Efficacy of Albendazole against Certain Parasites in Pigs

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ABSTRACT

Gastrointestinal parasites cause economic losses to the swine industry. A variety of anthelmintics are being used by the pig rearers to eliminate the gastrointestinal parasites. Hence, it is imperative to carry out investigations on anthelmintic efficacy in the pig industry to enable the swine rearers to adopt the suitable control measures. A study was undertaken to study the efficacy of Albendazole against certain internal parasites in pigs (Yorkshire, Landrace, Duroc and their crosses) using Faecal Egg Count Reduction Test (FECRT) in swine farms with long time exposure for anthelmintics. The present study revealed the prevalence of highest infection rate of *Ascaris suum*,(83.30 and 76.60% in farms I and II) followed by *Eimeria* spp., (60% and 40 % in farms I and II) and *Trichuris suis* (16.60 and 10.00% in farms I and II) Albendazole (10mg/kg B.W) was found to be more than 95% effective in reducing the helminth infection.

Key words: Pig, *Ascaris suum*, Albendazole, Efficacy

Gastro intestinal helminthes affecting the pigs are of zoonotic and public health importance and attracted more economic losses in swine rearing. The effect of GI parasitism range from stunted growth, unthriftiness, diarrhoea, anaemia and death (Blood and Radostits, 1995). Gastrointestinal parasites are responsible for substantial losses of productivity in swine and livestock industry as well. Since losses encountered in pigs due to helminth parasites are enormous and it is imperative to carry out the investigations on anthelmintic performance in the pig industry and their usage to minimize the losses to the extent possible.

Infections with internal parasites are mostly subclinical, causing huge economic losses to pig industry. Infected animals though have a higher feed conversion rate, but thelower daily weight gain and lower meat quality makes the industry to grow on negative side.

Albendazole, a broad spectrum anthelmintic drug, marketedunder different trade names belongs to benzimidazole group and is being used for the treatment of a variety of parasitic worm infestations in general and nematodes in particular.The present investigation was carried out to evaluatethe performance of Albendazole using the Faecal Egg Count Reduction Test (FECRT). Although several reports have been published on nematode parasites of pigs from different parts of India (Yadav and Tandon, 1989), there is scanty of information on the prevalence and efficacy of Albendazole against GI parasites from Hassan district of Karnataka.

MATERIALS AND METHODS

Selection of animals and study area: Two farms located in Hassan district owned by private with Thirty (30) pigs in each farm were selected for the present study. The pigs belong to breeds of Yorkshire, Landrace, Duroc and their crosses aged 2-2 ½ years. The farms had a history of unthriftiness, weakness, lowered weight gain and even death of piglets. The pigs in these selected farms had a history of deworming with Fenbendazole.

Dosing of animals: The animals that were tested positive for parasitic infestation were dewormed as a single dose withAlbendazole - 10mg/kg B.W. Ten pigs in each farm served as control group.

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Collection and Processing of faecal sample: Using disposable hand gloves faecal samples were collected directly from the rectum. The collected feacal samples were transferred into glass bottles, were well labeled and filled upto to the brim to exclude air as much as possible with the idea of reducing the rate of development and hatching of the eggs. In conditions where the laboratory examination could not be carried out soon after collection, the samples were kept in the refrigerator at 4° C for not more than 24 hours. The faecal samples were collected before treatment and 2 weeks after treatment. The eggs per gram (EPG) was determined using a McMaster method (Soulsby, 1982).

Statistical analysis of the data: The FECRT(%) is calculated as per the below mentioned formula:

FECRT (%) = 100 x (1- arithmetic mean of post DA FEC/ arithmetic mean of pre DA FEC)

Post DA FEC - post drug administration faecal egg count

Pre DA FEC - pre drug administration faecal egg count

The prevalence rate of the parasite was calculated by formula, p=d/n where 'd' is the number of pigs diagnosed as having a given parasite at that point of time and 'n' is the no. of pigs examined at that point of time and 'p' is the prevalence of the pig harboring each parasite.

Grading of EPG: Less than 500 – Low infection rate, 500 – 2000 – moderate infection rate, 2000 - 5000 – high infection rate

RESULTS AND DISCUSSION

In the present study it was possible to recover eggs of *Ascarissuum, Trichuris suis* and oocysts of *Eimeria*spp., and *Balantidium coli* cyst. Out of 60 pigs screened 10(33%) and 11(36.6%) of pigs had multiple infection (*Ascaris, Trichuris and Eimeria spp.)* in Farm I and II respectively (Table 1). The intensity of *Ascaris suum* infection was more in farm I than in farm II. Twenty animals (66%) in farm I had high EPG count ranging from 2000 to 5000. In farm II, 16(53.33%) animals had high infection rate, three (10%) had moderate infection and one (3.33%) animal with low infection rate.

Table 1: .Prevalence rate of Gastrointestinal parasitic infectionsin two different farms

Comparison of infection rates in two farms	Farm I –(30)		Farm II – (30)
Ascarissuum	25 (83.3%)		23 (76.6%)
Trichurissuis	5 (16.6%)		3 (10%)
Eimeria spp.	18 (60%)		12 (40%)
Mixed infection (Ascarissuum Trichurissuis, Eimeriaspp)	10(33.3%)		11 (36.6%)
Pre treatment infection rate, EPG	High (2000-5000)	20 (66.00%)	16 (53.33%)
	Moderate (500-2000)	-	3 (10%)
	Low (Less than 500)	-	1 (3.33%)
Post treatment infection rate, EPG	High (2000-5000)	-	-
	Moderate (500-2000)	2 (6.66%)	1 (3.33%)
	Low (Less than 500)	2 (6.66%)	2 (6.66%)

Ascaris suum was the most common helminth in all age groups(2-2 ½ years with piglets)of pigs examined and the present observation are in agreement with similar reports in extensively managed pigs in India (*Yadav and Tandon*, 1989 *Tomasset al.*, 2013, Ebibeni*et al.*, 2013).

The prevalence of the nematode parasite *Trichuris suis* was low(16.60 and 10.00% in farms I and II) in the present study and is in agreement with the findings of Keshaw *et al.*, 2009, who found prevalence of *Trichuris suis* in 38% of 221 pigs reared in sub-tropical and high rainfall area of India. This study further supports the speculation that *Trichuris suis* eggs are highly susceptible to environmental factors like high temperature which was around 35-38°C during the present study in the months of April and May 2014.

The prevalence of the oocysts of Eimeriaspp., and *Balantidium coli* was observed in 18 pigs (60%) of Farm I wheras12 pigs (40%)of farm II, the present findings are in agreement with the findings of earlier studies which indicated Eimeria oocysts are often found in pig feces and reported in 93% of herds evaluated (Lindsey et al., 1984).

After treatment with Albendazole (10mg/kg B.W), among 20 pigs, infected pigs with high

intensity parasite rate 16 had completely recovered fromthe parasites but 4 exhibited low to moderate intensity of infection in farm I. In farm II, Sixteen highly infected animals became free of infection, 4 animals with low to moderate rate of infection became 3 and one was free of infection after treatment with Albendazole at a dose rate of 10mg/kg B.W. The results indicated the efficacy of 97.6% and 96% in farm I and II respectively. (Table 2).

Table 2: Efficacy of albendazoleagainst helminths and differences in mean EPG of pre and post treatment

Farm no	*Ascaris suum*	*Trichuris suis*	*Eimeria, spp*	Pre treat-ment	Post treat-ment
I	97.60%	96.86 %	89.25 %	7520	180
II	96.00 %	98.45 %	88.74 %	2500	100

The results are in agreement with Adugna *et al*., 2007 whose findings reported that Albendazole revealed a cure rate of 83.9% and egg reduction rate of 96.3% against *Ascarislumbricoides.* In spite of the fact that anthelmintics remove the worms 100% effectively it is very difficult to control *Ascaris suum* due to its high reinfection rate, improper anthelmintic dosing and lack of suitable sanitary measures. Kipper *et al.,* 2011 concluded that *Ascaris suum* infections in pigs are mostly subclinical, .are often overlooked by the concerned personnel.

In conclusion, the present study indicated that despite the extensive use of anthelmintics**,** the GI nematode infections in pigs were existing in the area.. Though Piperazine is generally effective against ascariasis, Albendazole was equally effective and economical against all internal parasites including ascariasis. It is suggested to use Albendazole judiciously and rotate with different antihelmenthics to avoid drug resistance. To increase productivity of pigs in the study area it is suggested that control of GI nematodes be coupled with proper management practices such as planned appropriate strategic anthelmintic treatments and proper execution regular and frequent removal of dung from sites along with cleaning of floors with antimicrobials and premises. In locals where community rearing of pigs is involved pork is consumed by a large part of the population, they shouldbe educated about the scientific rearing of pigs and zoonotic potentiality of certain diseases. This study gave a scope tostudy the possible impact of parasitic infestations of pigs on public health in Hassan district.

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Haemato-Biochemical Changes in Dogs Suffering from Renal Failure*

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ABSTRACT

Kidney function tests are useful to detect the nature, magnitude and the extent of impairement of renal function. A total of 80 dogs were investigated. Group I (n=10) consisted of healthy animals, Group II (n=60) consisted of suspected cases of renal failure and non azotemic based on creatinine value (< 1.4 mg/dl) and Group III (n=10) consisted of renal failure cases. The present study reports the changes in haematology, serum creatinine, Blood urea nitrogen and microalbuminuria in these three groups. There was statistical significant difference in haemoglobin, PCV and TEC in Group III when compared to Group I and Group II ($P \leq 0.05$).There was a difference in TLC between three groups and was statistically significant ($P \leq 0.05$). Most important findings were the significantly increase in the values of Creatinine, BUN and microalbuminuria values in renal failure dogs compared to healthy dogs.

Key words: Haematology, Renal failure, Kidney function test, Biochemical change

Chronic kidney disease is one of the important problems that causes considerable morbidity and mortality in dogs. Majority of the old dogs suffer some degree of kidney damage thus it is one of the major causes of death in older animals. The underlying cause of the disease may have occurred previously and remains unknown in most cases. The prevalence of renal disease is high in dogs and cats especially in aged population (Polzin *et al.*, 2000). Kidneys are one of the vital organs in the body which perform multiple functions to keep the system in homeostasis. They get rid of the body waste that are either ingested or produced by metabolism. Renal failure is characterized by decline of glomerular filtration rate. Renal failure results when more than three quarters of nephrons in both kidneys are not functioning. Renal function tests, such as serum creatinine, blood urea nitrogen and endogenous creatinine clearance rates are indices of glomerular filtration rate. Serum creatinine and urea concentrations are widely used biochemical parameters for rapid estimation of glomerular filtration rate. Recent studies reported that microalbuminuria seems to be a good indicator of early renal disease in dogs, particularly those diseases that involve the glomerulus (x- linked hereditary nephropathy, heart worm disease) (Grauer *et al.*, 2002 and Lees *et al.*, 2002). Continually improving veterinary care has resulted in an expanding geriatric pet population and an increasing need to identify and understand conditions of importance in older animals. This paper reports haemato-biochemical changes in dogs suffering from renal failure by comparing them with control and suspected cases of renal failure.

MATERIALS AND METHODS

The present study was conducted using three groups of animals. Group-I (n=10) consisted of 10 healthy animals. Group-II (n=60) consisted of 60 suspected cases of renal failure. Group-III (n=10) consisted of 10 renal failure cases. Cases were selected based on history, physical examination, hematology, biochemistry and urine analysis.

Blood was collected with and without EDTA in vacutainers. Serum was separated and used for estimation of blood biochemical parameters immediately. Blood collected with EDTA was used for various hematological studies immediately after collection. Total leukocyte count, Haemoglobin, Packed cell Volume and total erythrocyte count were determined as per the standard procedure outlined by Schalm *et al.* (1975). Creatinine and BUN were measured by Artos® biochemical semi auto analyzer using commercially available kits. Urine sample was

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collected using sterile catheter in to sterile vials and were processed. Microalbumin in urine was estimated using commercially available human kit by turbidometric immunoassay using Artos ® biochemical semi auto analyzer. Statistical analysis was performed using one way analysis of variance and Tukey's multiple comparison test by using Graph pad prism software.

RESULTS AND DISCUSSION

In the present study TLC in renal failure cases was 22.43 ± 4.023 × 10^3 cells /µl, There was significant statistical difference (P≤0.05) in the mean between Group III and Group I. It is evident that there is leucocytosis in renal failure cases. Increased white blood cell count generally indicates the presence of inflammation, which may either have an infectious or a non infectious cause. In patients with renal failure, leucocytosis often is mainly due to inflammation in one or more organ system. When leucocytosis is caused by inflammation within the urinary system, the site of inflammation generally is in parenchymal tissue rather than in the excretory pathways (Osborne and Finco, 1995; Hurley, 1998). Leucocytosis has been documented by Adin and Cowgill (2000) in azotemic cases caused by leptospirosis. Polysystemic diseases such as bacterial endocarditis, leptospirosis and pyometra which involve urinary system are often associated with leucocytosis as indicated by Osborne *et al*. (1972). There was a significant difference in the total leukocyte count between Group I and Group II. This may be because of infection that caused leucocytosis. However, in the present study the reason for leucocytosis could not be ascribed as the work did not involve insight into etiological aspects.

In the present study mean ± SE of haemoglobin, PCV and TEC in renal failure cases (Table 1) were 7.89 ± 0.52 g%, 25.20 ± 1.48 per cent, 4.003 ± 0.25× 10^6 cells /µl. There was a statistical significant difference (P ≤ 0.05) noticed in the haemoglobin, packed cell volume and total erythrocyte count of renal failure cases to that of normal dogs indicating anemia. These findings are similar to that found by Robinson *et al.,* (1989). Anemia is a feature in CRF due to reduced erythropoietin secretion Michell (1988); Polzin *et al.* (1995), or decreased RBC life span, uremic inhibitors of erytropoiesis and external blood loss (Cowgill 1995, Osborne and Finco, 1995). Anemia can also be seen in concurrent infection like leptospirosis. There was no statistically significant (P ≥ 0.05) difference in haemoglobin, PCV and TEC between Group I and Group II.

Table I: Mean ± SE values of TLC, haemoglobin, PCV and TEC in Group I, Group II and Group III dogs.

	Group I (n=10)	Group II (n=60)	Group III (n=10)	F-ratio
Total Leukocyte Count (× 10^3 cells /µl)	8.005 ± 0.68^a	15.16 ± 1.041^b	22.43 ± 4.023^c	**7.509****
Haemoglobin (g%)	12.06 ± 0.65^a	12.00 ± 0.27^a	7.89 ± 0.52^b	**15*****
Packed Cell Volume (%)	43.00 ± 2.4^a	38.00 ± 1^a	25.00 ± 1.5^b	**17*****
Total Erythrocyte Count (× 10^6 cells /µl)	7.00 ± 0.40^a	4.00 ± 0.25^a	6.40 ± 0.17^b	**18*****

*** Significant at 5 % level (P≤0.05)
Common superscript Row = ab
Means bearing any one common superscript in a row do not differ significantly with each other.

In the present study the mean ± SE of Creatinine and Blood urea nitrogen in Group III animals (Table 2)) were found to be 3.978 ± 0.47 mg/dl and 63.72 ± 7.8 mg/dl respectively, which is much higher than Group I and indicates renal damage. There was significant difference (P ≤ 0.05) in mean of creatinine and BUN between Group III and Group I. These findings are similar to the observations made by Grauer and Lane, 1995 and Haller, 2002.The finding of hypercreatinemia and azotemia in dogs of Group III may be attributed to the impaired filtration process as a consequence of loss of physiological function of the nephrons. About 70-75 % of the nephrons must be nonfunctional before its values rise above the normal range (Krawiec *et al.,* 1986). Creatinine is efficient for monitoring the progression of CRF or the efficiency of treatment with the critical difference of 0.4 mg/dl in the range of normal values. The increased value of urea might be due to the facts that urea cannot be utilized or excreted to

any significant degree by organ other than kidney, low rate of urine flow enhance tubular absorption of urea (Osborne et al.,1972) and endogenous source such as rapid catabolism of body tissue (fever, infection etc.) or GIT bleeding.

Table 2: The mean ± SE values of Creatinine, BUN and Microalbuminuria in Group I, Group II and Group III dogs

	Group I (n=10)	Group II (n=60)	Group III (n=10)	F-ratio
Creatinine (mg/dl)	0.84 ± 0.064^{a}	0.9853 ± 0.038^{a}	3.978 ± 0.47^{b}	**119.9*****
BUN (mg/dl)	13.78 ± 1.91^{a}	16.39 ± 1.37^{a}	63.72 ± 7.8^{b}	**61.62*****
Microalbuminuria (mg/dl)	4.70 ± 1.5^{a}	16.00 ± 2.3^{a}	70.00 ± 26.0^{b}	**13*****

*** Significant at 5 % level (P≤0.05)
Common superscript Row = ab
Means bearing any one common superscript in a row do not differ significantly with each other.

The mean value of microalbumin in urine in renal failure dogs was 70 ± 26 mg/dl, further out of 10 renal failure cases 8 cases had overt albuminuria (> 30 mg/dl), 2 cases had microalbuminuria (1-30 mg/dl). This is similar to observation made earlier by Grauer (2007), who indicated that albumin in the range of 1-30 mg/dl is microalbuminuria and > 30 mg/dl overt albuminuria. Microalbuminuria could be encountered in diseases like leptospirosis, tick borne diseases, diabetes mellitus, Cushing's disease, hyperthyroidism, hypertension and some neoplasm as indicated by Cleland (2004). It can also be encountered in conditions including infectious, neoplastic, metabolic and cardiovascular diseases as indicated by Whittemore *et al.* (2003). Out of 10 healthy animals that is in group I dogs, 4 cases had albumin level in urine <1 mg/dl and 6 cases had 1-30 mg/dl (microalbuminuria). However workers like Jensen *et al.* (2001b) and Jensen *et al.* (2003) have indicated microalbuminuria in 19 per cent and 25 per cent respectively in healthy animals. Based on these observations in the presence study it can be construed that estimation of microalbuminuria alone is not a true indicator of renal damage in view of the fact that microalbuminuria have been observed in healthy animals.

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Reproductive Traits of Osmanabadi Goats in the Karnataka Maharashtra Border Region

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ABSTRACT

The reproductive parameters like age at puberty, body weight at puberty, gestation period, age at kidding, post partum anoestrus period, kidding interval, twinning and triplets ability of osmanabadi goats were studied and found to be 349.8±6.9 days, 17.45±0.23 kgs, 152.24±0.24 days, 494.4±8.1 days 67.34±6.31 days, 232.62±5.45 days, 11.99% and 0.75% subsequently. Breeding season was found to be about 4 months, commencing from the month of June and extending up to September and the kidding season was extending from November to February.

Key words: Reproductive traits, Osmanabadi goat

In India over 54 millions goats are slaughtered every year with an average meat yield of 9.63 kg per goat. The annual population growth of goat has been 0.22%. The goat population has increased with an annual growth rate of 0.6% in spite of 38% annual slaughter rate and approximately 15% mortality. The rate in increase in goat population during the last five decades has been the highest among all ruminants. Among the states west Bengal has the largest goat population of 18.77 million, followed by Rajasthan (16.80 million), Uttar Pradesh (12.94 million), Maharashtra (10.68 million), Bihar (9.49 million) and Karnataka (4.84 million) according to Livestock census India, 2003

Osmanabadi goat is an important indigenous breed of the south western part of India as per the Livestock census carried out in 2007. Its estimated population is about 1.32 millions and is extremely popular for its delicate meat. However, many of its reproductive traits are still to be documented. It was therefore the objectives of the present study to establish the reproductive traits of Osmanabadi goats and to make an effort to improve the reproductive efficiency.

MATERIALS & METHOD

The data of various reproductive parameters of Osmanabadi goats were generated from records maintained at Tuljapur and Ambajogai goat farms located at the border area of Maharashtra and Karnataka state during the year 2005 to 2007. The reproductive health records of 50 does at each farm was selected and screened and clubbed together and combined data was used to arrive the normal reproductive parameter of the Osmanabadi does. The normal reproductive parameters analysed were age at puberty, weight at puberty, age at first kidding, gestation period, postpartum anoestrus period, inter-kidding period, frequency of single and multiple births, frequency of ovarian cyclic activity during the different months of the year and distribution of kiddings during different month of the year. Completely Randomized Design had been used for the statistical analysis (Rangswamy, 2000). The comparison of proportions was estimated by chi-square test (Snedecor and Cochran 1968).

RESULTS AND DISCUSSION

The reproductive parameters studied in the Osmanabdi goats reared in the border area of the Karnataka and Maharashtra state were presented in Table.

Age at puberty: the mean age at puberty was recorded as 349.8±6.9 days and ranged between 180-510 days. Kamble *et al.* (2009) reported similar findings 335.3±13.0 days while Lawer *et al.* (2008) reported 219.34±0.72 days pubertal age in Osmanabadi goats. Nevertheless the review of available literature suggests that the puberty is a breed dependent trait.

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Table: Reproductive traits of Osmanabadi goats (n=100)

Sr. No.	Trait	Mean with SE	Range
1.	Age at puberty (Days)	349.8 ± 6.9	180-510
2.	Weight at puberty (Kgs.)	17.45 ± 0.23	14.5-20.5
3.	Gestation period (Days)	152.24 ± 0.24	137-158
4.	Age at first kidding (Days)	494.4 ± 8.1	330-650
5.	Postpartum anoestrus period (Days)	67.24 ± 3.61	29-120
6.	Interkidding period (Days)	232.62 ± 5.45	181-310
7.	Frequency of single and multiple births	Type of birth	Obser-vations
		Single	233 (267) (87.27%)
		Twins	32 (267) (11.99%)
		Triplets	02 (267) (0.75%)

Weight at puberty: In the present study puberty in Osmanabadi goats occurred when the does attained an average body weight of 17.45±0.23 kg. Smith (1997) stated that, Angora goats should weigh 32-41 kgs before being bred and recommended that breeding should be delayed until the animal has attained 60% or more of its adult body weight. It was also evident from the study that the age at puberty ranged between 6-17 months and it is highly probable that Osmanabadi goats attaining puberty at an early age had a better growth rate resulting in a better body weight cumulating in the onset of puberty.

Gestation period: The average gestation period in Osmanabdi goats was determined as 152.24±0.24 days and it ranged from 137 to 158 days. The average duration of gestation in goats is generally reported as 147-155 days (Asdell, 1929).

Age at first kidding: The first kidding in Osmanabadi goats occurred as early 330 days in some does and as late as 650 days in few others. The mean age at first kidding was determined as 494.4±8.1 days similar to the reports in other breeds (Rao and Pattnayak, 2007).

Post partum anoestrus period: The average duration of 67.34±6.31 days was recorded as postpartum anoestrus period in the present study. Reasonably close values are recorded in native Katjang goats (92 days) by Devendra, (1962). However, considerably lengthier postpartum anoestrus period (120.70-137.92 days) was recorded in Teddy goats (Azhar *et. al.*,1992) and local Assam goats (Das *et. al.*, 2004).

Inter-kidding period: The mean kidding interval in Osmanabadi Does was recorded as 232.62±5.45 days and ranged between 181 to 310 days. A similar kidding interval has been reported by Markendeya and Devanagare (1997) in Osmanabadi goats. Mabari goats also appear to have a kidding interval similar to Osmanabadi does (Raja and Mukundan, 1977). It was observed in the present study that the average postpartum anoestrus period in osmanabadi goats was only 67 days and it perhaps was the major factor for Osmanabadi does to have kidding interval of around 232 days.

Frequency of single and multiple births: In Osmanabadi does, a majority of kidding resulted in single births (87.27%) and with only 12.73 percent of multiple births. Sahare *et al.*(2009) also recorded a similar twinning ability in Osmanabadi goat under farm conditions. The results of present study suggested that the Osmanabadi does are not a breed known for its high fecundity.

Breeding activity in Osmanabadi goats during different months of the year: Osmanabadi does appear to have distinct breeding season of about four months commencing in the month of June and extending up to September. During these months 77.19 percent does were observed to exhibit oestrus activities. Wani *et al*. (1981) reported that more than 50 percent of oestrus activity in Jamunapari goats was observed in the month of June. Similarly Misra *et al*. (1983) reported the breeding season of Indian goats to be from June to October. The actual time of onset and termination of cyclic activity varies with several factors, including genetic background, latitude and presence of male (Bretzlaff, 1997). In these areas cyclicity is related more to rainfall and nutrition.

Distribution of kiddings in Osmanabadi goats during different months of the year: In the present study found that 69.29 percent Osmanabadi doe's kidding occurred between the month of

November to February as reported in Beetal goat (Bhadulu and Prasad, 1987) and Jamunapari goats (Wani *et. al.,* 1980).

CONCLUSION

Osmanabadi goats reared in the Maharashtra Karnataka border region had been analysed with reproductive parameters and found that, the female kids faced puberty at the age of 349.8±6.9 days with 17.45±0.23 kgs body weight. The average gestation period found was 152.24±0.24 days. The mean age at first kidding was found to be 494.4±8.1days. The average duration of post partum anoestrus was 67.34±6.31 days which was responsible for short inter-kidding length which shows high profile reproductive efficiency. The mean kidding interval recorded as 232.62±5.45 days. Majority of kidding resulted in single births (87.27%) and with only 12.73 percent of multiple births. Breeding season was observed from the month of June to September as a major while kidding season was observed between the months of November to February as a major.

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Reproductive Traits of Kenguri Ewes

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ABSTRACT

The present study on reproductive traits of Kenguri sheep was carried out at the veterinary college, Bidar, KVAFSU campus. Reproductive traits like birth weight, age at puberty, weight at puberty, oestrus duration, oestrus cycle length, breeding season, gestation period, lambing period and lambing interval were studied and found to be 2.26kg, 300 days, 25.5 kg, 18-26 hrs, 17 days, June to October, 148 days, November to December and January and 215 days subsequently.

Key words: Kenguri sheep, reproductive traits.

India is reach source of diverse ovine germplasm with 74 million sheep, which constitutes as 6.8% of world population (FAOSTAT 2010). There are 60 sheep breeds in India including well recognized, lesser known and some wild species. Karnataka is third largest sheep rearing state of India with 9.5 million sheep population (BAHS 2010). Kenguri is purely mutton purpose breed of sheep and also known as Tenguri (Tenguri the name of coat colour, "Teng" meaning coconut).

Reproductive efficiency affects the overall productivity as well as net returns of the sheep rearing. Moaeen-ud-Din *et al.* (2008) stated that reproductive efficiency of goats can be established based on parameters such as number of live born kids, mass of kids at birth and weaning, kidding interval and duration of reproductive cycle. Whereas Song *et al.* (2006) stated that reproductive efficiency of goats is determined by age of goats at first kidding, kidding interval, type of birth, litter size and weight of kids at birth and weaning. There is paucity of literature on the reproductive performance of the Kenguri ewes. Hence the present study was conducted to record the reproductive parameters of Kenguri sheep.

MATERIALS AND METHODS

Total of 50 Kenguri sheep maintained at Veterinary College, Bidar were studied for their reproductive traits. Animals were observed for birth weight, age at puberty, weight at puberty, oestrus, oestrus cycle, breeding season, gestation period and interlambing period and observations were documented. Animals maintained at University farm with all scientific managemental practices were carefully studied to record proper reproductive data. Observations were tabulated and interpretated.

RESULTS AND DISCUSSION

Reproductive parameters were recorded in Kenguri ewes and analysed. Average birth weight of Kenguri lamb was found to be 2.26±0.04 with the range of 1.90 to 2.60 kgs (n=37). Birth weight of Marwari sheep lambs was 2 to 3 kgs irrespective of sex (Verma *et. al.,* 2005), whereas birth weight in Mecheri sheep was observed as 2.18 to 2.30 kgs (Thiruvenkadan *et. al.*,2008).

Age at puberty of female lamb was observed to be 300.6±3.52 days with the range of 280 to 328 (n=20) age at puberty in Marwari sheep documented by Verma *et al*. (2005) was 18-24 months where as Rajanna et al. (2012) found it to be 610.00±3.81 days in Nellore ewes. Average body weight at pubertal age was found to be 25.5±0.40 kg with the range of 22 to 29 kgs (n=20).

Oestrus signs were observed in 46 Kenguri ewes. Predominantly external signs were recorded as swollen vulva and a transparent thick discharge at the vulval opening. In the estrus behaviour, ewes which were in oestrus were found to be isolated from the flock. Mamming (vocal sound) was observed frequently to attract the male and frequent

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urination was evident. Ewes which were in oestrus found restless and showed reduced feed intake. Upon teasing by ram, ewes were static and accepting the ram to get mounted. Teasing and mounting by ram was observed throughout the day of oestrus with interval of half to one hour. Oestrus signs and acceptance of male was found up to 18-26 hrs. The oestrus duration in Marwari sheep was 24 hrs as reported by Verma *et al.* (2005).

The average length of oestrus cycle was observed to be 17 days (16-19) (n=10). Major breeding activities were observed in month of June and July (n=30). At the same time minor breeding activities were also observed in the months of August to October. The average gestation period was found to be 148.97±0.21 days with the range of 146 to 151 days in Kenguri ewes (n=37).

The peak lambings were observed in November and December months and least lambings were observed in January month in the present study. The lambing period in Mecheri sheep was reported between September and February months by Thiruvenkadan *et al.* (2008).

Average lambing interval in Kenguri sheep was as 215.25±5.24 days with the range of 186-265 days (n=20). The lambing interval in Sangamneri strain of Deccani ewes was reported 256.40±4.63 days by Mandakmale, (2013) but 292.58±2.56 days in Telangana region by Rajanna et *al.* (2013). Verma *et al.* (2005) found 300 to 365 days lambing interval in Marwari sheep.

CONCLUSION

In the present study, reproductive traits have been recorded viz. Birth weight of lamb (2.26 kg), age at puberty (300 days), weight at puberty of female lamb (25.5 kg), oestrus duration (18-26 hrs), oestrus cycle length (17 days), breeding season (June to October), gestation period (148 days), lambing period (November to January) and lambing interval (215 days). Present paper puts on record important information on reproductive record of Kenguri ewes and it is necessary to record field data regarding the similar reproductive parameters.

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Development of Single Serum Dilution ELISA and Flow Through Assay Using Chicken Anaemia Virus VP1 Recombinant Antigen*

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ABSTRACT

The antibody status of breeder flock is important in assessing the level of protection conferred on hatchlings through maternal antibodies. The single serum dilution ELISA (SSD ELISA) developed is statistically validated and reduces the cost of reagents and saves time. The chicken anaemia virus (CAV) VP1 ORF3 recombinant clone was constructed in pPROEXHTb plasmid and expressed in DH5α *E.coli*. The recombinant plasmid was analyzed with regard to size, PCR amplification, sequencing of PCR product, restriction enzyme digestion using *Sal* I and *Xho* I enzymes and immuno blotting. A positive negative threshold (PNT) line was developed using 10 negative serum samples. The formula for prediction of titre was developed using 51 serum samples by subtraction method. The correlation coefficient at 1:1000 dilution of serum is 0.90 and other constants *viz*., slope and intercept were 0.06 and 0.106 respectively. With developed prediction formula, 302 serum samples were screened for the presence of CAV antibodies at 1: 1000 dilution of serum. A flow through assay was developed for the detection of recombinant viral antigen and the assay was able to detect 3 µg/µl of CAV antigen.

Key words: CAV – Recombinant Antigen – SSD ELISA – Flow through assay

Chicken anaemia virus (CAV) was first isolated from the contaminated Marek's disease vaccine (Yuasa *et al*., 1979), belonging to family *Circoviridae*, genus *Gyrovirus* and an emerging viral pathogen of poultry. Chicken anaemia virus causes disease in flocks between 2-4 weeks of age. In these infected flocks, growth is retarded and mortality is generally between 10-20% (Engstorm, 1988; Chettle *et al*., 1989a; Brentano *et al*., 1991 and Connor *et al*., 1991). This immunosuppressive viral disease brings about loss to the farming community directly or indirectly. The direct losses can be attributed to the mortality which in turn depends on the age and breed of the birds, dose of inoculum and presence or absence of passive immunity. Indirect losses are due to the acquired immunodeficiency, impaired growth or interaction following co-infection. The virulence of Marek's disease virus (MDV) is enhanced by CAV and the pathogenicity of CAV is increased by dual infection with IBDV. The immunosuppression caused by CAV and / or IBDV lead to the infection of hydropericardium syndrome virus (HPSV), which is sometimes considered as opportunistic pathogen (van den Berg, 1995). But, chicks develop resistance to experimentally induced disease due to CAV as age advances. This age resistance may be due to ability of the bird to develop humoral antibody (McNulty, 1991) and lack of specific target T cell – immature thymocytes. As a consequence of CAV infection, average weight per bird was lowered by 3.3% and average percentage mortality was more than 2% higher than that of unaffected flocks (McIlroy *et al*., 1992). This infection can be controlled by transferring maternal antibodies to hatchlings, so that conferring resistance to CAV till bird develops age- resistance. Hence, a serological assay which gives valid

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information regarding breeder flock immune status helps to develop hyperimmunization schedule and to transfer good quality maternal antibodies. The indirect enzyme linked immunosorbent assay (ELISA) helps in the determination of antibody titres and such an assay if performed at single serum dilution, saves reagents and time instead of performing the assay at different dilutions and assessing the titre.

MATERIALS AND METHODS

The chicken anaemia virus VP1 protein clones obtained from the Department of Animal biotechnology, Madras Veterinary College, Chennai were used for the recombinant antigen production. The clones received from department of Biotechnology were further confirmed for presence of CAV gene by analyzing plasmid profile, PCR amplification, restriction enzyme digestion and sequence analysis. The VP1 clones of CAV gene was constructed in pPRO EX HT b vector and expressed in *E.coli* DH5α by inducing the cultures with isopropylthio-β-D-galactoside (IPTG) (1mM concentration), after attaining approximately an OD of 0.4 to 0.6 at 560 nm in Luria-Bertani (LB) broth. Initially at hourly intervals the aliquots from LB broth were collected up to 5 hours and the aliquots were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for the expression of proteins. The expressed VP1 protein was purified by using the Ni-CL (Nickel) agarose column (M/s Bangalore Genei, Bangalore; No. PC137). The expressed VP1 protein of CAV was produced in bulk in LB broth and the cell lysate was suspended in PBS (pH 7.2). The lysate was subjected to sonication and again centrifuged at 5000 rpm for 30 min. The supernatant was collected and subjected for purification in above column. For the purification of VP1 protein linear gradient of buffer from pH 5.8 to 3.8 was used and 1 ml fractions were collected. The fractions were analyzed by PAGE for molecular weight and the fractions showing the suitable molecular weight were pooled, concentrated, protein content was estimated and stored in -60^{o}C for further use as antigen. The recombinant antigen was analyzed for immunodominant protein by Western blot (Burnette, 1981). The Western blot analysis of CAV VP1 recombinant protein was performed using CAV specific antiserum (CR Laboratories, USA). Later, for the detection of antibodies to CAV an indirect ELISA was followed with modifications (Otaki *et al*., 1991).

Estimation of PNT line: The positive negative threshold line to find out the titre was carried out as per the procedure (Snyder *et al*., 1983). For construction of PNT line ten serum samples, which were negative by dot ELISA, were selected**.** Serial dilutions of the ten sera were made *viz*., 500, 1000, 2000, 5000, 10000, 50000 and 100,000 and indirect ELISA was performed. The resultant OD values were plotted on Y-axis against dilutions in X-axis. The resultant line is referred as PNT line and used for finding out the observed titre (OT).

