014-15) in HF x Sahiwal crosses.

The variations in the present and earlier reports might be attributed to differences in size of data, type of analysis, and due to the prevalent management conditions at the different farms.

Data compiled on 1638 Frieswal cows maintained at MFs Belgaum and Secunderabad were utilized for the present study. The overall mean obtained in the present study was 915.41±8.24 days. Genetic grades and period of birth was found to have significant effects on AFC in the present study. HF x Sahiwal crosses with higher HF inheritance (≥ 75% HF, ≤ 5% Sahiwal) had lower (887.64±10.61 days) AFC than in genetic group (≤ 50% HF, ≥ 50 % Sahiwal) and genetic group (62.5% HF, 37.5% Sahiwal) with respective mean values of 904.12±15.86 days and 954.18±6.97 days. Present investigation revealed that, AFC of Frieswal cows varied significantly with the level of HF inheritance. AFC of recently born Frieswal cows had significantly lower AFC than the cows born during the first period.

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The Effect of Feeding Detoxified Karanj (honge) (*Pongamia Glabra* Vent) Seed Meal on Carcass Characteristics of Broiler Rabbits*

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ABSTRACT

A feeding trial of 70 days was conducted using broiler rabbits to evaluate detoxified karanj seed meal (KSM) as a potential animal feed source by replacing groundnut cake (GNC). T_1(Control), with solvent treated karanj seed meal (SKSM) at 25 (T_2) and 50% (T_3) or with alkali (2% NaOH) treated karanj seed meal (AKSM) at 25 (T_4) and 50% (T_5) in the total mixed ration (TMR) containing napier hay and concentrate in the ratio of 81:19. The average live weight (g) of rabbit at slaughter ranged from 1662 ± 46.3 (T_3) to 1844 ± 59.8 (T_1) and the dressing percentage was 54.32 ± 0.54 (T_1), 52.88 ± 0.60 (T_2), 52.65 ± 0.13 (T_3), 53.29 ± 0.90 (T_4) and 52.04 ± 0.60 (T_5) and the difference were not. The meat:bone ratio ranged from 2.99 ± 0.14 (T_1) to 3.15 ± 0.07 (T_5). The weight of heart, spleen, caecum, intestine, skin and head did not differ significantly whereas the weight of liver increased by 16.5% in KSM fed (T_3 and T_5) rabbits. The residual toxic factor might be the reason for the enlargement of liver but it was not supported by the biochemical profile of the serum. The proximate composition of meat of all the treatments was analyzed. The score card for sensory meat quality parameters such as appearance, texture, aroma, tenderness, flavor, juiciness and overall quality was carried out on 5-point Hedonic scale. Overall quality values ranged from 3.04 ± 0.15 to 3.15 ± 0.13. The carcass characteristics revealed that there was no adverse effect on taste or odour of rabbit meat due to feeding of detoxified KSM containing diets.

Keywords: Karanjseed meal, Broiler rabbit, TMR diet, Meat quality

Several studies have been conducted with unconventional feeds in livestock and poultry to evaluate their possibility of inclusion as alternate feed for sustaining optimum production and to extrapolate the same method of feeding system to the 'Micro-livestock' such as rabbits. Rabbitry is considered as an integral part of animal husbandry and agriculture to improve the socio economic status of the rural poor especially, landless laborers to provide food security. Realizing these facts, Government of India had included rabbit as an important livestock species for the first time in the livestock census 2003 (Anonymus, 2004).

Among various unconventional feeds, Karanj(honge) (*Pongamia glabra* vent) seed meal (KSM), a by-product of karanj seed, (24-32% CP) is a potential alternative to conventional feedstuffs. The annual production of honge seeds in India is estimated to be 1.37 lakh tones and 9 to 90 kg seed/tree/annum (Dwivedi *et al.,* 2011). Utilization of raw honge seed meal as such has limitation due to presence of various toxic factors such as karanjin, a furano flavonoid (Roy *et al.,* 1977; Prabhu *et al.,* 2002; Vinay and Sindhu Kanya, 2008), furanodiketone, chromenoflavones (Naikstam and Bringi, 1973), tannins and trypsin inhibitors (Vinay and Sindhu Kanya, 2008).

For higher level of incorporation of KSM in diet, further chemical treatments are necessary to minimize residual toxic factors. Efforts are required to utilize this unconventional agro industrial by-product in rabbit ration as an alternative to the expensive conventional protein supplement by evolving economically and easily adaptable processing technology to make wholesome protein supplement.

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Hence, the present investigation was taken to evaluate solvent treated karanj seed meal (SKSM) or alkali treated karanj seed meal (AKSM) as substitute for GNC by studying the effect on broiler rabbit meat quality characteristics.

MATERIALS AND METHODS

A 70-day feeding trial was conducted using broiler rabbits to evaluate the nutritive value of SKSM and AKSM in TMR based diet. Thirty weaned broiler rabbits of the similar age (6-7 weeks old), weight and breed were randomly allotted to 5 groups comprising of 6 rabbits in each treatment group. Five iso-nitrogenous and iso-caloric complete diets were formulated as per NRC (1977) with concentrate and napier hay in the ratio of 81:19 (Table 1).

Napier hay was ground to 1-2 mm particle size to make complete diet in mash form. The SKSM was soaked in 2 per cent NaOH (W/V) solution and kept for 24 hrs under airtight container, followed by sun drying. The SKSM and AKSM were ground to 1-mm size and stored before incorporation into complete feed (Table 2).The GNC of the control diet (T_1) was replaced by SKSM or AKSM to form five diets such that in T_2 and T_3 SKSM replaced 25% and 50% and in T4 and T5 AKSM replaced 25% and 50% of GNC nitrogen (Table 3).

At the end of 70-day trial, four rabbits from each treatment were selected randomly and slaughtered to assess the carcass characteristics and sensory evaluation of meat. Official permission was taken from the Institutional Animal Ethical Committee for the slaughter studies. Rabbits were fasted overnight with free access to water, sacrified and processed as described by Ramayyan (1977).

Dressing percentage was expressed on fasted pre slaughter body weight. Bone and muscle in the thigh were separated from each other and their

Table 1: Ingredient composition (%) of experimental diets

	Treatments				
	T_1	T_2	T_3	T_4	T_5
Maize	23.00	28.05	27.00	28.05	27.00
Deoiled rice bran	22.20	11.00	7.60	11.00	7.60
Groundnut cake	25.00	18.75	12.50	18.75	12.50
SKSM	-	8.00	16.50	-	-
AKSM	-	-	-	8.00	16.50
Sunflower cake	6.00	10.40	12.60	10.40	12.60
Napier hay	19.00	19.00	19.00	19.00	19.00
Molasses	2.50	2.50	2.50	2.50	2.50
Dicalcium phosphate	0.80	0.80	0.80	0.80	0.80
Salt	0.50	0.50	0.50	0.50	0.50
Mineral Mixture[1]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Additives[2]	0.05	0.05	0.05	0.05	0.05

1 Mineral mixture contained Ca-23; P-12; Mg-6.5; Fe-0.5; Zn-0.38%. Mn-120ppm; Cu-77ppm; I-26ppm; Co-12ppm.

2 Additives (g/kg) included Ecare-Se –10 (contained 10%, a-tocopherol and 200 ppm Se), Doxycycline – 10, Vitamin AB_2D_3K – 30.

Table 2: Chemical composition[1] of SKSM and AKSM on dry matter basis

	SKSM	AKSM
Proximate composition (%)		
DM	91.49	95.58
Organic matter	93.76	93.02
Crude protein	32.00	32.00
Crude fibre	7.97	9.67
Ether extract	0.98	0.78
Nitrogen free extractives	52.81	50.57
Total ash	6.24	6.98
Fibre Fractions (%)		
Neutral detergent fibre	36.97	38.47
Acid detergent fibre	13.90	14.60
Hemicellulose	23.07	23.87
Cellulose	11.48	12.05
Acid detergent lignin	2.42	2.55

[1]Mean of three replicates.

individual weights were recorded to find out the meat: bone ratio. Meat from loin region was selected for sensory evaluation. Representative deboned meat sample from each group was made into 3-4 g weighing pieces. Meat sample was cooked in boiling water with salt (1% w/w) for 20 minute before subjecting to sensory evaluation on 5-point hedonic scale by a panel of 25 trained panelists to assess the appearance, texture, aroma, tenderness, flavor, juiciness and overall quality of meat. The minced fresh meat from each group was analyzed for moisture, protein, fat and ash content according to AOAC (2000) methods.

The data were subjected to statistical analysis using analysis of variance techniques as described by Snedecor and Cochran (1980) and accordingly results were interpreted.

RESULTS AND DISCUSSION

Carcass Characteristics

1. Physical Characters

The average live weight of rabbits (Table 4)at slaughter were1844 ± 59.8 (T_1), 1742 ± 93.4 (T_2), 1662

Table 3: Chemical composition[1] of the experimental diets on dry matter basis

Particulars	Experimental diets				
	T_1 (Control)	T_2 (25%SKSM)	T_3 (50%SKSM)	T_4 (25%AKSM)	T_5 (50%AKSM)
Proximate composition (%)					
Dry matter	91.22	90.29	91.47	90.92	90.67
Organic matter	89.87	89.63	90.15	90.09	89.59
Crude protein	18.12	18.39	18.32	18.52	17.98
Crude fibre	13.12	13.34	12.97	11.98	12.22
Ether extract	3.51	3.47	3.12	3.47	3.02
Nitrogen free extractives	55.12	54.43	55.74	56.12	56.37
Total Ash	10.13	10.37	9.85	9.91	10.41
Fibre fractions (%)					
Neutral detergent fibre	33.48	32.47	32.57	31.27	31.33
Acid detergent fibre	17.76	16.99	18.72	17.28	16.82
Hemicellulose	15.72	15.48	13.85	13.99	14.51
Cellulose	15.09	14.87	16.51	14.83	14.85
Acid detergent lignin	2.67	2.12	2.21	2.45	1.97

[1]Mean of three replicates. Variation in triplicate measurement was within ± 5.0 % of the mean.

± 46.3 (T_3), 1767 ± 59.4 (T_4) and 1764 ± 23.6 (T_5) g. The average carcass weights were 1002 ± 34.9 (T_1), 921.3 ± 51.0 (T_2), 875.3 ± 26.1(T_3), 941.8 ± 35.8 (T_4) and 917.8 ± 14.5 (T_5) g.The dressing percentage of different treatments was 54.32 ± 0.54 (T_1), 52.88 ± 0.60 (T_2), 52.65 ± 0.13 (T_3), 53.29 ± 0.90 (T_4), 52.04 ± 0.60 (T_5).

There were no significant differences with respect to above physical characters among treatments. Rajendiran (2002) reported that dressing percentage ranged from 49.40 to 56.00, in rabbits fed on unconventional feedstuffs.The observation of the present study was in agreement with earlier report of Gowda (1994) and Igwebuike *et al.* (2003). The dressing percentage in the range of 52.1 to 57.0 and 52.83 to 54.81 in rabbits fed with neem seed cake and *Acacia albida* pods, respectively.

The meat: bone ratio values (Table 4) were ranged from 2.99 ± 0.14 (T_1) to 3.15 ± 0.07 (T_5) and there was no significant difference among the treatments. The meat: bone ratio was slightly lesser than values of Umapathy (2001) and Rajendiran (2002) and Igwebuike *et al.* (2003), could be due to the variation in body weight, age and genetic makeup.

2. Organometry

The weight of heart, spleen, caecum, intestine, skin and head did not differ significantly except for liver. Weight of liver in the present study ranged from 2.72± 0.05 (T_1) to 3.68 ± 0.11 (T_3).