Estimation of observed titre (OT): The OT of 51 serum samples was calculated using the subtraction method (OD value for sample – OD value of conjugate control) as specified (Snyder *et al*., 1983) using the PNT line. The point where the sample line cuts the PNT line is taken as titre of the sample.

Development of titre calculation formula: The formula to find out the titre from single serum dilution instead of serial logarithmic dilution was developed by applying the principle of linear regression. The procedure described briefly, the OD values obtained for every logarithmic dilution was compared with OT and correlation coefficient was obtained. The dilution that gave maximum positive correlation was selected for predicting the titre from that dilution. The other parameters like slope and intercept were arrived by plotting a scatter chart with OD values in Y-axis and OT in X-axis. The linear regression formula **Y=ax+b** is reversed as **x=(y-b)/a** to find out the log titre and antilog of titre is referred as predicted titre of the sample.

Single serum dilution: The log ELISA titres were arrived by applying the formula. The log10 titre = (Corrected absorbance + 0.106)/ 0.06 and the titre = antilog (log10 titre).

Flow through assay for detection of VP1 protein: After production of hyperimmune serum for VP1 recombinant protein in rabbits, flow through assay for the detection of CAV antigen was performed as

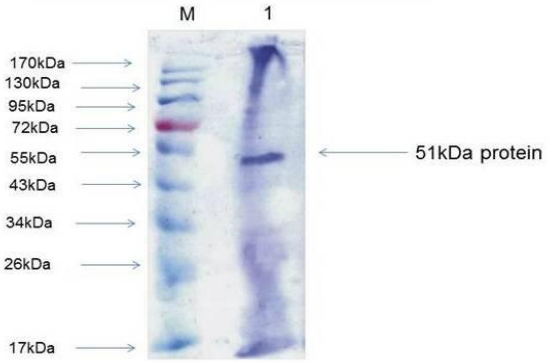
per the method described with modifications (Wang *et al*., 2005). The cellulose acetate membrane (mdi, Ambala Cantt) was placed above the absorbent pads in a flow through module. One µl (3 µg / µl) of CAV recombinant antigen was placed in the middle and 1 µl of rabbit serum was placed in a corner which acted as control. The membrane was dried in an incubator at 37^{o}C for 1 hour.

The membrane was washed twice with 200 µl of wash buffer and allowed to be absorbed through the membrane. Then, 200 µl of 1:10 diluted anti rabbit serum in wash buffer was added and allowed to be absorbed through the membrane. The membrane was washed again twice with wash buffer. Followed by 200 µl of wash buffer diluted (1:2) protein A colloidal gold conjugate was added and allowed to be absorbed through the membrane. The membrane was washed again twice with wash buffer and appearance of pink color dot indicated presence of antigen.

RESULTS AND DISCUSSION

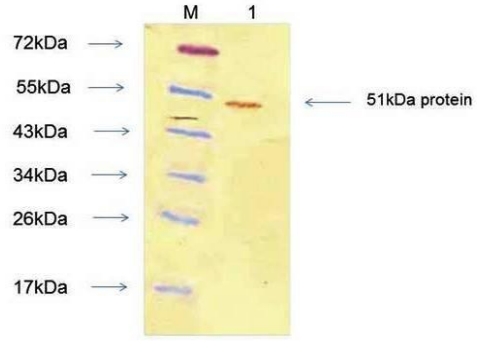
The extracted plasmid DNA from the recombinant clones was approximately 6 kbp in size which comprises pPROEX HT b vector with a size of approximately 4779 bp and insert of CAV –VP1 amplicon of approximately 1360 bp size. The pPRO EX HT prokaryotic expression system was designed for the expression of foreign protein in *E.coli*. The protein was expressed as a fusion protein to 6-histidine sequence for affinity purification. The recombinant clone was made expressed by inducing with IPTG at a concentration of 1mM and observed to be maximum at 4-5 hours after induction. The expressed recombinant protein of CAV VP1 was further purified by using Ni-CL agarose column and by linear gradient pH elution from 5.8 to 3.8. The maximum elution of purified protein took place with pH 5.8 of elution buffer and all the fractions at this pH were pooled and concentrated to make a final volume of one ml. The purified protein was analyzed by SDS-PAGE (Fig.1) which revealed 51 kDa band and these findings are in agreement with other workers (Todd *et* al., 1990). The Western blot analysis of CAV VP1 recombinant protein blotted on nitrocellulose membrane, after reacting with CAV antiserum showed a single band with a size of 51kDa (Fig. 2). Similar findings with CAV VP1 gene recombinant protein expressed in prokaryotic system were reported (Noteborn *et al*., 1992; Todd *et al*., 1990 and Pallister *et al*., 1994). In the present study, the *E.coli* cells growth after attaining an OD of approximately 0.4 to 0.6 at 560 nm was induced with 1 mM IPTG. This is in agreement with other workers (Pallister *et al*., 1994 and Dantas *et al*., 2007). The expressed protein after induction was analyzed by SDS-PAGE. The region of interest was the largest ORF-3 which coded for a potential protein of 51 kDa. The recombinant protein was expressed as a fusion protein to 6-histidine sequence for affinity purification. As the recombinant protein was having the tag of histidine, that could be purified alone by using affinity purification. The Ni-CL agarose columns purified the recombinant proteins effectively. In the present study, the recombinant protein was eluted serially from range of buffer pH from 5.8, 4.8 and 3.8. But, maximum protein was eluted at pH 5.8 and scanty at 4.8. This was confirmed by SDS-PAGE analysis of fractions collected at different pH. This expression of proteins is a useful method for obtaining proteins in bulk that can be used further in diagnostic laboratories. This recombinant proteins production reduces the risk of handling organisms *in vitro*. Some organisms may revert to virulence or can change the virulence pattern while handling in the laboratories. Some organisms cannot be cultivated *in vitro* due to many reasons. One of such problem faced during this present study was difficulty in getting the chicken lymphoblastoid cell line (MSB1) that allows the growth of CAV. This cell line was difficult to maintain and not available in India. This made the workers difficult to work with CAV. Unlike the native virus, the recombinant proteins will not react nonspecifically in serological assays. The PCR product was purified and used for sequence analysis. The CAV isolate used for this study was named as MIBTANUVAS and the NCBI, Gen bank accession number obtained was EU 661362. The PNT line was constructed using ten serum samples which are negative for CAV antibodies by dot ELISA (Fig.3).

Fig. 1
Protein profile of purified recombinant protein



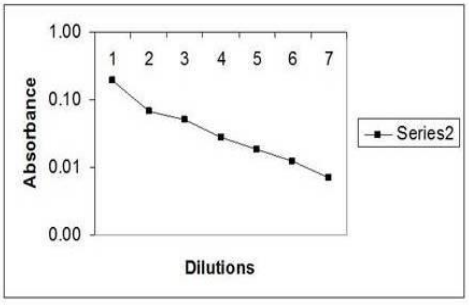
Lane M : Molecular weight marker
Lane 1 : Purified recombinant protein

Fig. 2
Western blot analysis of recombinant VP1 protein with CAV hyperimmune serum



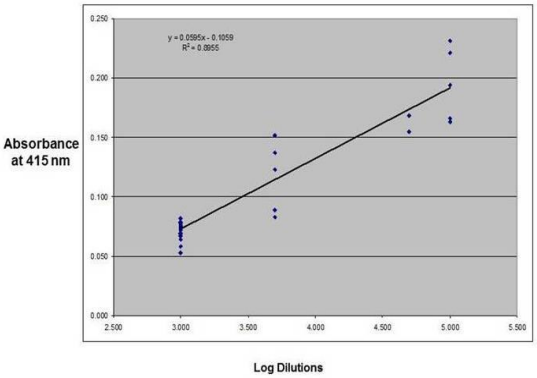
Lane M : Molecular weight marker
Lane 1 : Purified recombinant protein

Fig. 3 Positive Negative threshold line (PNT) line for CAV



Single serum dilution: The dynamic working range with regard to antigen and conjugate was determined by checker board titration. By this titration, coating antigen of CAV was found to be 7.5 ng / µl and the conjugate was 1 in 3000. By PNT line the correlation coefficient at 1: 1000 was 0.90, and which was more than that of all other dilutions. Hence, 1:1000 was preferred to predict the titre. The other two constants required to predict titres from serum dilution of 1:1000 *viz*., slope (A) and intercept (B) were estimated by drawing a scatter chart (Fig.4). The slope and intercept were 0.06 and 0.106 respectively. For prediction of titres formula was developed using 51 serum samples. After developing the prediction formula, 302 serum samples were screened for the presence of CAV antibodies at 1: 1000 dilution of serum. In the present study, an indirect ELISA to detect the CAV antibodies was standardized. In this assay recombinant protein was used as coating antigen. The purified recombinant antigen did not give any non-specific reaction in plate ELISA as the purification of recombinant protein using nickel column affinity chromatography had allowed only elution of the recombinant protein as this was having His tag and enabled to avoid the non-specific reactions. The virus isolation is possible, but usually not recommended as it is time-consuming and expensive (McNulty, 1991). Although the virus neutralization test (VNT) is more sensitive than other tests, it is cumbersome and it cannot be performed on large numbers of samples. For the first time single serum dilution ELISA was developed and standardized using recombinant protein as antigen. The developed ELISA reduced the cost of reagents, time and interpretation of results was easy (Snyder *et al*., 1983). The single serum dilution ELISA was highly useful for screening breeder flock samples which helped in assessment of flock status and there by the vertical transmission of the CAV infection could be checked.

Fig. 4 Scatter chart for prediction of titre using indirect ELISA for CAV



Flow through assay for VP1 protein detection: For the first time flow through assay was used to detect the CAV antigen. This assay was able to detect 3 µg / µl of purified recombinant protein (Fig. 5). This assay highly useful to detect CAV antigen, within no time and can be performed at farm premises. The result can be interpreted very easily without any technical expertise and equipment.

Fig.5
Flow through assay for CAV antigen detection

Positive sample showing dot development
With CAV antigen (dot in the centre)
And control antigen (dot in the periphery)

Negative sample showing dot development only
with control antigen (dot in the periphery)

CONCLUSIONS

The single serum dilution ELISA was standardized using recombinant antigen of CAV VP1. The PNT baseline was constructed using different field serum samples and the formula to predict the titre from single serum dilution was developed. The single serum dilution ELISA for CAV found to be 1:1000. By using developed SSD ELISA, 42 serum samples can be screened at a time. Flow through assay for the detection of recombinant viral antigen was developed. This is an ideal pen-side test for the detection of antigen. This assay is easy to perform and not requiring any sophisticated instruments. By using this test 3 µg/µl concentration of recombinant antigen could be detected.

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Standardization of Single Serum Dilution ELISA and Flow through Assay for Hydropericardium Syndrome Virus in Poultry

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ABSTRACT

The hydropericardium syndrome virus (HPSV) antigen after purification was used as coating antigen for single serum dilution Enzyme Linked Immunosorbent Assay (SS ELISA) and flow through assay (FTA). The HPSV was purified and characterized with regard to viral proteins. The single serum dilution ELISA was developed for HPSV at 1:1000 dilution of serum and for prediction of titres formula was evolved based on testing 77 serum samples. The other two constants required to predict titres from serum dilution of 1:1000 *viz*., slope (A) and intercept (B) were 0.041 and 0.027 respectively. After development of formula for prediction of titre, 302 serum samples were screened and the observed and predicted titres were in agreement. The flow through assay was developed for the detection of viral antigen and this could detect 200 ng/ µl of purified viral protein. The developed SS ELISA saves reagents by diluting serum samples at a particular dilution and 42 serum samples can be screened in a single plate. The FTA can be employed at the farm level, without much expertise and equipment for detection of virus.

Key words: *HPSV – Purification – SS ELISA – Flow through assay*

Hydropericardium syndrome virus is an adenovirus infection causing "Angara disease" and an emerging poultry syndrome with high mortality. It was first reported in broilers at Angara Goth, Karachi of Pakistan (Khawaja *et al*., 1988; Anjum *et al*., 1989). The etiological agent of hydropericardium syndrome has been identified as a group I aviadenovirus and serotype 4 (Chandra *et al*., 1997). The Indian isolates of inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) of poultry from different geographical parts of the country were typed as serotype 4 (Jadhao *et al*., 1997). In India, the disease was first noticed in poultry farms of Jammu in 1994, and the condition was known as "Leechi disease" because of the heart lesions resembling skinned Leechi fruit. Subsequently, the condition was reported from Punjab and Delhi (Sreenivas Gowda and Satyanarayana, 1994), West Bengal (Bhowmik, 1996), Himachal Pradesh (Asrani *et al*., 1997) and Uttar Pradesh (Ravikumar *et al*., 1997).

The disease commonly occurs among broilers (Shane, 1996) and broiler breeders (Anjum *et al*., 1989; Toshiaki Abe *et al*., 1998). However, sporadic incidences of hydropericardium syndrome have also been reported among layer poultry (Anjum *et al*., 1989; Qureshi, 1997; Shukla *et al*., 1999). Moreover, adenoviruses are ubiquitous in nature. The different species and strains of adenoviruses, can rapidly be opportunistic when the health of the bird is compromised as in co-infection with other pathogens like chicken anaemia virus (CAV) or infectious bursal disease virus (IBDV). The immunosuppression caused by CAV and / or IBDV lead to the infection of hydropericardium syndrome virus (HPSV), which is sometimes considered as opportunistic pathogen (van den Berg, 1995). The maternally derived protection of hatchlings in the form of antibodies is very important till they develop resistance. If the protection conferred by maternal antibodies is good, the vaccination programme can be

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rescheduled, or sometimes in case of good maternal antibody level, one dose of the vaccine can be avoided. This helps both the bird and the farmer, as the bird is relieved from the vaccination stress and at the same time the farmer can avoid expenditure on vaccine. In this direction, to monitor the immune status of breeder flocks, a SS ELISA for antibody titre and FTA for antigen detection were developed.

MATERIAL AND METHODS

The HPS virus used in the present study was an isolate from a field outbreak in the Coimabatore area of Tamil Nadu. The hydropericardium syndrome virus antigen was prepared from liver tissues of affected chicken (Roy *et al*., 2001). Fifty and twenty five per cent liver tissues were prepared in PBS (pH 7.4) and thoroughly mixed with chloroform (4:1). After 15 minutes, the mixtures were centrifuged and clear supernatants were used for further work. The presence of virus was confirmed by agar gel immunodiffusion and counter immunoelectrophoresis (Roy *et al*., 2001) with antiserum obtained from Central University Laboratory, Madhavaram, TANUVAS, Chennai.

Isolation in cell culture: The liver samples which were reacted positive by both AGID and CIE were used for the virus isolation after treating with antibiotics *viz*., Penicillin -10,000 units / ml, Streptomycin - 10 mg/ml and Gentamicin - 250 µg/ml and kept at 37^0C for 2 hrs. Then antibiotic treated material was filtered through 0.22 µm filter and aliquoted into 0.5 ml and stored at -70^0C. For the isolation of HPSV in cell culture, chicken embryo liver (CEL) cultures were used as described with modifications (Frank and Sheila, 1975). The virus was adapted to CEL cultures by four passages and by using fifteen per cent foetal calf serum in the growth medium.

Purification and concentration: The CEL adapted HPSV was partially purified (Ahmad and Burgess, 2001). A 30% ammonium sulphate solution (v/v) was used to precipitate the antigen from infected cell culture supernatant. The precipitate was resuspended in PBS and lipoproteins were removed with 2 parts of chloroform to 1 part of antigen. The suspension was subjected to ultracentrifugation at 150, 000 x g for 4 hours and the resultant pellet was resuspended in PBS and used for further studies.

Characterization of viral proteins: The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for HPSV proteins was performed by using 12.5% acrylamide gel (Laemmli, 1970). After purification and characterization of virus, hyperimmune serum was produced in chickens and rabbits as per standard immunization procedure.

Enzyme linked immunosorbent assay for antibodies: The ELISA for the detection of antibodies to HPSV was performed as per Dawson *et al*., (1979) with modifications. Prior to performing the ELISA, checker board titration was carried out to arrive at the optimum dilutions of antigen and conjugate (Sigma Cat No. A9046) (Rose *et al*., 1997). The wells in polystyrene microtitre plates were coated with 100 µl of 2.5 ng / µl concentration of stock antigen in carbonate / bicarbonate coating buffer, pH 9.6. The plates were incubated overnight at 4^0C in refrigerator. After overnight incubation, the plates were washed three times with PBS-T (Tween-20 @ 0.05% v/v) and each well was added with 100 µl of blocking buffer (PBS – 100 ml, Skim milk powder 5 g and Tween-20 5 µl) and incubated at 37^0C for one hour by keeping appropriate controls. The plates were washed with PBS-T for three times and 100 µl of each serum dilution in serum dilution buffer was added to wells and the plates were incubated for 1 hour at 37^0C. The plates were washed with PBS-T again for three times. Then, 100 µl of anitichicken HRP conjugate was added to all wells and incubated for 1 hour at 37^0C. The unreacted conjugate was washed again with PBS-T for three times and finally 100 µl of freshly prepared ABTS (2,2'-Azino-bis-3 ethyl benzthiazine 6-sulfonic acid) and hydrogen peroxide substrate prepared in citrate substrate buffer was added in subdued light. The plates were incubated in the dark for 15- 30 minutes at 37^0C for colour development. The colour

reaction was stopped by adding 100 µl of 1% SDS in distilled water. The optical density (OD) values were recorded using an ELISA reader at 415 nm.

Estimation of positive negative threshold (PNT) value: The positive-negative threshold value was estimated to find out the titre (Snyder *et al*., 1983). In brief, ten serum samples, which were negative by AGID, were selected**.** Serial dilutions of the serum were made from 500, 1000, 2000, 5000, 10000, 50000 and 100,000. The resultant OD values were plotted on Y-axis against dilutions in X-axis. The resultant line is referred as PNT line and used for finding out the observed titre (OT).

Estimation of observed titre: The OT of 77 serum samples was calculated using the subtraction method (OD value for sample – OD value of conjugate control) (Snyder *et al*., 1983) using the PNT line. Serial dilutions of the serum samples as mentioned in PNT value estimation were carried out and ELISA was performed with these samples. The resultant OD values were plotted on Y-axis against dilutions in X-axis. The point where the sample line cuts the PNT line was taken as titre of the sample.

Estimation of titre: The formula to find out the titre from single serum dilution instead of serial logarithmic dilution was developed by applying the principle of linear regression. Briefly, The OD values obtained for every logarithmic dilution was compared with OT and correlation coefficient was obtained. The dilution that resulted in maximum positive correlation was selected for predicting the titre from that dilution. The other parameters like slope and intercept were arrived by plotting a scatter chart with OD values in Y-axis and OT in X-axis. The linear regression formula **Y=ax+b** is reversed as **x=(y-b)/a,** to find out the log titre and antilog of titre is referred as predicted titre of the sample.

Single serum dilution: The log ELISA titres were arrived by applying the formula. Log10 titre = (Corrected absorbance + 0.0275)/ 0.041 and titre = antilog (Log10 titre).

Flow through assay for antigen detection: The flow through assay for the detection of HPS antigen was performed as per the method described (Wang *et al*., 2005) with little modifications. For the standardization of this assay, hyperimmune serum in rabbits was produced as per standard immunization procedure. The cellulose acetate membrane (M/s mdi, Ambala Cantt) was placed above the absorbant pads in a flow through module. One µl (100, 150 and 200 ng / µl) of HPSV was placed in the middle and 1 µl of rabbit serum was placed in a corner as control in three different FTA modules. The membranes were dried in an incubator at 37^0C for 1 hour. The membranes were added with 200 µl of wash buffer and allowed to be absorbed through the membrane. This was repeated once again. Then the 200 µl of wash buffer diluted (1:5, 1:10 and 1:15) antirabbit serum was added and allowed to be absorbed through the membranes. The membranes were washed again, twice with wash buffer. Then the 200 µl of wash buffer diluted (1:1, 1:2 and 1:5) protein A colloidal gold conjugate was added and allowed to be absorbed through the membrane. The membranes were washed again twice. Appearance of pink color dot indicated presence of antigen.

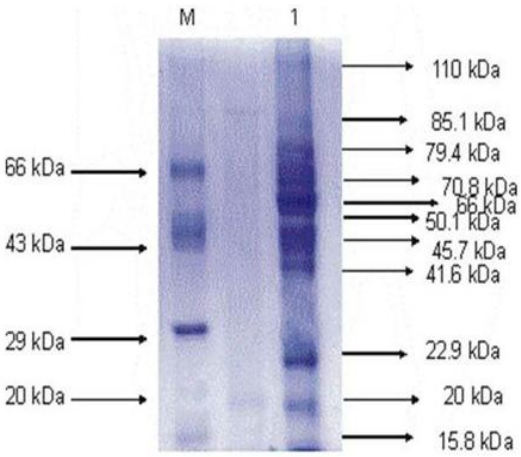
RESULTS AND DISCUSSION

Isolation in cell culture: The HPSV obtained from liver suspension was passaged four times in CEL cultures. The CPE was noticed in the first passage itself. The CPE was characterized by rounding and degeneration of cells at 48 hours post inoculation. Eventhough HPSV can be cultivated in different systems like embryonated eggs, chicken embryo kidney cells, the chicken embryo liver cells are highly sensitive to the virus and are most preferred system for the cultivation of virus. As the virus readily adapted to liver cells, there was no necessity for blind passages. In the first passage, changes like rounding of cells and degenerative changes were noticed in the monolayer. The same was reported by other workers (Rajesh Kumar *et al*., 2003; Toro *et al*., 1999).

Purification and concentration of virus: The HPSV propagated in CEL was purified by ammonium sulphate precipitation and chloroform extraction. The ammonium sulphate was removed after chloroform extraction by dialysis of precipitate against PBS (pH 7.2). A total volume of 500 ml was concentrated to get a final volume of one ml. The HPSV was concentrated by precipitating with ammonium sulphate, followed by removal of lipoproteins by chloroform. As the liver is a highly active organ and the liver cells are metabolically active, if the extraneous material other than virus is present, they will definitely affect the diagnostic tests, especially the sensitive tests like ELISA. Hence, the liver suspensions from natural cases or the liver cells from monolayer should be treated carefully to remove the unnecessary material. Initial purification of viral proteins was not satisfactory. Hence, the cell culture fluid was clarified again and subjected to ultracentrifugation. The pellet thus obtained was suspended in PBS (pH 7.2) and contained purified virus particles.

Characterization of viral proteins: The purified HPSV when subjected to protein analysis by SDS-PAGE resolved into various polypeptides of the virus. After staining the gel with Coomassie brilliant blue, the viral protein bands were visible. The HPSV resolved into 12 structural polypeptides (Fig.1) with molecular weights of 110 kDa (II - hexon), 85 kDa (III – penton base), 79 kDa (IV), 70 kDa (V – long fibre), 66 kDa (VI – short fibre), 50 kDa (VII), 45 kDa (VIII), 41 kDa (IX – core protein 1), 22 kDa (X), 20 kDa (XI – core protein 2), 15 kDa (XII) and 13 kDa (XIII). In the present study, the polypeptides of purified virus were resolved by SDS-PAGE. In 12.5% acrylamide gel, the purified virus yielded 12 polypeptides. This finding was in agreement with some of the workers (Rajesh Kumar and Rajesh Chandra, 2004). On the contrary, some workers got 8 polypeptides on resolution in 10% gel (Balamurugan *et al*., 2002). This difference in number of polypeptides might be attributed to difference in concentration of resolving gel and various other physical conditions.

Fig. 1 Protein profile of purified HPSV



Lane M – Mol.wt.Marker
Lane 1 – Viral polypeptide

Enzyme linked immunosorbent assay for antibodies: The dynamic working range with regard to antigen and conjugate was determined by checker board titration. By this the optimal concentration of HPSV coating antigen was found to be 2.5 ng / µl and the conjugate was1 in 5000 dilution. The present study evolved single serum dilution method for screening antibodies to HPSV for the first time. In case of adenoviruses and in particular HPSV, most of the studies were confined to the serotyping and classification of adenoviruses. On an average, the ELISA test can be completed in 4 hours of time on average, when compared with one week for the VNT (Dawson *et al*., 1980). This is a major advantage when screening large number of serum samples. The non - specific colour development in ELISA was a major problem during the initial standardization of system. But, by increasing the detergent concentration *i.e*., Tween-20 and by increasing the concentration of skim milk powder during initial blocking steps relieved the problem greatly. The same measures were taken up by other workers (Dawson *et al*., 1979).

The other major problem with adenoviruses is the serological cross reactivity as they are ubiquitous in nature. In VNT, the monospecific antisera discriminated among the various serotypes of virus and the ELISA showed cross reactivity. In this study, the antigen used for

ELISA was from disrupted liver cells and contained group-specific and type-specific antigens. The group-specific antigen was responsible for cross-reactions. Some workers adsorbed the sera with healthy liver tissue powder to reduce the non-specific reaction and thereby increasing the sensitivity of the assay (Dawson *et al*., 1979). The adsorption of gamma globulin preparation with liver and kidney powder of SPF chicks reduced the non-specific reaction (Saifuddin and Wilks, 1990).

Single serum dilution: The PNT line with ten serum samples was constructed (Fig.2). The correlation coefficient at 1: 1000 was 0.96, which was more than that of all other dilutions. Hence, 1:1000 was preferred to predict the titre. For prediction of titres formula was developed using 77 serum samples. The other two constants required to predict titres from serum dilution of 1:1000 *viz*., slope (A) and intercept (B) were estimated by drawing a scatter chart (Fig. 3). The slope and intercept were 0.041 and 0.027 respectively. Using these two constants, by applying the linear regression equation (y= ax+b), the formula for calculating titre was obtained which is read as follows x = (y-b) / a. The log 10 titre (x) = (Absorbance – intercept) / slope. The bar graph was drawn with observed and predicted titres of serum samples used for the construction of formula (Fig. 4). After developing the prediction formula, 302 serum samples were screened for the presence of HPSV antibodies at 1: 1000 dilution of serum.

Fig. 2 Positive Negative threshold line (PNT) line for HPSV

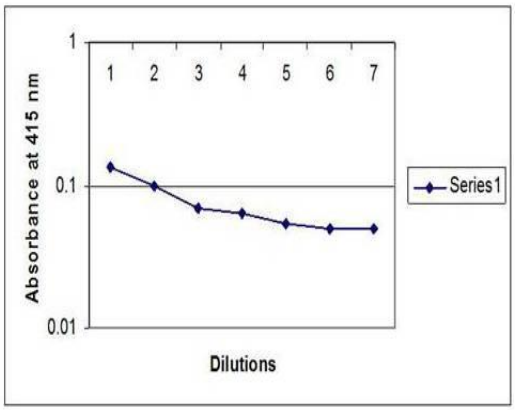


Fig. 3 Scatter chart for prediction of titre using indirect ELISA for HPSV

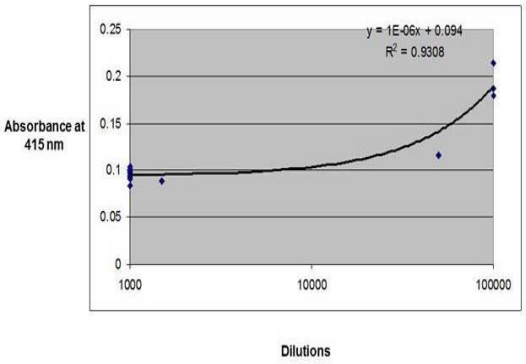
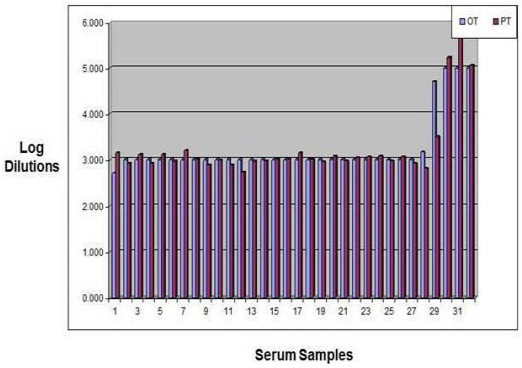
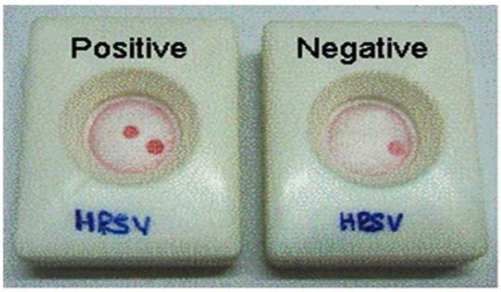


Fig. 4. Graph showing the observed and predicted titres of HPSV



Flow through assay for antigen detection: Out of different concentrations, dot with optimum intensity was observed with 200 ng / µl concentration of HPSV antigen and the dot developed in five minutes with 1:10 diluted antirabbit serum and 1:2 diluted protein A colloidal gold conjugate (Fig. 5). For the first time flow through assay was used to detect the HPSV antigen. This assay was able to detect 200 ng of purified virus.

Fig. 5 Flow through assay for HPSV antigen detection



Positive sample showing dot development With HPSV antigen (dot in the centre) And control antigen (dot in the periphery)
Negative sample showing dot development only with control antigen (dot in the periphery)

CONCLUSIONS

The single serum dilution ELISA developed in present study was highly useful for monitoring breeder flocks for antibodies to HPSV. This saves time and reagent cost and more number of samples could be screened. The vaccination of breeder flocks could be fine-tuned using this SS ELISA, to ensure maximum protection to hatchlings in the form of maternal antibodies. The FTA to detect virus antigen, can be performed at farm premises without much sophistication.

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Comparative Gross Morphology of Ruminal Papillae in Wild and Domestic Ruminants*

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ABSTRACT

A comparative gross morphological study was conducted on the ruminal papillae of wild and domestic ruminants. The density, length, width and surface enlargement factor (SEF) of the ruminal papillae in the wild (Spotted deer, Black buck and Nilgai) and domestic ruminants (buffaloes, sheep and goats) were compared. The papillae were tongue or conical shaped in black buck, nilgai and buffalo, while they were spatula or leaf shaped in spotted deer, sheep and goat. The colour of papillae was brown in black buck, buffalo and sheep while it was dark brown or black in nilgai, spotted deer and goat. The ruminal papillae were evenly distributed throughout the rumen in the spotted deer and goat whereas they were uneven in the others. The density of ruminal papilla was highest in blackbuck (87.7±3.09) and buffalo (69.3±1.79) while it was least in spotted deer (62.1±2.58) and goat (29.9±1.77). the surface enlargement factor (SEF) was highest in nilgai (29.4±4.97) and sheep (44.1±14.5), but least in blackbuck (10.1±1.67) and buffalo (9.75±0.29). Based on these morphological parameters buffalo and blackbuck had characteristics of the grazers, goat and spotted deer of browsers, whereas sheep and nilgai showed the overlapping morphology of both grazers and browsers, hence they are classified as intermediates.

Keywords: Comparative gross morphology, Rumen Papilla, Ruminants

Ruminants are classified based on their foraging behaviour as grazers, browsers or intermediates. Grazers, such as cattle, consume mostly low quality grasses while browsers such as moose and mule deer stay in the woods and eat highly nutritious twigs and shrubs. Intermediates, such as sheep, goats and white tail deer, have nutritional requirements midway between grazers and browsers. Of this group, sheep are more of a grazer, while goats and deer are browser type.

Comparing gastrointestinal characteristics of deer is one method to understand their relationship with forage quality and quantity (Hofmann, 1989; Ramzinski and Weckerly, 2007). The forestomach of deer, like other ruminants, contains a mucosal membrane that is studded with papillae. Papillae absorb volatile fatty acids that are products of microbial digestion, and their growth is stimulated by the production of these volatile fatty acids (Tamate *et al*., 1962; Hofmann, 1989). The size, density and distribution of papillae can be affected by the availability, quality and quantity of forage (Hofmann, 1988) and can be used to compare habitats and seasons as well as species, age and gender differences (Zimmerman *et al*., 2006). Research on rumen papillation of cervids has been conducted on Moose (*Alces alces*) (Hofmann and Nygren, 1992), Red Deer (*Cervus elaphus*) (Lentle *et al.*, 1996), Reindeer (*Rangifer tarandus*) (Knott *et al.*, 2005), and sympatric Mule Deer and White-tailed Deer (Zimmerman *et al.*, 2006) and Mule Deer and White-tailed Deer (Gerald and Hudson, 2008). The present study adds to the growing body of knowledge by comparing ruminal papillae characteristics of wild and domestic ruminants in India.

MATERIALS AND METHODS

The rumen from six black bucks (*Antelope cervicapra*), six nilgais (*Boselaphus tragocamelus*) and six spotted deers (*Axis axis*) were collected during postmortem at Bannerghatta Biological Park, Bengaluru, Sri Jayachamaragendra Zoological Gardens, Mysore and Lion Safari, Tyavarekoppa, Shimoga. The cause of death for all animals was ascertained before collection of the

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specimens and was found unrelated to any affections of the gastrointestinal system. Further, healthy rumen from six buffaloes (*Bubalus bubalils*), six sheep (*Ovis aries*) and six goats (*Capra hircus*) were collected after slaughter from the local slaughter house. The specimens thus obtained were subjected to different anatomical parameters.

The shape, colour and surface of papillae were observed using a stereozoom microscope (Lynx, Lawrence and Mayo). A square centimetre sample was used to determine the papillae density (No. of papillae/ square centimetre of rumen mucosa). Ten papillae were chosen randomly to measure the maximum length and width. The width was measured as the distance at midpoint of papillae using a Digimatic caliper (Mitutoyo®). The means of various parameters were calculated and used for subsequent statistical analysis. The surface enlargement factor (SEF) was then calculated according to Hofmann and Nygren, (1992).

$$SEF = \frac{(2 \text{ X Papillae Surface Area}) \text{ X } [\text{Papillae number} + \text{Base surface}]}{\text{Base surface}}$$

Papillae Surface Area = Papillae length X Papillae width

Base surface = Area of subsample (A square centimetre sample which was used to determine papillae density)

The photographs were taken with a Nikon digital camera (Nikon, MH 611 COOLPIX P5100, and Japan) attached to Lynx, Lawrence and Mayo stereozoom microscope. The statistical analysis of biometrical parameters of ruminal papillae in different species was depicted in a tabular form. The data was presented as mean±standard deviation. Anatomical terms were used in agreement with the Nomina Anatomica Veterinaria (2005).

RESULTS AND DISCUSSION

The ruminal mucosal surface of the ruminants studied was studded with many papillae giving it a 'turkey towel' appearance.

Shape of papillae: The papillae were tongue or conical shaped in blackbuck, nilgai and buffalo (Fig 1a, 1b and 1d). Spatula or leaf shaped papillae were seen in spotted deer, sheep and goat (Fig 1c, 1e and 1f). Rasha (2007) recorded a complete sequence of transitional forms of ruminal papillae under the influence of diet. These ranged from small, smooth tongue-shaped papillae in hay-fed group to large, heavily keratinized, finger-, foliate- or mushroom-shaped papillae in concentrate-fed sheep. The shape of the papillae was tongue or conical shaped in blackbuck, nilgai and buffaloe (as in the hay-fed group) while they were spatula or leaf shaped in spotted deer, sheep and goat (as in the concentrate-fed group). The results of Rasha (2007) may be indirectly correlated to hay-fed group as grazers and concentrate-fed group as browsers. The present study showed that the forage type might have affected the shape and colour of the papillae.

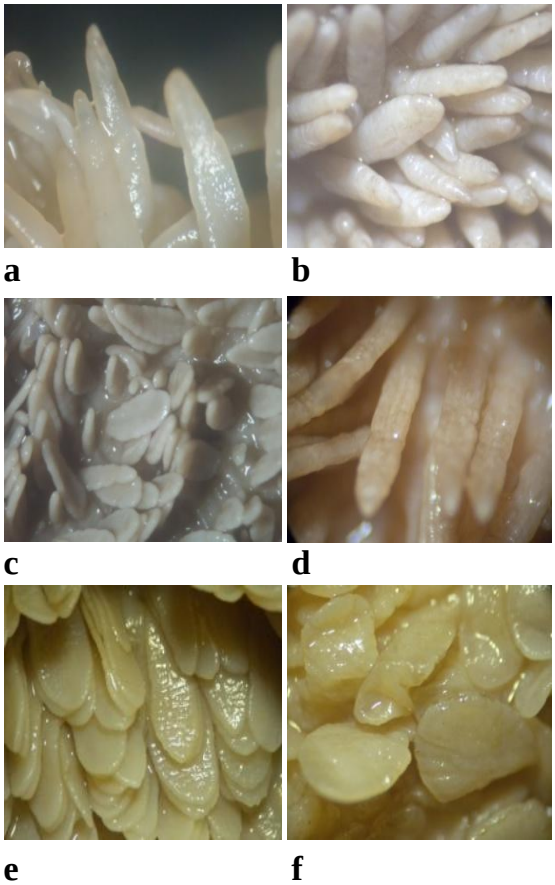

Fig 1: Stereophotograph showing ruminal papillae in a) Blackbuck b)Nilgai c) Spotted deer d) Buffalo e)Sheep and f)Goat (x 30)

In this context, the sequential changes from tongue or conical to spatula or leaf shapes might be due to forage type dependent grazer and browser ruminants respectively, which was an area that showed large alteration in the absorptive surface area of the papillae in relation to the feeding regimes as reported by Gabel *et al*. (1987). Possibly, the various forms of papillae were initially shaped by mechanical forces which acted on the mucosa, which could be from epithelium expansion on the one hand and from muscular contraction on the other hand (Wardrop, 1961). Nevertheless, subsequent development of papillae has been shown to have depended on the nature of the feed stuffs (Brownlee, 1956) whose metabolized products were short chain fatty acids (SCFA), which are mainly butyric and to a lesser extent propionic acids (Sander *et al.,* 1959).

Colour: The colour of papillae was brown in blackbuck, buffaloe and sheep while it was dark brown or black in nilgai, spotted deer and goat. The variations in colour of papillae could be due to the type of forage in ruminants. Radostits *et al*. (2007) observed that the ruminants such as cattle, sheep and goats which were fed mainly on grains had short, narrow and dark ruminal papillae. According to Ellenberger and Baum (1912); Habel (1975) and Church (1979) the ruminal epithelium of cattle was dark brown to blackish except on the margins of the pillars and the upper part of the dorsal sac where the colour gradually turned to pale brown. According to May (1964) the ruminal epithelium of sheep was brown and became paler towards and on the pillars.

Nockels *et al*. (1966) predicted that the dark brown colour of papillae appeared to be a combination of a supply of keratinized tissue, resulted from rapid growth and limited abrasion, high supply of iron, and an acid pH. Rasha (2007) also revealed that the diet changed the colour of papillae. Papillae from hay-fed sheep or sheep fed concentrate for 4 weeks had light brown colour. However, dark brown coloured papillae were observed in 6 and 12 weeks in concentrate-fed groups.

Surface of papillae: A stereozoom microscopic observations revealed that the surface of papillae was smooth in blackbuck and buffalo while it was rough with serrated borders in nilgai and spotted deer. In sheep and goat, the surface of the papillae was rough with a ridge-like appearance. These findings can be compared with the observations made by Rasha (2007) where he revealed that the surface of papillae was not smooth in hay-fed sheep but it possessed shallow grooves, which gradually became deeper and increased in number with duration of concentrate feeding (more obvious in 4-12 weeks concentrate-fed groups). These deep grooves increased the surface of papillae and offered a place for the ingesta to be settled for long time, hence, it increased the absorptive capacity of the epithelium. High magnification of these ridges and grooves revealed highly keratinized squamous cells on the surface of the epithelium.

Dimensions of papillae: The length, width and surface area of papillae was comparatively high in nilgai, spotted deer, sheep and goat as compared to black buck and buffalo. These observations were in accordance with those of Zitnan *et al*., (1999) in calf and Rasha (2007) in sheep. In ruminants, it was demonstrated that the development of papillae was stimulated by the presence of volatile fatty acids (VFA) (Sander *et al*., 1959; Sakata and Tamate, 1979). Because VFA production is also a function of diet quality, the number and size of forestomach papillae reflect the variation in it, e.g. within a species between seasons (Hofmann, 1973;Langer, 1974; Konig *et al*., 1976; Hofmann and Schnorr, 1982; Hofmann *et al*., 1988; Hofmann and Nygren,1992; Kamler, 2001), or between free-ranging and captive individuals (Hofmann and Nygren, 1992).

Although papillae served as absorptive structures, the total ruminal volume and surface area had a significant influence on nutrient transport (James *et al*., 1983), so changes in papillary size indicate a marked increase of relative rumen epithelial absorptive surface. The intake of high levels of protein and carbohydrate appeared to increase the papillary

size and density via butyrate and propionate regulation of IGF-1 production in goat (Shen *et al*., 2004) and was partially due to SCFA dependent increase in the mitotic index of the rumen epithelium of calf. However, Lentle *et al*. (1996) reported that the quality of ingested food affected the size of ruminal papillae in red deer. The results of this study clearly demonstrated the effect of the type of forage selected on the development of the ruminal papillae. The morphometrical evaluation revealed significant differences in the development of papillae between grazing and browsing type of feeding.

Density of ruminal papilla: The density of ruminal papilla was highest in blackbuck (87.7±3.09) and buffaloe (69.3±1.79) while it was least in spotted deer (62.1±2.58) and goat (29.9±1.77) (Fig. 2A and Table). According to Hofmann (1989) these data corresponded with the classification of food specialization of the studied species. The variations in density of rumen papillae observed between species corresponded with the presumption that the development of rumen papillation depended on the quality of the ingested forage-on production of volatile fatty acids (VFA) which stimulated papillary growth (Tamate *et al*., 1962 and Hofmann, 1989). The high density of papillae in blackbuck and buffalo in the preset study was in accordance with the observations of Hofmann (1973) in African antelope.

Rasha (2007) demonstrated that the decrease in the number of papillae per cm^2 mucosa was due to increased duration of concentrate feeding in sheep. But there was no indication of fusion of several papillae into one papilla. In the present study, the least density of papillae might be in animals of concentrate feed selectors (browsers). The reduction in the number might be due to increase in thickness of individual papillae, thus, accommodating fewer papillae per unit area. Tiwari and Jamdar (1970) and Aafjes (1967) demonstrated that areas of the rumen wall with large number of papillae absorbed more volatile fatty acids than do areas with few papillae. However, Lentle *et al*. (1996) reported that the quality of ingested food affected the density of rumen papillae in red deer.

Surface enlargement factor (SEF): The surface enlargement factor (SEF) was recorded highest in nilgai (29.4±4.97) and sheep (44.1±14.5), least in blackbuck (10.1±1.67) and buffalo (9.75±0.29) (Fig. 2B and Table). Josefsen *et al*. (1996) recorded the surface enlargement factor (SEF) in reindeer calves as 5.8-18.6 and reported that the papillar length and number of papillae per cm^2 had the largest influence on SEF, and these two parameters together accounted for 76-84% of the variation in SEF. The least SEF was recorded in blackbuck and buffalo which were corroborating with the observations made by Clauss *et al*. (2009) where

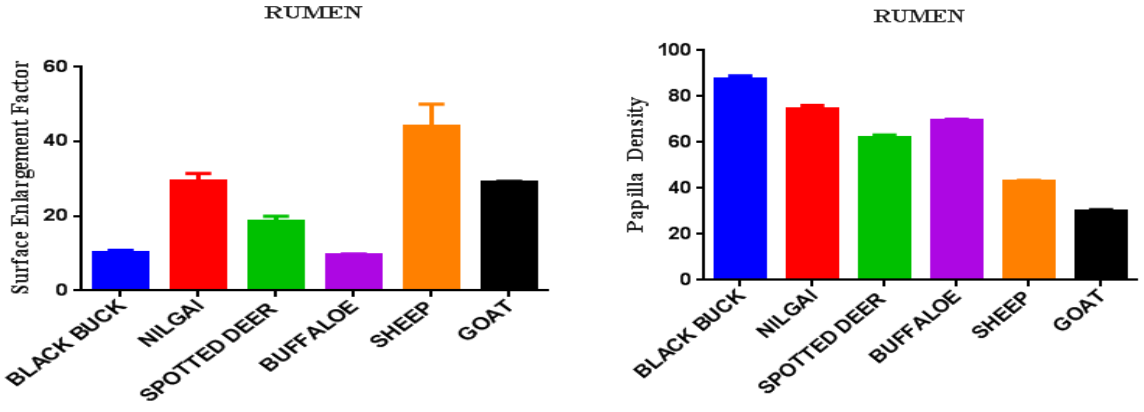

Fig 2: Bar diagram showing comparison of A) Surface enlargement factor (SEF) and B) Ruminal papillae density (Mean±SD) in different ruminants studied (Data from table 1).

Table: Gross parameters and measurements of ruminal papillae of different animals studied (Mean±SD, n=6).

Sl. No.	Parameters	Black Buck	Nilgai	Spotted Deer	Buffaloe	Sheep	Goat
1	Shape	Tongue or conical	Tongue or conical	spatula or leaf	Tongue or conical	spatula or leaf	spatula or leaf
2	Colour	brown	Dark brown	Dark brown to black	brown	brown	Dark brown
3	Surface of papillae (stereo zoom)	smooth	Rough with serrated borders	Rough with serrated borders	smooth	Rough with ridge appearance on surface	Rough with ridge appearance on surface
4	**Dimensions of papillae** Length(mm)	3.36±0.26	3.78±0.31	4.37±0.04	3.57±0.09	6.33±0.05	3.97±0.05
5	Width(mm)	0.76±0.11	2.33±0.28	1.44±0.06	0.81±0.01	2.88±0.04	2.83±0.09
6	Surface area of papillae (mm^2)	2.7±0.426	8.79±1.05	6.31±0.32	2.88±0.07	18.2±0.37	11.2±0.36
7	Density of papillae (No. of papillae/cm^2 mucosa	87.7±3.09	74.5±3.83	62.1±2.58	69.3±1.79	42.8±1.57	29.9±1.77
8	Surface enlargement factor (SEF)	10.1±1.67	29.4±4.97	18.5±3.64	9.75±0.29	44.1±14.5	29±0.96

they stated that the percentage of grass in their diet significantly affected the SEF.

Hofmann and Schnorr (1982) studied European roe deer (*Capreolus capreolus*), European red deer (*Cervus elaphus*) and the fallow deer (*Cervus dama*). In the roe deer and the red deer, but not in the fallow deer, significant correlations were observed between the factor of increase in surface area (f.i.s.a.) and the mitotic index (m.i.) could be found. Both f.i.s.a. and m.i. were highest in the largest species, the red deer, and much lower in the other two deer species. The large species had large papillae with intensive mitotic activity. Nutritional differences between these three species might have played an important role in this.

Hofmann *et al.* (1988) investigated the morphology of rumen and reticulum in roe deer (*Capreolus capreolus*) and did not find any significant differences between forest and field ecotypes. There were, however, differences in rumen papillary development, related to seasonal differences in forage quality and availability. Forest roe deer showed a wider range of the papillary surface enlargement factor than field roe deer in summer and had their optimal papillary development in autumn.

Dyce *et al.* (2002) explained that changes in the papillary prominence in wild ruminants could be due to the availability of the forage quality which was dependent upon seasonal changes. These changes were restricted in domestic ruminants whose diet was under the control of human influence to a greater degree.

CONCLUSIONS

Based on these morphological parameters of ruminal papillae in the present study, buffalo and blackbuck could be classified as grazing ruminants, goat and spotted deer represented typical characteristics of browser ruminants whereas sheep and nilgai showed the overlapping morphology of

both grazer and browsers, hence, they were classified as intermediate feeders. These parameters will help to schedule the ration (feeding schedule) for these wild animals in zoos and game sanctuaries.

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A Modified Ovsynch Protocol Improves Conception Rate in Repeat Breeder Cows*

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ABSTRACT

The objective of the present study was to compare the effectiveness of Ovsynch-48 and modified Ovsynch protocol (Ovsynch-56) on the conception rate in repeat breeder cows when initiated during the early luteal phase of the estrous cycle. Thirty three repeat breeder cows were randomly selected and divided into three groups with 11 cows in each group. Group-I (Control) was subjected to single A.I. during the observed estrus. Group-II was subjected to Ovsynch-48 and Group-III was subjected to Ovsynch-56 during the early luteal phase of the estrous cycle. The mean serum progesterone concentration did not differ significantly between Group-II and Group-III when the first GnRH was administered. Similarly, the mean serum progesterone concentration did not differ significantly between the control (Group-I) and treatment groups (Group-II, III) at the time of A.I. A conception rate of 18.18, 36.36 and 45.45 per cent was obtained for Group-I, Group-II and Group-III, respectively. Conception rate obtained between the control and treated repeat breeder cows differed significantly.

Key words: Ovsynch-56, repeat breeder cow, diestrus, conception rate

Fertility status of dairy cattle is generally decreasing. Overall estrus detection efficiency in lactating dairy cattle is low, as expression of estrus is often compromised. Consequently, undetected estrus, low AI-submission rates, and long inter-breeding intervals are the main contributors to poor reproductive efficiency. Although, failure to become pregnant is the most common reason for culling dairy cattle, pregnancy rates could be improved by increasing the AI-submission rates through increased estrus detection efficiency or timed artificial insemination (TAI). Ovsynch is a protocol based on the administration of GnRH, $PGF_{2\alpha}$ and GnRH (Pursley *et al*., 1995) to schedule the insemination time. Ovsynch protocol with FTAI has been developed, tested and intensively used in dairy cows and it yielded overall conception rates similar to those obtained after breeding to detected estrus (Pursley *et al*., 1997; Stevenson *et al*., 1999). However, conception rate is usually lower in Ovsynch-treated cows because ovulation is not adequately synchronized in approximately one third of the animals (Colazo and Ambrose, 2013). The variability in synchronization with the Ovsynch protocol is mainly due to the stage of the estrous cycle at the time of the first GnRH treatment (Vasconcelos *et al*., 1999; Moreira *et al*., 2000). Studies have amply demonstrated that the success of Ovsynch program is influenced by the number of follicular waves or length of the follicular wave (Pursley *et al*., 1997) and the stage of the estrous cycle when the first GnRH is administered (Vasconcelos *et al*., 1999; Moreira *et al*., 2000). The best time to initiate the Ovsynch treatment is between day 5 to 12 of the estrous cycle (early diestrus) with higher probability of cows responding to the treatment and become pregnant (Vasconcelos *et al*., 1999; Moreira *et al*., 2000).

A common question among dairy producers is the optimal interval between $PGF_{2\alpha}$ and the second GnRH treatment, and the optimal time to inseminate cows that have been synchronized with the Ovsynch protocol. In a preliminary study, Peters *et al*. (1999) examined the effect of delaying the second GnRH on synchrony of ovulation in dairy cows subjected to a 7-day Ovsynch and observed that ten out of 11 cows treated with the second GnRH between 56 and 60

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hours after administration of $PGF_{2\alpha}$ ovulated within 24 h (between 72 and 96 hours after administration of $PGF_{2\alpha}$). They concluded that GnRH administration between 56 and 60 h following $PGF_{2\alpha}$ administration resulted in the tightest synchrony of ovulation. Brusveen *et al*. (2008) evaluated altering the time of the second GnRH injection and AI during Ovsynch in lactating dairy cows and observed that P/AI was lower in cows receiving GnRH at 48 h (Cosynch-48, 29.2 %) or 72 h (Cosynch-72, 25.4 %) after $PGF_2\alpha$ and inseminated concurrently that in those given GnRH at 56 h and inseminated 16 h later (Ovsynch-56, 38.6 %).

Although, Ovsynch-56 yielded better conception rates in lactating dairy cattle, the efficacy of the same has not been attempted in repeat breeder cows when initiated during the early diestrus stage. Hence, the present study was conducted to evaluate the efficacy of Ovsynch and modified Ovsynch protocol (Ovsynch-56) on the conception rate in repeat breeder cows when initiated during the early diestrus stage.

MATERIALS AND METHODS

The study was carried out on 33 randomly selected repeat breeder cows aged 3-8 years in their first to fourth lactation with BCS between 2.5 and 3.5. The total number of services for repeat breeder cows in the present study ranged from 3-7 with an average number of 4.5 services. The number of days these animals were not pregnant at the time of presentation for treatment ranged from 150 to 240 days. All the cows were subjected to thorough clinico-gynaecological examination to ascertain the stage of estrous cycle based on trans-rectal palpation for the presence of CL and correlating with the history obtained from the owner regarding the day of last estrus.

These 33 repeat breeder cows were randomly divided into three groups with 11 cows in each group. Group-I (Control) repeat breeder cows were subjected to single A.I during the observed estrus. Group-II repeat breeder cows were subjected to Ovsynch-48 with FTAI. Repeat breeder cows in this group received 10 µg GnRH intramuscular on day 0 (day of start of the treatment) and 500 µg of $PGF_{2\alpha}$ intramuscular on day 7. A second dose of 10 µg of GnRH was administered on day 9 and FTAI was carried out at 16-20 h after the second GnRH injection. Group-III cows were subjected to Ovsynch-56 with FTAI. 10 µg of GnRH was administered to all the repeat breeder cows on day 0 (day of start of the treatment). 500 µg of $PGF_{2\alpha}$ was administered intramuscular on day 7. A second dose of 10 µg of GnRH was administered at 56 h after the $PGF_{2\alpha}$ injection and FTAI was carried out at 16-20 h from the second GnRH. Repeat breeder cows in Group-II and III were initiated with synchronization protocol anywhere between day 5-12 of the estrous cycle.

Blood samples were collected from all the three groups for estimation of serum progesterone both at the time of induction of treatment (Group-II and Group-III only) and at the time of A.I (Group-I, II and III). Endometrial cytology was performed in all the repeat breeder cows before A.I. by uterine lavage technique as described by Salasel *et al*. (2010) to rule out subclinical endometritis. The diagnosis of subclinical endometritis was arrived when the endometrial cytology revealed the threshold of 10 % PMNs in the uterine smears (Kasimanickam *et al*., 2004). Repeat breeder cows in the three groups were subjected to pregnancy diagnosis by trans-rectal ultrasound scanning using linear array probe with 7.5 MHz frequency on day 30 post A.I. and confirmed by rectal examination at day 45 post A.I. to evaluate the conception rate.

Mean values (± SE) for serum progesterone levels for repeat breeder cows of three groups were computed and analyzed statistically using one way analysis of variance under completely randomized design (Steel and Torrie, 1980). The conception rates for the three groups were analyzed by Chi-square test. Results were considered to be statistically significant when "P" values are less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSIONS

Serum progesterone concentration: The mean serum progesterone concentrations at the time of first GnRH injection in the treated repeat breeder cows (Group-II and III) were recorded as 2.95 ± 0.6 and 2.36 ± 0.41 ng/ml, respectively (Table). The

mean serum progesterone concentrations among the treatment groups showed no significant variations (P>0.05). The observation on serum progesterone concentrations at first GnRH in the two treatment groups confirm that all the repeat breeder cows in these groups were in diestrus stage of the estrous cycle when estrus synchronization protocol were initiated. Vasconcelos *et al*. (1999) and Moreira *et al*. (2000) initiated Ovsynch protocol during different stages of the estrous cycle and concluded that the best stage to initiate the Ovsynch protocol was the early luteal phase of the estrous cycle to obtain better conception rate.

The mean serum progesterone concentrations at A.I. were 0.61 ± 0.06, 0.56 ± 0.08 and 0.39 ± 0.07 ng/ml in Group-I, II and III, respectively (Table). Analysis of the data revealed that there was no significant differences (P>0.05) in the mean serum progesterone concentrations between the control and treatment groups at the time of A.I. This is in agreement with the findings of Kim *et al*. (2007), Celik *et al*. (2009) and Ravikumar *et al*. (2014) who also reported no significant differences in serum progesterone concentrations on the day of AI in the untreated and treated repeat breeder cows. Further, the serum progesterone concentrations in every animal assigned to untreated and treated repeat breeder groups was less than 1 ng/ml confirming the complete luteolysis and the onset of estrus.

Endometrial cytology: None of the 33 repeat breeder cows were found positive for subclinical endometritis by lavage technique at the time of A.I.

Conception rate: The conception rates obtained were 18.18, 36.36 and 45.45 per cent for Group-I, Group-II and Group-III, respectively (Table). Analysis of the data revealed that there was a significant difference in the conception rate (P<0.05) between the groups.

The conception rate (18.18%) obtained for untreated repeat breeder cows (Group-I) is in agreement with the values of 20 per cent (Awasthi *et al*., 2002), 20.56 per cent (Amiridis *et al*., 2009), 25 per cent (More *et al*., 2012) and 25 per cent (Vijayarajan and Meenakshisundaram, 2013) reported for untreated repeat breeder cows. The low conception rate of 18.18 per cent obtained for the untreated repeat breeder cows (Group-I) could be due to the small sample size, varying age of the cows, parity and the suprabasal levels of progesterone (0.61 ± 0.06 ng/ml) recorded in this group.

The conception rate of 36.36 per cent obtained for repeat breeder cows subjected to Ovsynch-48 with FTAI (Group-II) agrees with the earlier reported values of 31.5 per cent (Bartolome *et al*., 2000) for postpartum dairy cows. However, the conception rate is lower than the reported values of 50 per cent (Vijayarajan *et al*., 2009), 66.67 per cent (Ravikumar, 2014) in repeat breeder cows. On the other hand, the conception rate obtained in this group is much higher than the reported values of 22.3 per cent (Kasimanickam *et al*., 2005) and 27.2 per cent (Celik *et al*., 2009) for repeat breeder cows.

Five out of 11 repeat breeder cows subjected to Ovsynch-56 became pregnant with a conception rate of 45.45 per cent. This is in close agreement with the earlier reported value of 44.8 per cent (Brusveen *et al*., 2008) in dairy cows. Higher conception rates have been reported for cows subjected to Ovsynch-56 than Ovsynch protocol alone (Brusveen *et al*., 2008; Mecitoglu *et al*., 2012). The present study also reported a significantly (P< 0.05) higher conception rate of 45.45 per cent for Ovsynch-56 group than 36.36 per cent for Ovsynch-48 in repeat breeder cows. The increase in conception rate for repeat breeder cows subjected to OVS-56 could possibly be subscribed to tight synchrony of ovulation and allowing more time for the recruited follicle to undergo maturation. Even though, Ovsynch-56 increases fertility compared with Ovsynch-48, the difficulty for dairy managers in implementing Ovsynch-56 will be handling cows in both the morning and evening hours during reproductive management protocol. Nevertheless, the improved conception rate obtained using Ovsynch-56 in the present study is likely to offset any increase in labor costs needed to implement this change.

Hence, it can be concluded that Ovsynch-56 can be effectively implemented to improve conception rate than Ovsynch-48 in repeat breeder

cows when initiated during the early diestrus stage of the estrous cycle. They can be implemented in dairy herds as well as in cows of individual dairy farmers with high milk yield. Such a treatment would definitely reduce the number of days open and enable the repeat breeder cows to conceive at an early stage.

Table: Mean serum progesterone concentrations and conception rate in control and treated repeat breeder cows

Groups	Mean Serum Progesterone Concentration (ng/ml)		Conception rate (%)
	At first GnRH of Synchronization Protocol	At the time of A.I	
Group-I (Control) n= 11	***	0.61 ± 0.06	18.18[c] (2/11)
Group-II (Ovsynch-48) n=11	2.95 ± 0.60	0.56 ± 0.08	36.36[b] (4/11)
Group-III (Ovsynch-56) n=11	2.36 ± 0.41	0.39 ± 0.07	45.45[a] (5/11)

The values with different superscript in column differs significantly (P< 0.05)

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Comparative Histomorphology of Conducting Portion of Lung in Bidri Goat and Deccani Sheep*

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ABSTRACT

The present comparative histological study of lung in Bidri goat (*Capra hircus*) and Deccani sheep (*Ovis aries*) was carried out to know the structural differences between these animals. The structures of conducting portion in both species had mucosa lined by pseudo-stratified ciliated columnar epithelium with goblet cells and basal cells in bronchi, ciliated columnar and non-ciliated columnar epithelium in bronchiole and simple low columnar to cuboidal epithelium in terminal bronchiole. Lamina propria contained collagen, reticular and elastic fibres. Lamina muscularis had varying amount of smooth muscle fibres in different regions. Submucosa of primary and secondary bronchi had serous dominant bronchial glands. The fibroelastic layer had large hyaline cartilage plates in primary bronchi, small isolated plates in secondary bronchi and these were absent in distal conducting portion. No major histomorphological differences were noticed between these animals except more number of bronchial glands in Bidri goat.

Kay words: Lung, Goat, Sheep, Histomorphology

Sheep and goat are the important sources of milk, meat and fibres as well as products of high economic value. Many farmers especially small and marginal farmers depend mainly on goat and sheep husbandry but heavy mortality is reported due to respiratory tract diseases, developmental deformity, faulty branching of bronchiolar tree and defective development of respiratory alveolar system. These become the challenging problems in the growth of goat and sheep husbandry. In order to gain complete understanding of cause and their possible elimination, the knowledge of histomorphology of conducting and respiratory portion of lung very is essential. There are no studies on the comparative histomorphology of conducting portion of lung in Bidri goat and Deccani sheep.

MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Anatomy and Histology, Veterinary College, Bidar, Karnataka. The samples were taken from medial, central and lateral portion of all six lobes of lung from eight adult Bidri goat and eight adult Deccani sheep immediately after slaughter from local slaughter house and they were fixed in different fixatives like 10% Neutral buffered formalin, Zenker's fluid and Bouin's fluid. The samples were processed in Isopropyl alcohol-Xylene sequence and embedded in paraffin by routine method (Luna, 1968) and sections were cut at 6-8µm thickness. The following staining techniques were carried out to study histomorphological feature of lung. Harries Haematoxylin and Eosin stain (Luna, 1968), Van Geison's stain for collagen fibres (Bancroft *et al*., 2008), Verhoeff's method for elastic fibres (Singh and Sulochana, 1996), Gomori's method for reticular fibres (Luna, 1968) and Azan's Trichrome method for connective and muscle fibres (Singh and Sulochana, 1996).

RESULTS AND DISCUSSION

The conducting portion consisted of intra pulmonary bronchi, bronchioles and terminal bronchioles in both adult Bidri goat and adult Deccani sheep. This finding is similar to earlier reports of Banks (1993), Trautmann and Fiebiger (2002) in domestic animals.

In the present study, three types of intrapulmonary bronchi were noticed in both adult Deccani sheep and adult Bidri goat. This classification is in accordance with Trautmann and Fiebiger (2002) who reported primary, secondary and tertiary bronchi based on abundance of smooth

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muscle fibres in lamina muscularis and presence of hyaline cartilage plates in fibro elastic layer in domestic animals.

Primary bronchi of both adult Bidri goat and adult Deccani sheep consisted of folded mucosa lined by pseudo-stratified ciliated columnar epithelium, goblet cell and basal cell in the epithelial lining (Fig.1 and 2). In the secondary and tertiary bronchi the epithelial mucosa was lined by pseudo-stratified ciliated columnar epithelium, goblet cells and basal cells but the goblet cell number reduced gradually from primary bronchi to tertiary bronchi. The present findings are similar to earlier findings of Mariassy and Plopper (1983) in sheep, Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals. It was observed that the height of epithelial mucosa of primary bronchi was 54.14±2.97µm, secondary bronchi 32.62±1.34µm and tertiary bronchi was 27.58±1.56µm in adult Bidri goat whereas, in adult Deccani sheep it was 51.63±2.55µm, 30.50±0.20µm and 28.78±1.31µm respectively. These studies showed a gradual reduction in height of mucosal epithelia from primary bronchi to tertiary bronchi. This finding is similar to Attar Singh *et al*. (2001) who reported reduction in height of mucosal epithelia from 57.43±2.98µm in more proximal bronchi to 22.15±0.73µm in most distal bronchi in buffalo.

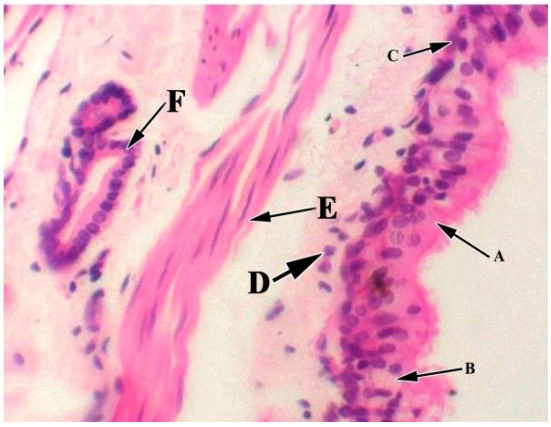

Fig.1: Photomicrograph of primary bronchi showing pseudo-stratified ciliated epithelium (A), goblet cell (B) and basal cell (C) in epithelial lining, fibroblast in lamina propria (D), smooth muscle fibres in lamina muscularis (E) and bronchial gland in submucosa (F) of adult Bidri goat (H&E, X 40)

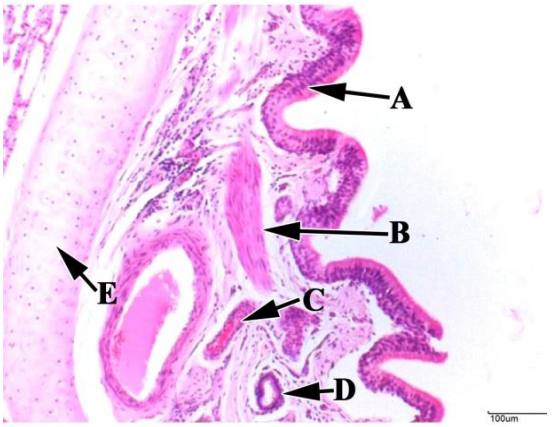

Fig. 2: Photomicrograph of primary bronchi showing mucosal epithelial lining (A), isolated bundles of smooth muscle fibres (B) in lamina muscularis, capillary (C) and bronchial gland (D) in submucosa and large hyaline cartilage plates (E) in fibroelastic layer of adult Deccani sheep (H&E, X 10)

Lamina propria of primary bronchi, secondary bronchi and tertiary bronchi was made up of loose connective tissue consisted of fine network of elastic, reticular, collagen fibres and fibroblasts in both adult Bidri goat and adult Deccani sheep (Fig. 3 and 4). It is similar to earlier reports of Mariassy and Plopper (1983) in sheep, Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals.

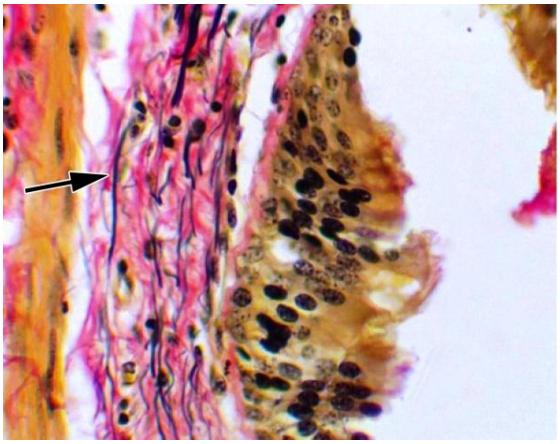

Fig.3: Photomicrograph of primary bronchi showing elastic fibres in lamina propria (arrow) of adult Bidri goat (Verhoeff's stain, X 40)

The muscularis mucosa of both adult Bidri goat and adult Deccani sheep was discontinuous and had bundles of smooth muscle fibres in primary bronchi. In secondary bronchi, abundance of smooth muscle increased relatively in lamina muscularis and in tertiary bronchi lamina

muscularis consisted of continuous layer of smooth muscle fibres (Fig. 4). The observations are similar to earlier reports of Mariassy and Plopper (1983) in sheep, Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals and Kalita *et al.* (2003) in yak.

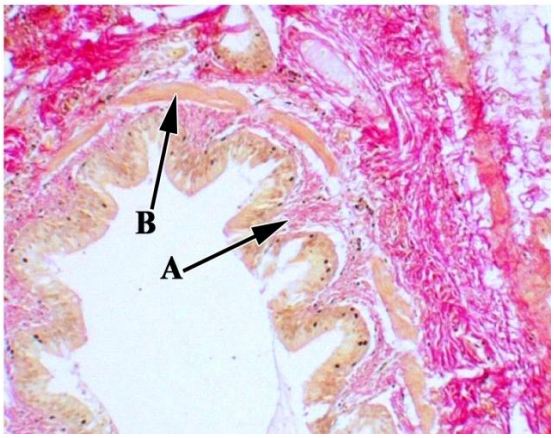

Fig.4: Photomicrograph primary bronchi showing collagen fibre (A) in lamina propria and smooth muscle (B) fibres in lamina muscularis of adult Deccani sheep (Van Geison's stain, X 40)

The submucosa of primary bronchi, secondary bronchi and tertiary bronchi was wider and composed of loosely arranged connective tissue fibres in adult Bidri goat and adult Deccani sheep. Presence of serous type of bronchial glands were observed in submucosa of primary and secondary bronchi only and it was also noticed that number of bronchial glands were less in secondary bronchi than in primary bronchi. Bronchial glands were not observed in submucosa of tertiary bronchi in both adult Bidri goat and adult Deccani sheep (Fig. 3). This finding is similar to observation of Mariassy and Plopper (1983) in sheep, Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals. In the present study, it was noticed that there was a difference in the distribution of bronchial glands since they were more in both primary and secondary bronchi of adult Bidri goat than that of adult Deccani sheep. This may be due to breed variations.

The primary bronchi consisted of fibroelastic layer made up of large hyaline cartilage plates which completely surrounded the submucosa layer (Fig. 5) and in secondary bronchi fibro elastic membrane contained isolated plates of hyaline cartilage and no cartilage plates or strips were observed in fibroelastic layer of tertiary bronchi. The present findings were in agreement with the earlier reports of Trautmann and Fiebiger (2002) Dellmann (2006) in domestic animals.