Samantha and Sasmal (1986) observed that on feeding neutral part of karanj oil, liver had enlarged, showing red infarction, fatty liver and enhanced SGOT activity in chicks. Natanam *et al.* (1989) found that

Table 4: Carcass characteristics of broiler rabbits fed with KSM

	T_1	T_2	T_3	T_4	T_5	SEM
Physical Characteristics of Carcass						
Live wt. (g)^{NS}	1844 ± 59.8	1742 ± 93.4	1662 ± 46.3	1767 ± 59.4	1764 ± 23.6	27.62
Carcass wt. (g)^{NS}	1002 ± 34.9	921.3 ± 51.0	875.3 ± 26.1	941.8 ± 35.8	917.8 ± 14.5	16.69
Dressing %^{NS}	54.32 ± 0.54	52.88 ± 0.60	52.65 ± 0.13	53.29 ± 0.90	52.04 ± 0.60	0.3
Meat:bone^{NS}	2.99 ± 0.14	2.94 ± 0.07	3.00 ± 0.11	3.13 ± 0.17	3.15 ± 0.07	0.05
Organometry(% of Body wt.)						
Liver*	2.72± 0.05^{bc}	3.03 ± 0.10^{b}	3.68 ± 0.11^{a}	2.90 ± 0.04^{bc}	3.37 ± 0.14^{a}	0.24
Heart^{NS}	0.34 ± 0.05	0.35 ± 0.01	0.36 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.09
Spleen^{NS}	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.01
Caecum^{NS}	6.22 ± 0.09	6.21 ± 0.56	8.12 ± 0.32	6.65 ± 0.45	7.46 ± 0.61	0.00
Intestine^{NS}	12.93 ± 0.14	12.88 ± 0.29	14.91 ± 0.35	13.40 ± 0.67	14.50 ± 0.64	0.13
Skin^{NS}	10.92 ± 0.40	10.74 ± 0.43	10.33 ± 0.80	10.18 ± 0.72	10.89 ± 0.39	0.25
Head^{NS}	9.27 ± 0.38	8.82 ± 0.40	8.90 ± 0.09	8.27 ± 0.17	8.49 ± 0.13	0.27
Chemical Composition (%)						
Dry matter^{NS}	25.43 ± 0.79	27.14 ± 0.36	26.80 ± 0.57	27.01 ± 0.17	26.88 ± 0.12	0.24
Organic matter^{NS}	24.45 ± 0.80	26.14 ± 0.40	25.81 ± 0.57	25.99 ± 0.16	25.89 ± 0.10	0.25
Crude Protein^{NS}	20.07 ± 0.31	19.93 ± 0.27	19.87 ± 0.24	19.81 ± 0.43	19.83 ± 0.43	0.13
Fat^{NS}	4.37 ± 0.11	4.39 ± 0.13	4.29 ± 0.13	4.30 ± 0.16	4.26 ± 0.11	0.05
Ash^{NS}	0.99 ± 0.00	1.00 ± 0.03	0.99 ± 0.00	1.02 ± 0.03	1.00 ± 0.02	0.01

NS = Non-Significant* Means bearing different superscript in a row differ significantly at $P \leq 0.05$.

Table 5: Sensory evaluation[1] of rabbit meat

Meat Quality Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
Appearance[NS]	3.84 ± 0.08	3.76 ± 0.11	3.76 ± 0.17	3.36 ± 0.14	3.52 ± 0.18
Texture[NS]	3.64 ± 0.16	3.88 ± 0.18	3.72 ± 0.20	3.28 ± 0.16	3.24 ± 0.23
Aroma[NS]	3.32 ± 0.15	3.36 ± 0.15	3.04 ± 0.14	3.20 ± 0.18	3.08 ± 0.19
Tenderness[NS]	3.72 ± 0.17	4.00 ± 0.15	3.72 ± 0.19	3.56 ± 0.14	3.36 ± 0.21
Flavor[NS]	3.56 ± 0.17	3.76 ± 0.15	3.40 ± 0.16	3.28 ± 0.17	3.12 ± 0.18
Juiciness[NS]	3.16 ± 0.17	3.56 ± 0.14	3.20 ± 0.17	2.88 ± 0.21	2.96 ± 0.19
Overall quality[NS]	3.15 ± 0.13	3.15 ± 0.14	3.07± 0.15	3.04 ± 0.15	3.07 ± 0.14

[1]Mean of 25 observations NS = Non-Significant

dietary inclusion of 1% karanj oil and 10% karanj cake in the diet of broiler chicks significantly increased the weight of liver. This could be due to presence of karanjin and other toxic factors in the oily portion of karanj cake. Even though liver weight had increased in present feeding trial, the serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activity was within normal range and all the rabbits were in healthy condition. The residual toxic factor might be the reason for the enlargement of liver but it was not supported by serum biochemical profile.

3. Chemical composition of meat

The chemical composition of fresh meat (Table 4) did not vary significantly among different treatments and in agreement with the results of Gowda (1994), Umapathy (2001) and Rajendiran (2002).

4. Sensory evaluation of meat

The results with regard to sensory evaluation of rabbit meat (Table 5) for various meat quality parameters such as appearance, texture, aroma, tenderness, flavor, juiciness and overall quality, carried out on a 5-point Hedonic scale revealed quality values ranging from 3.04 ± 0.15 to 3.15 ± 0.13.

Similarly, there was no adverse effect on meat quality parameters, which has been already reported by Panda (2004) in broiler chicken fed karanj based diets. Gowda (1994), Rajendiran (2002) and Fanimo *et al.* (2003) also did not find any variation in meat quality parameters of rabbits fed with unconventional feeds.

The present study clearly demonstrated that no adverse bitter taste or odour in the meat of rabbits due to feeding of processed KSM containing diets.

CONCLUSION

Incorporation of detoxified KSM in TMR of rabbit's ration did not have any adverse effect on the carcass and sensory qualities of meat.

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Study of Effect of Whey Protein Concentrate (WPC) on the Sensory and Chemical Composition of Date Syrup Blended Yoghurt*

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ABSTRACT

In this study an attempt has been made to incorporate WPC at 1, 1.5 and 2 per cent levels into date syrup blended yoghurt. Among these levels 1.5 per cent blended product was found to have better sensory characteristics. Higher sensory score of 8.50 out of 9.00 for overall acceptability of yoghurt was obtained. On further addition of WPC at above 1.5 per cent level significant reduction in the sensory scores was observed. An increase in protein and total solids content in the yoghurt was observed with increase in the levels of WPC addition. The total solids and protein content increased from 15.0 and 3.30 per cent in plain yoghurt to 24.64 and 4.60 per cent respectively when WPC was added at 1.5 per cent level.

Keywords: Yoghurt, date syrup, WPC, body and texture, proteins, total solids

Yoghurt is a fermented milk product with a good reputation due to its probiotic cultures and its reported beneficial effects on health. In theory, only milk and starter culture activity are needed to make a yoghurt product. However, in practice, the total solids content of yoghurt needs to be increased to prevent syneresis (Henriques *et al.,* 2013).

Dates or date products provide unique functionality when used with other products including sweetening, flavoring, and increasing nutritional quality.

Date syrup as a natural and nutritional additive is one of the best choices for milk flavoring and a safe alternative to added sugar to produce dairy products. Moreover, most of the carbohydrates in this product are in the form of fructose and glucose, which are easily absorbed by the human body (Mousazadeh, 2011).

Whey is one of the major byproducts of dairy industry. Whey contains nearly 50 per cent of total milk solids, and thus it is of great significance to utilize whey solids in human food chain. Whey protein products have been attempted in yoghurt and various cheeses to improve the yield, nutritive value and consistency. WPC could be used to replace dried skim milk in yoghurts without adverse effect on sensory properties.

Whey and whey products are used as potential food ingredients due to their excellent nutritional and promising functional properties in variety of foods like ice-creams and frozen desserts, bakery products, soups, sauces, snack foods, confectionaries and beverages (Morr and Foegeding, 1990; Jayaprakasha and Brueckner, 1999). Whey proteins provide a wide range of functional properties such as solubility, viscosity, water binding, whipping, emulsification and gelation (Zadow, 1986). Incorporation of WPC in channa spread not only improves the functional properties but also increases the nutritional quality of the spread (Arun Kumar *et al.*, 2012).

The addition of only date syrup leads to slight thinning of the curd due to dilution and to overcome this problem, WPC can be added to obtain the required consistency of the yoghurt. Also, addition of whey protein concentrate will increase the total amount of nutrients in the yoghurt and also enhances the quality of the product. Therefore, an attempt has been made to manufacture the yoghurt by blending date syrup and WPC.

*Part of M.V.Sc Thesis submitted by the first author to Karnataka Veterinary and Animal Sciences University, Bidar.

MATERIALS AND METHODS

Materials:

Whole milk: Pasteurized and homogenized cow milk was procured from the Student Experimental Dairy Plant (SEDP). **Date syrup:** "Lion Date Syrup" brand manufactured by Lion Dates Impex Pvt. Ltd., Trichy, India, was procured from the local market. **Whey Protein Concentrate:** Fresh spray dried Whey Protein Concentrate having 80 per cent protein was procured from "Saputo" brand, DKSH, Bengaluru. **Starter cultures:** Yoghurt cultures such as *Streptococcus salivarious ssp. thermophilus* and *Lactobacillus delbrueckii* var- *bulgaricus* in the form of freeze dried direct Vat set (FD-DVS) was obtained from Chr. Hansehs Laboratories, Denmark.

Method of manufacture of WPC enriched date syrup blended yoghurt: Yoghurt samples were prepared by adding date syrup into the milk along with the addition of WPC at the rate of 1 per cent, 1.5 per cent and 2 per cent and the control yoghurt was prepared by using cow milk. The yoghurt was manufactured as described by Lee and Lucey (2010). The resultant yoghurt was subjected for various chemical analysis and served to a panel of judges along with the control to judge the sensory characteristics and overall acceptability on 9-point hedonic scale. Based on sensory evaluation the best combination was selected.

Cow milk (3.5% fat, 8.5% SNF)
↓
Addition of date syrup (@ 8%)
↓
Addition of WPC (@ 1.0, 1.5 or 2.0%)
↓
Homogenization (60±1°C and 2000 psi at 1st stage and 500 psi 2nd stage)
↓
Heating (90±1°C for 10 min)
↓
Cooling (43±1°C)
↓
Addition of starter culture (*Streptococcus salivarious ssp. thermophilus* and *Lactobacillus delbrueckii* var. *bulgaricus)* (@ 2% level)
↓
Filling into cups
↓
Incubation (43±1°C/4 h)
↓
Storage (5±1°C)

Figure 1: Flow diagram for preparation of WPC enriched date syrup blended yoghurt

Analytical Procedure: The fat, protein, total solids content and titratable acidity of the samples were determined as per IS: SP 18 (Part XI) 1981.

Statistical analysis: The data were analyzed using one-way ANOVA, two factor ANOVA depending on the experiment and the number of treatments. The results were analyzed statistically for test of significance by using two and three factorial ANOVA as per the procedure of Sundarraj *et al.* (1972) in SAS 9.2 Version and R software.

RESULTS AND DISCUSSION

The effect of addition of WPC on the sensory characteristics of date syrup blended yoghurt:

The sensory characteristics of date syrup blended yoghurt prepared by adding WPC from 1.0 to 2.0 per cent levels are presented in Table (1). Addition of WPC at 1.5 per cent level increased the sensory

Table 1: Effect of addition of different levels of WPC on the sensory characteristics of date syrup blended yoghurt

Levels of WPC(Per cent)	Sensory scores (Max. 9.0)				
	Color and Appearance	Body and Texture	Flavour	Sourness	Overall Acceptability
Control (0)	8.30[a]	7.70[a]	7.50[a]	8.00[a]	8.20[a]
1.0	8.30[a]	7.70[a]	7.80[a]	7.80[a]	8.20[a]
1.5	8.50[a]	8.10[b]	7.85[a]	8.30[a]	8.50[b]
2.0	7.50[b]	7.10[c]	7.80[a]	7.00[b]	7.20[c]
CD(P≤0.05)	0.40	0.35	NS	0.60	0.28

Note : All the values are average of 3 trials

Similar superscripts in a row indicate non-significance at corresponding critical difference (CD)

scores for colour and appearance, body and texture, flavour and overall acceptability of yoghurt. But addition at higher levels (above 1.5 per cent) showed significant difference in sensory scores for all attributes except flavour. The highest sensory score of 8.50 out of 9.00 was secured for colour and appearance at 1.5 per cent WPC level as against control (8.30). Further addition lowered the sensory scores significantly for colour and appearance of date syrup blended yoghurt. The reduction in score could be due to the formation of brownish yellow/dull colour of the product with slightly less shiny surface. Steinsholt and Holth (1991) reported an increase in intensity of yellow colour with increase in concentration of WPC. Similar results were reported by Rashmi (2014), who used 2 per cent WPC in the development of carrot based yoghurt drink. Also, Priyatam Reddy (2015) reported the similar results with 5 per cent WPC incorporation in development of low fat set yoghurt.