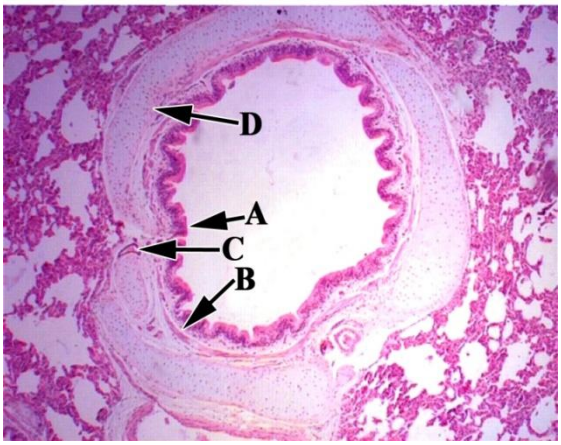

Fig. 5: Photomicrograph of primary bronchi showing mucosal epithelial lining (A), isolated bundles of smooth muscle fibres (B) in lamina muscularis, bronchial gland (C) in submucosa and large hyaline cartilage plates (D) in fibroelastic layer of adult Deccani sheep (H&E, X 4)

Peribronchial layer of primary, secondary and tertiary bronchi consisted of loose connective tissue mainly of collagen and little elastic fibres in both adult Bidri goat and adult Deccani sheep and it was also noticed the presence of blood vessels nerve fibres, ganglions and lymphatic aggregations in all region of intrapulmonary bronchi. These observations were similar to the reports of Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals, whereas Singh and Mariappa (1981) observed aggregation of lymphoid tissue in the connective tissue wall of the bronchi in buffalo and Anderson *et al.* (1986) noticed the more number of bronchus associated lymphoid tissue in adult bovine as compare to young animals.

In the present study, the epithelial mucosa of bronchioles in adult Bidri goat and adult Deccani sheep was folded and lined by simple columnar epithelium consisted of ciliated columnar cells and non-ciliated columnar cells (Fig.6). The present findings were similar to earlier reports of Suman *et al.* (2005) in goat, whereas Baskervilli (1970) reported four type of cells viz., pseudo-stratified ciliated columnar epithelium, non-ciliated, goblet and brush cells in

pig and Bouljihad and Leipold (1994) reported bronchioles were consisted of basal cells, intermediate cells, ciliated cells and non-ciliated cells in sheep. Epithelial mucosa of second and third generation of bronchioles were less folded and lined by few tall columnar epithelium with cilia and large number of short columnar epithelium in both adult Bidri goat and adult Deccani sheep. The present finding were similar to the reports of Baskervilli (1970) in pig, whereas Smith *et al.* (1979) observed more number of Clara cells in distal bronchioles of mammals, Dellmann (2006) reported small bronchioles were distinguished from the large bronchioles by smaller lumen, lower mucosal folds and lined by more cuboidal epithelial cells in domestic animals. However, it differs with reports of Attar Singh *et al.* (2001) in buffalo where epithelium of bronchioles comprised of 2 layers of cuboidal epithelial cells which decrease further to simple cuboidal cells in most distal bronchioles. The height of epithelial mucosa in large bronchiole and small bronchioles were, 22.26±1.12µm and 17.45±0.81µm in adult Bidri goat, whereas, in adult Deccani sheep it was 21.26±0.91µm and 16.45±0.56µm respectively indicating reduction in the height of epithelial mucosa in small bronchioles as compared to large bronchioles. Same observation was reported by Baskervilli (1970) in pig, Bouljihad and Leipold (1994) in sheep, Attar Singh *et al.* (2001) in buffalo and Suman *et al*. (2005) in goat.

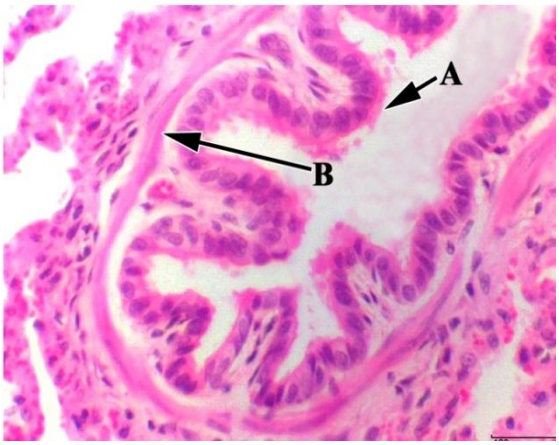

Fig. 6: Photomicrograph bronchiole showing simple columnar epithelial cells in mucosal lining (A) and continuous layer of smooth muscle fibres (B) in lamina muscularis of adult Deccani sheep (H&E, X 10)

Lamina propria of bronchioles had reticular, elastic and collagen fibres in adult Bidri goat and adult Deccani sheep in the present study are similar to earlier reports of Baskervilli (1970) in pig, Bouljihad and Leipold (1994) in sheep, Attar Singh *et al*. (2001) in buffalo, Suman *et al*. (2005) in goat and Dellmann (2006) in domestic animals.

Lamina muscularis of bronchiole was composed of continuous circularly arranged smooth muscle fibres and the thickness of smooth muscle fibres supporting epithelial mucosa was reduced in most distal bronchioles in both adult Bidri goat and adult Deccani sheep (Fig.7). The present findings is in agreement with the earlier reports of Baskervilli (1970) in pig, Bouljihad and Leipold (1994) in sheep, Attar Singh *et al*. (2001) in buffalo, Suman *et al*. (2005) in goat and Dellmann (2006) in domestic animals.

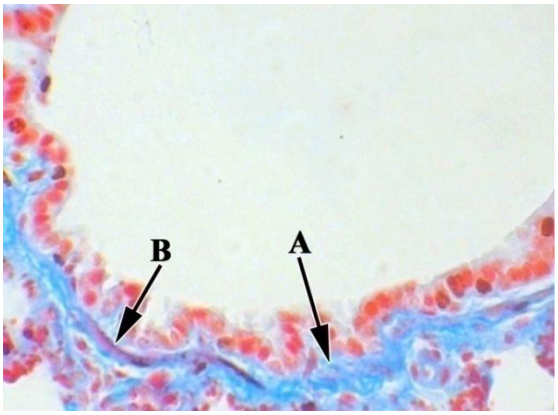

Fig.7: Photomicrograph of bronchiole showing collagen fibres (A) in lamina propria and smooth muscle fibre in lamina muscularis (B) of adult Bidri goat (Azan's Trichrome stain, X 40)

Submucosa of large bronchioles had loose elastic, reticular and collagen fibres and it was devoid of bronchial glands (Fig.7). The thickness of submucosa was found to be reduced in small distal bronchioles as compared to large bronchioles in both adult Bidri goat and adult Deccani sheep. This observation is similar to the earlier reports of Baskervilli (1970) in pig, Bouljihad and Leipold (1994) in sheep, Suman *et al*. (2005) in goat and Dellmann (2006) in domestic animals.

The fibroelastic layer was absent and peribronchial area had compact connective tissue fibres of collagen, elastic and reticular. Large blood vessels and lymphatic aggregation was observed.

The thickness of the peribronchial area was found to be reduced in small distal bronchioles as compared to proximal bronchiole in both adult Bidri goat and adult Deccani sheep in the present study. These findings were similar to findings of Bouljihad and Leipold (1994) in sheep, Suman *et al.* (2005) in goat and Dellmann (2006) in domestic animals.

In the present study the epithelial mucosa of terminal bronchioles had smaller lumen compare to bronchioles and were lined by low columnar to cuboidal cells and also consisted of few ciliated cuboidal epithelial cells but number of non ciliated cuboidal epithelial cells were more in both adult Bidri goat and adult Deccani sheep (Fig. 8). The present findings are similar to earlier report of Baskerville (1970) in pig, Mariassy and Plopper (1983) and Bouljihad and Leipeld (1994) in sheep and Wright *et al.* (1983) in dog, Bhatacharyya and Bishya (1995), Kahwa and Purton (1996) and Khawa and Atwal (1997) in goat and Trautmann and Feibiger (2002) in domestic animals. The Mean±SE height of epithelial mucosa of terminal bronchiole was 12.90±0.49µm in adult Bidri goat, whereas, it was 10.45±0.45µm in adult Deccani sheep. The earlier reports were not available with respect to the measurement of height of epithelial mucosa of terminal bronchioles, whereas, Baskerville (1970) in pig, Mariassy and Plopper (1983) and Bouljihad and Leipeld (1994) in sheep, Kahwa and Purton (1996) in goat and Trautmann and Fiebiger (2002) in domestic animals reported the reduction in the height of epithelial mucosa in terminal bronchiole as compared to proximal bronchioles.

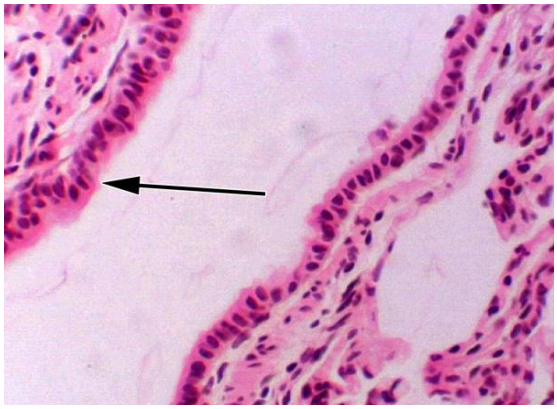

Fig.8: Photomicrograph of terminal bronchiole showing cuboidal epithelial cells (arrow) in epithelial mucosa of adult Deccani sheep (H&E, X 40)

Basement membrane of terminal bronchioles was indistinct and cells lining were supported loose connective tissue consisted of small collagen, reticular, elastic fibres (Fig.9) and bundle of smooth muscle fibres and the smooth muscle fibres were seen more in proximal terminal bronchioles than that of distal terminal bronchioles in both adult Bidri goat and adult Deccani sheep in the present study. These findings were similar to earlier reports of Baskerville (1970) in pig, Mariassy and Plopper (1983) in sheep, Khawa and Atwal (1997) in goat and Trautmann and Fiebiger (2002) in domestic animals and it was observed that the distal terminal bronchioles gave 2-3 generations of respiratory bronchioles in both adult Bidri goat and adult Deccani sheep. These findings are almost similar to earlier reports of King (1982) and Banks (1993) who reported that the terminal bronchiole gave off 3-5 generation of respiratory bronchioles in pig and ruminants respectively.

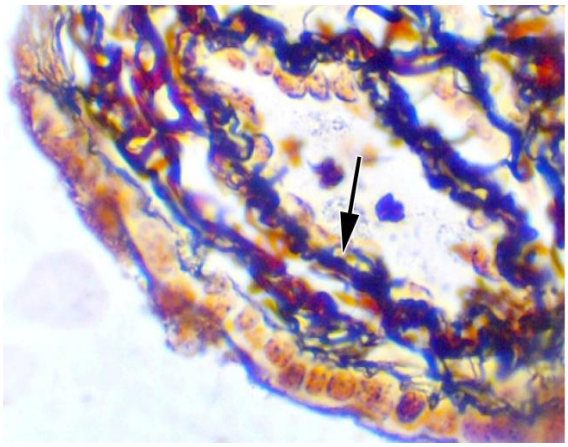

Fig.9: Photomicrograph of terminal bronchiole showing reticular fibres in lamina propria (arrow) of adult Bidri goat (Gomorie's stain, X 40)

CONCLUSION

In the present study, it was observed that little histomorphological variation in conducting portion in lung of adult Bidri goat and adult Deccani sheep than that of other domestic animals. There was no major structural difference in the conducting portion in lung of adult Bidri goat and adult Deccani sheep whereas, the numbers of bronchial glands were more in primary and secondary bronchi of adult Bidri goat than that of adult Deccani sheep.

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Haematobiochemical and Ultrasonographical Studies of Hepatic Disorders in Dogs*

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ABSTRACT

Haemato-biochemical and ultrasonographical findings were analyzed in dogs with hepatic disorders. Haematological investigations revealed reduced Hb, PCV and neutrophilic leukocytosis. Serum biochemical investigation revealed moderately elevated ALT, marked ALP and bilrubin elevation in icteric dogs, hypoprotenaemia and hypoalbuminaemia (marked in ascitic dogs) and hypoglycemia. Ultrasonographic findings were diffuse / focal hyperechlogenicity of liver parenchyma, ascetic shadow in the abdomen and biliary sludge.

Key Words: Hepatic disorders, hyperechoic, ALT, ALP.

Hepatic disorders are not uncommon in dogs due to various insults to the liver. Early and accurate identification of hepatic disorders is important to improve the long-term outcome (Cooper, 2006). Any insult to liver causes damage to hepatobiliary system, which inturn causes leakage of enzymes (ALT, AST), increase in enzymes because of accelerated production stimulated by bile retention (ALP, GGT) affects cell synthesis (proteins, albumin and fibrinogens) and pigment metabolism (Bilrubin stasis).

During recent years, hepatic ultrasonography has become an important diagnosic tool in the differentiation of canine hepatopathies (Nyland and Mattoon, 1995). The present study describes haemato-biochemical and ultrasonographical changes in hepatic disorders in dogs.

MATERIALS AND METHOD

Clinical cases of dogs (22) which were confirmed to be positive for hepatic disorders (German shepherd 8, Mongrel 5, Spitz 4, Labrador retriever 3, Dalmatian 1 and Dachshend 1) among the dogs presented or referred to the Teaching Veterinary Clinical Complex and College Hospital of College of Veterinary Science, Tirupati, during the study period of one year formed the material for the present study. They were confirmed to be positive for hepatic disorders based on dogs exhibiting few or all the clinical signs of hepatic disorders like anorexia / inappetance, loss of body weight, jaundice, ascites, vomiting, diarrhea, dullness and lethargy and haematobiochemical parameters. Six apparently healthy dogs were kept as control for comparison. Haematology was carried out on these dogs by using whole blood for the parameters, Hb, PCV, TLC and DLC. Liver enzymes ALT, ALP; total protein, albumin, glucose, bilrubin were estimated in serum samples by using spectrophotometer. Hepatic ultrasonography was done for 9 dogs with hepatic disorder (base on clinical signs and haematobiochemical readings) and in 6 dogs of control group. It was performed with LOGIQ and 100 cls scanner using 3.5 / 7.5 MHL mechanical transducer as per the guidelines of Nyland and Matton (1995) and the images were recorded on thermal printing paper.

RESULTS AND DISCUSSION

Haematological findings in dogs with hepatic disorders revealed anaemia as out of 22 dogs, 15 were found to be truly anaemic, i.e., with Hb < 8 gm / dl (Schalm, 1975). Anaemia could be due to elevated bile acid content increasing the fragility of the membrane of RBCs (Rothuizen and Meyer, 2000). Mean PCV was significantly low in dogs with hepatic disorders (29.83 ± 1.76) compared to

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that of normal dogs (44 ± 2.08) (Table). In the 15 anaemic dogs, PCV was <25%, Hunt *et al.,* (1993) and Rallis *et al.,* (2005) reported reduced PCV in non-fibrosing liver disease and chronic hepatitis respectively. The present findings are in agreement with the above authors. Dogs with hepatic disorders showed neutrophilic leukocytosis. Leukocytosis in chronic liver diseases of dogs might be interpreted as an inflammatory response to acute phase stimulation and or as a stress response (Sevelius, 1995). Lucena *et al.,* (2001) and Neill *et al.,* (2006) reported neutrophilic leukocytosis in hepatic cirrhosis and bacterial cholangitis/cholangio-hepatitis respectively.

Serum biochemical estimations showed elevated hepatic enzymes ALT and ALT (Table 1). Amino transferases are commonly elevated in hepatocellular disease, hepatitis, hepatic trauma, anaemia, toxaemia etc., (Jones, 1986) ALT, a leakage enzyme, the magnitude of elevation is roughly proportional to the number of injured hepatocytes (Rothuizen and Meyer, 2000). ALP elevation was marked in icteric dogs. Elevation in ALP is subsequent to accelerated production stimulated by bile retention (Meyer *et al.,* 1992). Marked elevation of ALP was noticed in cholestatic dog and reported by Armstrong *et al.,* (2000); Vijayakumar *et al.,* (2001), and Udayasree *et al.,* (2006). The findings of the present study are in concurrence with above reports.

Hypoproteinaemia and hypoalbuminaemia was observed in the dogs with hepatic disorders (Table) which was even marked in 3 ascitic dogs (Total protein : 3.33, 3.72 and 2.28 g/dl and Albumin : 1.84, 1.87 and 0.49 g/dl). Low serum proteins and albumin could be attributed to decreased production from liver, besides anorexia / inappetance. Decreased total protein and albumin levels in the present study are in agreement with the reports of Sevelius (1995), Varshney and Hoque (2002), Rutgers *et al.,* (1993) and Lucena *et al.,* (2001).

Almost all the dogs with hepatic disorders were hypoglycemic, which could be due to impaired carbohydrate metabolism in the liver and was a rare finding that glucose is a bad prognostic marker (Strombeck *et al.,* 1988), Vijayakumar *et al.,* (2001) and Varshney and Hoque (2002) reported hypoglycemia in canine hepatic disorders.

The mean total bilrubin value in the affected dogs revealed slight elevation and with marked elevation in 4 icteric dogs (3.37, 8.37, 10.78, 12.18 mg/dl). Presence of hyperbilirubinaemia with equal amounts of direct and indirect bilirubin as seen in first icteric dog among above 4 is an indicative of hepatocellular damage (Jones, 1986). And the remaining 3 icteric dogs had more proportion of direct bilurubin suggestive of cholestasis (Kaneko *et al.,* 1999). Dalton *et al.,* (1975), Vijayakumar *et al.,* (2001), Saini *et al.,* (2003) and Udayasree *et al.,* (2006) reported hyper bilrubinaemia in intrahepatic cholestasis, cholecystitis, jaundiced dog of hepatic origin and cholestatic hepatitis respectively. But Lucena *et al.,* (2001) reported severe hyperbilrubinaemia (100 mg/dl) in hepatic cirrhosis.

Ultrasonography findings of liver in apparently healthy dogs showed uniform echo pattern of low to medium echogenicity. The diaphragm was seen as a continuous curvilinear hyperechoic structure along the boundary of liver. Liver was hypoechoic to spleen and isoechoic to slightly hyperechoic to renal cortex. The findings are in agreement with the findings of Voros *et al.* (1991) and Nyland and Mattoon (1995) and Varshney and Hoque (2002). Whereas in 9 dogs with hepatic disorders it was observed that hypercehogenicity of liver (6 dogs) i.e., hyperechoic liver of normal size (Fig.1), suggestive of lymphosarcoma, hepatic lipidosis and chronic hepatitis (Voros *et al.,* 1991, Nyland and Mattoon, 1995). In addition to hyper echogenicity of liver, there was free abdominal fluid in 2 ascitic dogs (Fig.2). Cartee *et al.,* (1995) and Nyland and Mattoon (1995) mentioned massive accumulation of free fluid separating intra abdominal organs by large anechoic spaces. Focal hyperechoic parenchymal changes were noticed in two dogs which may indicate nodular hyperplasia / calcification (Cartee *et al.,* 1995 and Biller & Black Welder, 1998). Biliary sludge (Fig.3) was noticed in one dog but the wall of gall bladder did not show

any thickening. Biliary sludge is a common age related finding as opinioned by Tiwari *et al.*, (2005), but the above dog had clinical signs suggestive of hepatitic disorder viz., anorexia, anaemia, poilydipsia and laboratory findings revealed ALP elevation (49.7 KA units). So the condition was diagnosed to be suggestive of cholangitis / cholangithepatitis. The finding was similar to the observations of Neill *et al.*, (2006).

Table: Haemato biochemical changes in dogs with hepatic disorders

Sl. No.	Parameter	Apparently healthy dogs (n = 6)	Dogs with hepatic disorders (n = 22)
1.	Hb (g/dl)	15.17 ± 0.83	8.5 ± 0.37**
2.	PCV (%)	44 ± 2.08	29.82 ± 1.76**
3.	TLC (Cells / µl)	9596 ± 908	15297 ± 895**
4.	Neutrophils (%)	65.33 ± 2.12	78.77 ± 2.51**
5.	Lymphocytes (%)	23.5 ± 1.78	15.77 ± 2.03**
6.	Monocytes (%)	8.33 ± 0.42	3.82 ± 0.63**
7.	Eosinophils (%)	2.83 ± 0.4	1.45 ± 0.27**
8.	Basophils (%)	---	0.09 ± 0.06^{NS}
9.	ALT (U/L)	21.78 ± 2.09	117.1 ± 6.45**
10.	ALP (KA Units)	5.79 ± 1.76	39.8 ± 9.07**
11.	Total Serum Protein (g/dl)	6.79 ± 0.26	4.3 ± 0.21**
12.	Serum Albumin (g/dl)	3.52 ± 0.24	1.9 ± 0.14**
13.	Glucose (mg/dl)	95.93 ± 4.97	56 ± 3.25**
14.	Total Bilirubin (mg/dl)	0.2 ± 0.03	2.7 ± 0.8**

Fig-1. Ultrasonogram of liver showing hyperchogenicity

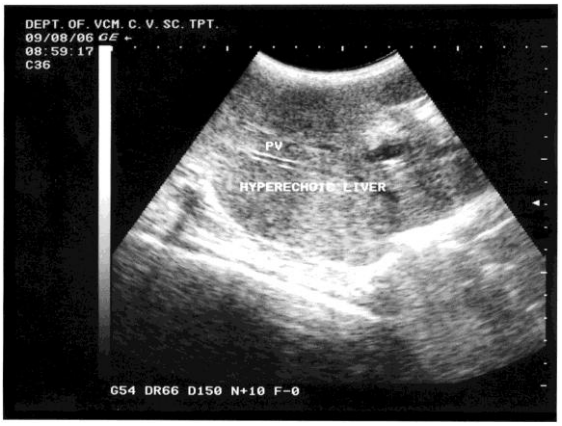


Fig-2. Ultrasonogram of dog with ascites

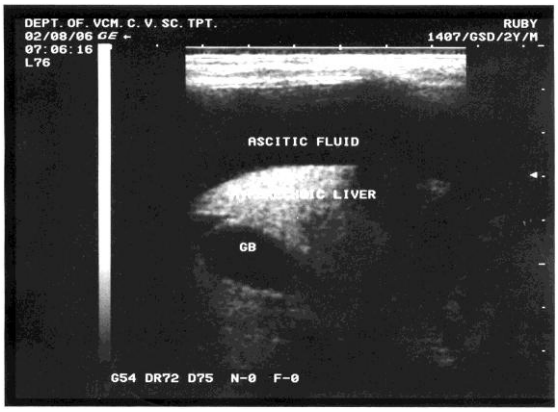
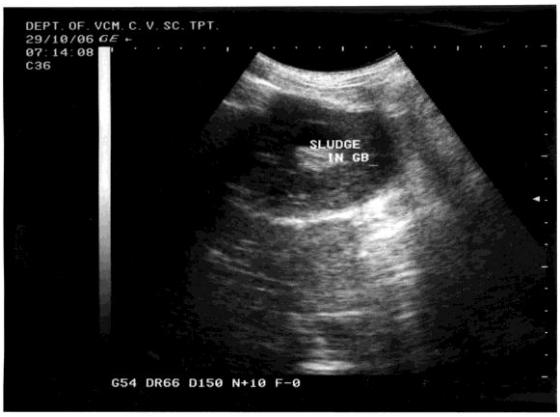


Fig-3. Ultrasonogram of liver showing sludge in gall bladder



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Socioeconomic and Psychological Profile of Dairy Farmers of Kolar and Chikkaballapur Districts of Karnataka*

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ABSTRACT

India owns the largest livestock population in the world. Dairy farming is one of the important activities of the rural population of our country. It provides continuous income and reduces unemployment to a large number of the rural poor. A study was undertaken to analyse the socioeconomic and psychological profile of dairy farmers in Kolar and Chikkaballapur districts. Two taluks viz. Kolar and Malur from Kolar district, Chintamani and Gudibande taluks from Chikkaballapur district were selected for the study. A total of 120 dairy farmers were randomly selected and data were collected using an interview schedule. The analysis of socio economic and psychological profile revealed that majority (61.67%) of the dairy farmers were of middle age group (35-50 years), and most of them had middle school education. Majority lived in nuclear family with small family size (3-8 members) and had agriculture as their main occupation. Most of them were large farmers (>4 acres) with low annual income and low material possession, had low livestock possession and medium extension contact. Majority of the respondent dairy farmers had medium economic motivation, medium risk and scientific orientation.

Key words: Socio-economic Profile, Psychological profile, Dairy farmers

Indian livestock industry makes up for a significant amount of world's livestock resources. National economy and socio-economic growth of the country is backed by the livestock sector. It also plays important role in the rural economy as it improves the family income and generates a gainful employment in the rural sector, particularly among the landless labourers and small and marginal farmers. Animal husbandry sector provides large self-employment opportunities and is proved to be a boon for sustaining livelihood of the landless and marginal farmers. In India, dairying is recognized as an instrument for social and economic development. Milk production in India is dominated by small, marginal farmers and landless labourers who, in aggregate, own about 70 per cent of the national milch animal herd and are dispersed throughout the rural areas. These farmers maintain an average herd of one or two milch animals comprising cows and buffaloes. (BAHS, 2012)

Dairy sector in India has acquired substantial growth momentum from 9th Plan onwards as a result of which we now rank first among the world's milk producing nations, this represents sustained growth in the availability of milk and milk products for our growing population. Dairying has become an important primary source of income for millions of rural families and has assumed the most important role in providing employment and income generating opportunities particularly for marginal and women farmers (FAO, 2010). The per capita availability of the milk has reached a level of 290 grams per day during year 2013-14 which is slightly more than the world average. Dairying is a major livestock activity in Kolar and Chikkaballapur districts, because of regular and sustainable income generation to the farmers and also there is less agricultural activity due to low underground water table. The present study was undertaken to get an insight into the socio-economic profile of the dairy farmers of this region.

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MATERIALS AND METHODS

The exploratory research design was adopted to formulate a problem for more precise investigation and to develop working hypothesis from an operational point of view. The Kolar and Chikkaballapur districts were selected purposively for the study since it has got a good network of Kolar milk union limited and majority of the farmers depend on dairying due to lack of water facility for agricultural activities. Two taluks viz; Kolar and Malur from Kolar district and two taluks viz; Chintamani and Gudibande from Chikkaballapur district were purposively selected for the study. These taluks were selected purposively as they were the highest and the lowest milk producing taluks, respectively. From each taluk a total of 30 respondents, 15 respondents related to Kolar milk union limited and 15 related to private dairy were randomly selected for the study. Thus a total of 120 respondents were selected. The data were collected from the dairy farmers through a structured interview schedule. The collected data were coded, tabulated and analysed by using standard statistical procedures.

RESULTS AND DISCUSSION

Socio-economic characteristics of dairy farmers: A glance at Table I indicated that majority (61.67%) of the dairy farmers belonged to the middle age group, followed by old age group (23.33%) and young age group (15.00%). The findings are in line with the findings of Gautam *et al*., (2007) and Rupeshkumar and Chandawat ., (2011). The reasons for the above results may be due to the fact that the income from dairy is an assured source unlike agriculture. Therefore, more of middle age dairy farmers are taking up dairying as income generating activity and have more work efficiency than younger and older ones.

The data presented in Table 1 revealed that majority (35.00%) of the respondents possessed middle school education followed by high school (23.33%), primary (19.17%), P.U.C (9.17%) and 2.50 per cent of the respondents completed their graduation, while 10.83 per cent of the respondents are illiterate. The formal schooling helped the dairy farmers to gather scientific information required for dairy farming which in turn might have increased the knowledge and improved the dairy farming activity. It was a well-known fact that an educated person turned out to be rational in his thinking and imagination which in turn developed competency in

Table 1: Distribution of respondents based on socio-economic characteristics of dairy farmers (n=120)

Characteristics	Category	Respondents	
		F	%
Age group	Young	18	15.00
	Middle	74	61.67
	Old	28	23.33
Education	Illiterate	13	10.83
	Primary	23	19.17
	Middle	42	35.00
	High	28	23.33
	P.U.C	11	9.17
	Degree	3	2.50
Family type	Nuclear	84	70.00
	Joint	36	30.00
Family size	Small	97	80.83
	Medium	19	15.83
	Large	4	3.33
Main occupation	Agriculture	96	80.00
	Animal husbandry	21	17.50
	Others	3	2.50
Landholding	Landless	6	5.00
	Marginal	14	11.67
	Small	19	15.83
	Medium	30	25.00
	Large	51	42.50
Annual income	Low	110	91.67
	Medium	8	6.67
	High	2	1.67
Material possession	Low	104	86.67
	Medium	16	13.33
	High	-	-
Livestock possession	Low	90	75.00
	Medium	26	21.67
	High	4	3.33
Extension contact	Low	13	10.83
	Medium	73	60.83
	High	34	28.33

the society. The findings of the study are not in agreement with the findings of Chauhan *et al*., (2004), Ranuji (2006).

The data related to family type revealed that 70.00 per cent of the respondents lived in a nuclear family followed by joint family which constituted 30.00 per cent. The probable reasons behind these findings could be that young and middle aged people would prefer to live in nuclear families and old age people prefer joint family. These findings are in accordance with the findings of Punam and Grover, (2012) who reported that 60 per cent men and 55 per cent women had nuclear family and remaining had joint family.

The data collected with respect to the family size of the respondents presented in Table 1 revealed that 80.83 per cent of the respondents belonged to small family size followed by medium family size (15.83%) and large family size (3.33%). The results are in conformity with the findings of Gautam *et al*. (2007). The probable reason might be due to awareness about problems of large family size. Further, awareness and formal education of respondents might have helped them to maintain a small family.

The data regarding occupation revealed that majority (80.00%) of the respondents had agriculture as their major occupation, while 17.50 per cent had animal husbandry and 2.50 per cent had other occupation like business and service as their main occupation. The results are in conformity with the findings of Naik *et al.* (2013). This may be due to the continuation of ancestral traditional occupation of agriculture along with livestock rearing.

It was observed from the table I that majority (42.50%) of the respondents were large farmers, followed by medium farmers (25.00%), small farmers (15.83%), marginal farmers (11.67%), whereas 5 per cent of the respondents were landless. These findings are not in line with the findings of Ranuji (2006), Biradar (2006), and Rupeshkumar and Chandawat., (2011). The probable reasons for these findings may be majority of the dairy farmers had agriculture as their main occupation and were involved in commercial crops for getting high income.

The data related to income revealed that 91.67 per cent of the respondents had an annual income of between Rs.16,000 to 70,667, 6.67 per cent of the respondents had an income between Rs.70,667 to Rs.125,334 and 1.67 per cent of the respondents had annual income above Rs.1,25,334. The results are in line with findings of Rathod *et al.* (2009). The probable reason which could be attributed for varied income levels of the respondents might be their size of the land holdings and practicing of subsidiary occupations like dairying and other animal husbandry contributed much to their total income.

It is evident from Table I that 86.67 per cent of the respondents had low material possession followed by medium material possession (13.33%) and none had high material possession and 75.00 per cent of the respondents had low livestock possession followed by medium (21.67%) and high (3.33%) livestock possession. These findings are not in consonance with the findings of Nishi *et al*. (2011) and Singh *et al*. (2012). The probable reason for possessing low livestock by dairy farmers might be due to lack of fodder and constraints in management of livestock. And majority (60.83%) of the respondents had medium extension contact, followed by high (28.33%) and low (10.83%) extension contact. This might be due to the fact that the dairy farmers had education level more than middle school education, medium economic motivation and scientific orientation and had more zeal to gather recent information regarding dairying.

Psychological characteristics of dairy farmers: The data furnished in the Table 2, revealed that majority (66.67%) of the respondents had medium economic motivation followed by low (20.83%) and high (12.50%) economic motivation. This trend was in line with the findings of Gautam *et al.* (2008). The reason for medium economic motivation of the dairy farmers might be due to their average economic position, land holding and possession of low livestock.

Table 2: Distribution of dairy farmers based on psychological characteristics dairy farmers (n=120)

Characteristics	Category	Respondents	
		F	%
Economic motivation	Low	25	20.83
	Medium	80	66.67
	High	15	12.50
Scientific orientation	Low	14	11.67
	Medium	61	50.83
	High	45	37.50
Risk orientation	Low	9	7.50
	Medium	88	73.33
	High	23	19.17

The data regarding scientific orientation revealed that majority (50.83%) of the respondents had medium scientific orientation, followed by 37.50 per cent and 11.67 per cent of the respondents had high and low scientific orientation respectively. The above findings were in line with the findings of Ranuji (2006) and Gautam *et al.* (2008). The probable reason for this may be higher formal education helps the respondents to use scientific practices in dairying.

The data related to risk orientation revealed that majority (73.33%) of the respondents had medium risk orientation, followed by high (19.17%) and low (7.5%) risk orientation. The results of this study are not in line with results of Bhagyalaxmi *et al,* (2003), Suresh (2004) and Ranuji (2006). It might be due to ability of dairy farmers under small, marginal and medium land holding category and landless to face risk in dairy activity, along with other activities.

The present study concluded that the respondents had different level of education and occupations with different economic and psychological conditions and to improve the socioeconomic status of dairy farmers in the study area, there is a need for technical and institutional intervention to alleviate the education and income levels of the dairy farmers through dissemination of appropriate technologies and extension strategies.

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Influence of Suckling Pattern on Pre-Weaning Growth Performance in Mandya Lambs

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ABSTRACT

The present study investigated the influence of suckling pattern on pre - weaning growth performance in Mandya lambs. Ninety one week old suckling lambs were randomly distributed into one of the three experimental groups comprising 30 lambs each. In Group I and II, lambs were allowed free suckling until one month and two months of age, respectively. After completion of respective free suckling period, lambs were switched to restricted suckling pattern until weaning. In Group III, lambs were restricted from suckling by way of separation from dams for a minimum gap of at least three hours between consecutive sucklings. Lambs in all the three groups were allowed to stay together with dams during night hours and weaned at three months of age. In all the groups, biweekly live body weights were recorded till weaning. The mean of live weight at weaning in Group I, Group II and Group III was 11.17 ± 0.39, 10.53 ± 0.40 and 9.06 ± 0.44 kg, respectively. The 0 - 3 month Average Daily Gain (ADG) for Group I, Group II and Group III lambs was 101.70 ± 4.25, 94.36 ± 4.26 and 78.72 ± 4.60 g, respectively. ADG in both Group I and II lambs was significantly higher ($P \leq 0.05$) than Group III lambs but significant difference was observed in ADG between Group II and Group I lambs. This study demonstrated improved growth performance of Mandya lambs that had free suckling or continuous access to their dams during early part of growth phase.

Key words: Mandya lambs, Suckling, Growth performance

Pre-weaning growth performance in young lambs is greatly influenced by the quantity of maternal milk available and as well as the pattern of suckling. It is essential to understand the suckling pattern of young lambs in order to expedite optimal growth performance and early weaning, thereby reduced input costs or better economic returns. A strong mother - lamb communication is established immediately after lambing and this has particular importance for the growth characteristics of the lamb. It is also evident that significant improvement occurs in live weight of lambs, which were allowed free suckling and longer stay with their dams (Napolitane *et al.*, 2002). In view of the above, the present study was undertaken to investigate the influence of suckling pattern on pre - weaning growth performance in Mandya lambs reared under semi-intensive system of management.