Higher sensory scores of 8.10 and 8.50 out of 9.00 for body and texture and overall acceptability of yoghurt were secured at 1.5 per cent WPC level. Further addition of WPC (above 1.5 per cent) had shown significant difference in the sensory scores. This may be partially due to slight increase in whey separation. Hence, at higher levels of WPC addition, the product had poor consistency with whey separation which led to lower sensory score of 7.10 out of 9.00. Similar observations were made by Jayaprakasha *et al.* (2000) in the preparation of frozen yoghurt by replacing the MSNF at 50 per cent level, which secured lower sensory scores.

The flavour score of 7.85 was obtained with addition of WPC at 1.5 per cent level and was found to be non-significant when compared to control (7.50) and the treated samples. This indicates that the addition of WPC had no significant effect on the flavour of the product. Similar findings were reported by Jayaprakasha *et al.* (2000) where yoghurt prepared by replacing the MSNF with WPC at higher levels (70 per cent) secured lower sensory scores with respect to flavour.

The mean sensory scores for sourness of the yoghurt increased from 7.80 to 8.30 with increase in WPC level upto 1.5 per cent. Further increase in addition of WPC showed significantly decreased score to 7.00. This could be due to development of bland taste (due to low sourness). The results are in agreement with Thapa and Gupta (1992).

Table 2: Effect of addition of WPC on chemical composition of date syrup blended yoghurt

Levels of WPC (%)	Protein (%)	Fat (%)	TS (%)	Acidity (%L.A.)
Control (0)	3.30[a]	3.50[a]	15.00[a]	0.85[a]
1.0	4.20[b]	3.49[a]	24.16[b]	1.13[b]
1.5	4.60[b]	3.49[a]	24.64[b]	1.19[b]
2.0	5.04[b]	3.48[a]	25.12[b]	1.24[b]
CD (P≤0.05)	0.85	NS	1.25	0.20

Note: All the values are average of 3 trials

Similar superscripts in a row indicate non-significance at corresponding critical difference (CD)

The effect of addition of WPC on chemical composition of date syrup blended yoghurt:

The chemical composition of date syrup blended yoghurt prepared by adding WPC from 1.0 to 2.0 per cent levels are presented in Table (2). The protein and total solids content of yoghurt increased with increased level of WPC addition. The protein content of 4.60 per cent and total solids content of 24.64 per cent were recorded when WPC was added at 1.5 per cent levels. This could be mainly due to added WPC which itself contains 80% protein. The results of the present investigation are in agreement with the observations made by Opdhal and Baer (1991).

The acidity of experimental yoghurt samples ranged from 1.13 to 1.24 per cent lactic acid with the increase in addition of WPC levels. There was a progressive increase in acidity in the yoghurt samples. The higher amounts of phosphates and citrates in the added WPC which are more readily utilized by starter organisms and also the lactose content of WPC contributed to higher acidity. Similar results were observed by Venkateshaiah (1995), when whey powder was used in place of skim milk powder in the preparation of frozen yoghurt. Also, Rashmi (2014) reported the similar results, where 1, 2 and 3 per cent WPC was used in the development of carrot based yoghurt drink.

CONCLUSION

The consumption of fermented dairy products is increasing in recent years, mainly due to their excellent nutritional and therapeutic properties. Date syrup as a natural and nutritional additive is one of the best choices for milk flavouring and a safe alternative to added sugar to produce dairy products.The results of this study have shown that it is possible to prepare yoghurt with WPC while enhancing textural properties such as water holding, hardness and viscosity. Additionally, whey protein addition improved the protein content and reduced syneresis. Yoghurt can be made with whey protein concentrate as added solids without compromising the chemical properties and sensory characteristics. Thus, the yoghurt prepared with 10 per cent date syrup and 1.5 per cent WPC not only improves the nutritional aspects but also enhances the therapeutic benefits.

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Surgical Management of Extensive Ventral Hysteroenterocele in a Holstein Friesian Cow

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ABSTRACT

A cross bred Holstein Friesian cow aged 8 years was presented to the Hospital, with the history of swelling at the left lower flank region with a stepping gait (Abduction). Animal had a history of accident with motor vehicle 20 days back and it had a small swelling in the left ventral abdominal region which increased gradually. On rectal examination of the animal revealed eight and half months pregnancy and there was a large swelling on left lower flank extending ventro-laterally below the stifle. Ultrasonographic examination of the swelling was performed which revealed discontinuity in the muscle layer, under which caruncles and loops of intestines suggestive of hysteronenterocele were observed. Hernioplasty was performed and a live male calf was removed. Then uterus was sutured with lamberts followed by cushings pattern using chromic catgut no.2. The sterilized double layer nylon mesh was sutured to the torn muscle by vertical matrix pattern using silk no-2.

Keywords: Hysteroenterocele, Ultrasonography, Hernioplasty

Abdominal hernia is the protrusion of organ or tissue through an opening that may be natural opening or tear in the abdominal wall (Krishnamurthy, 2010). Repair of large abdominal hernia is a clinical challenge to surgeons and use of prosthetic material for hernioplasty is indicated when size of hernia ring exceeds 3 cm diameter (Venclauskas *et al.,* 2008). Singh *et al.* (2011) reported abdominal hernia repaired by hernioplasty using polypropylene mesh in cattle. In the present paper reports successful surgical management of large ventral hysteroenterocele in a cross breed Holstein Friesian cow by hernioplasty using double layer nylon mesh.

CASE HISTORY AND OBSERVATION

A cross bred Holstein Friesian cow aged 8 years was presented to the Hospital, with a history of swelling at the left lower flank region with stepping gait (Abduction). Animal had a history of an accident and swelling that increased gradually. On rectal examination, animal was found to be eight and half months pregnant and swelling on left lower flank extending ventro-laterally below the stifle skin fold (Fig. 1). Haematological and biochemical parameters were within the normal range. Ultrasonographic examination of the swollen area revealed discontinuity in the muscle layer, uterine caruncles and also loops of intestines (Fig. 2), based on clinical and ultrasonographic examination the case was diagnosed as hysteroenterocele and it was decided to perform caesarean section and hernioplasty using a double layer nylon mesh.

TREATMENT AND DISCUSSION

The animal was sedated with XBK combination (Xylazine @ 0.01mg/kg BW, Butorphenol @ 0.02mg/kg BW, Ketamine @ 0.04mg/kg BW) and restrained in right lateral recumbency after epidural anesthesia with Lignocaine hydrochloride, surgical site was aseptically prepared, desensitized by local infilteration of lignocaine hydrochloride. Uterus was exposed by making oblique incision from the stifle joint to the lateral milk vein. The right gravid horn was exteriorised towards left and upon incising the uterus a live male calf was removed. The uterus was sutured with lamberts followed by cushings pattern using catgut no.2. Discontinuity of the muscle (hernial ring) was large (about 30 cm) and irregular, therefore the incision was extended towards the dorsum from the middle of previous incision to expose the hernial ring. The

Fig. 1: Cross breed Holstein Friesian cow showing large ventral hernia

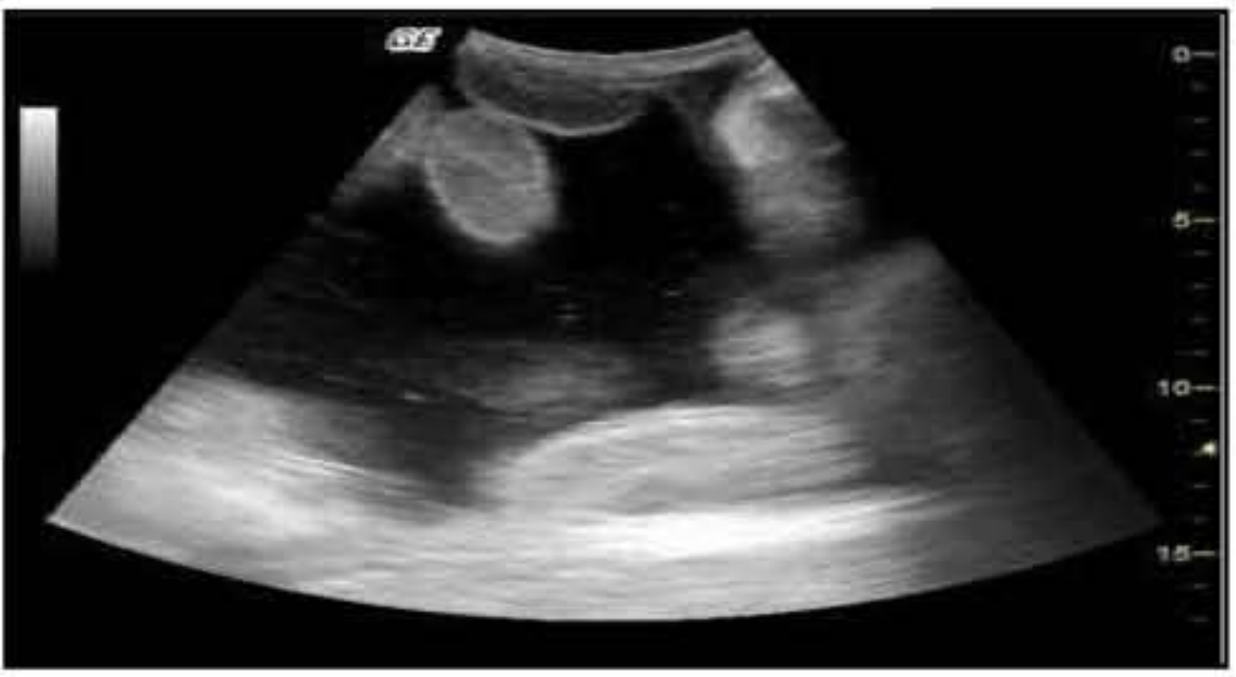

Fig. 2: B-mode ultrasonography image showing caruncles just below the skin

Fig. 3: Hernioplasty with nylon mesh

sterilized double layer nylon mesh was sutured to the torn muscle by vertical matrix pattern using silk no-2 (Fig. 3), subcutaneous tissue was sutured with a simple interrupted pattern no-2 catgut, followed by skin suture with cross mattress using polyamide no-1. Post operatively Normal saline 5 litres daily for 2 days, Ceftriaxone 10 mg/kg body weight twice daily for 5 days and Meloxicam 10 ml I/M for 3 days were administered. Wound dressing was performed every day, slight seroma formation was observed for 4 days. Animal recovered uneventfully on the 10th post operative day without any complications.

Any break in continuity of abdominal wall results in abdominal hernia high or low flank hernias are usually caused by violent force such as from the impact of blunt objects but may also result from overstretching of the abdominal muscle (Krishnamurthy, 2010). Where as in the present case ventral hernia was due to automobile accident compounded by advance pregnancy. Hernia in a calves can be diagnosed by palpation of hernia ring and use of real time B-mode ultrasonography (Sutaria *et al.*, 2015). Kumar *et al.* (2012) reported decrease in abdominal wall thickness and superficially located abdominal viscera which help in the diagnosis of hernia. In the present case hernia was too voluminous to palpate hernial ring, ultrasonography revealed discontinuity in the muscle layer and presence of caruncles and loops of intestine just below the skin were diagnostic.

Sutaria *et al.* (2015) used sublay pre-peritoneal mesh plasty using polypropylene mesh to repair large hernial ring (approx 15cm). In the present case the hernial ring was measured about 30 cm therefore a double layer nylon mesh was sutured to the torn muscle by vertical matrix suture. Post operatively slight seroma formation was took place and the wound healed without any complication. These finding are in accordance with the finding of Singh *et al.* (2011) in surgical repair of large congenital lateral abdominal hernia. It was concluded that large ventral abdominal hysteroenterocele in cross breed Holstein Friesian cow was surgically managed successfully by hernioplasty using double layer nylon mesh.