MATERIALS AND METHODS

The study was carried out at Livestock Research and Information Centre (Sheep), Karnataka Veterinary, Animal and Fisheries Sciences University, Nagamangala, Mandya District, Karnataka. The birth weight of lambs was recorded within 24 hours of birth using digital weighing machine. The respective dam and lambs were kept in separate pens for a week and thereafter lambs were allocated randomly to one of the three treatment groups *viz*., T1 (lambs were allowed for free suckling till one month of age) ; T2 (lambs were allowed for free suckling till two month of age) and T3 (lambs were restricted from suckling by way of separation from dams for a minimum period of three hours between consecutive sucklings). Lambs in T1 and T2 were allowed together with dams during their respective experimental period after which, lambs were switched to restricted suckling pattern until weaning. Lambs were housed together with dams during night hours. Beginning from day 0 (day of birth) all the lambs were weighed during early hours of the day, twice in a month, until three months of age. The lactating ewes were maintained in a standard management condition with the

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supplement of compounded sheep feed. All the lambs were encouraged to consume roughage *ad libitum* during experimental period to stimulate their ruminal activity.

The data of live body weight and ADG were analyzed by least square method using GLM procedure using "Statistical Analysis Software" (SAS 9.3) and Duncan test was used to compare the mean of different treatment groups. In the statistical model, suckling pattern was considered as fixed effects.

RESULTS AND DISCUSSION

The data on live weights of lambs recorded at different age interval *viz.*, birth weight, II week, IV week, VI week, VIII week, X week and XII week are presented in Table 1. Table 2, illustrates the ADG of lambs recorded at different time intervals *viz.*, 0 - II wk, II - IV wk, IV - VI wk, VI - VIII wk, VIII - X wk, X - XII wk and overall 0 - XII wk for the lambs in T1, T2 and T3 groups.

Table 1: Least squares mean of body weight (kg) recorded during pre-weaning stage of Mandya lambs subjected for different suckling pattern

Parameter	Birth wt.	II wk	IV wk	VI wk	VIII wk	X wk	XII wk
P value	**3.514**	**0.0004**	**0.0023**	**0.0046**	**0.0023**	**0.0008**	**0.0016**
1 month free suckling	2.02 ± 0.05 (30)	4.08 ± 0.13[a] (30)	5.56 ± 0.19[a] (30)	6.99 ± 0.24[a] (30)	8.53 ± 0.32[a] (30)	9.89 ± 0.33[a] (30)	11.17 ± 0.39[a] (30)
2 month free suckling	2.05 ± 0.06 (30)	4.15 ± 0.15[a] (30)	5.53 ± 0.23[a] (30)	6.87 ± 0.32[a] (30)	8.23 ± 0.35[a] (30)	9.47 ± 0.41[a] (30)	10.77[a] ± 0.45 (30)
Restricted suckling	1.99 ± 0.07 (30)	3.44 ± 0.11[b] (30)	4.68 ± 0.18[b] (30)	5.85 ± 0.22[b] (30)	7.01 ± 0.28[b] (30)	7.94 ± 0.36[b] (30)	9.06 ± 0.44[b] (30)

Figures in parentheses are number of observations. Means bearing common superscript in the same column do not differ significantly.

Data analysis revealed that birth weight had no significant effect between treatment groups T1, T2 and T3 (2.02 ± 0.05, 2.05 ± 0.06 and 1.99 ± 0.07, kg). From week II, there was a highly significant difference in growth performance, which prevailed between the treatment groups. The growth pattern of lambs in T1 and T2 found to be similar, whereas the lambs in T3 group recorded a significantly lower body weight in contrast to the lambs in T1 and T2, which was in accordance with the findings of Ozdal *et al.* (2006) in Karakas lambs.

The lower body weight of lambs in T3 groups may be attributable to the fact that they might have consumed lesser quantity of milk due to interruption or gap during successive suckling, which could have been further complimented by decreased milk secretion from the dams. Long - term nursing and stimulation of the mammary gland is important for milk production (Hatfield *et al.*, 1995) as the decrease in number of daily milking (or suckling) during early lactation results in reduced milk yield in ewes (Knight and Gosling, 1994), due to decreased alveolar diameter (Li *et al.*, 1999) and/or reduced functional udder capacity (Wojtowski *et al.*, 2006). It has been demonstrated that suckling would stimulate the mammary gland (Bruckmaier and Wellnitz, 2008) and facilitates secretion of neuro - pituitary peptides by neuro - endocrine mechanisms (Marnet and Mckusick, 2001), which in turn release oxytocin for induction of milk ejection (Negrao *et al.*, 2001). In addition, frequency of suckling and distribution of milk in the udder is known to affect the milk composition significantly (Jackuliakova and Tancin, 2011). Mskusick *et al.* (2001) reported significant drop (30 to 40 %) in milk production due to less frequent udder evacuation and lacking of the mother - young one bonding. Extended suckling or milking intervals increases the intra - mammary pressure, decreases alveolar drainage and increases the mammary tight junction permeability, all of which affects the milk secretion and consequently daily milk yield (Peaker, 1980), especially in ewes with smaller cisterns of the udder (Jackuliakova and Tancin, 2011). Castillo *et al.* (2009) reported that ewes with large udder cisterns were better adaptive

Table 2: Least squares mean of ADG (g) recorded during pre-weaning stage of Mandya lambs subjected for different suckling pattern

Parameter	0 – II wk	II – IV wk	IV – VI wk	VI–VIII wk	VIII–X wk	X – XII wk	Overall 0 - XII wk
P value	**<0.0001**	**0.4097**	**0.2412**	**0.1016**	**0.0961**	**0.6578**	**0.0013**
1month free suckling	137.90 ± 6.69[a] (30)	98.09 ± 6.26 (30)	95.58 ± 6.84 (30)	102.4 ± 9.13 (30)	90.51 ± 9.90 (30)	85.61 ± 11.33 (30)	101.70 ± 4.25[a] (30)
2 month free suckling	140.00 ± 8.34[a] (30)	92.09 ± 9.03 (30)	86.69 ± 7.98 (30)	90.65 ± 7.51 (30)	82.27 ± 9.87 (30)	86.54 ± 9.39 (30)	96.87 ± 4.76[a] (30)
Restricted suckling	96.74 ± 5.22[b] (30)	82.33 ± 9.48 (30)	78.13 ± 7.29 (30)	77.46 ± 7.74 (30)	62.05 ± 8.48 (30)	74.90 ± 9.03 (30)	78.60 ± 4.62[b] (30)

Figures in parentheses are number of observations. Means bearing common superscript in the same column do not differ significantly

to extended milking interval than ewes with small cisterns of the udder.

In the present study, the overall ADG in restricted suckling group T3 (78.60 ± 4.62 g) was significantly lower compared to the ADG in free suckling group T1 (101.70 ± 4.25 g) and T2 (96.87 ± 4.76 g), which is in congruous with the findings of Cimen and Karaalp (2009) in non - dairy sheep. The bi - weekly ADG differed significantly during initial days (0 – II wk of age) and thereafter there was a non-significant decrease of ADG levels in T3 lambs in contrast to the ADG levels of lambs in T1 and T2 group. During first few weeks of early life ADG levels were higher than the subsequent weeks, which may be attributable to the overall composition of milk. Ewe milk production increases from birth and reaches its peak between two and four weeks and there after it declines slowly (Cardellino and Benson, 2002). Milk yield, proteins and total solids would be higher at the early stages of lactation, then decreases during mid lactation stage and it increases gradually during the late stage of lactation, whereas fat, lactose and ash were more stable throughout lactation stages (Mahmoud *et al.,* 2014).

The ADG in T1 group were lower during IV – VI week interval (95.58 ± 6.84 g) than week II – IV week interval (98.09 ± 6.26 g). The ADG of T2 group had a similar pattern at VIII – X week (82.27 ± 9.87 g) and VI - VII week (90.65 ± 7.51g) interval, which may be attributable to the stress due to switching over from free suckling to restrict suckling mode. However, such lambs would regain the ADG levels during subsequent weeks. Lambs in T3 recorded lower ADG levels throughout their experimental period.

In conclusion, this study demonstrated an improved growth performance of Mandya lambs that had free suckling or continuous access to their dams during early part of their pre - weaning growth phase. Further it may be concluded that lambs with sustained access to milk and continuous bonding with dams would enhance the weaning weight of lambs thereby improved economic output.

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Evaluation of Some Tropical Feedstuffs on Fermentation Characteristics to Assess their Fodder Potential*

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ABSTRACT

Fifteen tropical feedstuffs comprising eight roughages *viz.*, sugarcane bagasse; (SCB) sorghum stover (SS), fresh banana plant waste (BPW) and fresh banana plant waste silage (BPWS), Banana pseudostem silage (BPS), BPS mixed with SS (50:50), Banana plant (85%) mixed with SCB (13%) and ground sorghum grain (2%):BJS, 10% urea treated BJS silage and seven concentrates *viz.*, ground sorghum (GS), maize (GM), wheat bran (WB), soybean meal(SBM), concentrate feed mixture (CFM), 2% urea treated maize, 1% urea treated wheat bran, which are commonly used for ruminant feeding were analyzed to evaluate rumen fermentation characteristics and microbial biomass indices to assess their fodder potential. The fermentation characteristics were studied by *in vitro* gas production technique by fitting cumulative gas production against time of incubation in the exponential model, $Y=D(1-e^{-k*t})$, where Y is gas volume at time *t*, *D* is potential gas production(ml/g DM) and *k*(h^{-1}) is rate at which gas is produced. The microbial biomass indices were obtained by determining the ratio of truly digested OM and gas production at $t_{1/2}$. The ME predicted based on chemical composition (Summative equations of detergent system of feed analysis: SEDS) was compared with gas production technique (Rumen *in vitro* incubation by gas production; RIVIGP). Among roughages, CP (g/kg) content was highest in banana plant waste (88) and lowest in sugarcane bagasse (21.7). Whereas in fermented feedstuffs (BPWS), due to proteolysis, the protein content was decreased than the parent (BPW) feedstuff (88*vs* 68g/kg). In concentrates, CP was highest in wheat bran (158.1 g/kg) and lowest in sorghum grain (77.4). Among roughages the NDF content exceeded 600(g/kg) in all feedstuffs reaching as high as 819 in SCB. While in concentrates, NDF (g/kg) was varied from 123.9 in sorghum to 432.5 in wheat bran. Remaining values are between these two extremities. The *D* and *k* values were higher for concentrates than roughages and so on for PF values. The ME derived from SEDS had a close association with RIVIGP (R^2 0.89). It can be concluded that SS, PS+SS and WB were superior feedstuffs with high PF among roughages, fermented feedstuffs and concentrates respectively. But, SBM ranked first with lowest gas, higher TDOM and PF when compared to other feedstuffs.

Key words: Tropical feedstuffs, *In vitro* gas production, Fermentation characteristics

The demand for feed resources for ruminant production in India is increasing day by day because of rising costs and limited supplies. It is, therefore, imperative to use fully the locally available feed resources such as crop residues, non-conventional feedstuffs to alleviate feed shortage. Such an attempt does not require additional arable land for cultivation. In order to throw a light on usage of such unconventional feed stuffs in large scale, feed evaluation is essential. A range of feed evaluation techniques is available to predict the nutritional value of ruminant feedstuffs. A gas production technique (GPT) is one such method to predict ME value of ruminant feed stuffs, OM degradation and microbial biomass synthesis. Thus, the present study was undertaken to evaluate few crop residues and concentrates by *in vitro* techniques as potential feed for ruminants.

MATERIALS AND METHODS

The feed samples were divided arbitrarily into roughages *viz*.. sugarcane bagasse (SCB) sorghum stover (SS), fresh banana plant waste (BPW) and fresh banana plant waste silage (BPWS), banana pseudostem silage (BPS), BPS mixed with SS (50:50), banana plant (85%) mixed with SCB (13%) and ground sorghum grain (2%):BJS, 10% urea treated (UT) BJS silage; concentrates *viz*..ground sorghum (GS), maize (GM), wheat bran (WB), soybean meal (SBM) and concentrate feed mixture (CFM), 2% urea treated maize, 1% urea treated wheat bran. All the feedstuffs were dried in hot air oven at 60^0C until constant weight, ground to pass 1mm sieve in Willey mill and stored in airtight bottles at room temperature. Ground samples were analyzed for proximate analysis

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(AOAC,1995) and detergent system of analysis (Goering and Van Soest,1970). Predicted energy was calculated based on chemical composition of feedstuffs by summative equations of detergent system (SEDS) for roughages as proposed by Goering and Van Soest, (1970) ; Van Soest, (1971) and for concentrates as per Giger – Reverdin *et al*.(1994).

Donor cow and collection of rumen fluid: A lactating dairy cow producing 9.0kg of milk per day, fitted with a flexible rumen canula of large diameter (Bar Diamond, Inc. USA), receiving a basal diet consisting of 50% ragi straw, 50% maize silage and CFM (maize-50, WB-45, urea-2% mineral mixture-2%, salt-1%,) was used as the donor cow for rumen fluid. The roughage and CFM were fed separately. The roughage was offered 8 kg in proportions six times in a day, starting at 9.00 a.m. The CFM was offered 4.0 kg per day in two equal portions at 6.00 a.m. and 2.30 p.m. Rumen fluid was collected in the morning between 9.00 a.m. and 9.30 a.m. before offering feed.

Metabolisable energy (ME) determination: Using proximate composition and rumen *in vitro* gas production (RIVIGP) the net gas production (corrected for blanks and reference standard hay and concentrate) at 24 h incubation, ME and *in vitro* organic matter digestibility (IVOMD) in roughages and concentrates were calculated by equations 1 and 1a ; 2 and 2a according to Menke and Steingass (1988).

Roughage:

$ME = 2.2+0.1357GP+0.0057CP+0.0002859\ EE^2$ ……..(1)

IVOMD= 16.49+0.9042GP+0.0492CP+0.0387TA……...(1a)

Concentrate ingredients and CFM:

ME= 1.06+0.1570GP+0.0084CP+0.022EE-0.0081TA…..…..(2)

IVOMD = 9+0.9991GP+0.0595CP+0.0181TA…… (2a)

Where,

GP=Gas, ml/200mg DM

CP, EE, TA are crude protein, ether extract, total ash respectively, in g/kg DM

ME=Metabolisable energy, MJ Kg^{-1}; IVOMD=In vitro organic matter digestibility.

The ME derived by RIVIGP and SEDS were compared and interpreted accordingly.

Kinetics of gas production: Air equilibrated feed samples (200± 10 mg) of roughages and concentrates were incubated in 100ml calibrated glass syringes in triplicate according to Menke and Steingass (1988) with 30 ml mixed rumen suspension with three blank incubations and standards. Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, 72, 84 and 96 h of incubation. The rate and extent of gas production were calculated by non–linear regression using the exponential model $Y= D\ (1-e^{-k*t})$ Where, Y is gas volume (ml) at time *t, D* is potential gas production (ml) and *k* is rate (per hour) at which gas is produced (Krishnamoorthy *et al*., 1991). The time at half asymptotic gas production ($t_{1/2}$) was calculated as In2/k. Gas production at 24 h, corrected for the blank and standards were used for determination of ME.

Microbial biomass synthesis: The microbial biomass synthesis of feedstuffs was obtained by determining the ratio of truly digested organic matter (TDOM) and gas production at $t_{1/2}$ described by Blummel *et al*. (1997). Incubation was run for all feed stuffs in triplicate to determine PF valued at $t_{1/2}$. Air equilibrated feed samples (500±10mg) were weighed into 100ml calibrated syringes and incubated with 40ml of mixed rumen suspension at 39^0 C with parallel incubations of blanks (Makkar *et al*.,1995). Incubations were terminated by recording gas production at $t_{1/2}$ for respective feed samples by immersing in ice water bath to prevent further microbial activity. The contents of the syringes were rinsed with 100ml neutral detergent solution (NDS) into the syringe each time. Refluxed the incubation residue for 1h followed by filtration on preweighed gooch crucibles (Grade-2) to recover true undigested matter (Goering and Van Soest,1970). Crucibles with undigested organic matter (OM) of feed origin recovered in the residue. The PF was calculated as the ratio of mg TDOM to ml gas produced at $t_{1/2}$.

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of roughages, fermented feedstuffs and concentrates is summarized in Table 1. The OM (g/kg) content of roughages, fermented feedstuffs and concentrates

varied from 844.8 to 958.9, 842.8 to 880.6 and 855.5 to 977.4, respectively. Among roughages, CP (g/kg) content was highest in banana plant waste (88) and lowest in sugarcane bagasse (21.7). While in fermented feedstuffs (BPWS), due to proteolysis (McDonald *et al*., 2002) the protein content was decreased further than the parent (BPW) feedstuff (88 *vs* 68g/kg). It is obvious that, due to urea enrichment the protein content of feedstuffs increases further. Again, protein content of mixed feedstuff (BJS) decreases further due to addition of SCB. In concentrates, CP (g/kg) was highest in wheat bran (158.1) and lowest in sorghum grain (77.4) except in urea treated concentrate and CFM. Among roughages, the NDF (g/kg) content exceeded 600 in all feedstuffs reaching as high as 819 in SCB. While in concentrates, NDF was varied from 123.9 in sorghum to 432.5 in WB. Other nutrients such as the EE, CF, ADF and ADL varied to some extent. The estimated values were on par with earlier reported values (Sheikh, 2000; Kiran and Krishnamoorthy, 2007).

Metabolizable energy and in *vitro* organic matter digestibility (IVOMD): The gas production kinetics, metabolisable energy and organic matter digestibility are given in Table 2. In general, gas production was recorded higher in concentrate supplements followed by dry roughages and lowest in fermented feedstuffs. The difference in the gas production was due to varied level of fermentable carbohydrates and available nitrogen. Therefore, the feedstuffs with higher fermentable substrate tend to produce more gas and the protein supplements tend to produce less gas compared to the energy supplements. Among roughages, the predicted ME (MJ/kg DM) obtained from RIGVP ranged between 5.35 in SCB to 8.1in SS whereas in fermented feedstuffs, it varied from 5.33 (PS) to 7.64 (PS+SS). In concentrates, wheat bran had lowest ME content (10.9) against highest in maize (13.13**).** However, the gas production was decreased in 10% urea treated BJSS. Similar observations with urea supplementation to straw were also reported by Pichad and Metha (2010).

Table.1: Chemical composition (g/kg) of some tropical feedstuffs on dry matter basis

Feedstuffs	OM	CP	EE	CF	TA	NDF	ADF	BS	ADL
Roughages									
BPW	888.60±2.0	88.00±1.8	12.70±0.5	303.00±4.9	111.40±2.0	622.10±4.2	392.00±5.7	09.30±0.28	73.10±1.8
BJS	879.50±1.8	79.10±1.8	11.00±0.7	328.90±8.4	120.50±1.8	648.00±5.0	443.10±5.8	04.00±0.5	92.50±1.4
SS	844.80±0.6	27.30±1.0	17.50±1.5	350.8±11.9	93.90±0.6	663.90±8.5	496.20±9.8	12.40±1.4	94.20±1.0
SCB	958.90±1.6	21.70±1.9	06.10±0.5	455.00±7.7	41.10±1.6	819.00±5.5	626.50±2.7	01.70±0.6	103.00±0.9
Fermented feedstuffs									
BPWS	880.60±0.23	68.00±1.2	13.30±1.5	361.30±7.0	119.40±2.3	643.20±5.5	467.80±4.2	04.00±0.5	92.50±1.4
PS	842.80±0.19	49.50±2.0	20.30±1.1	361.30±16.6	157.20±1.9	600.30±2.0	382.50±5.0	01.80±0.2	96.50±2.1
PS+SS	873.10±0.20	38.40±1.7	18.50±0.8	332.10±5.5	126.90±2.0	632.90±5.2	439.10±0.9	06.40±0.10	95.30±1.9
10%UTBJS	841.10±0.39	335.40±4.5	12.10±0.3	378.60±13.3	158.90±3.9	690.10±5.8	539.9±9.1	05.90±0.5	98.90±2.3
Concentrates									
Sorghum	977.40±2.6	77.40±2.3	20.40±0.9	18.60±4.4	22.60±2.6	123.90±2.5	058.9±1.2	4.2±0.6	11.20±0.5
Maize	956.70±0.3	89.10±2.0	29.70±0.3	17.80±5.0	43.30±0.3	135.40±3.2	35.6±0.3	0.37±0.3	11.80±0.7
2%UTM	953.30±3.1	145.30±3.3	30.10±0.3	20.10±7.5	46.70±3.1	137.80±5.8	31.7±8.8	04.2±0.9	12.10±1.9
Wheat bran	906.60±0.2	158.10±1.3	25.00±0.5	91.40±3.6	93.40±0.2	431.50±1.4	115±1.8	05.6±0.14	28.10±1.0
1%UTWB	906.60±4.1	186.20±3.7	24.50±0.1	91.40±11.2	93.80±4.1	432.50±10.5	121.1±5.9	8.50±0.8	27.30±2.9
CFM	939.60±1.5	166.90±2.9	23.10±0.9	37.30±2.0	60.40±1.5	235.00±6.0	93.7±1.1	6.5±1.00	17.70±1.3
SBM	855.50±1.2	471.50±1.0	11.70±1.0	49.70±4.1	144.50±1.2	223.60±6.6	97.9±0.8	10.1±0.6	13.10±0.7

The ME estimated by SEDS was 3.95, 5.19 5.05 5.46 (MJ/kg DM) in BPS, BPWS BJS, and SCB, respectively. Among concentrates tested, WB had lower ME than sorghum (11.46 *vs* 13.13). Remaining values were in between these two extremities**.** Among different feedstuffs tested, SBM had highest ME (13.38MJ/kg), CP (471.5g/kg). Although ME content of roughages and CFM predicted by RIVIGP and SEDS differed, the relationship between ME (RIVIGP) (y) and ME (SEDS) (x) was y=2.60+0.75x(*r* 0.94, P<0.01). Thus, in the present observation ME predicted from RIVIGP and SEDS, were almost closely associated (R^2 0.89) with each other (Graph-1). Therefore, energy estimation by SEDS had the advantage of easy adaptability of feed analysis. The obtained ME content of different feedstuffs were in concurrence with the reported values of Elahi *et al.*, 2012; Krishnamoorthy *et al*.,1995 with some exceptions.

The IVOMD varied from 39.30 (SCB) to 58.71 (SS) in dry roughages while in fermented feedstuffs it ranged from 43.18 (BPS) to 56.72 (PS+SS) and in all the concentrate feedstuffs tested, the predicted IVOMD exceeded 75%. In general *IVOMD* was lowest in fermented feedstuffs than any dry roughages except sugarcane bagasse and PS+SS. These IVOMD of banana plant waste values reported by previous study (Viswanathan *et al*.,1989) was higher than the values obtained in this study. Variety difference could only be possible reason between reported and observed values. Urea treatment increased CP content of BJSS but the ME, IVOMD were not affected by urea treatment much.

***In vitro* rumen gas production kinetics:** The gas production kinetics, substrate degradation, PF and MBP are summarized in Table 3. The *D* (ml/g DM)

Table 2: Gas production (GP-24h), predicted ME (MJ/kg DM) and *in vitro* organic matter digestibility (*IVOMD* %) of some tropical feedstuffs

Feedstuffs	GP-24h	ME (RIVGP)	ME (SEDS)	*IVOMD* (%)
Roughages				
BPW	134.62±2.54	6.37±0.07	5.41±0.07	49.25±0.46
BJS	130.38±02.10	6.19±0.06	5.05±0.07	48.39±0.38
SS	210.55±3.42	8.10±0.09	5.32±0.27	58.71±0.51
SCB	112.40±01.95	5.35±0.05	5.46±0.04	39.30±0.35
Fermented feedstuffs				
BPWS	105.79±1.97	5.48±0.05	5.19±0.06	43.40±0.35
BPS	101.48±1.50	5.33±0.04	3.95±0.03	43.18±0.27
PS+SS	187.22±4.07	7.64±0.11	4.79±0.19	56.72±0.42
10%UTBJSS	53.52±0.84	5.62±0.02	3.92±0.15	48.90±0.15
Concentrate				
Sorghum	356.09±5.15	13.05±0.16	13.13±0.02	84.46±1.02
Maize	354.57±4.96	13.13±0.15	13.03±0.04	84.16±0.98
2%UTM	376.01±1.37	14.28±0.04	13.15±0.09	92.86±0.27
Wheat bran	280.44±2.67	10.90±0.08	11.46±0.03	75.58±0.53
1%UTWB	281.42±4.05	11.17±0.13	11.54±0.08	77.44±0.80
CFM	303.18±3.36	11.91±0.10	12.79±0.07	80.00±0.67
SBM	217.19±2.41	10.86±0.08	13.81±0.02	82.62±0.48

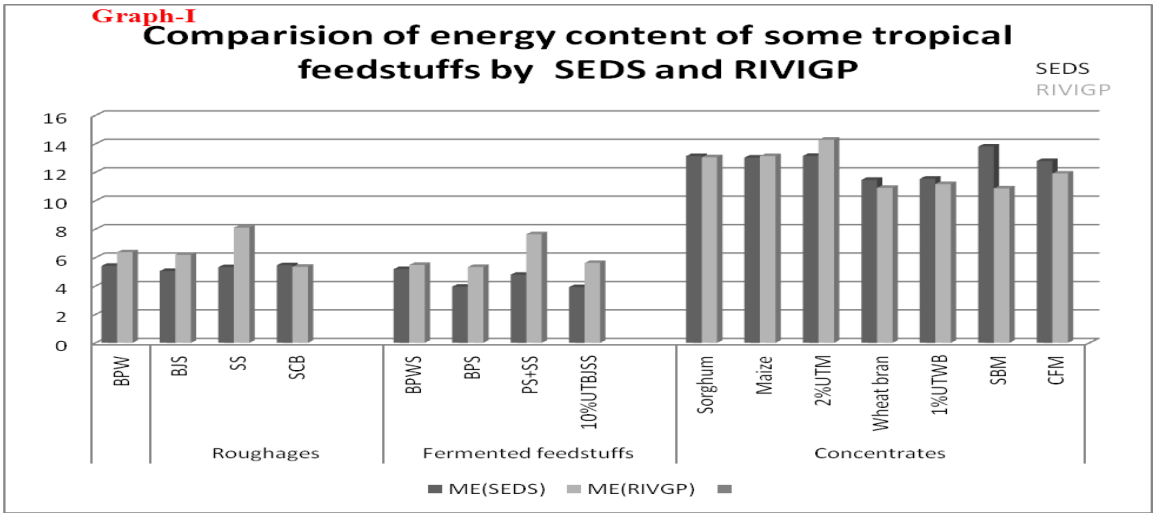
value for roughages, fermented feedstuffs and concentrates were ranged between 141.05 to 337.7, 172.55 to 304.3 and 344.4 to 409.6, respectively. The corresponding feedstuffs had *k* values between 0.03361 to 0.05224, 0.03462 to 0.0416 and 0.07293 to 0.0928, respectively.

The PF (ml/mg) ranged from 3.24 to 5 in roughages while in fermented feedstuffs varied from 4.52 to 5.24 but in concentrate it exceeded 5 in all feedstuffs. However, lower volume of gas measured in 10% urea treated BJSS could be attributed to highest PF but the TDOM was low as

Table 3. Gas production kinetics, microbial biomass production (MBP) and efficiency of microbial biomass synthesis (EMBS) of different feedstuffs

Feedstuff	$t_{1/2}$ (hr)	*D* (ml/g)	$k(h^{-1})$	Gas at t1/2 (ml/g)	TDOM (mg/g DM)	PF (mg/ml)	MBP at $t_{1/2}$(mg)	EMBS at $t_{1/2}$ (g/kg)
Roughages								
BPW	13.3	189.1	0.05224	101	440.93	4.37	218.73	496.07
BJS	14.1	141.05	0.03869	86.72	356.34	4.11	165.56	464.60
SS	15.9	337.7	0.04351	79.03	395.78	5.01	221.91	560.70
SCB	20.6	193.95	0.03361	88.43	286.61	3.24	92.06	321.22
Fermented feedstuffs								
BPWS	20	182.8	0.03462	86.35	388.9	4.52	198.93	511.52
BPS	18.5	172.55	0.03738	50.58	251.98	4.99	140.70	558.39
PS+SS	16.7	304.3	0.0416	71.78	375.89	5.24	217.97	579.89
10%UTBJSS	31.1	139.6	0.02229	35.66	329.55	9.27	251.10	761.94
Concentrates								
GS	7.5	406.4	0.0928	156.52	801.81	5.12	435.55	543.21
GM	7.8	409.6	0.08878	167.68	886.81	5.31	494.43	557.54
2%UTM	7.5	430.25	0.09273	122.44	883.66	7.22	597.15	675.76
WB	9.5	344.4	0.07293	117.02	713.45	6.1	439.62	616.19
1%UTWB	9.1	342.55	0.07578	109.71	735.78	6.71	479.05	651.08
CFM	7.9	351.6	0.08825	106.73	790.35	7.43	540.60	684.0
SBM	9.2	265.5	0.07504	110.3	828.27	7.52	570.16	688.38

MBP= [TDOM – (gas at t1/2 x SF)], where SF (stoichiometric factor) was considered as 2.20 for roughages and 2.34 for concentrate. EMBS = (MBS/TDOM) x 1000



compared to other banana plant based feedstuffs indicating medium quality feedstuff. The PF of the banana plants in this study was consistent with the results of previous study (Amarnath and Balakrishnan,2007). In all the feedstuffs tested, the PF and EMBS for the concentrate supplements was higher (5.12 mg/ml and 543.21g/kg) than other feedstuffs. The PF of feedstuffs can theoretically vary from 2.75 to 4.41 (Blummel *et al*., 1997). Therefore, PF exceeding 4.41 is likely an analytical artefact. Any factor that increases TDOM, such as soluble unfermented OM, or that decreases gas production, such as the degraded protein, contribute to higher PF (Blummel *et* al.,2003). In this study since the incubations were terminated at $t_{1/2}$ as recommended by Blummel *et al*.(2003) and for concentrate sources was less than 12h, the possibilities of unfermented OM contributing to TDOM cannot be ruled out. Thus, using the GPT in combination with substrate degradation may be used to rank feeds of interest but for full tool in the feed evaluation with whole rumen models are more appropriate (Dijkstra *et al*., 2005).

CONCLUSION

The feedstuffs used in this study have their own nutritive value and fermentation characteristics. Among roughages which were evaluated for their fodder potential to ruminants, SS ranks first with highest ME, PF, MBP, lowest gas production than BPW and BJS. Whereas for fermented feedstuffs PS+SS ranks first with higher ME, PF, MBP and lowest gas production than PS and BPWS. In concentrates WB, CFM and maize were decreasing order of feed quality. But, SBM ranked first with lowest gas, higher TDOM and PF when compared to other feedstuffs.

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Genetic Polymorphism of Calpastatin Gene in Bandur Sheep

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ABSTRACT

The present study was carried out to determine the genetic polymorphism of Calpastatin gene in Bandur sheep, a native breed of Karnataka. Blood samples were collected from randomly chosen 100 Bandur ram lambs born at Livestock Research and Information Centre (Sheep), KVAFSU, Nagamangala and from different farmer's flocks in the home tract. Genomic DNA was extracted by following Miller's High salt method. Using gene specific primers, ovine Calpastatin sequence of 620 bp comprising of exon 1C/1D (including the intervening intron) was amplified. Further, PCR-RFLP analysis was carried out using *MspI* and *NcoI* restriction enzymes, which differentiated three genotypes *viz*., MM, MN and NN having genotypic frequency of 0.24, 0.59 and 0.17, respectively. The frequency of M and N allele were 0.535 and 0.465, respectively. Alignment of M and N allele sequences of Calpastatin by CLC Main Workbench 6.8.1. showed 5 SNPs; G→A transition at 286 and 352 bp, C→T transition at 373 and 526 bp and A→G transition at 443 bp position. Chi square test confirmed the existence of Hardy Weinberg equilibrium in the studied population. Genetic variability observed in the calpastatin gene could be used to find association of observed genotypes and SNPs with growth, carcass and meat quality traits.

Key words: Calpastatin, Restriction enzymes, SNPs, Bandur sheep

Bandur sheep is an other popular name of Mandya sheep, which has been acclaimed as one of the best mutton type indigenous sheep breed of Karnataka and in India. Besides, its meat is being treated at National and International spectrum of sheep products as the most nutritious and of high quality in the diets of human beings. Bandur phenotype is typically meat type with inverted 'U' shaped conformation from the rear. Although it is highly rated for its meat quality/ taste, there is scant literature on the carcass traits and mutton quality traits of Bandur sheep. While traditional methods to improve carcass and meat quality traits turned out to be inefficient, recent development of molecular genetics tools eased the recognition of genes that may have a large effect on specific traits. One of the ways of identifying genes that determine a given trait is to evaluate the polymorphism in a candidate gene underlying the trait and contributing to a specific phenotype (Gregula-Kania, 2012).

Ovine calpastatin gene is considered as one of the potential candidate gene for growth, carcass traits and to improve slaughter lamb production (Palmer *et al*., 1999). Calpastatin gene is of 100 kb length; includes 4 exons and located at 5q15 region on chromosome no. 5 (Gabor *et al.*, 2009). The 'Calpain-Calpastatin System (CCS) is found in most of the animal tissues and influences many important processes including muscle development and degradation, regulation of protein degradation and rebuilding, cell cycle, organogenesis, post mortem meat tenderization, cataract formation and cell death (Merin *et al.*, 1998). The CCS is constituted of three well characterized proteins, which include two ubiquitous Ca^{2+} dependent proteolytic enzymes (μ and m calpain). Whereas, Calpastatin is the third member capable of inhibiting more than one calpain molecule**.** Calpastatin is a variable component of the calpain system and skeletal muscle calpastatin activity is highly related to rate of muscle protein turnover and post-mortem tenderisation. All these factors are main concerns for livestock industries to improve rate of growth and meat quality (Goll *et al*., 2003).

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In recent years, increasing and improving quality of meat have been highlighted, by various reports confirm relationship of calpastatin polymorphism with growth and meat quality traits *viz*. Nassiry *et al*. (2006) found that AB genotype had higher daily weight gain in Iranian Kurdi sheep and whereas, Palmer *et al*. (1999) reported that AC genotype (123 g/ day) having increased live weight at 18 per cent more than AA genotype in the crossbred Dorset and Coopworth sheep. In view of these evidences from the literature, the present study was carried to identify the possible genetic polymorphism of calpastatin gene, as a productive marker related to growth and meat quality traits in Bandur sheep.

MATERIALS AND METHODS

Collection of Blood Samples and Isolation of DNA: Blood samples were collected from 100 Bandur ram lambs distributed over the villages of Malavalli taluk, Mandya district as well as from Livestock Research and Information Centre (Sheep), KVAFSU, Nagamangala. Approximately 5 ml of blood were collected in vacutainer containing EDTA and stored at 4 $^{\circ}$C. The blood samples were processed within 24 hours of collection. Genomic DNA was isolated by adopting High salt method as described by Miller *et al*. (1988) with necessary modifications. The purity of the isolated DNA was ascertained by Spectrophotometer reading and electrophoretic analysis using 0.8 per cent agarose gel.

Polymerase Chain Reaction amplification of Ovine Calpstatin sequence: Using sequence specific primers, exon 1C/1D and the intervening intron from a portion of the first repetitive domain of the ovine CAST gene was amplified by Polymerase Chain Reaction (PCR). Further, the PCR amplified product was subjected for RFLP analysis using *MspI* and *NcoI* restriction enzymes. Oligonucleotide primers were procured from Amnion Biosciences Pvt. Ltd., Benagluru, India. Details of primer sequences, size of expected PCR amplicons and related references are presented in Table 1.

Table 1: Details of Primers used for amplification of Calpastatin gene in Bandur Sheep

Primer sequence	Expected product size (bp)	Reference
CAST 1C- Forward (5' TGGGGCCCAATGA CGCCATCGATG 3')	620	Palmer *et al*., 1998; Nassiry *et al.*, 2006; Mohammadi *et al*., 2008
CAST 1D - Reverse (5' GGTGGAGCAGCAC TTCTGATCACC 3')		

Thermal Cycler (S1000, Bio-Rad, USA) was used for PCR amplification of CAST gene using Red PCR Master Mix (Amnion Biosciences Pvt. Ltd.). The reaction mixture of 25 µl consisted of 12.5 µl of Red PCR Master Mix, 1 µl (10 pmol/ µl) each of Forward (Exon 1C) and Reverse (Exon 1D) primer, 9.5 µl of PCR grade water and 1 µl (100 ng) of template DNA. The PCR reaction was carried out with an initial denaturation temperature of 95 $^{\circ}$C (5 min), 33 cycles of 94 $^{\circ}$C (1 min), 60 $^{\circ}$C (1 min) and 72 $^{\circ}$C (2 min), followed by final extension at 72 $^{\circ}$C (8 min). The PCR amplified products were confirmed by resolving on 1.5 per cent agarose in parallel with 100 bp DNA ladder. Gel electrophoresis was carried out at a constant voltage of 100 V for 60 min in 1X TAE buffer.

Restriction Fragment Length Polymorphism (RFLP) Analysis: RFLP analysis of PCR amplified CAST gene was carried out using *Msp*I and *NcoI* restriction enzymes. The details of the restriction enzymes and recognition sites are mentioned in Table 2.

Table 2: Restriction enzymes used for digestion of PCR amplicons

Sl. No.	Restriction enzymes	Recognition site
1.	*Msp*I (*Morexella species*)	5'…C↓CGG…3'
		3'…GGC↑C…5'
2.	*Nco*I (*Nocardia corallina*)	5'…C↓CATGG…3'
		3'…GGTAC↑C…5'

Restriction enzyme digestion was carried in a total reaction mix of 10 µl, which consisted of 3.5 µl of autoclaved triple distilled water, 1 µl of 10 X assay buffer for RE, 0.5 µl of restriction enzyme (10 U/µl) and 5 µl of PCR product. The digestion

mixture was incubated overnight at 37 °C. The digested products were resolved along with 100 bp DNA ladder on 2.5 per cent agarose gel electrophoresis and the resultant fragments that were stained with ethidium bromide were analysed under Gel documentation system (Geldoc XR+, Bio-Rad, USA).

Statistical analysis: The gene and genotypic frequency was calculated based on the fragments resolved by digested PCR products. Presence or absence of Hardy Weinberg equilibrium in the population was determined by Chi square test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The PCR yielded 620 bp fragment comprising the exon 1C/1D and the intervening introns of the ovine CAST gene (Fig. 1). RFLP analysis of PCR products yielded two alleles *viz.*, M and N. The presence of these two alleles in CAST gene was differentiated using two restriction enzymes having differing restriction sites. The enzyme *MspI* digested only M amplimer but not the N whereas, enzyme *NcoI* digested only N amplimer. *MspI* digestion of M amplimer yielded digestion products of 284 and 336 bp whereas, *NcoI* digestion of N amplimer resulted in two fragments of size 370 bp and 250 bp (Fig. 2).

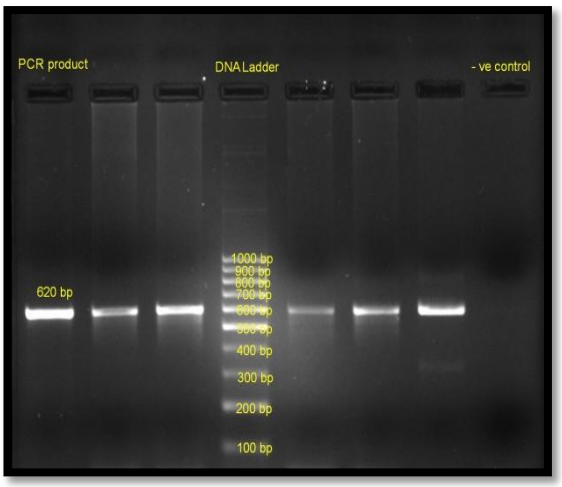

Fig.1: Agarose gel electrophoresis (1.5 %) showing the amplification of Calpastatin gene (Exon 1C/ 1D and the intervening intron) under gel documentation system.

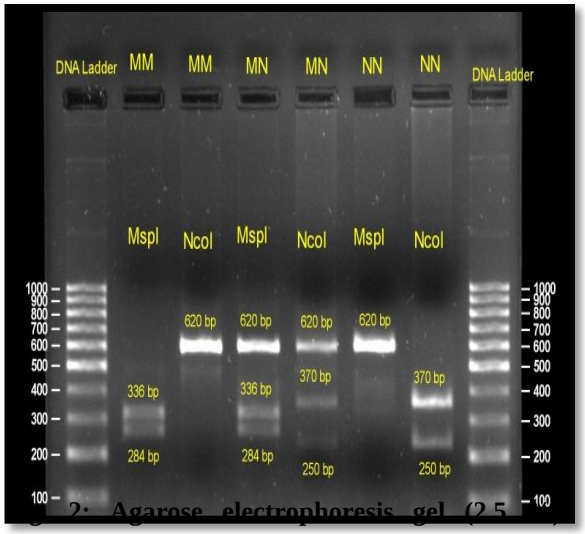

Fig. 2: Agarose electrophoresis gel (2.5 %) showing the PCR-RFLP pattern of Calpastatin gene under gel documentation system.

Lane 1 & 8 – 100 bp Ladder Lane 2, 4, 6 – CAST/MspI genotypes MM, MN & NN and
Lane 3, 5, 7 – CAST/NcoI genotypes MM, MN & NN

Thus, the PCR-RFLP pattern for animals that were homozygous for M allele (MM) produced two and one bands by *MspI* and *NcoI*, respectively and the animals that were homozygous for N allele (NN) produced one and two bands by *MspI* and *NcoI*, respectively. The animals which were heterozygous (MN) yielded three bands each for both *MspI* and *NcoI*.

In the present study the Bandur ram lamb population showed three genotypes MM, MN and NN with frequency of 0.24, 0.59 and 0.17, respectively. The allelic frequencies were 0.535 and 0.465 for allele M and N, respectively (Table 3). The selected PCR product corresponding to three genotypes: MM, MN and NN were got sequenced and the expected size of 620 bp fragment was confirmed. Further, restriction site analysis of these sequences revealed presence of a single restriction site each for *MspI* in M allele (Fig. 3) and *NcoI* in N allele (Fig. 4). Each restriction cleavage yielded two fragments of 284 and 336 bp and, 370 and 250 bp length for M and N allele, respectively.

Table 3: Gene and Genotypic frequency of Calpastatin gene in Bandur sheep

No. of Samp -les	Allele frequency		Genotypic frequency			Chi square value (P < 0.05)
	M	N	MM	MN	NN	
100	0.535	0.465	0.24	0.59	0.17	3.4526^{NS}

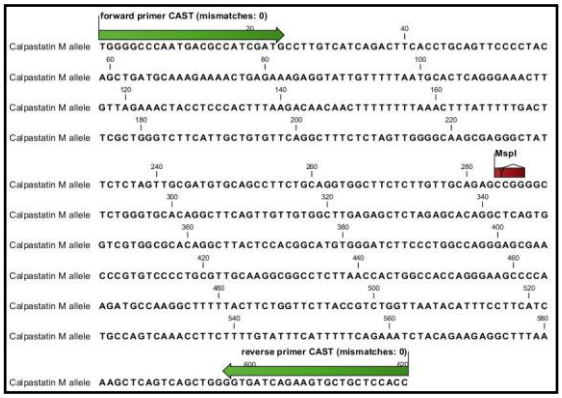

Fig. 3: Annotated sequence of Calpastatin M allele showing restriction site for MspI and Primer position

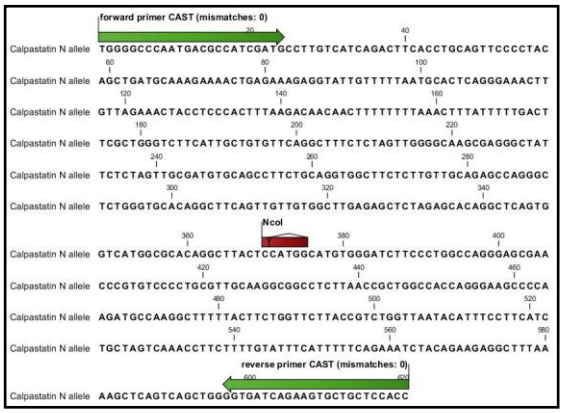

Fig. 4: Annotated sequence of Calpastatin N allele showing restriction site for NcoI and Primer positio

Alignment of M and N allele sequences of CAST by CLC Main Workbench 6.8.1. showed 5 SNPs: G→A transition at 286 and 352 bp (Fig. 5), C→T transition at 373 and 526 bp (Fig. 6) and A→G transition at 443 bp position (Fig. 7). Further, the PCR amplified sequence of CAST gene (620 bp) was used as query and subjected to nucleotide blast at NCBI (http://blast.ncbi.nlm.nih.gov/ blast/Blast.cgi), which revealed 100, 94 and 98 per cent identity with *Ovis aries* (AF016006.1), *Bos taurus* (AY834771.1) and *Capra hircus* (JQ739234.1), respectively.

Palmer *et al.* (1998) described two allele systems of polymorphic variants (M and N) in a region (Exon 1C/1D) of the ovine *CAST* by the PCR-RFLP method in Dorset Down sheep. They concluded that digestion with restriction enzymes *MspI* and *NcoI* differentiates alleles M and N. The present study confirm the presence of these alleles as observed by previous workers (Gabor *et al*., 2009; Gharahveysi *et al*., 2012; Khan *et al*., 2012; Mohammadi *et al*., 2008; Nanekarani *et al*., 2011a; Nanekarani *et al*., 2011b; Shahroudi *et al*., 2006; Suleman *et al*., 2012 and Szkudlarek- Kowalczyk *et al*., 2011) in different sheep breeds.

The results from present study are in agreement with the observations from various studies involving different breeds of sheep. Azari *et al*. (2012) reported an allele frequency of 55.45 and 44.55 per cent for A_1 (M) and A_2 (N), respectively in Dalagh sheep of Iran. Elyasi *et al*. (2009) observed an allele frequency of 69, 48 and 50 per cent for M allele in Ghezel, Arkhamerino and Ghezel x Arkhamerino sheep, respectively. Nanekarani *et al*. (2011a) found frequency of 63.8 and 36.2 per cent for M and N allele, respectively in Lori Sheep, whereas Ata and Cemal (2013) observed allele frequency of 54.4 and 45.6 per cent for M and N alleles, respectively in synthetic Karya sheep of Turkey. Further, higher MN genotype was observed in Arkhamerino (47.62 %) and Ghezel x Arkhamerino sheep (46.67 %), Lori sheep (46.2 %) and Dalgh sheep (38 %).

However, the allele frequencies observed from the present study were in contrast to that of observations reported by Palmer *et al.* (1998) wherein, M and N allele frequency were 77 and 23 per cent, respectively in Corriedale rams. Similar results as per Palmert *et al.* (1998) were also reported in different sheep breeds of Iran (Shahroudi *et al*., 2006 and Gharahveysi *et al.*, 2012), Slovakia (Gabor *et al*., 2009), Pakistan (Suleman *et al.*, 2012) and Poland (Szkudlarek-Kowalczyk *et al*., 2011). Further, with respect to the genotypic frequency in the above studies, MM genotype was found to be higher followed by MN and NN. However, breeds like Berrichon du Cher,

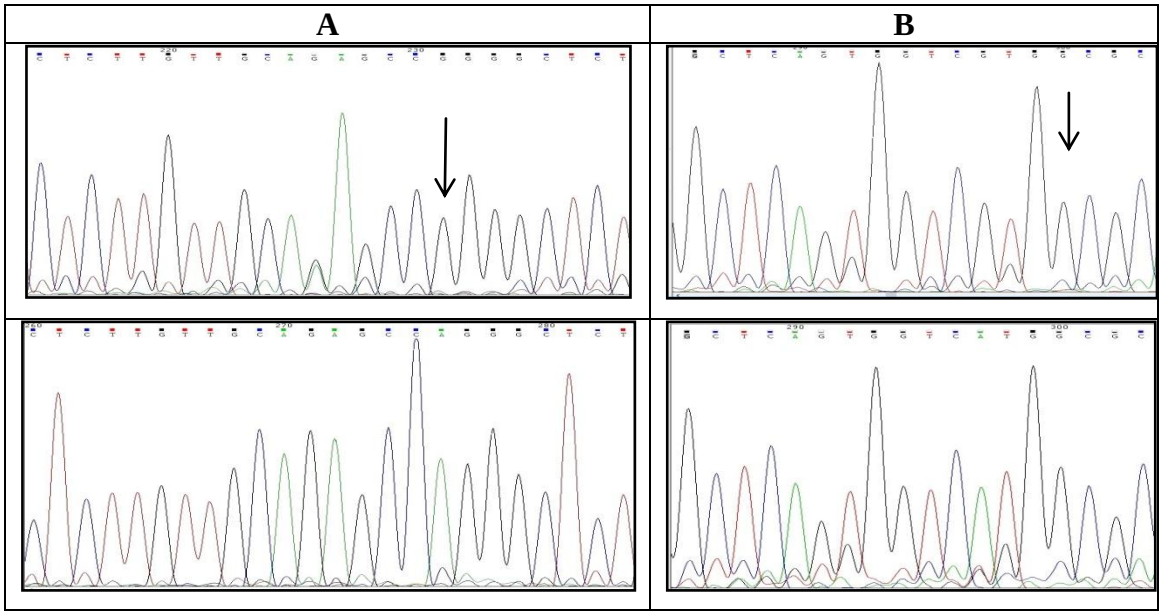

Fig. 5:Chromatograms showing G→A transition at 286 (A) and 352(B) bp position

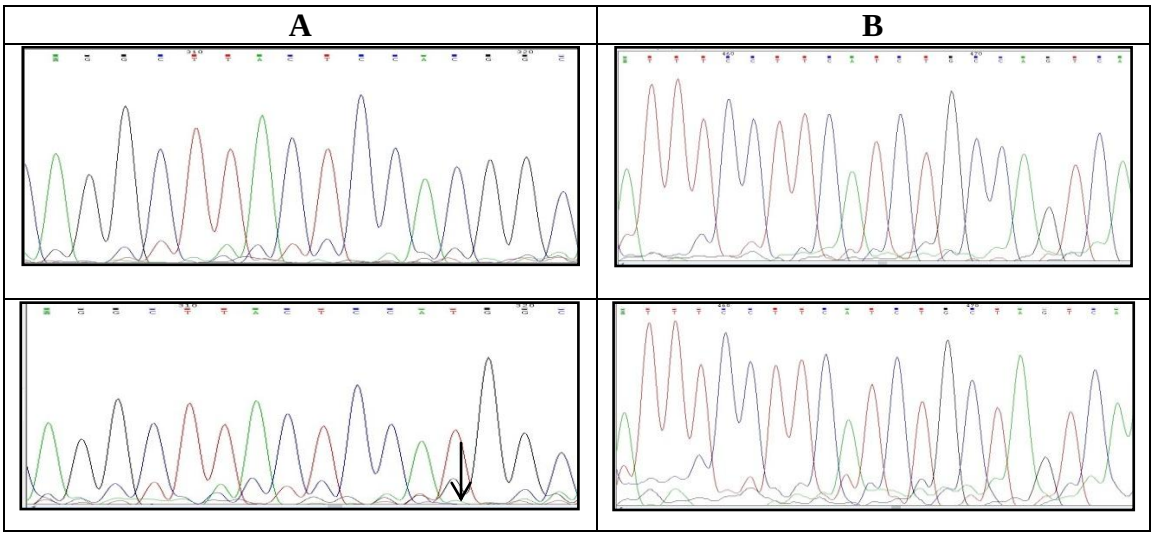

Fig. 6: Chromatograms showing C→T transition at 373 (A) and 526 (B) bp position

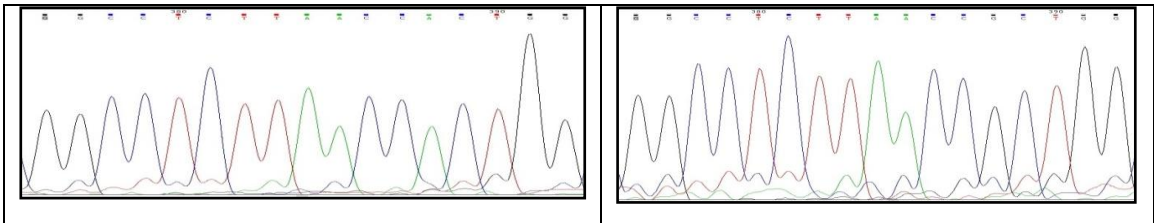

Fig. 7: Chromatograms showing A→G transition at 443bp position

lle de France (Szkudlarek-Kowalczyk *et al*., 2011) and Slovakia (Gabor *et al*., 2009) showed only two genotypes; MM and MN, whereas in the present study, the frequency of MN genotype was higher followed by MM and NN.

CONCLUSION

The present study successfully demonstrated the molecular genetic polymorphism of CAST gene (Exon 1C/1D and the intervening intron) in Bandur sheep through PCR-RFLP analysis. It was

concluded that the PCR-RFLP method is suitable for assessing genetic variability of CAST gene and detects single nucleotide changes after DNA sequence analysis of the investigated genomic region. Further the results unwrap remarkable prospects for future selection programmes, especially marker assisted selection. The genetic variability observed in the calpastatin gene could be used to find association of observed genotypes and SNPs with growth, carcass and meat quality traits.

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Moisture Adsorption Characteristics of Whey Protein Concentrate

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ABSTRACT

Water activity is considered as one of the most important parameters in food preservation and processing. Sorption isotherms of Whey Protein Concentrate of 35% & 70% protein at 25, 35 and 45^{0}C were determined using a static-Gravimetric method. Sorption isotherms followed a type II (sigmoidal) shape. Equilibrium moisture content decreased with increase in temperature at constant water activity. The GAB, Modified Mizrahi, Caurie, Oswin, and Halsay models were applied to the isotherm data. The GAB model, which was having least RMS% value, found as best fitted equation. Net isosteric heats were estimated from equilibrium sorption data using the Clausius–Clapeyron equation. The heat of sorption was found to increase initially and attain a peak value of 19.28 kJ/kg in WPC (35%) and 17.6 kJ/kg in WPC (70%) at 5% moisture level and then decreased.

Keywords: *Whey Protein Concentrate (WPC), Isotherms, Sorption models.*

Water is the main component of all the food products and stability of product depends upon the interaction of water with other components present in the product. The status of water in the product plays a crucial role in the preservation of biological materials in both the raw and processed states. The simplest way for expressing such a status is the concept of water activity.

Water activity (a_w) has become one of the most important intrinsic properties used for predicting the survival and growth of microorganisms in food (Scott, 1957), due to its direct influence on product stability and quality. The understanding and control of a_w contributes to safer food storage conditions in general and forms the basis of much modern food formulations, especially for intermediate-moisture foods. The relationship between water activity and the equilibrium moisture content of food is known as moisture sorption isotherm. The moisture sorption isotherm is an extremely valuable tool for food scientists and technologists to predict potential changes in food stability. It can be used for the determination of storage conditions, packaging requirements and ingredient selection.

MATERIALS AND METHODS

Test Sample: Whey protein Concentrate (35% and 70% protein content) was chosen as test sample for the present study. Test sample was procured from a commercial firm "M/S Modern Dairies Ltd", Karnal. Chemical composition of WPC was determined using standard methods:

Moisture content (IS 1981), fat content (Mojonnier method), total protein by Micro Kjeldhal (AOAC, 2000), lactose (IS: SP-18, 1981) and ash content was calculated by difference. Average composition of WPC (35%) was found to be protein 37.4%, moisture 4.4% (dry basis), fat 3.3%, lactose 46.7%, ash 8.3% and that of WPC (70%) was protein 70.7%, moisture 3.9% (dry basis), fat 4.6%, lactose 14.5% and ash 6.3%.

Establishing Moisture Sorption Isotherm

Sorption Apparatus: The standard sorption apparatus recommended by COST 90 Project was used in this study. The sorption device consisted of a wide mouth glass bottle (200 ml) with vapor tight lid used as sorbostat. Inside each sorbostat was a support for weighing beaker (25 ml) and the weighing beaker (10 ml) in which the sample material was exposed to the humid atmosphere in the container maintained by the saturated salt slurries.

Selection of Salts for Maintaining Different Humidity: Eight reagent grades salt slurries, viz., Lithium chloride, Magnesium chloride, Sodium bromide, Ammonium sulphate, Potassium chloride, Potassium chromate, Barium chloride and Potassium sulphate were chosen. The salts were dissolved in distilled water at 100°C and cooled to

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test temperatures (25, 35 and 45^{0}C) for crystallization to form saturated slurries. Water activities of these salts ranges from 0.11 to 0.97 at different temperature are given in the Table 1.

Table 1: Water Activity of saturated Salts solutions at Different Temperature

Salts	Water Activity		
	25^{0}C	35^{0}C	45^{0}C
$LiCl.H_2O$	0.11	0.11	0.11
$MgCl_2.6H_2O$	0.32	0.32	0.31
NaBr	0.57	0.57	0.56
$(NH_3)_2SO_4$	0.81	0.80	0.79
KCl	0.84	0.83	082
K_2cro_4	0.87	0.84	0.80
$BaCl_2.2H_2O$	0.90	0.88	0.86
K_2SO_4	0.97	0.96	0.97

Maintenance of Temperature: A set of three temperatures viz., 25, 35, and 45^{0}C were selected for the study. Constant temperature cabinets were used for maintaining these temperatures. Effect of temperature on water activity of whey protein concentrate was studied by comparing moisture gain or loss of sample inside the sorbostats at different water activity and at corresponding temperatures.

Sorption Procedure: The sorption apparatus were allowed to equilibrate at respective temperatures for four days before the samples were placed into them. For adsorption isotherm, samples were completely dehydrated by placing them over P_2O_5 for 24hours. Weighed quantity of the dry test samples (1.00 +/- 0.05g) was taken into tared sample beakers, which was then transferred to the sorbostat, containing saturated salt slurry.

To prevent mold growth, about 0.1mg of potassium sorbate was added to each WPC sample. At each designated controlled temperature, the samples were weighed at intervals of 3 days and equilibrium was judged to have been attained when difference between three consecutive sample weighing was less than 1 mg/ g of solids. The equilibration period ranged from 12 to 14 days. Measurements for each experiment were done in duplicate and average value of the equilibrium moisture content was taken for further analysis. Equilibrium moisture content (EMC) at the designated temperature in terms of grams water / 100 g of solid was obtained for each sample by using the equation (1). The EMC were plotted against equilibrium relative humidities / water activities (a_w) to establish moisture sorption isotherm.

$$M = \frac{(W_2 - W_1) + m_1}{(W_1 - m_1)} \times 100 \quad \ldots\ldots \quad (1)$$

Where,

M = Moisture content (g water/100g solids, d.b)

W_1 = Initial weight of the sample (g)

W_2 = Final weight of the sample (g)

m_1 = Initial moisture content of sample (g water/100g solids, d.b)

Fitting Of Isotherm Equations: The five isotherm equations that were used to fit the data are presented below. The linearized forms of the two parameter models were used for evaluating the best-fitted values of constants using a regression programme. The sorption data was analyzed according to the models and the corresponding constants were determined. The goodness of fit of each model was computed in terms of root mean square percent error (RMS %).

(Halsey) $a_w = e^{\left[\frac{-a}{W^b}\right]}$ ……. (2)

(Oswin) $W = a\left\{\frac{a_w}{(1-a_w)}\right\}^b$ ……. (3)

(Caurie) $\ln\frac{1}{W} = \ln\frac{1}{CW_o} + \frac{2C}{W_o}\ln\frac{1-a_w}{a_w}$ ..... (4)

(Mizrahi) $W = \frac{a + a_w(C.a_w + b)}{a_w - 1}$ ..……. (5)

(GAB) $W = W_0 + \frac{GKa_w}{(1-Ka_w)[1-Ka_w+GKa_w]}$ …. (6)

Where,

W = Equilibrium moisture content (g /100g dry solids)

W_o = Monolayer moisture content (g /100g dry solids)

a_w = Water activity, decimal fraction

C = Density of sorbed water (g/ml)

G = Guggenheim constant

K = Correction factor

a, b, and c are constants

The GAB equation could be rearranged into a second-degree polynomial equation

$$\frac{a_w}{W} = a.a_w^{\,2} + ba_w + c \quad \text{..... (7)}$$

Where,

$$a = \frac{k}{W_0}\left[\frac{1}{G-1}\right] \quad \text{..... (8)}$$

$$b = \frac{1}{W_0}\left[1 - \frac{2}{G}\right] \quad \text{..... (9)}$$

$$c = \frac{1}{W_0.G.k} \quad \text{..... (10)}$$

W_0 is the moisture content corresponding to saturation of all primary adsorption sites by one water molecule (equivalent to monolayer in the BET theory), G is the Guggenheim constant and k is the factor correcting for properties of multilayer molecule with respect to the bulk liquid. A non-linear regression analysis of a_w/w vs a_w yields a polynomial of second order. The coefficients a, b and c were obtained from this polynomial equation and substituted one by one to obtain GAB constants W_m, C and K.

Accuracy of Fit: The accuracy of fit was evaluated by calculating root mean square percent error (RMS %). (Lievonen and Roos, 2002):

$$RMS\% = \sqrt{\frac{1}{n}\left[\sum_1^n \left(\frac{W_{exp} - W_{cal}}{W_{exp}}\right)^2\right]} \times 100 \quad \text{.... (11)}$$

Where,

W_{exp} = EMC experimentally obtained

W_{cal} = EMC predicted by the model

n = Number data points

2.7 Calculation of net isosteric heat of sorption: The net isosteric heat of sorption was evaluated from the following generalized equation of Clausius- Clapeyron (Aguerre et al., 1988):

$$ln\left(\frac{a_w(T_1)}{a_w(T_2)}\right)_W = \frac{\Delta H_W}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \quad \text{..... (12)}$$

Where,

ΔH_W = Net isosteric heat of sorption (kJ/ mol)

R = Gas constant (8.314 J/ mol-K)

The isosteric heat of sorption was calculated from above equation by plotting log (aw) vs. 1/ T and determining slope of curve, which is equal to -Δ Hw/ 2.303 R. The slope of each line was found out by linear regression and the net isosteric heat of sorption was calculated.

Temperature dependence of isotherm parameters: Temperature dependence of sorption phenomenon provides the valuable information about the changes related to the energetics of the system. The constants in moisture sorption isotherm equations, which represent either temperature or a function of temperature, are used to calculate the temperature dependence of water activity. Clausius-Clapeyron equation was used to predict water activity at any temperature. The variation in water activity with temperature was predicted by incorporating temperature terms into sorption equations.

$$W_m(T) = W_m' \exp(\Delta H/RT) \quad \text{..... (13)}$$

$$G(T) = G' \exp(H_1 - H_m)/RT \quad \text{..... (14)}$$

$$K(T) = K' \exp(H_L - H_m)/RT \quad \text{..... (15)}$$

The above equations together, with G.A.B. equation were used to calculate the equilibrium water content W (T) of *WPC* at a known water activity and temperature by means of GAB (T) constants.

RESULTS AND DISCUSSION

Sorption Isotherms: Moisture adsorption isotherms for Dried Whey Protein Concentrate with 35% and 70% protein content at 25, 35 and 45^0C were established and are shown in fig 1 & 2 respectively. All sorption isotherms demonstrate an increase in equilibrium moisture content with increasing water activity and beyond 0.8 a_w the increase in EMC was sharp. This behavior is manifested in the form of a sigmoidal shaped curve, thus reflecting a **Type II** isotherm which is typical to the most of the foods.

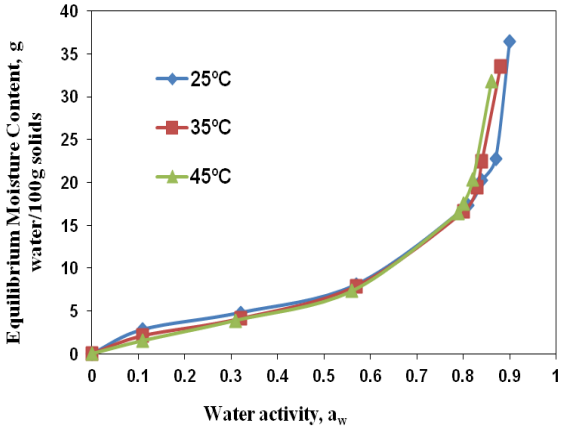

Fig. 1: Adsorption isotherm of WPC (35%) at various temperatures

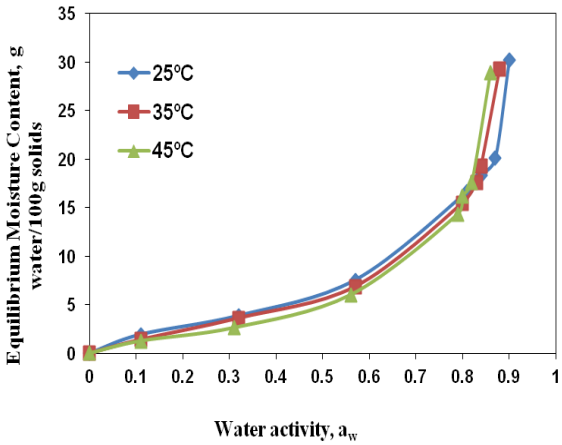

Fig. 2: Adsorption isotherm of WPC (70%)at various temperatures

Similar types of observations were found by Lin *et al.* (2005) for milk powders (SMP, WMP), Sahu (2006) for SMP, Govardhan (2007) for *Gulabjamun* mix powder.

Effect of temperature on isotherms: It may be observed from fig. 1 that in case of WPC (35%), up to $0.5a_w$ all the three curves are slightly distinguishable. After that the curves are superimposing on each other. Where as in WPC (70%), the curves (fig. 2) are distinguishable upto to $0.8a_w$, above that the curves were superimposed. It was also noted that equilibrium moisture content was lower at higher temperature. The equilibrium moisture content decreased with increase in temperature at the same a_w, or the a_w increased with decrease in temperature at the same equilibrium moisture content. A number of workers have suggested explanations for this observation and explained this trend as being due to the increased state of excitation of molecules at higher temperatures, leading to an increase in distance and corresponding decrease in attractive forces between them. This gives rise to the observed decrease in degree of water sorption at a given relative humidity as the temperature is increased.

The analysis of variance (ANOVA) revealed that the effect of temperature on moisture content was statistically not significant ($p > 0.05$) over temperature range 25- 45^0C. However beyond 0.8 a_w the effect of temperature on equilibrium moisture content was reverse, that is, the equilibrium moisture content increased with increase in temperature at the same water activity.

Isotherm Models: The two and three parameter models as shown in equations from 2 to 10 were used for evaluating the best-fitted values of constants using a linear regression programme. Values of different parameters and RMS at different temperatures for different models have been given in table 2 and 3.

Table 2: Statistical parameters and %RMS values of Adsorption of WPC (35%) for selected equations at different temperatures

Equation	^{0}C	Constants			% RMS
		a	B	c	
Halsey	45	1.3424	1.0619	-	9.6746
Oswin		1.9002	0.7423	-	13.8403
Caurie		-1.9002	0.7423	-	13.8403
Modified Mizrahi		-1.2568	-2.9486	-0.2563	11.4720
GAB		0.0477	0.2174	-0.2772	6.7242
Halsey	35	1.4826	0.9336	-	6.2097
Oswin		1.9837	0.6625	-	11.2973
Caurie		-1.9837	0.6625	-	11.2973
Modified Mizrahi		-1.4165	-4.6257	2.4193	6.1256
GAB		0.0323	0.2246	-0.2594	5.2974
Halsey	25	1.6516	0.7961	-	6.9028
Oswin		2.0878	0.5732	-	12.8047
Caurie		-2.0878	0.5732	-	12.8047
Modified Mizrahi		-2.1627	-4.1478	3.2933	6.1701
GAB		0.0148	0.2445	-0.2543	6.4388

Table 3: Statistical parameters and %RMS values of Adsorption of WPC (70%) for selected equations at different temperatures.

Equation	^{0}C	Constants			% RMS
		a	b	c	
Halsey	45	1.1334	1.1150	-	6.3543
Oswin		1.7197	0.7782	-	12.2404
Caurie		-1.7197	0.7782	-	12.2404
Modified Mizrahi		-0.9034	-2.5038	-0.6724	7.2392
GAB		0.0657	0.2503	0.3374	4.9398
Halsey	35	1.2697	0.9968	-	9.3212
Oswin		1.8016	0.7124	-	7.1051
Caurie		-1.8016	0.7124	-	7.1051
Modified Mizrahi		-0.6931	-6.2721	3.9307	6.0153
GAB		0.0623	0.1574	-0.2152	5.2593
Halsey	25	1.4281	0.8613	-	7.5248
Oswin		1.8964	0.6256	-	7.3204
Caurie		-1.8964	0.6256	-	7.3204
Modified Mizrahi		-1.0012	-7.0699	5.6074	5.2334
GAB		0.0385	0.2009	-0.2304	5.9926

At all the three temperatures 25, 35 and 45ºC GAB equation showed the best fit as exemplified by the percentage RMS values of 6.4338, 5.2974 and 6.7242 at 25ºC, 35ºC and 45ºC respectively for WPC (35% protein content) and 5.9926, 5.2593 and 4.9398 for WPC (70% protein content). This was followed by modified Mizrahi with % RMS between 6.1701 and 11.472 for WPC (35%) and between 5.2334 and 7.2392 for WPC (70%). The Halsey, Oswin and Cauri models were inadequate for representing the adsorption isotherm of whey protein concentrate giving % RMS above 13.8403 for WPC (35%) and 12.2404 for WPC (70%). (Table 2 and 3). Since Modified Mizrahi equation is not defined for the whole range of water activity. this study resulted in the GAB as best fitted equation for adsorption isotherms. GAB equation was fitted precisely up to 0.90 a_w as well as it provided the better evaluation of amount of water tightly bound by primary adsorption sites.

The parameters of GAB equation at different temperatures for adsorption isotherms for WPC (35% & 70%) are given in the table 4 and 5. The values of GAB sorption constant for multilayer moisture content (K) increased with increasing temperature, where as the monolayer moisture content decreased with increasing temperature. This decrease in monolayer with increasing temperature has also been reported for different foods and food systems Govardhan (2006), Sahu (2005), Lin *et al.*, (2005) and Foster *et al.*, (2005).