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Enterolith and its Surgical Management in a Labrador – A Case Report

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ABSTRACT

A 14 month old, Labrador male dog was presented to Teaching Veterinary Clinical Complex, APMC yard, Gandhi Gunj, Bidar with a history of projectile vomiting, anorexia and unable to pass faeces since 3 days and not responded to the routine medical treatment undertaken by a local Veterinarian. On clinical examination, all the physiological parameters were within the normal range. On abdominal palpation a hard mass was felt near the anterior portion of the abdomen hence subjected for radiography. Radiographical examination revealed round smooth edged radio opaque foreign body in the anterior portion of small intestine which confirmed it to be a case of intestinal obstruction. The enterotomy operation was performed and incision was closed using polyglycolic acid 2-0 by simple interrupted pattern and omentopexy was undertaken and animal recovered uneventfully.

Keywords: Labrador, Enterolith, Enterotomy and Omentopexy

Proximal intestinal obstructions are acute conditions that require early diagnosis and surgical treatment for the better prognosis and high survival rate. In canines, proximal intestinal obstructions occur mainly due to foreign bodies like trichobezoars, stones, sponge ball and mango corns. These cases need rapid diagnosis either by radiograph or ultrasound examination to undertake quick and appropriate surgical intervention for better survivability (McNeel, 1986 and Saini *et al.*, 2002).

CASE HISTORY AND OBSERVATIONS

A 14 month old, Labrador male dog was presented to Teaching Veterinary Clinical Complex (City Hospital), APMC yard, Gandhi Gunj, Bidar with a history of projectile vomition, anorexia and unable to pass faeces since 3 days and not responded to the routine medical treatment undertaken by a local Veterinarian. On clinical examination, all the physiological parameters were within the normal range. On abdominal palpation the animal evinced pain and a hard mass was felt near the anterior portion of the abdomen hence subjected to radiography. Radiographical examination revealed round smooth edged radiopaque foreign body in the anterior portion of small intestine with gastric dilatation which might be due to aerophagia confirming the intestinal obstruction. The animal was prepared aseptically for enterotomy operation.

TREATMENT AND DISCUSSION

Initially animal was premedicated with atropine sulphate (Atral®) @ 0.04mg/kg body weight, intramuscularly and diazepam (Calmpose®) @ 0.25mg/kg body weight, intravenously. Preoperatively, inj. Ceftriaxone (Intacef®) @ 20mg/kg body weight, intravenously and tramadol (Domodol®) @ 2mg/kg

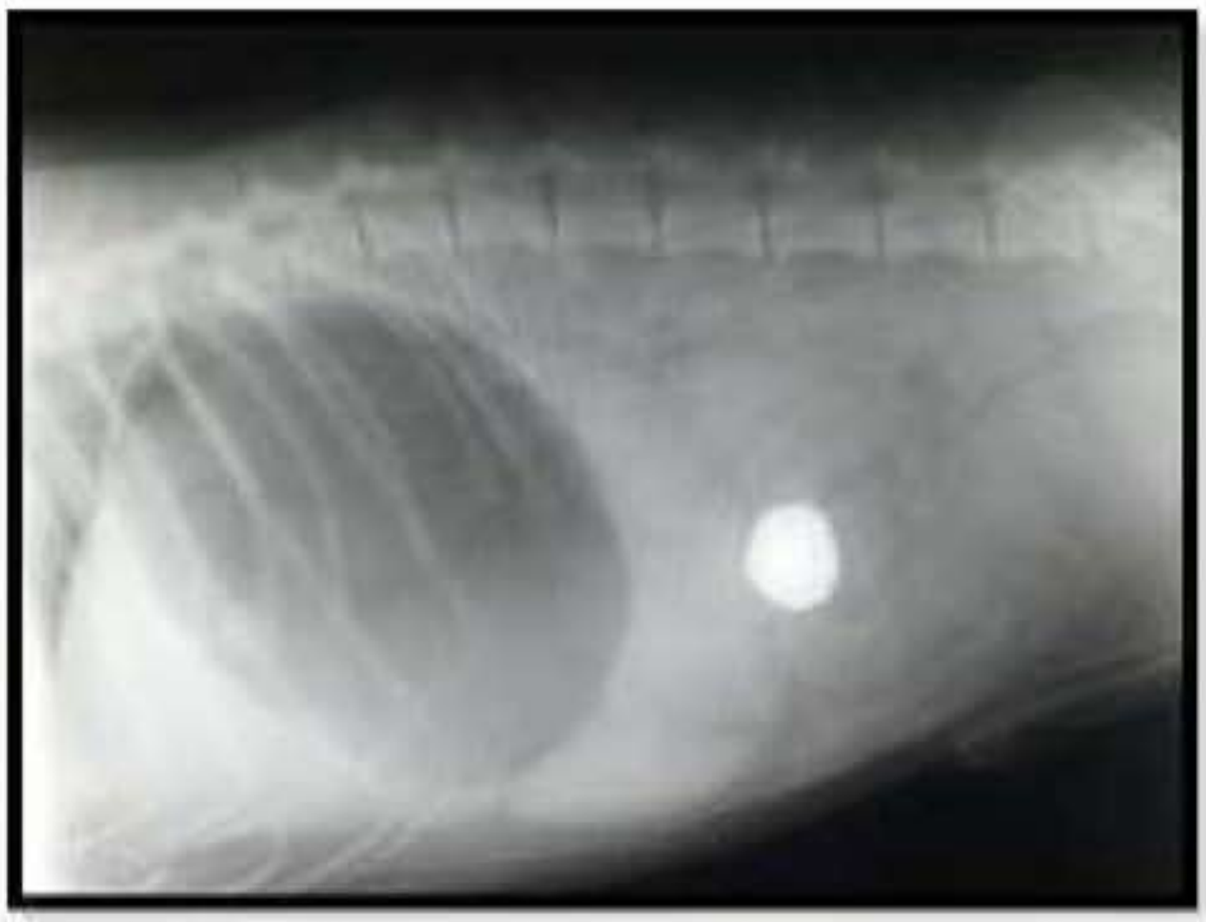

Fig. 1: Skiagram showing radiopaque FB with gastric dilatation (aerophagia)

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body weight, intravenously were administered. Anaesthesia was induced and maintained with Propofol (Neorof®) @ 4mg/kg body weight administered intravenously. The surgical site, ventral midline from the xiphoid region to the penis, was aseptically prepared with repeated scrubbing using povidone iodine (Pideen®) solution.

Surgical skin incision was done starting from the umbilicus on linea alba and extended caudally to the paraprepucial region. After opening the abdominal cavity, careful examination of intestinal segments revealed hard mass at the jejunum. The distal part of the obstruction site was slightly cyanotic and large in diameter as compared to the proximal intestinal segment. Enterotomy was performed by giving incision proximal to the obstruction site on the ante mesenteric site. Enterolith was retrieved from the site which measured about 6cm in diameter. The enterotomy incision was closed using polyglycolic acid 2-0 by simple interrupted pattern and omentopexy was performed by covering the free omentum over the sutured site. Laporatomy incision was closed as per the standard operating procedure using PGA 1. Post operatively pet was maintained on intravenous fluid therapy with liquid diet for 5 days along with Inj. ceftriaxone @ 25mg/kg B.wt. intravenously for nine days and Inj. meloxicam (Melonex®) @ 0.2mg/kg B.wt. intramuscularly for five days with alternate dressing of the wound with Pideen® solution and Pideen® ointment. Pet started passing faeces next day after surgery without no vomition. Skin sutures were removed after 10days**.** Animal recovered uneventfully without any complications.

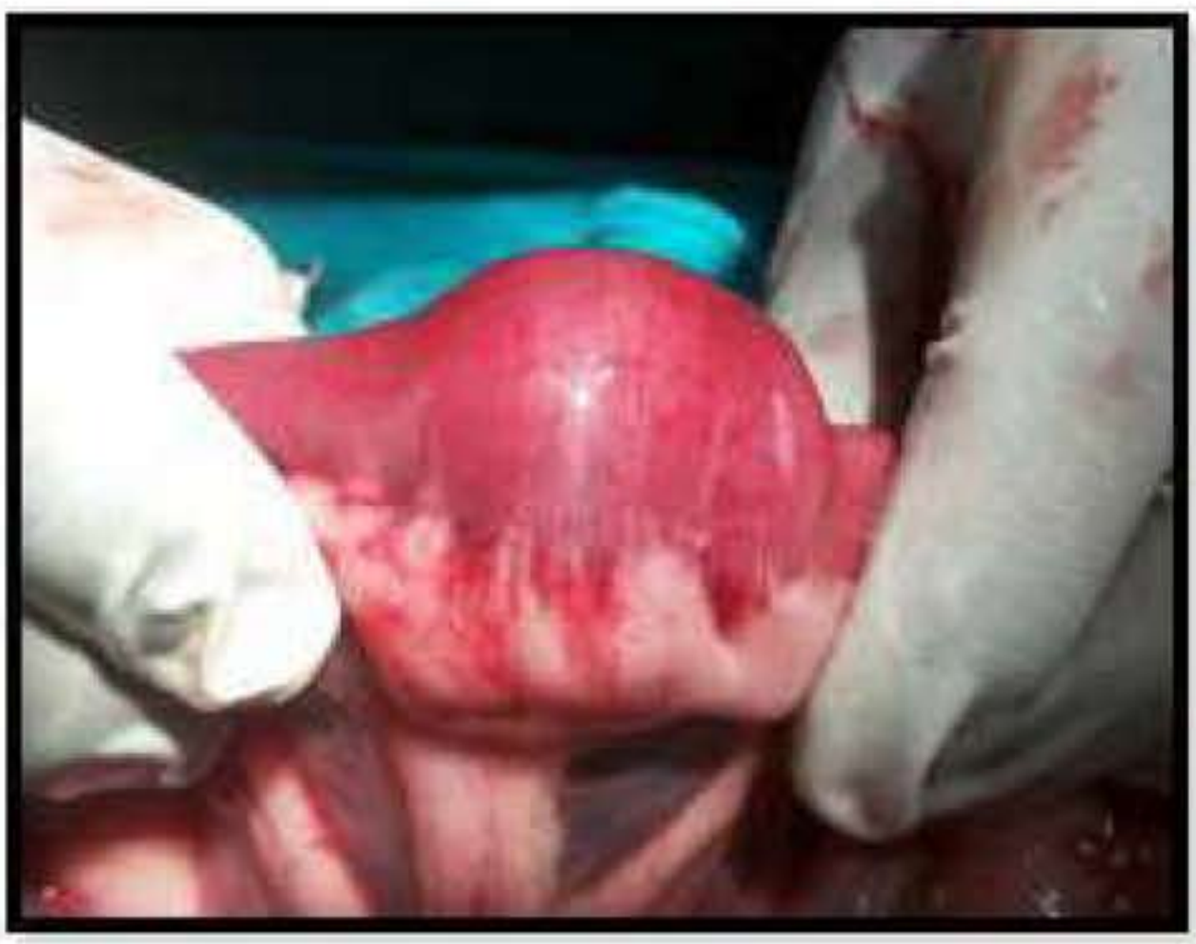

Fig. 2: Foreign Body at the proximal jejunal segment

Fig. 3: Retrieved enterolith from the obstruction site

Rao *et al.* (2010) reported successful treatment of eight cases of intestinal obstructions and summarized that clinical signs would vary with the degree of obstruction, location, duration, and type of foreign body. However, vomition with no response to the routine antiemetics, loss of appetite, absence of defecation, weight loss, lethargy and abdominal pain are commonly noted with the proximal intestinal obstructions. These findings correlated with the present case.The diagnosis of intestinal obstruction is of crucial importance and could be done based on history, careful physical examination of abdomen, clinical signs and radiographic findings (Lamb, 1994; Gahlot *et al*., 2005 and Rao *et al.*, 2010). The authors also opined that early and quick diagnosis along with surgical intervention has better survival rate.

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Refractory Epilepsy in a Golden Retriever: A Case Report

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ABSTRACT

Refractory epilepsy is often more difficult to manage using standard therapy. Around 5% of all dogs and 1% of all cats will have a seizure at some point in their life. In the present report 4-year-old male Golden Retriever dog with refractory epilepsy was successfully managed with combination of Phenobarbitone sodium and Levetiracetam.