Table 4: Set of parameters of GAB equation of Adsorption of WPC (35%) at different temperature

Temperature (^{0}C)	GAB Parameters		
	W_m	G	K
25	3.78	12.85	0.971
35	3.45	10.52	1.008
45	3.15	8.59	1.038

Table 5: Set of parameters of GAB equation of Adsorption of WPC (70%) at different temperatures

Temperature (^{0}C)	GAB Parameters		
	W_m	G	K
25	4.10	25	4.10
35	5.57	35	5.57
45	2.84	45	2.84

Evaluation of Net Isosteric Heat of Sorption: Net isosteric heat of sorption was calculated from the generalized equation of Clausius Clapeyron as given in equation 12 by plotting ln a_w v/s inverse of absolute temperature & determined its slope. The sorption isosters of WPC (35% & 70%) at different moisture content were shown in fig. 3 & 4. The slope of each isosters decreased as the moisture content increased. The net isosteric heat of sorption of the WPC (35% & 70%) as a function of the moisture content was given in the table 6. The heat of sorption was found to increase initially with increase in moisture content and attain a peak value of 19.2812kJ/kg in *WPC (35%)* and 17.6039 KJ/Kg in WPC (70%) at 5% moisture level, and then decreased.

Table 6: Values of net isosteric heat of sorption of WPC (35% & 70%) at different moisture contents

Moisture content (g/100g solids)	Net isosteric heat of sorption (KJ/Kg)	
	WPC 35%	WPC 70%
5	19.2812	17.6039
7	3.7509	8.7445
8	2.6787	6.7647
9	1.8658	5.6944
10	1.1555	4.5272
15	0.1304	2.1203

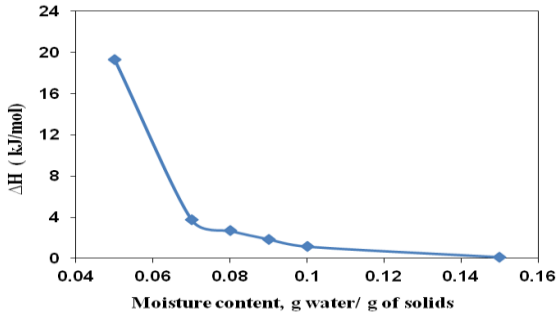

Fig. 3: Net isosteric heat of sorption (ΔH) of WPC (35%) at different moisture contents

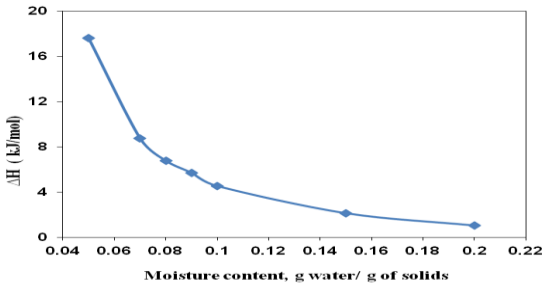

Fig. 4: Net isosteric heat of sorption (ΔH) of WPC (70%) at different moisture contents

Evaluation of Temperature Dependence of GAB Parameters: Temperature dependence of GAB constants were predicted by Arrhenius type relationship given by Weisser (1985). The constants W', G', K' and the corresponding exponents in the equations as shown in equations 13 to 15 were determined from the GAB constants of WPC (35% & 70%) by least square regression analysis are given below.

For WPC (35% protein)

$W_m(T) = 0.3646 \exp (5.725 \times 10^3 / RT)$ ……..(16)

$G(T) = 0.0001 \exp (4.274 \times 10^4 / RT)$ ……..(17)

$K(T) = 2.3977 \exp (-2.214 \times 10^3 / RT$ ……..(18)

For WPC (70% protein)

$W_m(T) = 0.0167 \exp (1.3465 \times 10^4 / RT)$ …….. (19)

$G(T) = 0.0731 \exp (1.1167 \times 10^4 / RT)$ …….. (20)

$K(T) = 3.7903 \exp (-3.4055 \times 10^3 / RT)$ …….. (21)

The parameters of GAB equations Wm, G and K at each temperature and Predicted values by Weissler equation GAB (T), W_m (T), G (T) and K (T) of adsorption isotherm of WPC(35% & 70%) with %RMS are summarized in the table 7 and 8. %RMS value of GAB and GAB (T) constants are nearly similar which showed the best fit of GAB equation to the experimental values.

Table 7: Sets of parameters of WPC (35%) calculated using GAB equation from moisture adsorption measurement

Temperature (°C)	Calculated from GAB			Calculated from Weissler Equation			RMS (%)	RMS (T) (%)
	W_m g water/100g solids	G	K	$W_m(T)$ g water/100g solids	G (T)	K (T)		
25	3.78	12.85	0.97	3.82	12.37	0.96	6.4388	8.5631
35	3.45	10.52	1.01	3.41	10.11	1.01	5.2974	6.8903
45	3.15	8.59	1.04	3.17	8.74	1.03	6.7242	5.3267

Table 8: Sets of parameters of WPC (70%) calculated using GAB equation from moisture adsorption measurement

Temperature (°C)	Calculated from GAB			Calculated from Weissler Equation			RMS (%)	RMS (T) (%)
	W_m g water/100g solids	G	K	$W_m(T)$ g water/100g solids	G (T)	K (T)		
25	4.10	7.24	0.95	4.2	7.16	0.94	5.9926	7.0361
35	5.57	5.61	0.99	5.72	5.73	1.00	5.2593	6.7423
45	2.84	4.86	1.05	2.72	4.99	1.04	4.9398	4.5391

CONCLUSIONS

Moisture adsorption isotherm of whey protein concentrate with 35% and 70% protein content obtained in the temperature range of 25- 45^{0}C were sigmoid type II , common to most foods. A negative temperature effect on EMC was evident as often observed in foods with high protein content, but it was not statistically significant. Adsorption data was best described by GAB model. The monolayer moisture content decreased with increase in temperature. Equations were developed by correlating the GAB constants with temperature. The results would be valuable in appraising the shelf life of the product under varying storage conditions and design of suitable packaging system.

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Effect of Filling Media on Heat penetration and Quality Characteristics of Canned Ribbon Fish (*Trichiurus lepturus)* in Tin Free Steel Cans (TFS)*

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ABSTRACT

In the present investigation the effect of different filling media on heat penetration characteristics and the quality changes of ribbon fish in tin free-steel (TFS) cans was assessed. About 110 g of ribbon fish meat was packed into 6-oz TFS cans (303 x 109) and filled with 60 g of different media such as masala and tomato sauce. The F_0 values of ribbon fish in masala pack and sauce pack were 15.16 min and 13.80 min respectively whereas process time for ribbon fish in masala pack and sauce pack was 48.08 and 52.20 min respectively. The results indicated that heat penetration is faster in masala than sauce. Total volatile base nitrogen (TVB-N) and tri-methylamine nitrogen (TMA-N) contents were found to be slightly higher but within the acceptable limit at the end of five months storage, while thiobarbituric acid (TBA) and free fatty acid (FFA) content increased slightly in masala and sauce pack at the end of the storage period. The sensory evaluation scores revealed that quality of the processed products was rated as good and the products were in acceptable condition even after storage period.

Keywords: Ribbon fish, TFS can, Heat penetration, Filling media and Quality.

Canning is one of the ideal methods among the various methods of food preservation, and has been playing an important role in long term preservation of foods (Chandra *et al*., 2014). One of the important advantages of thermally processed foods over foods processed by other methods is its longer shelf life at room temperature (Ali *et al.*, 2005). One of the important factors contributing to the success of a canning operation is an efficient container that can form a hermetic seal, protects the food from damage during storage and transport and which can provide an aesthetic appearance to the product (Sreenath *et al.*, 2009).

The awareness of health benefits of fish has created a higher demand for fish and fishery products. Further, the consumption of processed fish products like fillets, mince based products, surimi and canned products such as fish in brine, oil, curry, sauce and masala medium is increasing worldwide. However, it has become difficult to meet the demand due to short supply. Ribbon fish (*Trichiurus lepturus*) is one of the major species contributing to the 6% of total marine fish landings of India (CMFRI, 2013). In order to cater the supply of value added products, long term preservation by using canning is being used commercially.

Heat transfers in processed foods are affected by the filling medium used, resistance incurred at the container wall, size of the container and thermo physical properties of foods. F_0 values of the sterilization process highly depend upon the amount of raw material, cans and filling medium. Consistency of the medium affects the heat penetration characteristics of thermal processing. Therefore, for arriving at the heat penetration characteristics requirements, knowledge on the pattern of heat penetration in the food inside the can is very essential. Reports on the canning of ribbon fish are meagre and also much information regarding evaluation of thermal process is not available. Hence, in the present investigation the effect of different filling medium *viz.*, masala and *tomato sauce* on heat penetration characteristics and quality of canned ribbon fish (*Trichiurus lepturus)* in tin free steel cans was evaluated.

MATERIALS AND METHODS

Raw Material: Ribbon Fish (*Trichiurus lepturus)* caught by trawl net along the west coast of Mangalore (India) was used for the study. The

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mean total length of fish used was 64 ± 1 cm, weighing about 230-240g. The fish was iced immediately in the ratio of 1:1 (fish: ice) and transported to the laboratory under iced condition (0-4 ºC) in an insulated box. After reaching to the laboratory, the raw material was dressed and cut into pieces of 2.5 cm size followed by thorough washing in potable chilled water in order to make it free of blood and visceral remains after which it was subjected to brining. The brining was done by keeping the dressed fish in saturated brine for about 8 min at room temperature then it was immediately washed with water to remove adhering salt crystals on surface of fish pieces so as to reduce salt concentration and drained for 5 min.

Fish masala preparation: Fish masala was prepared as per the recipe given by Saralaya (1978), with some modifications. *Dressed fish (1kg), onion (30g), garlic (10g), garam masala (25g), Chili powder (30g), jeera (8g), mustard (2g), refined oil (150ml) and salt (10g)*

Heat penetration studies of ribbon fish in different filling media: For standardization of temperature and time for processing of ribbon fish in masala and sauce media was carried out according to the method described by Chandra *et al*, (2014). Freshly dressed ribbon fish pieces were brined in saturated brine solution for 8 min and then about 140±2 g of fish pieces were filled into the TFS cans (307 X109) of 6-oz capacity supplied by M/s Amtech packs, Mysore, India. The filled cans were pre-cooked in steam for 25 min. Cans after precooking were drained and about 110g of pre-cook weight of fish pieces was maintained in the cans. Later about 60g of hot filling medium either (1) fish masala or (2) diluted sauce (1:3):: sauce: water) was added to the respective cans. The filled cans were then steam exhausted for 10 min and immediately sealed in a double seaming machine. The cans had been instrumented with thermocouple glands by inserting thermocouple probes (Ellab SSA- 12050-G700-TS stainless steel) in which method the tip of the thermocouple gland was inserted into the ribbon fish piece. The position of perforation was such that thermocouple tip would be finally at the predetermined cold spot and at one third of the height of the can from the bottom so as to record the core temperature. The sealed cans were loaded inside the retort (John Fraser and Sons Ltd., UK. Model.No.5682) and processed at 115.6 $^{\circ}$C. Heat penetration data were recorded using Ellab (CTF 9008, Denmark) temperature recorder and process value integrator. Data were recorded for every min of processing. The process was carried out according to the optimized time and the lethality (F_0) value obtained is recorded. Time optimization was done based on the time taken for softening of the bone at a constant temperature. Heat penetration data was plotted on a semi logarithmic paper with temperature deficit (Retort temperature-core temperature) on log scale against time. Lag factor for heating (J_h), slope of the heating curve (f_h), time in min for sterilization at retort temperature (U), lag factor for cooling (J_c), final temperature deficit (g) and cook value (cg) were determined. Total process time was calculated as per the method given by Stumbo (1973). Actual process time was determined by adding process time (B) and the effective heating period during come-up time *i.e.* 58% of the come up time. After processing, the samples were drawn for quality analysis with an interval of one month period up to five months.

Proximate composition: The moisture and ash content were determined according to (AOAC, 2000). Total lipid content was determined by Bligh and Dyer (1959) method. The crude protein content was calculated by multiplying the nitrogen content with 6.25 which was determined by Kjeldahl digestion as described by AOAC (2000).

***Biochemical analysis*:** Total volatile base nitrogen (TVB-N) content and tri-methylamine nitrogen (TMA-N) content were determined using the Conway micro diffusion unit according to Siang & Kim (1992). The thiobarbituric acid (TBA) of fish sample was determined following the method

described by Tarladgis *et al,* (1960). The free fatty acid (FFA) content of canned fish samples was estimated by the method described in AOCS (1989). Peroxide value of the fish sample was determined using the method described in AOCS (1989). The pH of the sample was measured using a pH meter (Systronic 324 pH meter, Ahemdabad, India). Five gm of meat was macerated with 45 ml distilled water to measure the pH.

Sensory analysis: Sensory characteristics of canned ribbon fish in masala and tomato sauce were evaluated by minimum of 12 members trained taste panel on a ten point hedonic scale as per the guideline given by IS: 6273 (II)-1971. The product with equivalent scores of 9-10 was considered as an excellent, whereas, product with scores of 7-8, 5-6, 3-4 and 1-2 was considered as good, fair, poor and rejected respectively.

Statistical analysis: Significant difference between the means at 5% level of significance was determined using ANOVA and Duncan's Multiple Range test as described by Snedecor and Cochran (1962). Statistical package used in the study was SPSS Version 16.0.

RESULTS AND DISCUSSION

Proximate composition of the fish gives an idea about the nutritional quality of fish. The moisture content of ribbon fish was 75.86% which is in agreement with reported value (Chandra *et al.,* 2014). Fish muscle is a good source of protein. Most finfish muscle tissue contains about 18-22% protein (Sidwell, 1981). Ribbon fish meat had a crude protein content of 18.05 %. Ribbon fish had the fat content less than 2%, indicating that they are lean variety fish (Table 1A). The protein and ash content were little higher than that reported by Dileep *et al*. (2005). The contents of TMA-N and TVB-N are reported to be good indicators of freshness of fishes (Keay and Hardy, 1972). These two parameters are reported to get increased with thermal treatment of fish muscle (Gallardo *et al*., 1990).

Table 1: A. Proximate and biochemical composition of fresh ribbon fish (*Trichiurus lepturus)*

Parameters	***Mean value***
Moisture (%)	75.9 (0.8)
Fat (%)	1.6 (0.10)
Protein (%)	18.1 (0.5)
Ash (%)	1.52 (1.0)
pH	6.6 (0.80
PV(milliequi. of O_2 /kg of fat)	4.2 (0.10
TVB-N (mg/100gm)	4.6 (0.2)
TMA-N (mg/100gm)	1.1 (0.1)
FFA (as % of oleic acid)	0.7 (0.07)
TBA value (mg malonaldehyde/kg of fish sample)	0.10 0.02)

B. Proximate composition of processed fish products during storage periods

Pro-ducts	*Storage duration (months)*	*Moisture (%)*	*Protein (%)*	*Fat (%)*	*Ash (%)*
Masala	0	61.47 (1.2)	23.85 (1.1)	5.12 (0.1)	3.40 (0.3)
	1	62.02 (0.7)	23.70 (1.8)	6.08 (0.6)	3.15 (0.4)
	2	62.60 (0.9)	22.60 (1.2)	6.20 (1.1)	2.90 (0.1)
	3	62.54 (1.4)	23.42 (1.3)	5.90 (0.6)	2.65 (0.2)
	4	61.90 (1.0)	22.19 (1.5)	5.70 (0.7)	2.42 (0.1)
	5	61.86 (0.9)	22.65 (1.3)	6.28 (0.5)	2.36 (0.3)
Tomato sauce	0	67.43 (0.1)	22.64 (0.8)	2.33 (0.2)	2.82 (0.2)
	1	67.40 (0.3)	23.41 (1.0)	2.56 (0.5)	2.58 (0.4)
	2	68.53 (0.7)	22.66 (1.6)	2.35 (0.2)	2.35 (0.5)
	3	67.37 (0.9)	22.16 (0.9)	2.38 (0.2)	2.06 (0.3)
	4	66.37 (1.0)	22.77 (1.1)	2.45 (0.3)	2.10 (0.1)
	5	66.87 (0.5)	22.84 (0.5)	2.53 (0.7)	2.05 (0.1)

*Values in parenthesis represent standard deviation, n=3

In the present study the changes in proximate composition of the ribbon fish packed in TFS can with masala and sauce as a packing media, during the storage period is given in Table 1B. The higher fat and ash content in the respective masala and sauce packs were attributed to the media in which they were packed. Overall quality of the products was acceptable even after 5 months of storage.

Heat penetration studies of ribbon fish in different media: Severe sterility heat treatments are most often applied to low acid foods (pH > 4.6) packaged in hermetically sealed containers. Temperature of treatment in the case of low acid foods ranges from about 115 °C (Jaiswal, 2002). Heat penetration parameters of ribbon fish in different media are presented in Table 2A. In the present investigation a retort temperature of 115.6 ºC and process time of 90 min for masala and sauce pack were taken as optimum process required, the values got from process calculation to achieve the required lethality values of 15.16 min for masala pack and 13.80 min for sauce pack and total process time were 48.08 min and 52.20 min for masala and sauce respectively. Froot and Lewis, (1994) reported the F_0 value recommended for the thermal processing of fish and fish products ranged from 5-20 min. Ali *et al*. (2005) reported that, a F_0 at 121.1 ^oC was 10 min, sufficient to get the product with good sensory attributes and commercial sterility in case of tuna in oil, whereas F_0 for herring in tomato sauce with 6-8 min is sold in U.K. (Brennan *et al*., 1990). Hence, the final products were processed based on the values got from process calculation and was studied during the storage. The semi log heat penetration graph revealed f_h (The time required to attain 1 log cycle reduction) value was 25 min for masala and sauce products. This indicates that both the products filled with masala and sauce as a medium showed more or less same rate heat penetration. Ramaswamy and Grabowski (1999) reported that the j_h is highly influenced by the processing temperature and container shape. The log factor of cooling (j_c) shows a contrasting trend with j_h factor and increased with increase of processing temperature. Heat penetration studies showed a faster heat penetration rate in TFS can pack products as the body thickness is thin compared to tin cans (Sreenath *et al.*, 2008).

Table 2: A. Thermal process parameters of ribbon fish in different media

Products / *Parameter*	*Ribbon fish in Masala*	*Ribbon fish in sauce*
Heating rate of index f_h (min)	25	25
Heating lag factor (J_h) min	0.683	1.114
Cooling lag factor(J_c) min	1.08	1.138
F_0 value (min)	15.16	13.80
Temperature deficit (g) min	0.0138	0.0348
Cook value (C_g)min	213	208.7
Initial deficit temperature (I)	84.9	71.8
Number of minutes for sterilization at the retort temperature (U) min	61.75	55.5
Process time (B) min	44.08	47.56
Total process time (T) min	48.08	52.20

B. Sensory evaluation (overall acceptability) of thermally processed ribbon fish in masala and tomato sauce during storage at ambient temperature

Storage duration (months)	*Overall acceptance*	
	Ribbon fish in masala	*Ribbon fish in tomato sauce*
0	8.52 (0.5)^a	8.38 (0.14)^e
1	8.43 (0.1)^b	8.25 (0.12)^d
2	8.30 (0.1)^c	8.14 (0.29)^{ed}
3	8.11 (0.2)^d	8.01 (0.17)^c
4	7.86 (0.2)^e	7.85 (0.30)^b
5	7.58 (0.1)^f	7.50 (0.13)^a

*Values in parenthesis represent standard deviation, n=10. Different alphabetical letters indicate a significant difference ($p < 0.05$) between the storage periods.

Total process time (B+0.42 of come up time) taken to reach an F_0 value of 15.6 was 44.08 min for masala pack and F_0 value of 13.80 was 52.20 min for tomato sauce respectively. It may be attributed to more lethality resulting from more heat penetration. As more heat penetration takes place, the cold spot

temperature is closing towards the processing temperature of 115.6 ^{o}C. Tomato sauce medium has taken more time than the masala pack. The similar trend was also reported by Ali (2003) in tuna canned in oil, brine and curry media in aluminium cans.

A prescribed normal limits for TVB-N and TMA-N as 30-35 mg/100g and <15 mg/100g respectively (Connell, 1995). Changes in biochemical characteristics of ribbon fish in masala and tomato sauce packed in TFS can during canning and subsequent storage are depicted in Fig. 1, 2, 3 and 4. The present study revealed that, the volatile compounds such as TMA-N and TVB-N were found to increase slightly, but were within the acceptable limit. The TVB-N of raw ribbon fish flesh increased from 4.55 mg /100g to 22.10 mg /100g flesh in case of both the products during the storage period. The TMA-N content showed similar trend, increasing from 1.05 mg to 13.2 mg /100g at the end of the storage period of 5 months in both the canned products. Based on the results of biochemical characteristics, the ribbon fish canned in masala and sauce were found wholesome and acceptable.

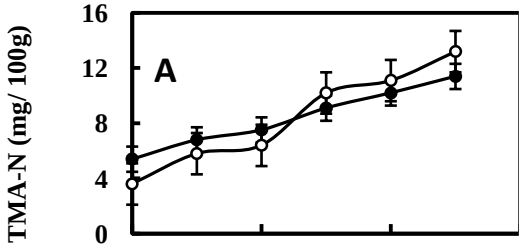

Fig 1: Changes in TMA-N content in masala and sauce packed ribbon fish in tin-free-steel can during storage.

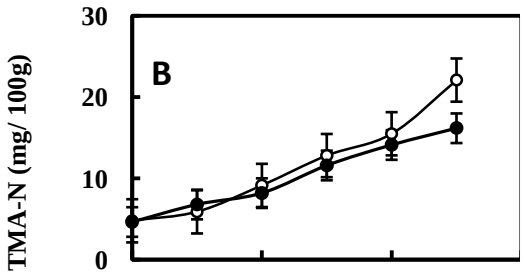

Fig 2: Changes in TVB-N content in masala and sauce packed ribbon fish in tin-free-steel can during storage

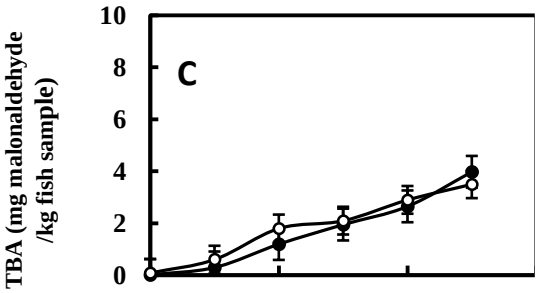

Fig 3: Changes in thiobarbutaric acid content in masala and sauce packed ribbon fish in tin-free-steel can during storage

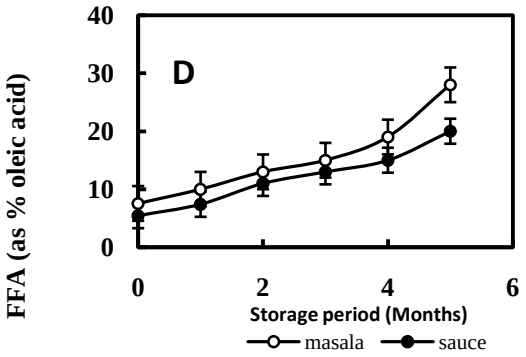

Fig 4: Changes in free fatty acid content in masala and sauce packed ribbon fish in tin-free-steel can during storage.

Changes in TBA value of ribbon fish in masala and tomato sauce during storage at room temperature are given in (Fig.1C). In ribbon fish packed in masala as a filling medium there was an increase in TBA value during storage from 0.09 to 0.58 mg malonaldhyde/kg of sample. Ribbon fish in sauce also showed an increasing trend in TBA value during storage from 0.095 to 0.483 mg malonaldhyde/kg of sample is given in (Fig. 1C). Even though the TBA values have been increased with the storage period they are within the limits of 0-39 mg malonaldhyde/kg suggested as being noticeable levels of rancid taste in foods for TBA (Shamberger *et al*., 1977). Therefore the results found in this study showed that there was a slight increase in TBA value throughout the storage period. The Free Fatty Acid (FFA) content varied between 7.55 to 17.1 % in masala packs and 5.43 to 19.4 % in sauce packs as oleic acid in fat respectively. Peroxide content was negligible throughout the storage period.

The overall acceptability for ribbon fish canned in masala and tomato sauce showed significant difference ($p < 0.05$) between storage periods (Table 2B). The fish masala was delicious with attractive reddish brown color and the fish pieces were distinctly present without breakage. It is seen from the results that ribbon fish in masala got higher overall acceptable score than sauce packs and the fish products were acceptable even after 5 months of storage at room temperature. The initial overall acceptability score of about 8.75 was gradually reduced to around 8.0.

CONCLUSION

It can be concluded that the heat penetration was highly influenced by the type of filling medium used for the canning purpose. Among the different media used for the study, heat penetration was found to be faster in masala than tomato sauce media. Hence the process parameters are found suitable for preparing value added products like the ribbon fish in masala and sauce medium in TFS cans. The biochemical and sensory attributes also indicate the quality of canned ribbon fish in masala and sauce medium were in acceptable condition even after 5 months of storage at room temperature.

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Growth, Survival and Body Composition of *Labeo Rohita* Fed with Different Levels of Nucleotide Supplemented Diets*

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ABSTRACT

Eight weeks feeding trial was conducted to evaluate the effect of dietary nucleotide on growth, survival and body composition of rohu (*Labeo rohita*). The nucleotide was supplemented at 0, 5, 10, 15 g kg^{-1} nucleotide diet. The test diets were fed for eight weeks in triplicate groups of rohu, which had initial weight of 1.3 g. At the end of the feeding trial, growth and survival of rohu was recorded. Fish fed with 10 g kg^{-1} nucleotide incorporated diets had significantly ($p<0.05$) greater weight gain, specific growth rate and protein percentage in body composition.

Keywords: Nucleotide, *Labeo rohita*, proximate composition, growth, survival

In recent years, increased importance has been given to health management in aquaculture. Use of drugs and antibiotics is discouraged in aquaculture in view of residual accumulation, destruction of gut micro flora, development of resistant bacteria and other various harmful effects. Use of immunostimulants is practiced as a potential route for the reduction in the widespread use of antibiotics

Immunostimulants have promising beneficial effects in aquaculture. They are known to enhance growth and disease resistance in fish (Robertsen, 1999) and in shrimp (Smith *et al*., 2003). Dietary supplements, including killed bacteria, β-glucans, lipopolysaccharides, and nucleotides have exhibited immunostimulatory effects (Sakai, 1999) and are known to enhance non-specific defense mechanisms in shrimp (Sung *et al*., 1994). Dietary nucleotides have been shown to have several beneficial effects in humans (Carver *et al*., 1990; Carver & Walker, 1995) as well as aquatic animals (Devresse, 2000; Burrells, 2001). Other benefits derived from administration of nucleotides include rapid intestinal repair improved mucosal gut flora and mucosal surfaces (Uauy, 1989; Gil, 2002), and elongation of the intestinal tract (Burrells *et al*., 2001) in aquatic animals. Nucleotides also have been shown to enhance the immune system (Quan, 1992) and disease resistance of various animals (Kulkarni *et al*., 1986a, 1986b). Most cell types can synthesize nucleotides from purines and pyramidines. However, *de novo* synthesis and salvage pathways are metabolically expensive processes. Further, sufficient quantities of nucleotides required to meet the metabolic requirements are presumably not synthesized by shrimp under stressful conditions (Devresse, 2000). Additional sources of exogenous nucleotides in the diet optimize function of rapidly dividing tissues (Burrells *et al*., 2001) and may enhance feed intake (Burrells *et al*., 2001), increase growth rate (Adamek *et al*., 1996), and elevate immune responses (Ramadan *et al*., 1994).

The present study was carried out to evaluate the effect of nucleotide on growth, survival and body composition of *Labeo rohita* fed with different levels of nucleotide supplemented diet.

MATERIALS AND METHODS

Experimental setup: *Labeo rohita* with an average weight of 1.3 g produced in the carp hatchery at the College of Fisheries, Mangalore, India, were used for the study. All the fingerlings were acclimatized by feeding control diet for two weeks. Rohu were randomly distributed into four groups. Four experimental

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groups, namely, control (T_0) (basal diet with no nucleotide), T_1 (5 g kg^{-1} nucleotide), T_2 (10 g kg^{-1}nucleotide) and T_3 (15 g kg^{-1} nucleotide) were arranged in triplicate groups following a completely randomized design (CRD) design and fed respective diets. The total volume of water in each tank was maintained at 100 l throughout the experimental period. Fish were fed with one of the four experimental diets at a feeding rate of 4% of body weight per day for eight weeks. The amount of feed for each tank was divided to two equal meals, which were offered to fish at 10:00 and 17:00 h. Fish in each tank were collected, counted and weighed at 2-week intervals, and the offered diets were subsequently readjusted. Three quarters of tank's water volume along with fish excreta was removed daily by siphoning and replaced with an equal volume of well-aerated fresh water. Dead fish if any were removed and recorded. At the end of the experiment, fish were collected from each tank, counted, and weighed.

Experimental diet: The composition of experimental diet is given in Table 1. Soybean meal and groundnut cake were used as protein sources and rice bran and tapioca flour were used as carbohydrate sources. Graded levels of dietary nucleotide (combination mixture of AMP, IMP,CMP, GMP in equal proportions, were procured from Sri Durga Laboratories, Mangalore) were added to the respective diet (Table I). Dry and powdered ingredients were thoroughly mixed and mixed with water to make soft dough. The dough was steam cooked for 10–15 minutes in a pressure cooker. Vitamin-mineral premix was added and thoroughly mixed with diets after cooling and diets were extruded through a laboratory pelletizer having 2 mm dia. Pellets were dried in hot air oven at 60°C till the moisture content was reduced to less than 10%. Diets were packed in high-density polythene bags and stored in refrigerator.

Table 1. Composition and proximate analyses (% on dry matter bases) of the experimental diets containing different levels of Nucleotide

	Treatment			
Ingredient	**T_0**	**T_1**	**T_2**	**T_3**
Soyabean Meal	47.95	47.95	47.95	47.95
Ground nut oil cake	20.0	20.0	20.0	20.0
Rice bran	26.05	25.55	25.05	24.55
Tapiocaflour	5.0	5.0	5.0	5.0
Vitamin and mineral mixture	1.0	1.0	1.0	1.0
Nucleotide	0	0.5	1.0	1.5
Total	100	100	100	100
Proximate composition of diets (%)				
Moisture	8.84 ±0.16	8.78 ±0.21	8.69 ±0.07	8.43 ±0.18
Dry matter	91.00 ±0.05	91.22 ±0.11	91.29 ±0.09	91.57 ±0.13
Protein	30.94 ±0.12	30.64 ±0.13	30.53 ±0.09	30.71 ±0.21
Fat	6.32 ±0.05	6.39 ±0.10	6.44 ±0.15	6.34 ±0.12
Fibre	9.67 ±0.11	9.24 ±0.24	9.72 ±0.21	9.59 ±0.16
Ash	8.88 ±0.16	8.63 ±0.09	8.49 ±0.19	8.70 ±0.21
NFE	35.27 ±0.2	36.32 ±0.10	36.11 ±0.09	36.23 ±0.14

Growth studies: Rohu were weighed at the beginning of the experiment and every 15 days interval thereafter till the completion of the experiment. The growth performance of rohu was assessed in terms of weight attained.

Specific growth rate (SGR): Specific growth rate was calculated by using the following formula

$$SGR = \frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{No of days}} \times 100$$

The calculated value gives the average percentage increase in weight per day over the experimental period.

Food conversion ratio (FCR): Food conversion ratio was calculated by using the following formula

$$FCR = \frac{\text{Dry weight of the feed given (g)}}{\text{Gain in wet weight of fish (g)}}$$

Protein efficiency ratio (PER): Protein efficiency ratio was calculated by using the following formula

$$PER = \frac{\text{Weight gain(g)}}{\text{Protein intake (g)}}$$

Survival: Survival was calculated as the difference between the number of live animals stocked at the beginning and those survived at the end of the experiment.

Table 2. Growth performance and survival (mean ± SD) of rohu fed different levels of nucleotide for 8 weeks

Items	Nucleotide levels (g/kg diet)			
	T_0	T_1	T_2	T_3
Final weight gain (g $fish^{-1}$)	3.55 ±0.11[a]	3.57 ±0.16[a]	3.88 ±0.14[b]	3.57 ±0.12[a]
Weight gain (% of initial weight)	273.58 ±8.58[a]	275.07 ±12.02[a]	298.46 ±11.61[b]	274.61 ±8.87[a]
SGR (% day^{-1})	1.68 ±0.05[a]	1.69 ±0.08[a]	1.79 ±0.07[a]	1.67 ±0.06[a]
Survival rate (%)	62.66 ±4.61[a]	64.0 ±6.93[a]	72.0 ±8.0[a]	65.33 ±6.11[a]
FCR (g feed g $gain^{-1}$)	2.86 ±0.15[a]	2.81 ±0.28[a]	2.60 ±0.36[a]	2.78 ±0.34[a]
Protein efficiency ratio	1.41 ±0.07[a]	1.44 ±0.05[a]	1.44 ±0.04[a]	1.44 ±0.04[a]

Means with the same letter in the same row are not significantly different at P<0.05.

Proximate analysis: Proximate composition of experimental diet before starting the experiment and fish muscle was estimated soon after completion of the experiment (Tables 1 & 3). Whole meat of the fish was taken for proximate analysis. The fish meat was minced thoroughly and dried at 60°C for 12 hours to obtain the dry matter. The dry matter thus obtained was powdered and stored in air tight containers for further analysis. The samples were analyzed for crude protein, crude fat, total ash and carbohydrate (NFE) employing standard methods (APHA, 1995).

Table 3: Proximate body composition of rohu fed with different levels of nucleotide for 8 weeks

Items	Nucleotide levels (g/kg diet)			
	T_0	T_1	T_2	T_3
Moisture (%)	77.99 ±0.56[a]	77.76 ±0.31[a]	77.12 ±0.12[a]	78.02 ±0.56[a]
Dry matter (%)	22.01 ±0.55[a]	22.23 ±0.31[a]	22.88 ±0.11[a]	21.98 ±0.56[a]
Protein (%)	14.72 ±0.26[a]	15.05 ±0.08[ab]	15.56 ±0.42[b]	14.74 ±0.25[a]
Fat (%)	2.52 ±0.13[a]	2.43 ±0.22[a]	2.36 ±0.10[a]	2.45 ±0.18[a]
Ash (%)	2.23 ±0.16[a]	2.19 ±0.08[a]	2.20 ±0.08[a]	2.25 ±0.13[a]
NFE (%)	2.52 ±0.32[a]	2.43 ±0.33[a]	2.76 ±0.21[a]	2.54 ±0.28[a]

Means with the same letter in the same row are not significantly different at P<0.05.

Analysis of physicochemical parameters of water: Water quality parameters were maintained within the normal range throughout the experimental period. Water samples collected on each sampling day were analyzed for pH, temperature, dissolved oxygen, free carbon dioxide, ammonia and total alkalinity. Digital portable kit model CK 704 was used to record pH, atmospheric temperature and water temperature. Dissolved oxygen was estimated by Winkler's method, total alkalinity, ammonia and free carbon dioxide were determined by following Standard methods (APHA, 1995).

In all treatments, dissolved oxygen concentrations ranged from 7.03 to 7.60 mgL^{-1}, free CO_2 ranged from 1.39 to 3.05 mgL^{-1}. The ambient water temperature varied from 26.4 to 27.3°C. The pH range was 7.43–7.83. Free carbon dioxide, and unionized ammonia concentration ranged from 1.22 to 3.08 mg L^{-1} and 0.040 to 0.279 mg L^{-1}. Total alkalinity ranged from 63.18 to 77.63 mg L^{-1} as $CaCO_3$. All the water quality parameters were within the acceptable ranges for growth (Boyd, 1982).