Keywords: Refractory epilepsy, seizure, levetiracetam, phenobarbitone sodium

Seizures are the most common neurologic problems encountered in small-animal practice. Recurrent seizure activity can lead to functional and pathological changes in the neurons that can potentiate refractoriness. Primary or idiopathic epilepsy is a disorder characterized by recurrent seizures for which the underlying structural cause in 25% to 40% of dogs is not clear (Podell *et al.*, 1995; Berendt *et al.*, 1999). Seizures are common in 1 to 5 years old dogs and its successful management requires lifelong medical attention. Sometimes it is difficult to control seizures. Different terms are used to describe seizures which include drug resistant epilepsy, intractable epilepsy and uncontrolled epilepsy. Management of refractory epilepsy is a real challenge. A recent study evaluated levetiracetam as an add-on medication in dogs with idiopathic epilepsy that was refractory to phenobarbital and potassium bromide (Volk *et al.*, 2008). In the present report the potential use of newer antiepileptic drug levetiracetam in combination with phenobarbitiol in a case of refractory epilepsy is discussed.

CASE REPORT

A four-year-old male Golden Retriever dog was presented to Veterinary College Hospital, Veterinary College, Bangalore with a history of repeated episodes of seizure activity, despite of use of high dose of phenobarbital.

On physical examination animal was found apparently healthy. Hematobiochemical examination revealed normal levels of ALT (Alanine amino Transferase), BUN (Blood Urea Nitrogen), creatinine, blood sugar, sodium, potassium, and calcium but total plasma protein level was 5.6 g%.

Electro encephalography studies, thoracic radiography and urinalysis findings were found normal but on ultrasound examination mild hepatic fibrosis was noticed. Based on physical examination, laboratory findings and radiographic findings the condition was diagnosed as idiopathic epilepsy, which is refractory to conventional therapy.

Animal was treated with levetiracetam @ 20 mg/kg, thrice daily and dose of phenobarbital was reduced gradually from 6 mg/kg body weight twice daily to 3 mg/kg body weight twice daily by about three weeks. Pet showed good response and the seizure episode activity disappeared completely at the end of first week. Pet did not show any seizure activity for next 8 months and combination of levetiracetam and phenobarbital was found effective in the management of refractory epilepsy.

DISCUSSION

Idiopathic epilepsy (seizure of unknown origin) is most commonly seen in healthy dogs aged between 1 and 5 years and may be inherited in certain breeds *viz.* Golden Retrievers, Beagles, Keeshonden, Irish Setters, Belgian Tervurens, Siberian Huskies, Springer Spaniels, and German Shepherds. (REF) Idiopathic epilepsy is diagnosed when other causes of

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seizures are ruled out by physical exam, blood parameter analysis, and any other necessary work up procedures. Recurrent seizure activity can lead to functional and pathological changes in the brain that can potentiate refractoriness.

In the present study low total plasma protein level was observed, which may be due to hepatic dysfunction caused by high dose phenobarbital. Phenobarbital and potassium bromide are the most commonly prescribed antiepileptic drugs in Veterinary practice. Phenobarbital decreases seizures by potentiating the effect of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) in neurons. Potassium bromide's antiepileptic mechanism of action is thought to involve hyperpolarization of the neuronal membrane by the movement of negatively charged bromide ion via GABA-activated chloride channels (Karen *et al.*, 2009). However, about 20% to 30% of epileptic dogs never attain satisfactory seizure control with these conventional antiepileptic drugs and are considered refractory to treatment (Lane *et al.*,1990). In addition, both the drugs have a narrow therapeutic index and a propensity to cause marked side effects. Only less than half of epileptic dogs receiving phenobarbital or potassium bromide maintain a seizure-free status without experiencing drug-related adverse effects (Podell *et al.*,1996). Side effects range from sedation, vomiting, polyuria, polydipsia, and polyphagia to more serious complications such as bone marrow suppression, hepatotoxicosis, and pancreatitis.

Levetiracetam (Keppra) is one of the recently approved human antiepileptic drugs, which selectively prevents hypersynchronization of epiceptiform burst firing and propagation of seizure activity (Donald *et al.*, 2008). Its unique mechanism of action is a potential advantage when the drug is used in combination with other antiepileptic drugs. Levetiracetam has minimal hepatic metabolism in dogs, with more than 80% of the drug excreted in the urine. It's half-life in dogs is three to four hours, which necessitates frequent administration. The recommended oral dose is 20 mg/kg every eight hours (Karen *et al.*, 2009; Patterson *et al.*, 2008).

In a study conducted by Volk *et al.* (2008) levetiracetam was well tolerated by all dogs and sedation was the only side effect reported. Intravenous levetiracetam was a good alternative to hepatically metabolised antiepileptic drugs for the management of cluster seizures and status epilepticus (Fryer *et al.*, 2011)

Levetiracetam was found to be effective as add-on therapy in dogs with refractory epilepsy in the present case study.

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Therapeutic Management of Feline Scabies - A Case Report

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ABSTRACT

Domestic short hair cat presented with the history of pruritus and alopecia was diagnosed as feline scabies based on skin scraping examination.The cat was treated with injectable ivermectin @ 200µg/kg. The cat recovered uneventfully by fourth week of treatment.

Keywords: Feline scabies, *Notoedres cati,* Ivermectin

Notoedric mange is also sometimes referred to as feline scabies, because the disease is similar to sarcoptic mange in dogs. These mites cause severe skin infections in cats, generally starting on the face and ears and spreading to the rest of the body, and are highly contagious (Scott *et al*., 2001). Mange can cause restlessness, intense itching and frantic scratching; symptoms that generally appear one week after exposure. It also typically results in patchy hair loss and a moth-eaten appearance to the skin.

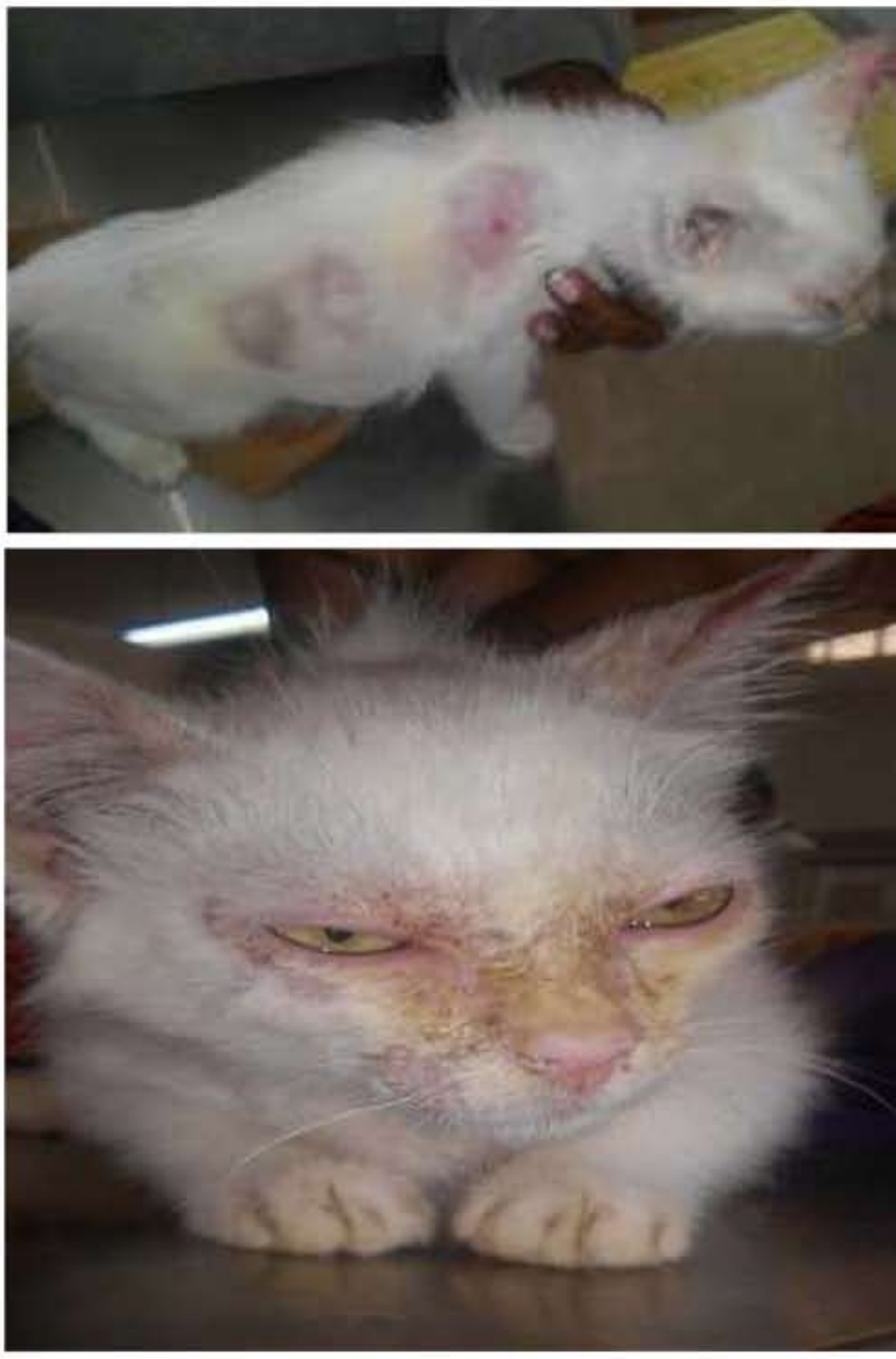
Diagnosis is based on the history of severe pruritus of sudden onset, possible exposure, and involvement of other animals. Making a definitive diagnosis is sometimes difficult because of negative skin scrapings. Concentration and flotation of several scrapings may increase chances of finding the mites, eggs, or feces. Several extensive superficial skin scrapings should be done of the ears, elbows and hocks.

A specific and sensitive commercially available ELISA to detect specific antibodies has been developed and may be useful. Treatment consists of both topical and systemic therapies (Malik *et al.,* 2006).

CASE HISTORY AND OBSERVATIONS

A 14 month old, domestic short hair, free roaming, intact male cat, initially treated by the owner with over the counter medications, was presented to Veterinary College Hospital, Veterinary College, Hebbal, Bangalore with intense pruritus, alopecia, scabbing, crust formations around the eyes, nasal bridge, ear margins and alopecia of caudal aspect of the external ear, moth-eaten appearance on right thoracic region and also on the right abdominal region.

The pet was normo-thermic with rectal temperature of 101°F, active, alert and oriented. the cat was on regular commercial feline diet, with normal appetite, regularly vaccinated and dewormed periodically. Complete physical examination revealed



absence of external parasites (fleas,ticks) and flea dirt. Skin scrapings revealed *Notoedres cati*, which were circular in shape with the adult having four pairs of legs. Hematological examination was normal.Based on physical examination, skin scrapings, the case was diagnosed as feline scabies.

TREATMENT AND DISCUSSION

Treatment was initiated with Inj. Ivermectin 200µg/kg body weight SC at weekly intervals (Scott *et al.*, 2001) along with multivitamin and mineral supplement daily. Taylor *et al.* (2007) suggested to use 1% selenium sulphide solution to soften the skin crusting, but in the present study liquid paraffin was used and found effective in softening skin crusts. Pet showed gradual improvement and recovered completely at end of fourth dose. Skin scraping examination revealed dead mites in the second week, but on the fourth week no mites were found in the skin scraping. Intensity of pruritus was reduced by one week, but the apparent clinical recovery was noticed after second treatment with Ivermectin. Ivermectin was found to be effective in the treatment of Notedric mange in cats, similar observations were made by Sudhakar and Sivajyothi (2014). Senthil *et al.* (2008) and Yathiraj *et al.* (1994). No adverse reactions were observed during the medications. Ivermectin has got large margin in safety in cats (Plumb, 2008).

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Naproxen Toxicity in a Dog – A Case Report

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ABSTRACT

A one-year-old Labrador Retriever was presented to the Department of Veterinary Medicine, Veterinary College, Bangalore with a history of severe vomiting and dehydration after accidental ingestion of Naproxen tablets. Successful management of Naproxen toxicity is discussed.

Keywords: Naproxen, Toxicity, Sucralfate

Naproxen is a popular non-steroidal anti-inflammatory drug (NSAID) that is used in the management of pain especially in cases of arthritis. Naproxen is a propionic acid derivative that possess analgesic and antipyretic properties which inhibit prostaglandin synthesis. (Ricardo *et al.,* 1989). Naproxen toxicity is well established in humans but reports are meager in veterinary practice. In preclinical toxicology studies, acute to sub-acute oral toxicities were studied in mice, rats, hamsters and dogs. Repeated oral doses of Naproxen were poorly tolerated by dogs. (Duane *et al.,*1973).

CASE REPORT

A one-year-old Labrador Retriever dog weighing 22 Kg was presented to the Department of Veterinary Medicine, Veterinary College, Bangalore with a history of accidental consumption of ten 500 mg Naproxen tablets, which were prescribed as an analgesic for human use. Clinical examination of animal revealed elevated body temperature (103°F), increased heart rate (136 bpm), congested and sunken eye balls, on abdominal palpation of animal severe epigastric pain was noticed. Hematological examination revealed normal leucocyte (11300 cells/µl) and platelet (2.3 Lakh cells/µl) count but the PCV (54%) was very high indicating dehydration.

Serum biochemical studies revealed mild increases in BUN (36 mg/dl) and creatinine (1.9 mg/dl). Condition was considered as naproxen induced gastritis and treated with Inj. Ondansetron (Inj. Emset®) @ 0.1mg/kg intravenously twice daily, Inj. Pantoprazole (Inj. Pantodac®) @ 1mg/kg intravenously once daily, Sucralfateoral suspension (1g/10ml) was given @ 10ml once in eight hours and pet was kept completely on intravenous dextrose for 72 hours. Pet started taking water from third day without any signs of vomition and hematobiochemical parameters were returned to normalcy after 5 days of continues therapy indicating complete uneventful recovery.

DISCUSSION

Naproxen is an over the counter drug available in acid or sodium salt form. In humans and dogs, it is used for its anti-inflammatory properties. (Safdar, 2015). Naproxen is given orally for treatment and has rapid absorption with oral bioavailability of 68 to 100% in dogs. The drug is highly protein bound and undergoes extensive enterohepatic recirculation in dogs which is the reason for long half-life of the drug in dog (Frey and Rieh, 1981). Naproxen is a NSAID that blocks the enzyme cyclooxygenase (COX) which prevents the synthesis of prostaglandins. Elevated BUN and creatinine in the present study may be because of reduction in PGE_2, which is essential for renal vasodilation and to maintain optimum circulation (DeClementi, 2012). There are two COXs *i.e.,* COX-1, which is important for normal physiologic function and COX-2, which mediate inflammation. Naproxen is a non-selective inhibitor of cyclooxygenases. So the protective function of COX-1 against the gastric acid will be lost due to Naproxen which may lead to gastric irritation and ulceration. (DeClementi, 2012).

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Pantoprazole is a potent proton pump inhibitor. Hence gets activated by acid and inhibits the gastric H^+, K^+-ATPase. (Jai and Nayoung, 2013). Ondansetron is a potent antiemetic drug that is being used to treat acute and drug induced nausea and vomiting in dogs. (Baek *et al.*, 2015). The drug is a highly potent and selective antagonist at 5-HT3 receptors and its antiemetic actions were revealed by its ability to antagonize vomiting induced by chemotherapy in animals and man. The antagonistic action on 5-HT3 receptors by Ondansetron is mediated by its action on two specific sites. i) centrally, in the area postrema/NTS; and ii) peripherally on vagus nerve terminals. (Naylor and Rudd, 1992). Sucralfate reacts with gastric acid to form a paste like substance, and binds to the proteins in the gastric ulcers to prevent further damage. (Safdar, 2015). Intravenous dextrose therapy was initiated to correct fluid and electrolyte loss and to supplement energy. In the present study combination of Ondansetron, Pantropazole, Sucralfate and intravenous dextrose therapy was found to be very effective in the treatment of naproxen induced gastritis.

CONCLUSION

Naproxen is a nonsteroidal anti-inflammatory drug used in humans as an analgesic and antipyretic agent. Naproxen toxicity in dogs can be managed successfully with antacids, anti-emetics, gastric protectants and fluids if presented early. If delayed animal may develop gastrointestinal perforation and renal insufficiency.

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Foetal Dystocia due to Hydrocephalic Red Khandari Calf and Its Successful Management

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ABSTRACT

Dystocia is a major cause of economic loss to the dairy herd. Foetal dystocia due to hydrocephalus in calf (Water head) is a rare condition that is attributed to genetic defect of calf caused by a lethal recessive autosomal gene. Prolonged dystocia is a danger to the survival of the cow due to complications like rupture of uterus and haemorrhages, this case describes incomplete and abnormal development of cranial tissue and hydrocephalus recorded in Red Kandhari cow calf. Per vaginal examinations revealed absence of any fetal reflex indicating that foetus was non-viable and there was enlargement over the head of foetus at the occipital regionand trocarization of the head of the foetus was done by trocar and cannula to evacuate the contents. The foetus was then delivered by the mutational procedures with application of hook on the inner canthus of eye along with the judicious application of traction on the fore limbs.

Keywords: Dystocia, hydrocephalus, Red Kandhari, trocarization and genetic defect

Dropsical conditions of fetus resulting in dystocia include hydrocephalus, ascites, hydrothorax and anasarca (Purohit *et al.,* 2006; Purohit *et al.,* 2012). Hydrocephalus is accumulation of excessive fluid in durameter or ventricles of brain (Noakes, 2009; Purohit *et al.,* 2012). It may be caused due to genetic, nutritional and environmental factors(Kalman, 1989). Internal hydrocephalus is collection of fluid in the cerebral ventricle and external hydrocephlous is collection of fluid outside the brain substance (Cole and Moore, 1942). Death of fetus occurs due to pressure on vital centres of brain (Purohit *et al.,* 2012). Congenital hydrocephalus has been described in various animal species including cattle (Mouli, 1987; Balasubramanian *et al.,* 1997; Sharda and Ingole, 2002), buffalo (Bhandari *et al.,* 1978; Bugalia *et al.,* 1990), mare (Sharma, 1996) and camel (Abubakr *et al.,* 1998). The condition results in dystocia and the fetuses are delivered by either excision of the head followed by traction (Bhandari *et al.,* 1978) or caesarean section (Bugalia *et al.,* 1990; Balasubramanian *et al.,* 1997). Per-vaginal delivery in cases of foetal hydrocephaly is difficult except in few cases by giving stab incision on foot ball shaped foetal mass and draining out the fluid to compress the head (Upasana *et al.,* 2012). The present communication describes the clinical management of congenital hydrocephalus in Red Kandhari cattle.

CASE HISTORY AND OBSERVATIONS

A six years old Red Kandhari cow was presented to TVCC, COVAS, Parbhani with a history of difficulty in parturition since 16 hrs.Cow was dull, depressed, anxious and exhausted. No fetal parts were observed at the vulval region of the cow. The case was earlier handled by local practionerand partial foetotomy was attempted. Pervaginal examination revealedanterior longitudinal presentation of the fetus withno suckling reflex or pedal reflex was elicited by the fetus. Both forelimbs stretched at the vulva and thedead calf had a soft, pliable, fluid filled large dome shaped head. Both ruptureof water bags was observed.

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DIAGNOSIS AND TREATMENT

The foetal presentation, position and posture were anterior-longitudinal, dorso-sacral respectively with downward deviation of head and partially foetotomised body parts. The case was diagnosed as a foetal dystocia due to hydrocephalus of the calf with malposture (Figure.1).

On per vaginal examinations revealed that absence of any fetal reflex indicated that foetus was dead. The enlargement over the head of foetus at the occipital region of fetuswas trocarized to evacuate the contents. The fetus was then delivered by the mutational procedures with application of hook in the inner canthus of eye along with the judicious traction on the fore limbs with full lubrication. The animal was administered calcium borogluconate (Inj Mifex 450 ml IV), Oxytocin (Inj pitocin 40 IU IM), dexamethasone (Dexona 40 mg IM), and fluid replacement therapy (Dextrose 4 litres IV). Parentral antibiotic (Dicrysticin 2.5gm IM) was administered; simultaneously intrauterine bolus (Clenex bolus 4nitrofurazone, metranidazole, urea, povidone iodine) was administered

Fig. 1: Hydrocephallic Red Khandari Calf

to combat any possible infection. Subsequent to fetal delivery animal stopped straining and was in good condition. The animal recovered uneventfully within 12 hrs.

DISCUSSION AND CONCLUSION

Autosomal recessive gene (Roberts, 1986) have been reported to be linked with hydrocephalus in cattle. And also it may be due to disturbances in normal circulation of cerebrospinal fluid resulting from its altered production or absorption (Fride, 1975). In all of these cases CSF collects passively inside or sometimes outside the ventricles, causing pressure atrophy of the cerebral tissues. Such accumulations of fluid may be due to obstruction of the foramen of Monroe, the cerebral aqueduct, or the foramina of the roof of the fourth ventricle, resulting in internal hydrocephalous. Jubb and Kennedy (1970) stated that congenital hydrocephalus is known to be inherited in cattle and exacerbated in its manifestation by a coexisting hypovitaminosis. Compression of the brain occurs in calves with hypovitaminosis A due to failure of growth and sculpturing of the cranial vault to accommodate the growing brain. The incidence of bovine hydrocephalus has been reported at 1.5 cases per 1000 calvings. Some affected foetuses are stillborn and many are born prematurely. Calves with pronounced cranial enlargement usually die within 48 hours but less severely affected calves may survive for several weeks or longer (Leech, 1978). The enlarged head cannot easily pass through the birth canal and results in dystocia as was seen in the present case, In more severe form, marked thinning of cranial bones – facilitates trocarization and compression of the skull to allow vaginal delivery.

Water head of foetus was located at the occipital region on the head of fetus and trocarization was done by strong trocar and cannula to evacuate the contents. Dystocia due to hydrocephallic Red Khandari male calf was delivered pervaginally with the help of mutational procedures.

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Successful Therapeutic Management of Bovine Fasciolosis

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ABSTRACT

Bovine fasciolosis (liver fluke) is a parasitic disease of cattle caused by trematodes usually *Fasciola gigantica* and *Fasciola hepatica*. The life cycle of these trematodes involves snail as an intermediate host (IH). The present abstract describes six cases presented to a Department of TVCC, Veterinary College, Hassan with a history of chronic diarrhoea, accumulation of fluid below the neck, restlessness, yellow coloured urine, recumbent and anorectic since last 10 days. On clinical examination there was pale conjunctival mucus membrane, bottle jaw appearance, weakness, mild anaemia and jaundice. On blood examination there was decreased TEC, Hb and PCV and increased neutrophils count compared to normal blood values. Diagnosis was done based on clinical signs, grazing history, seasonal occurrence and examination of feaces by laboratory tests. Cases were treated with anthelmintic oral solution, antihistaminic therapy, liver extract therapy, B complex injection. For confirmation the faecal examination was carried on day 7 and 14 which showed no presence of ova. Uneventful recovery was seen after seven to ten days.

Bovine fasciolosis is economically important parasitic disease of cattle caused by trematodes of genus *Fasciola* commonly referred as liver fluke. *F. hepatica* and *F. gigantica* are the most common species as etiological agents of the fasciolosis. *F. hepatica* has a worldwide distribution while *F. gigantica* is found on most primarily in tropical regions (Ahmed, 2009). *Fasciola hepatica*, the common liver fluke, causes fasciolosis (liver rot). It may lead to secondary bacterial infection such as bacillary icterohemoglobinuria (red water) in cattle (Marquardt *et al.*, 2000). Adult parasites are found in the bile ducts and the immature flukes in the liver parenchyma of infected final hosts. Chronic disease usually results in decreased production of meat, milk, wool and secondary bacterial infections, fertility problems, loss of weight (Alcaino, 1990). Clinical disease is usually characterized by weight loss, anaemia and hypoproteinaemia. Fasciolosis is a worldwide zoonotic disease (Talukder *et al.*, 2010).The clinical signs of acute disease are characterised by sudden acute deaths, weakness, anaemia and dyspnoea. Sub-acute and chronic fasciolosis is characterized by progressive loss of condition, anaemia, hypoalbuminaemia, emaciation, pallor of the mucous membranes, submandibular oedema and ascites. Anaemia is hypochromic and macrocytic and an accompanying eosinophilia is usually present. In milder infections clinical signs may or may not be readily observed, however, a decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on production.

CASE HISTORY AND OBSERVATIONS

Cases presented to the Department of TVCC, Hassan with a history of chronic diarrhoea, accumulation of fluid below neck, restlessness, yellow coloured urine, recumbent and anorectic were included in the study. On clinical examination there was pale conjunctival mucus membrane, bottle jaw appearance (Fig. 1 and Fig. 2), weakness, mild anaemia and jaundice.

On haematological examination there was normocytic normochromic anaemia indicated by decline

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Fig . 1: Bottle jaw

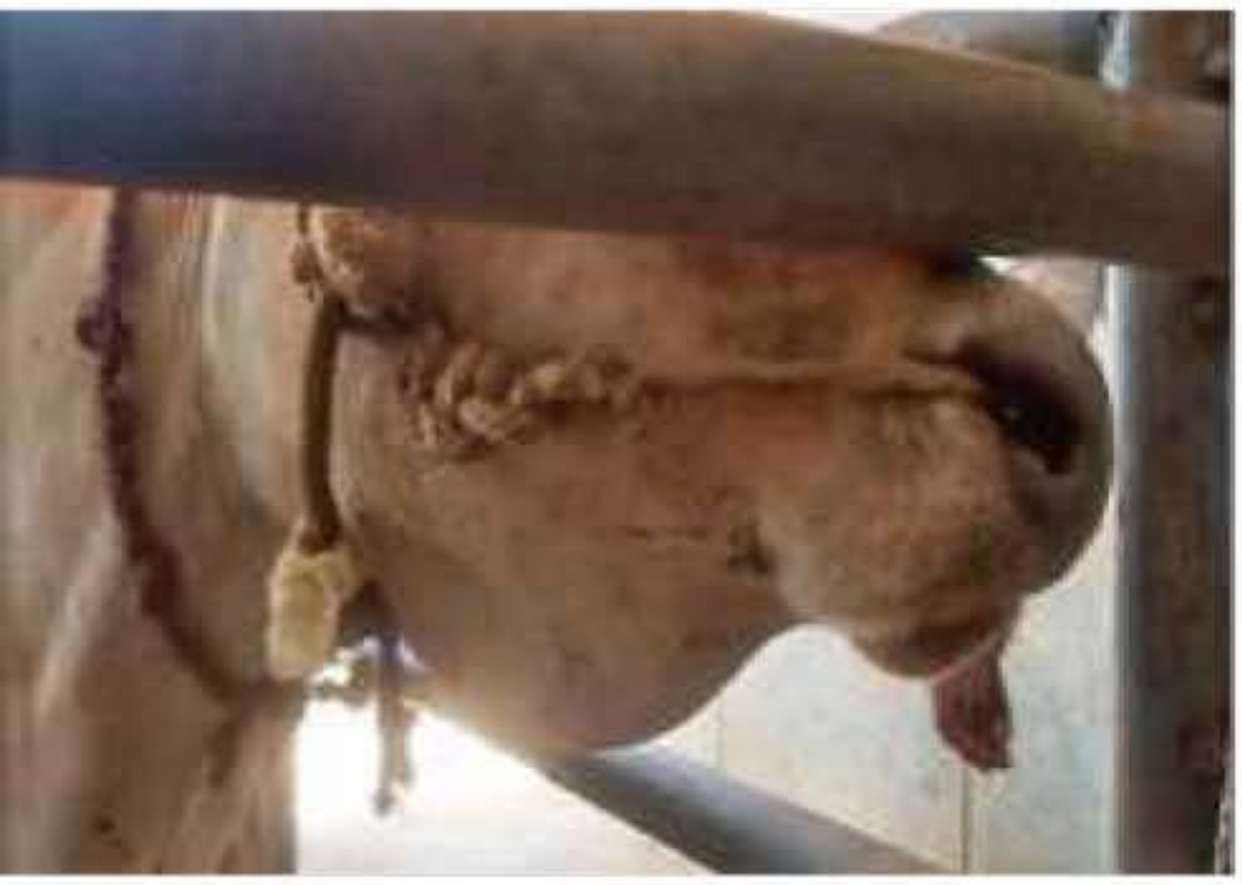

Fig. 2: Bottle jaw

in the TEC, haemoglobin level, packed cell volume, mean corpuscular haemoglobin concentration and increase in erythrocyte sedimentation rate.There was also significant increase in the eosinophil and neutrophil

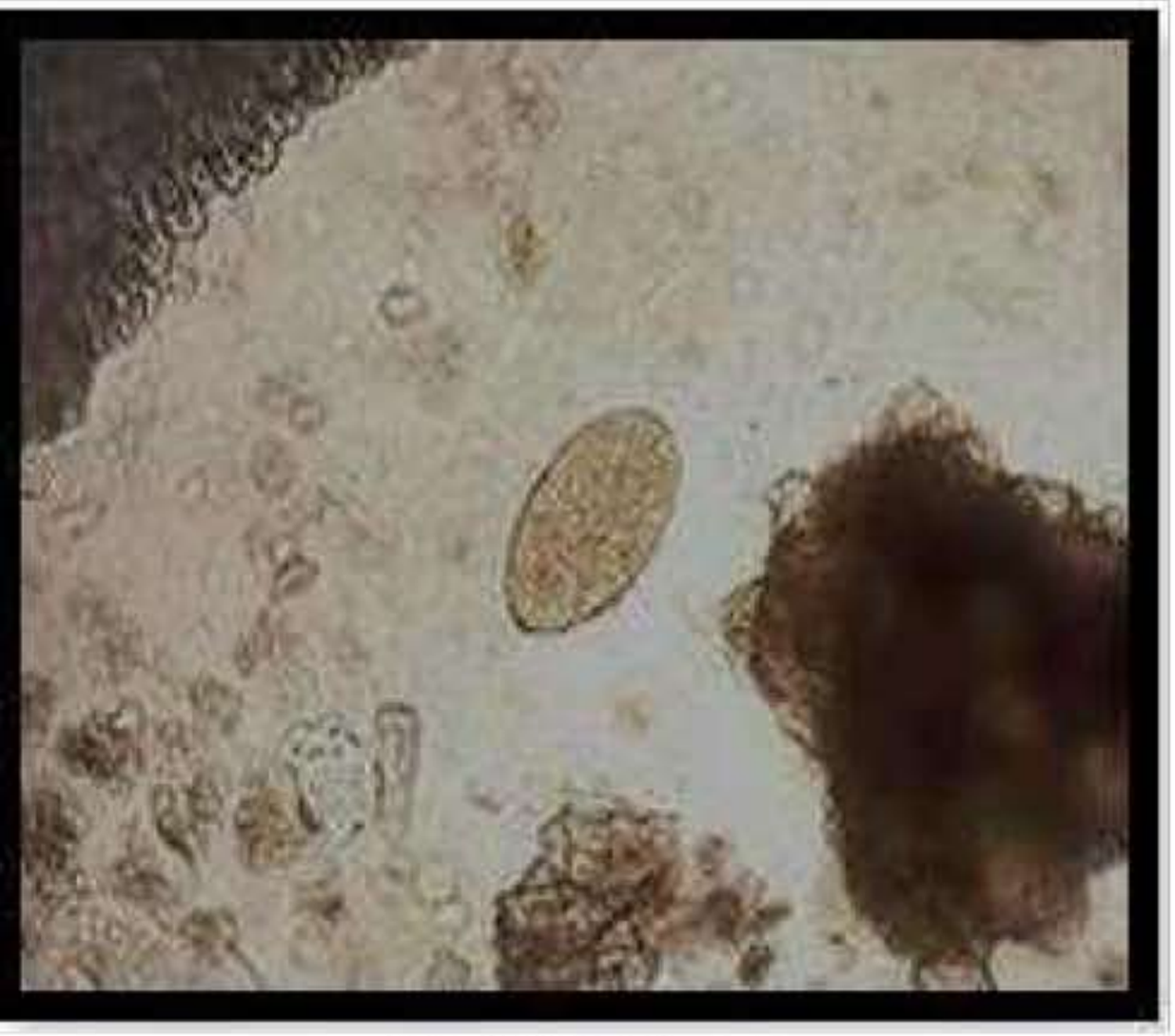

Fig. 3: Fasciola ova

counts and lower lymphocyte counts as compared to normal values.

Diagnosis of bovine fasciolosis was based on clinical signs, grazing history, seasonal occurrence and examination of faeces by sedimentation technique (Fig. 3).

TREATMENT AND DISCUSSION

Haematological observations of the present study were in correlation with the observations of Swarup *et al.* (1987) and Chaudri *et al.* (1988). The lower values of TEC might be due to loss of blood resulting from severe haemorrhage caused by extensive migration of the young flukes through the hepatic parenchyma and from blood sucking activity of the adult fluke, secretion of unknown toxic substance swhich depress the haematopoietic activity resulting in decreased production of erythrocytes. The continuous drainage of iron stores and reduction in the total number of erythrocytes were thought to be responsible for reduction in haemoglobin level. Decrease in PCV might be attributed to hemodilution demonstrated by the increase in plasma and blood volumes as well as to lower level of total erythrocytes.

Increased eosinophils in the present study were comparable with Haroun *et al.* (1986). Neutrophil level was also found significantly increased in infested animals and this might be due to secondary bacterial infections caused by migration of young flukes through the biliary parenchyma. This finding was in agreement with Furmaga *et al.* (1974).

Chronic hepatic fasciolosis develops only after the adult flukes establish in the bile ducts. Here they cause cholangitis, biliary obstruction, fibrosis and a leakage of plasma protein across the epithelium. Although this protein can be re-absorbed in the intestine, there, is poor utilization and retention of nitrogen leading to hypoalbuminaemia which is the reason for development of "Bottle Jaw" (Radostits *et al.*, 2006)

Chronic fasciolosis is diagnosed by finding eggs in the feces using sedimentation technique. However,

they must be distinguished from eggs of the other flukes especially the large eggs of paramphistomum. Fasciola eggs have high specific gravity and sedimentation is preferred to floatation. The oval operculated golden eggs of *F. hepatica* appear in the feces 10 weeks after infection, *F. gigantica* eggs only appear 15 weeks after infection. Excretion of fluke eggs shows considerable day to day and within day variation and the distribution of eggs in feces are irregular; single fecal egg count assay may lead to incorrect conclusion.

Cases were treated with a anthelmintic oral solution (Albendazole @10mg/kg BW) and antihistaminic, liver extract and B complex injection as a supportive treatment to ensure faster recovery. Albendazole is a broad-spectrum compound also active against nematodes and cestodes. It is ovicidal and is effective on *F. hepatica* eggs present in bile ducts or the alimentary tract at the time of treatment. For confirmation the faecal examination was carried on day 7 and 14 which showed no presence of ova. Uneventful recovery of the cases was seen after seven to ten days.

CONCLUSION

Successful treatment of Bovine fasciolosis can be done by anthelmintic oral solution, antihistaminic therapy, liver extract therapy and B complex injection as a supportive treatment to ensure faster recovery.

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Management of Pharyngeal Foreign Bodies in Three Buffaloes

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ABSTRACT

Three female buffaloesaged between five to eight years were presented to the TVCC with the symptoms of excessive salivation, regurgitation and anorexia for the last four to twenty days. Buffaloes were suspected for oesophageal obstruction. Plain radiograph of the cervical oesophagus revealed lodgment of metallic foreign bodies i.e. sewing needle, a linear wire and a folded wirein the pharyngeal region. These foreign bodies were retrieved manually through oral cavity under sedation. All animals recovered uneventfully within seven days.

Keywords: Buffalo, sewing needle, pharyngeal region

Buffalo commonly ingest foreign objects because of their indiscriminate feeding behaviour. Consequently, foreign bodies struck in reticulum. They may cause diaphragmatic hernia (Krishnamurthy *et al.*, 1985) and foreign body syndrome in bovines (Kohli *et al.*, 1982 and Singh and Nigam, 1981) and sometimes trauma or iatrogenic injury. The lack of sensitivity in prehensile organs such as lips and tongue in bovine is considered to be the main predisposing factor for such incidences. As a consequence, buffaloes reared close to human habitat, often swallow metallic objects such as nails and wires that have been unknowingly left in their feeding areas (Jones *et al.*, 1996; Desiye and Mersha, 2012). Entrance and migration of the ingested foreign object through the body cavities and tissues or its lodgment anywhere in the body may lead to various complications that differ according to the nature of the foreign body and its migration route (Cheel and Sethi, 1999; Calfee and Manning, 2002). Management of these clinical conditions sometimes requires time consuming surgeries with variable complications. Sometimes these foreign bodies in the pharynx can be retrieved per orally under sedation (Sharma *etal.*, 2014). Thus accidental ingestion of foreign body in dairy animals may prove to be of great economic importance due to its associated morbidity leading to loss of production and in some cases mortality as well (Radostits *et al.*, 2007). Though metallic foreign bodies lodged in upper gastrointestinal tract of bovine can be readily diagnosed through radiographs (Spouge *et al.*, 1990; Hunt *et al.*, 2004) but their retrieval may sometimes be quite challenging.

Three female buffaloes aged between five to eight years were presented to the Teaching Veterinary Clinical Complex, LUVAS with a history of anorexia and regurgitation in all casesbut excessive salivation in one buffalo.There was no sign of tympany in any case. On clinical examination all the animals were dull with congested ocular mucous membrane, dry muzzle and they were making efforts but unable to swallow feed and water properly. Rectal temperature was slightly elevated in one case and within normal range in two buffaloes, whereas heart rate, respiration rate and pulse rate were in normal range. Haematology revealed that there was slight rise in Hb indicating the dehydration and remaining parameters were in normal range. However, lateral radiograph of the neck region revealed lodgment of metallic potential foreign body

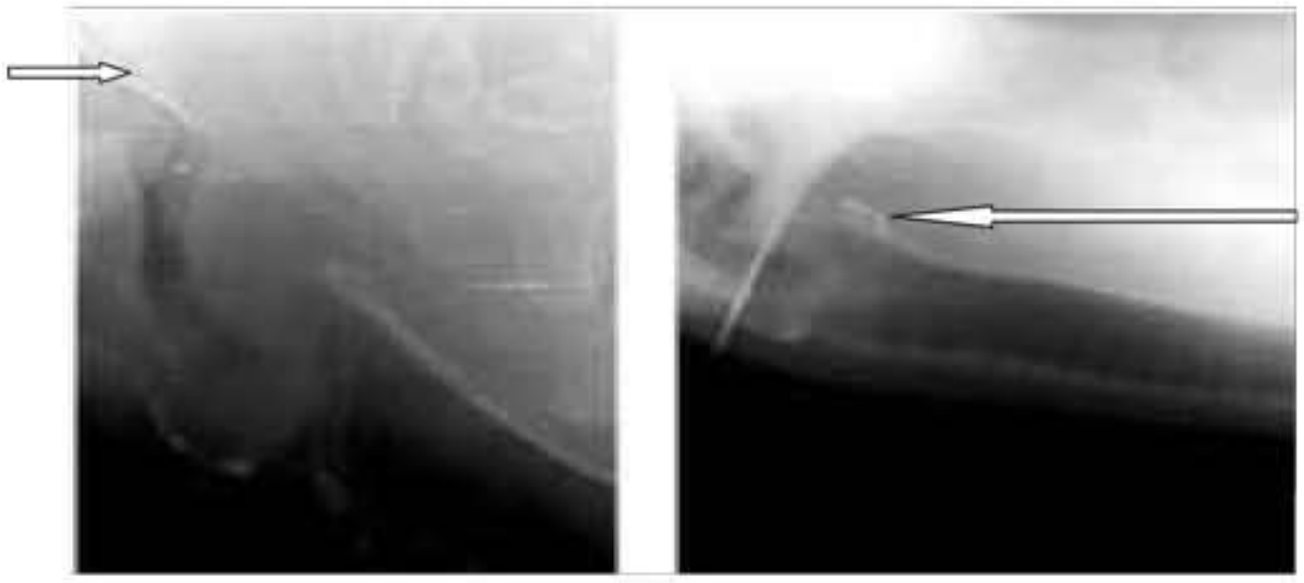

Fig. 1: Linear wire **Fig. 2: Curved wire**

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simulating a linear wire in two cases (Fig. 1) and a curved wire in one case in the pharyngeal region (Fig. 2).

It was decided to attempt retrieval of foreign body manually orally in all the cases and the buffaloes secured in the standing position. All the animals were sedated with inj. Xylazine hydrochloride at the dose rate 0.04 mg/kg body weight intravenously. The mouth gag was applied to open the mouth and to aid in searching the foreign bodies in pharyngeal area. There after, the hand was inserted into the mouth and the pharyngeal region of the buffalo was explored with

Fig. 3

Fig. 4

Fig. 5

the fingers. After feeling the foreign bodies *i.e.*, a linear wire (Fig. 3), a curved wire (Fig. 4) and a sewing needle (Fig. 5) in the pharyngeal region grasped between thumb and index finger and dislodged from soft tissue. Post operatively, treated with inj. Strepto-penicillin at the dose rate 2.5gm., inj. meloxicam at the dose rate15 ml and inj. B complex 10 ml intramuscularly for five consecutive days. Owner was advised to provide gruel like soft feed for three to four days. All the animals recovered uneventfully within a week. These case semphasize the importance of manual exploration of the pharyngeal area through the oral cavity of bovine to make an attempt to retrieve any lodged foreign body before undertaking more invasive surgeries for the purpose.

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Surgical Management of Deep Lacerated Tongue in Hallikar Bullock

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Tongue plays an important role like prehension, mastication, deglutition, sorting and intake of feed and water (Lobprise and Wiggs, 1993). Any major injury of the tongue causes severe pain and interference with feeding, requires emergency attention (Das and Konar, 2014). Laceration of tongue may occur due to injury caused by sharp foreign body ingestion such as nails, wire and needles (Singh *et al.,* 2010). In the present paper surgical management of lacerated tongue in Hallikar bullock was reported. A five-year-old Hallikar bullock was presented to Hospital, with the history of blood mixed saliva drooling from the mouth and not taking food and water since morning. Clinical examination of oral cavity revealed a transverse deep laceration just caudal to the tip of the tongue which exposed full length of tunica muscularis (Fig. 1). It was decided for emergencysurgical intervention.The animal was sedated with Xylazine hydrochloride 0.03mg/kg BW and restrained in the standing position.Upon glassopharangial nerve block with local infiltration of lignocaine HCL 2%, wound edges were derided. Using No1-0 polyglactin-910 tunica muscularis was sutured with simple interrupted pattern fallowed by outer layer which was sutured by mano-filament polyamide No 1-0 by vertical mattress suture pattern.

Post operatively animal was administered 5 liters of normal saline daily for three days, ceftriaxone3g intravenously twice daily for five days, meloxicam10 ml once daily for three days and tribivet 10 ml once daily for three days and externally boro-glycerine paste to tongue twice daily for 5 days. After three days animal was fed with liquid diet allowed to eat green fodder and the tongue healed within 10 days (Fig. 2) without any complication.

Deep laceration of tongue requires extensive surgery and in some cases extensive laceration, partial glossectomy was recommended (Singh *et al.,* 2010), whereas in the present case the tongue was sutured and preserved. Hernadez and Negro (1999) reported that most common tongue affections are traumatic injuries,whereas tongue laceration in this casemight be due to a sharp object. Das and Konar (2014) employed double layer suture pattern in repairing deeply

Fig. 1: Photograph showing lacerated tongue

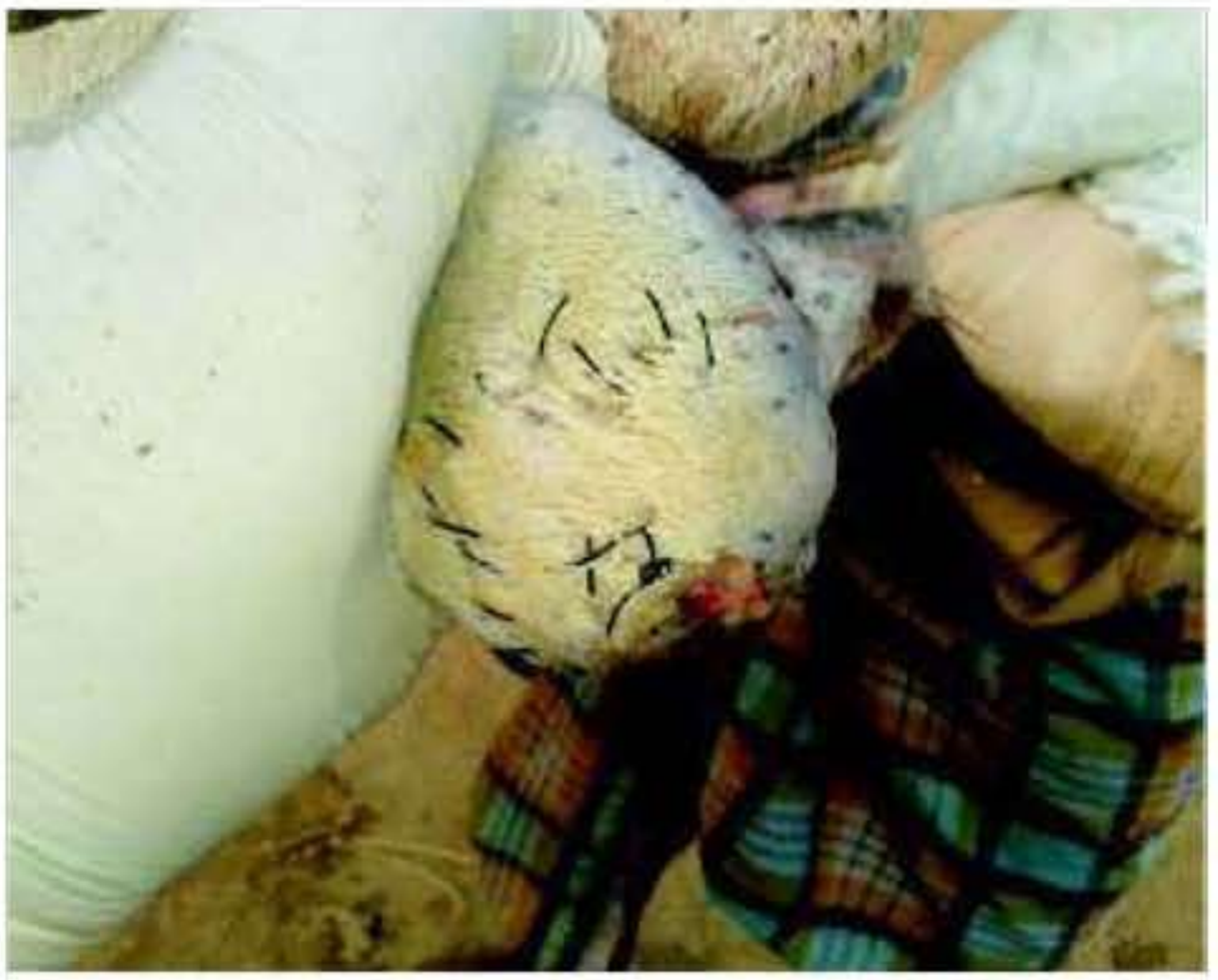

Fig. 2: 10th day post operativ photograph

lacerated tongue in a bull and the same technique was followed in this case. Orsini *et al.* (1992) recommended liquid diet on the day of surgery because tongue is a highly vascular organ and heals rapidly but in present

situation laceration of tongue was very severe and wound extended from one side to the other therefore liquid diet was offered on 3rd post-operative day. In the present paper successful surgical management of lacerated tongue in Hallikar bullock was reported and discussed.

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