Statistical analysis: The mean growth, survival and body composition of rohu were recorded, and significant difference among them was tested by one way ANOVA. A statistical package SPSS version 20.0 was used for data analysis.

RESULTS AND DISCUSSION

Effect of nucleotide on growth and survival of Labeo rohita: The final weight of rohu fed with supplemental nucleotides was found to be greater than that of rohu fed the basal (control) diet. However, significant (P=0.052) difference in growth was observed between rohu fed with nucleotide 10 g kg^{-1} diet (Table 2) and control. Survival over the 60 days feeding period was higher for rohu fed with 10 g kg^{-1} nucleotide compared to other treatment groups. Rohu had attained mean weight of 298.46% in T_2 group, which is 24.88% higher growth than the groups fed control diet. Highest survival was recorded in T_2 (72.0%) (P=0.051) (Table II). No significant difference (P=0.125) was observed in survival rate due to the inclusion of nucleotide in fish diets. The survival of rohu ranged from 62.66 to 72.0% overall.

Effect of nucleotide on proximate composition: No significant change in moisture content of flesh was observed among the different treatments (P=0.108) and it ranged from 77.12 to 78.02 % (Table 3). Crude protein increased significantly (P=0.023), while total lipids decreased with the increase of nucleotide level in fish diet (P=0.707). The highest content of crude protein (15.56±0.42%) was found in fish group fed 1.0% nucleotide diet, while the lowest crude protein level (14.73±0.27%) was seen in control group. The highest content of total lipid (2.52±0.14%) was found in the control group, while the lowest one (2.36±0.10%) was found in the fish group fed on 10 gkg^{-1} nucleotide diet, respectively. No significant difference (P=0.919) was observed in ash content among different treatments and it ranged from 2.19 to 2.25 %.

The main objective of the present investigation was to evaluate the efficiency of dietary nucleotide on growth, survival and body composition of rohu. Nucleotide incorporated diets in the present study enhanced the growth and survival of rohu (Table 2). The results are comparable with the published reports. Supplementation of fish diets with nucleotides influence a positive effect on growth in salmons (Burrells, 2001). The biologically active brewers yeast had been reported to serve as a probiotic and enhance growth in tilapia (Lara-Flores *et al*., 2002). It is reported that dietary supplementation of nucleotide enabled the fish to survive through salt water grower phase as a result of nucleotide supplementation (Burrells *et al*., 2001). The effect of dietary nucleotide is comparable to probiotics and other dietary supplements (Murthy *et al*., 2009; Li *et al.,* 2007). The survival of *P. monodon* brooders fed with a diet containing β-1; 3-glucan (from *Schizophyllum commune*) was significantly higher than that of control groups. *Labeo rohita* fed 0.3% brewers yeast had higher growth and survival (Mohseni *et al*., 2011).

Proximate analysis of the whole body of rohu at the end of the feeding trial indicated a decline in lipid content of fish fed with 10 g kg^{-1} nucleotide. There was an inverse relationship observed between total protein and lipid contents. Moisture and ash contents did not vary.

It can be concluded that the present investigation indicate that the inclusion of nucleotide at 10 g kg^{-1} diet improve the growth performance and body composition. A dietary level of 10 g kg^{-1} nucleotide diet was found optimal.

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Dystocia due to Dicephalus Monster in Murrah Buffalo

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ABSTRACT

Dicephalus monster is a congenital abnormality due to incomplete separation of head resulting from twining. The present paper involves a case report of a Murrah Buffalo with history of labor pain and difficulty in parturition. On per vaginal examination, the dead foetus was found to have duplicated cranial portion. Upon lubrication and forceful traction dystocia due to Dicephalus monster was relieved.

Key words: Dicephalus monster, Dystocia, Murrah Buffalo.

Congenital anomaly are caused by genetic or environmental factors or by interaction of both and is one of the cause for dystocia in buffaloes (Roberts 1971). Monstrosities are malformed foetuses, which are rare in buffaloes (Chauhan and Verma, 1995). Dicephalus monster is one such congenital abnormality which occurs due to incomplete separation of head resulting from twining (Unver *et al.,* 2007). The present paper reports a case of dystocia due to Dicephalus monster in Murrah buffalo.

CASE HISTORY AND OBSERVATION

A multiparous full term pregnant Murrah buffalo was presented to Department of Teaching Veterinary Clinical Complex, Veterinary College, Bidar with the history of labor pain, ruptured water bag with relaxed pelvic ligaments and difficulty in parturition. On per vaginal examination, dicephalic fetus in anterior longitudinal presentation, dorso-sacral position and oriented towards the pelvic inlet with the absence of fetal reflex was noticed. Based on the case history and observation, the present case was tentatively diagnosed as dystocia due to dicephalus monster.

TREATMENT AND DISCUSSION

Dystocia was relieved under caudal epidural anaesthesia with 2% lignocaine hydrochloride. The birth canal was lubricated with castor oil. The rope was applied to both the forelimbs of the foetus and then by forceful traction the dicephalus monster dead calf was delivered (Fig-1). The dam was administered with Rintose 3L, Calcium borogluconate 450 ml and Oxytocin 40 IU intravenously after delivery of monster calf. Meloxicam (Melonex®) was administered @ 0.3mg/kg body weight intramuscularly for two days and Ceftriaxone 3g (Intacef®) was administered intravenously for three days. The fetal membranes expelled after 5 hours of delivery and the dam made uneventful recovery.

Fig-1: Delivered calf with Dicephalus monster

The female fetus had two heads on a single neck, four eyes, three ears, two mandibles, two maxillas with two fore and hind limbs. Both the heads were united at 90^0 angle which was devoid of bone and skin covering (Fig-2).

Fig-2: Dicephalus monster calf brain devoid of bone and skin covering

Dicephalus monster had been reported in Jersey crossbred heifer (Napolean *et al*., 2008), buffalo (Shama *et al*., 2010) and in Osmanabadi goat (Daimi *et al.,* 2014). Abnormal duplication of the germinal area in the fetus gives rise to congenital fetal abnormalities with partial duplication of the body structure, duplication of cranial portion of fetus is more common that of caudal part (Roberts 2004). Incidence among all calves seems to range from 0.2 to 3.0 percent with 40 to 50 percent born dead (Bugalia *et al.,* 2001). Rao *et al.,* (2011) relieved the dystocia due to Dicephalus monster in a Graded Murrah buffalo by caesarian section, whereas in present case by lubrication and forceful traction to the Dicephalus monster calf was used to relieve the dystocia in Murrah buffalo.

CONCLUSION

Successful management of dystocia due to Dicephalus monster in Murrah buffalo was reported and discussed.

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Caudectomy (Epididymectomy) For Teaser Buffalo Bull Preparation – A Case Report

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ABSTRACT

Epididymectomy is generally a surgical technique used to produce teaser males or as an adjunct to vasectomy and it achieves infertility but does not alter the ability or desire of males to mate. The present paper reports teaser buffalo bull preparation by epididymectomy technique.

Keywords: Epididymectomy, Teaser, Tunica vaginalis & Buffalo bull.

Teasers are used to detect or aid in identification of females in estrus to allow them to be bred by natural or artificial means to males of greater genetic merit than the teasers. Ideal methods for creating teaser animals involve rendering the teaser both infertile and incapable of mating to prevent spread of venereal diseases. Epididymectomy is generally a surgical technique used to produce teaser males or as an adjunct to vasectomy and it achieves infertility but does not alter the ability or desire of males to mate (Gilbert and Fubini, 2004).

CASE HISTORY AND OBSERVATION

A two year old, Non Descriptive buffalo bull from an organized dairy farm was presented to large animal clinic, MVC, Chennai-07 for teaser preparation. Thorough clinical examination was performed and the physiological and haemato – biochemical parameters were well within the normal range. Epididymectomy was decided. Preoperatively streptopenicillin 2.5g, IM and meloxicam @ 0.2 mg/kg BW, IM were administered.

TREATMENT AND DISCUSSION

Epidural anaesthesia was achieved by using 10ml of 2% lignocaine, injected at sacro-coccygeal joint. Once the scrotal area was desensitized, the surgical site was aseptically prepared and tincture iodine was applied at the site.

A horizontal skin incision was made at the ventral aspect of left scrotum, fascia and tunica vaginalis were also incised (Fig. 1). The tail of the epididymis was exposed and separated carefully from the distal aspect of the testis (Fig. 2) with blunt dissection (Fig. 3) and ligated between two sites with 2cm apart with catgut 1-0 (Fig. 4), and the tail of epididymis was resected. The tunica vaginalis was closed by simple continuous pattern using catgut 1-0 (Fig. 5) and skin incision was closed with silk by horizontal mattress pattern. The same procedure was performed for the right side epididymis.

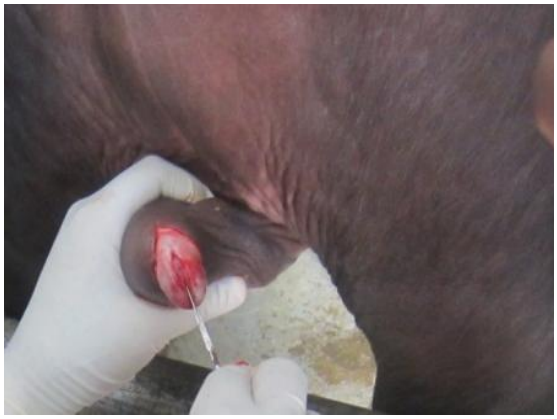

Fig. 1: Surgical incision

Fig. 2: Separation of epididymis

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Fig 3: Exposed tail of epididymis

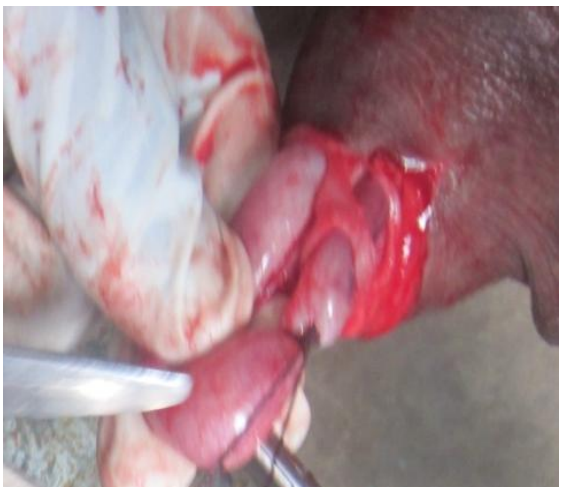

Fig. 4: Double ligation using catgut 1-0

Fig. 5: Suturing of tunica vaginalis

Weaver *et al.* (2005) stated after caudectomy sclerosing agents have also been injected into the testicle and epididymis of bulls to produce fibrosis and to abolish spermatogenesis and tease the bull weekly for three weeks and when no live sperm were seen, then the bull be considered suitable for work.

Gilbert and Fubini (2004) reported this procedure was performed as the main or supplementary method for producing teaser animals. It may be performed in ruminants of all species either in laterally restrained or standing animals. This technique should be performed at least 30 days before intended use of the teaser to allow sufficient time to achieve infertility.

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Successful Clinical Management of Pre-Parturient Haemoglobinuria in a Buffalo –A Case Report.

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ABSTRACT

Nutritional haemoglobinuria is one of the potent threats to the milking buffaloes and cows in India, occurring as post - parturient episode; however pre-partum reports are available. The present case report discusses successful clinical management of phosphorous deficient haemoglobinuria in a pregnant buffalo. An 8 month pregnant buffalo was presented to TVCC veterinary college Bidar, with a history of passing coffee decoction coloured urine since 4 days. Based on the thorough clinical examination and laboratory findings the case was diagnosed as pre- parturient haemoglobinuria. The animal was treated with Novizac^{R}, Intas pharmaceuticals Ltd. (kit of Buffered Phosphorous, Inosine and Pyruvate Injection) along with haematinics and liver extracts as supportive therapy. An uneventful recovery was observed with follow-up treatment for three days suggestive of efficacy of Novisac^{R} which can be used as the good alternative for phosphorous deficient haemoglobinuria.

Key words: Buffalo, Buffered phosphorous, Haemoglobinuria, Pregnant.

Parturient haemoglobinuria is one of the potent threats to the milking buffaloes and cows in India which is a non infectious haemolytic syndrome characterized by intravascular haemolysis, anaemia and haemoglobinuria (Akhtar *et al*., 2007). The exact pathogenesis is not yet known however associated risk factors include ingestion of cruciferous plants, saponin from berseem, dietary phosphorous deficiency, decreased serum copper and selenium whereas increased molybdenum (Radostits *et al*., 2007). The present case report discusses the successful clinical management of phosphorous deficient haemoglobinuria in a pregnant buffalo.

A 6 year aged female buffalo was presented to the TVCC Veterinary college Bidar, with history of not taking feed, passing coffee decoction coloured urine since 3 days (Plate: 1). The history revealed that the animal was kept on exclusive sugarcane top feeding since 3-4 months. Clinical examination of the animal revealed pale mucous membrane which was icteric at edge, slight hurried respiration with distress. Rectal temperature was normal (100.4ºF). On examination the body coat revealed no tick infestation. Examination of blood smear stained with Giemsa's stain confirmed that the animal was negative for *Babesia* spp. The serum sample was analysed for Phosphorous level revealing 2.5 mg/dl inorganic phosphorous against the normal range of 5.6-6.5 mg/dl. The haemoglobin content was hardly 5.5 g/dl (normal 8-13 g/dl). The case was diagnosed as phosphorous deficient haemoglobinuria. The animal was treated with Novizac^{R}, Intas pharmaceuticals Ltd. (Kit of Buffered Phosphorous, Inosine and Pyruvate Injection) 25 ml intravenously and 10 ml subcutaneously. Supportive treatment included haematinics (Ferritas^{R,} Intas pharmasuitical Ltd.) 7 ml and Liver extracts (Belamyl^{R}, Sarabhai zydus Pvt.Ltd) 15 ml by IM route and 50 grams of sodium bicarbonate powder PO.

Fig: Pregnant buffalo passing coffee decoction coloured urine.

Twelve hours after treatment animal passed the urine which was of almost normal colour indicating uneventful improvement in the condition. The treatment was continued for 3days

with Novizac[R] and Belamyl[R]. The normal appetite was resumed on third day onwards. Orally Ferritas[R] bolus as haematinics was given once a day for 10 days. Livotas[R] syrup (Intas pharmaceuticals Ltd.) was dispensed to owner with advice of giving 40 ml per day for one week.

Increased demand for phosphorous and calcium for developing foetus and subsequent dietary deficiency of these compounds leads to hypophosphataemia in peri-parurient periods (Digraskar *et al*. 1991). Pandey and Misra (1987) reported that hypophosphataemia leads to intravascular haemolysis due to impaired glycolytic pathway and depletion of ATP in erythrocytes that inturn alter functions and structure of RBC, causing a loss of normal formability and an increase in fragility, ultimately leading to haemolysis.

Novizac[R] supplies necessary phosphorous which helps in augmenting the cell activity through enhancing the production of ATP and also aids in relieving oxidative stress and stabilising RBC membrane.

Durrani *et al*. (2010) conducted clinical trials on post parturient haemoglobinuria with sodium acid phosphate, toldimfos sodium and tea leaves. They noticed the highest efficacy with toldimfos sodium (85%) followed by tea leaves (56%) while the lowest efficacy was observed with sodium acid phosphate (18%). The present clinical report showed that parturient haemoglobinuria can be successfully managed by Novizac[R] injections in accordance with earlier reports of Rashmi and Yadav (2014).

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Diagnosis of Intussusception by Ultrasonography in Pups - A Case Report

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ABSTRACT

Intussusception is recognized as a common cause of bowel obstruction in small animals and has been classified according to their location in the alimentary tract as gastroesophageal, pylorogastric, enteroenteric, enterocolic and colocolic had been reported in small animals. Ultrasonography and radiographic evaluation of abdominal region are very helpful in establishing a definitive diagnosis (Lewis and Ellison, 1987 and Oakes *et al*., 1994). Present case paper reports ultrasonography findings of intussusception in two Labrador pups were discussed.

Key words: Intussusception, Enteroenteric, Ultrasonography and Labrador pup.

Intussusception is one of the major surgical condition in canines most common at jejunojejunal and ileo-colic regions (Fossum *et al.,* 2002) and commonly observed in puppies less than one year of age, with more occurrence in German Shepherd and less commonly in cocker spaniels (Sivasankar, 2000 ; Khan *et al.,* 2011 and Vineet *et al.,* 2012). It is classified as high (proximal to the jejunum) and low (distal to the duodenum) intussusception (Dixon, 2004). Predisposing factors and aetiological agents responsible for intestinal intussusception were intestinal parasitism, linear foreign bodies, hyper motility of intestines and prior abdominal surgery (Wilson and Burt, 1974). Ultrasonography and radiographic evaluation of abdominal region are very helpful in establishing a definitive diagnosis (Lewis and Ellison, 1987 and Oakes *et al*., 1994). Present case paper reports ultrasonography findings of intussusception in two Labrador pups.

CASE HISTORY AND OBSERVATIONS

Case1: A six month old male, Labrador pup was presented to Teaching Veterinary Clinical Complex (City Hospital), APMC yard, Gandhi Gunj, Bidar with a history of recurrent rectal prolapse, inappetance and not passed stools since two days and owner had manually applied ice packs and replaced the rectum inside. On clinical examination the pet was dullwith congested mucus membrane and slightly distended abdomen. Rectal temperature and heart rate were 103.9°F and 106 beats/minute, respectively with tachypnea. Haemato–biochemical examination revealed leucocytosis with neutrophilia indicating underlying infection. On abdominal palpation, hard mass was felt at the left caudal abdominal part and suspected for intussusception or intestinal obstruction.

Case 2: A two and half month old female Labrador pup was presented to Teaching Veterinary Clinical Complex (City Hospital), APMC yard, Gandhi Gunj, Bidar with a history of not passing feces since two days with normal appetite. One week back pup was treated for acute enteritis by local veterinarian. On clinical examination pet was active with congested mucus membrane. Rectal temperature and heart rate were 101.5°F and 88 beats/minute, respectively. Haemato–biochemical values were within the normal physiological range. On abdominal palpation, firm hard mass was felt near the anterior portion of the abdomen and suspected for jejunal intussusception or intestinal obstruction.

Both the puppies were subjected to ultrasonographical examination for confirmation of the condition.

ULTRASONOGRAPHICAL FINDINGS AND DISCUSSION

Ultrasonography of both the cases revealed concentric rings on transverse section, hyperechoic outer (intussuscipiens-ensheathing layer) and hypoechoic (intussusceptum -entering layer) at the centre giving characterstic appearance of "Bull eye" at the ileo colic region for case 1(Fig.1 & Fig.2) where in ultrasonography probe was placed at the left mid abdominal region and at jejunojejunal segments for case 2 (Fig.3 & Fig.4) wherein the ultrasonography probe was placed at the lower anterior abdomen region.

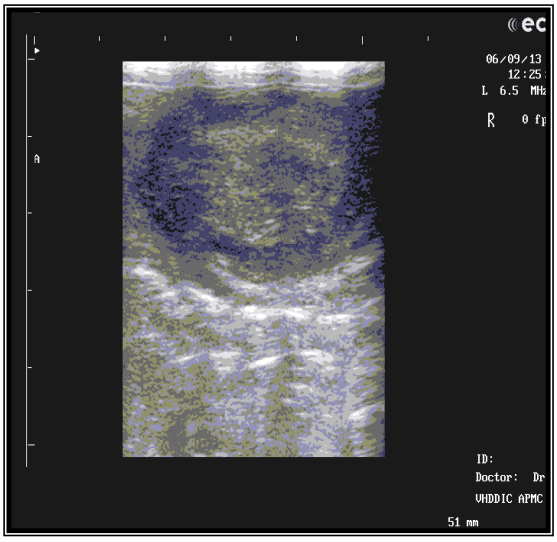

Fig.1: Concentric rings at the ileo-colic junction

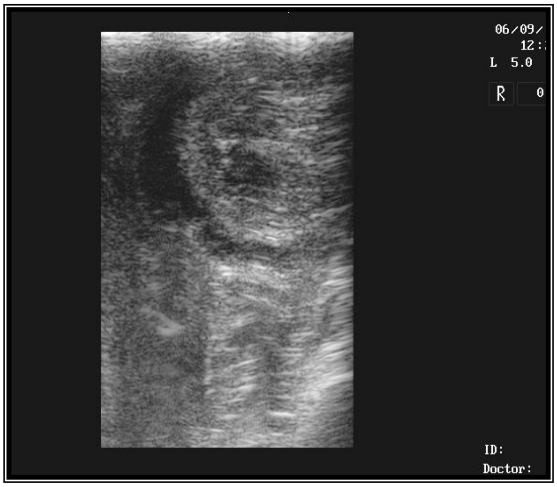

Fig.2: "BULL EYE" appearance of intussusception mass

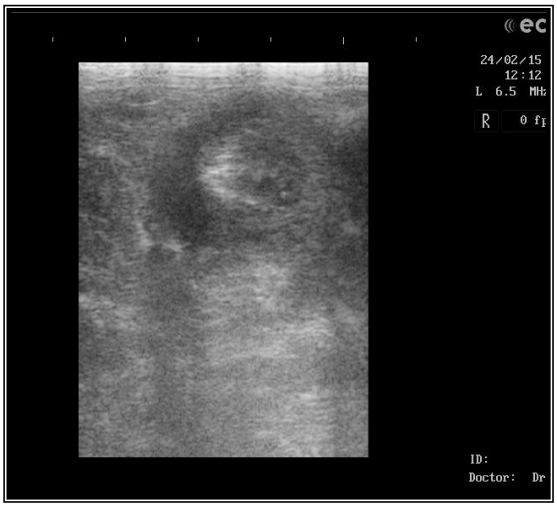

Fig.3: Concentric Hypo and hyperechoic part at the jejunojejunal part in a Labrador pup

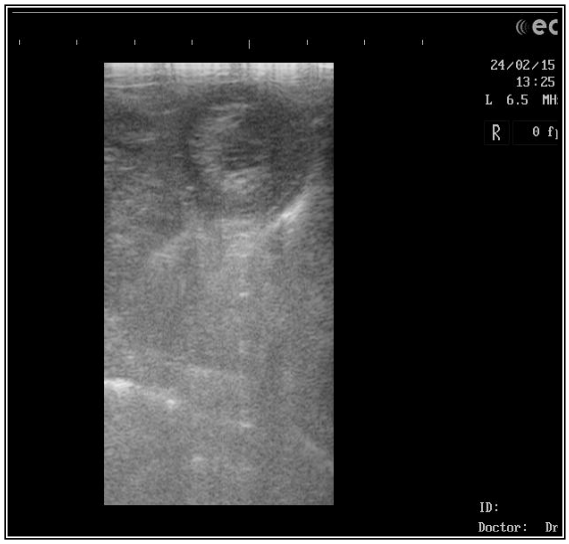

Fig.4: Concentric Hypo and hyperechoic part at the jejunojejunal part in a Labrador pup in transverse plane

Intussusception is recognized as a common cause of bowel obstruction in small animals and has been classified according to their location in the alimentary tract as gastroesophageal, pylorogastric, enteroenteric, enterocolic and colocolic (Wilson and Burt, 1974 and Lewis and Ellison, 1987). Acute enteritis or gastroenteritis has been demonstrated as the most likely predisposing factor for intestinal intussusceptions in young dogs (Rallis *et al.,* 2000). It forms as a result of abnormality within the intestinal wall that alters the intestinal pliability and motility (Sivasankar, 2000; Hedlund and Fossum, 2007). Puppies and kittens are most likely ones to develop intussusceptions. Although it can occurs in any age or species but more than 80 per cent of intussusceptions occurs at one year old puppies (Oakes *et al.,* 1994; Dixon, 2004; Hedlund and Fossum, 2007). Most frequently observed clinical signs in case of low intussusceptions were bloody mucoid diarrhea, tenesmus, intermittent vomiting and weight loss (Dixon, 2004). Intussuception at the enterocolic level appeared to be more at risk of developing adhesion than those involving only the enteroenteric region (Kumar *et al.,* 2011). Accumulation of gas proximal to the intussusception was observed on plain radiography

(Vineet *et al.,* 2012). On ultrasonography, intussusceptum and intussuscipiens appear as hyperechoic and hypoechoic concentric rings in transverse plane and as multiple hyperechoic and hypoechoic parallel lines in longitudinal plane (Lewis and Ellison, 1987 and Oakes *et al*., 1994). Ultrasonography of ileocolic intussusception showed concentric hyperechoic and hypoechoic rings in the transverse plane however, longitudinal plane revealed a sausage-shaped mass with folded layers of intestinal wall (Khan *et al.,* 2011). In the present cases characteristic circular hyperechoic and hypoechoic (bull eye) appearance of intestinal segments were noticed which concurred with the findings of Wilson and Burt (1974), Lewis and Ellison (1987), Oakes *et al*. (1994) and Khan *et al.* (2011).

CONCLUSION

Ultrasonography can be used as confirmative diagnostic tool for diagnosing intestinal intussusception in paediatric patients without any harmful effects.

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Incidence of Theileriosis in Cattle at Veterinary College Hospital, Bangalore*

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ABSTRACT

A study on the incidence of theileriosis at Veterinary College Hospital, Hebbal, Bangalore was undertaken for a period of six months from April to September 2004. The incidence was studied based on the clinical signs, blood smear examination and Dot-ELISA using schizont antigen. Eleven (14.66%) out of 75 cattle presented to Veterinary College Hospital, Hebbal had clinical signs of theileriosis. Out of these 11 cases, 6 were positive by blood smear examination and all were positive by Dot-ELISA. The clinical signs recorded in the present study included anorexia, pyrexia of 103° to 104.4°F, paleness of mucous membranes and swelling of superficial lymph nodes. In addition some cases showed reduced milk yield, panting, salivation, weakness and nasal discharge.

Key Words: Theileriosis, Incidence, Dot-ELISA

A study was carried out to study incidence of theileriosis in cattle brought to in Veterinary College Hospital, Bangalore based on blood smear examination and Dot-ELISA. The cattle brought to Veterinary College Hospital, Hebbal for a period of six months from April to September 2004 were included for the study. The cases were recorded based on clinical signs of theileriosis. Blood samples were collected for smear examination and serum was separated for detection of antibodies by Dot-ELISA.

Fresh smears were made and quickly dried by waving in the air and stained with Giemsa stain using standard procedure as described by Schalm *et al*. (1975). The stained smears were microscopically examined under oil immersion. Dot-ELISA test was performed using nitrocellulose strips according to the method described by Prasanna *et al*. (2001).

Based on clinical signs 11 (14.67 per cent) out of 75 were suspected to be suffering from clinical theileriosis. Among them 8 were HF cross and 3 were Jersey cross. The common clinical signs observed were anorexia (100%), pyrexia of 103º to 104.4° F (90.90%), paleness of mucous membranes *viz*., oral, conjunctival and vulval mucosa (63.64%) and swelling of superficial lymph nodes namely prescapular and prefemoral lymph nodes (54.55%). In addition some cases also exhibited reduced milk yield (36.36%), panting (27.27%), salivation (27.27%), weakness (18.18%) and nasal discharge (9.09%). Similar clinical signs were recorded by Sisodia and Mandial (1986) and Sundaram *et al*. (2003). However occasional signs of haemoglobinuria and urticarial lesions were reported by few workers.

In the present study, 6 (8.00%) out of 75 animals were positive for theileriosis by blood smear examination. Further, ring form of *T. annulata* organism was predominantly found on blood smear examination. However, Venugopal (1983) reported oval forms of the parasite in higher percentage as compared to rod and ring forms. Out of these 11 clinical cases, all were (14.47% of total 75 animals) were positive by Dot-ELISA.

Tanwar *et al*. (1984) reported an incidence of 42.86 per cent in Rathi calves in Bikaner, Rajasthan. Sundar *et al*., 1993 reported a prevalence of 53.19 per cent by ELISA, while Soundararajan *et al*., 2000 reported a prevalence of 22.8 per cent by blood smear examination and 66.4 per cent by Dot-ELISA in Madras Veterinary College, Chennai.

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PCR Amplification and Characterization of *RGN* Gene in Buffalo (*Bubalus bubalis*)

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ABSTRACT

Successful PCR amplification of the whole buffalo regucalcin (RGN) gene and restriction enzyme characterization was done. Single amplification product showed the primers designed were working well and the thermal protocol was standardized. The PCR product was further characterized using three different restriction enzymes (Cla I, Pst I and Kpn I) The RFLP pattern obtained proved that the product obtained is regucalcin and not any nonspecific product. This is the first report of PCR amplification of RGN from tissue of buffalo. The RGN amplicon can be further used for structure prediction of the RGN protein. It can be used for molecular cloning work. So far no buffalo RGN clones were reported to use in any studies.

Key words: Regucalcin, PCR, RFLP.

Regucalcin (*RGN*) gene is located on the X-chromosome of humans (region Xp11.3-q11.2), rats (region Xq11.1-12) (Fujita *et al*., 1995) and a number of other mammalian organisms, except in yeast (Shimokawa *et al*., 1995). The *RGN* gene consists of seven exons and six introns, encodes a protein of 299 amino acids that has a molecular weight of ~33kDa. A high degree of conservation in both genomic and protein levels has been shown by a comparison study made between gene sequences from seven different vertebrate species (Misawa and Yamaguchi, 2000). This high conservation of *RGN* protein throughout evolution, from invertebrates to vertebrates, indicates its involvement in basic and important biological functions. *RGN* regulates intracellular calcium homeostasis by modulating the activity of enzymes regulating Ca^{2+} concentration, and enhancing Ca^{2+} pumping activity through the plasma membrane, endoplasmic reticulum and mitochondria of several cell types (Yamaguchi, 2005). It is also demonstrated to have role in calcium signaling. *RGN* is shown to regulate apoptosis both *in-vivo* and *in-vitro* (Ishigami *et al*., 2002, Izumi and Yamaguchi, 2004). It regulates glycogenolysis and gluconeogenesis stimulated by Ca^{2+} in liver cells (Yamaguchi and Shibano, 1987). Here we have done the PCR amplification and RE characterization of buffalo *RGN*. This can be further used for cloning in to appropriate host for future research.

RNA was isolated from the testis tissue of buffalo using PROMEGA, Total RNA Isolation System, according to manufacturer's instruction. cDNA was synthesized from the RNA using Revert Aid™ H-Minus First Strand cDNA Synthesis kit (Fermentas). Forward and reverse primers (ExRGNF and ExRGNR) for the full length amplification of coding sequence of bovine *RGN* were designed using DNA star molecular biology software. PCR reaction mixture of 25ul, consisting of 1X *Taq* buffer with $MgSo_4$, 0.2mM of dNTP mixture, 10pmol of each of forward and reverse primers, 100ng of buffalo testis cDNA and 5U of *Taq* polymerase enzyme was subjected to initial denaturation at 95^0C for 5 min followed by 30 cycles of 1min of denaturation at 95^0C, 1min of annealing at 55^0C and 1min of extension at 72^0C, and final extension for 10min at 72^0C. The amplified PCR products were confirmed for their size by 1% agarose gel electrophoresis. The electrophoresis was carried out in a horizontal submarine gel electrophoresis at 50V for 2h with 100bp ladder and visualized in gel documentation system. The PCR product was loaded in a LMP agarose gel and given a long run at 50V. Gel extraction was done according to the standard protocol (Sambrook, 2001). Pellet was reconstituted

in NFW and further used for RE characterization using *ClaI, PstI, KpnI* enzymes. A 20 µl reaction was put for each enzyme containing 2µl of gel extract, 1µl of enzyme, 2µl of respective 10x buffer and volume made up by NFW. Overnight digestion at 37°C was done and checked by running a 2% agarose gel.

The PCR product was confirmed by running a 1% agarose gel. The expected size of ~893 bp of PCR product was confirmed. Gel extraction was done to remove the salts and buffers and used for RE characterization. RE digestion with *Cla1* yielded two fragments (~300bp and ~600bp), *Pst1* yielded three fragments (~548bp, ~357 and ~273bp) and *Kpn1* yielded two fragments (~350bp and 550bp). The attainment of predicted fragment sizes after RE digestion suggests the specificity of the product. The *RGN* amplicon can be further used for structure prediction of the RGN protein. It can be used for molecular cloning work. So far no buffalo RGN clones were reported to use in any studies.

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Effect of Genetic and Non-Genetic Factors on Litter Size in Pigs

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ABSTRACT

The present investigation was undertaken to evaluate the effect of genetic and non-genetic factors on litter size of various pig breeds. Least Squares Analysis of data has revealed the significant (P<0.01) effect of genetic factor and non-significant effect of environmental factors on litter size at birth and litter size at weaning. This result might be attributed to the uniform housing, feeding, and other managemental conditions provided to the different groups of pig. Also, the higher litter size at birth and at weaning, when compared to the previous reports, in some pig breeds under this investigation, suggest the importance of genetic factor in improving reproductive efficiency of sows than non-genetic factors. Although, this finding is of use in pig rearing practices, Least Squares Analysis of more data with additional environmental factors will prove the importance of reducing/ eliminating non-genetic factors in enhancing the returns on investments in pig husbandry.

Key words: Swine, Genetic factor, Non-genetic Factor, Litter Size

Swine industry is growing swiftly at present due to the change in food habits of a fairly good human population and also due to the change in perception towards pig rearing in our county, which earlier was solely an occupation of weaker sections of society. Pigs are the crux of enhancing rural economy through an account of high prolificacy, better efficiency of feed conversion, shorter generation interval, faster growth rate, low maintenance cost and high dressing percentage besides ability to utilize unconventional feed stuffs and human leftovers efficiently. Hence, they can be crucial in resolving the acute demand for protein of high biological value, comparatively at low cost. This minimized cost of production and eventual higher returns on investment is a good fortune for animal husbandry activities in rural masse. Although, India ranks 3rd in Asia for pig population, with 11.134 million (BAHS, 2013), only 21.46% are of improved genotype (crossbred) pigs and remaining 78.54% are of indigenous with poor reproductive efficiency. Therefore, superior germplasms of improved exotic pig breeds were introduced in our country to enhance important economic traits genetically in native pigs through crossbreeding. Also, the performance of purebreds was assessed in our environmental conditions. This performance of pigs is controlled by various genetic and non-genetic factors. Therefore, with dearth of information on the factors affecting the economic traits, the present study was undertaken with an objective to study the effect of various factors affecting reproductive traits in pigs.

Data on litter size of pigs at birth and at weaning spread over a period of 29 years from 1972 to 2000 were extracted from the available History cum Pedigree sheets maintained at Regional Pig Breeding station-cum-Bacon factory, Haringhata, West Bengal. This data was classified according to genetic group, period and parity of dams. Genetic factor considered in this study were Large White Yorkshire (LWY), Landrace (LR), LWY x LR and LR x Desi (D) pigs. Total period was divided into five periods, the first period of nine years duration and the remaining four periods of five years duration each and up to 5th parity of dams was considered in our investigation. The data was subjected to Least Squares Analysis as per Harvey (1966) and modified Duncan's multiple range (DMR) test (Kramer, 1957) was utilized for pair-wise comparison of the least squares means. Least Squares means ± SE of Litter Size at Birth and at Weaning in pigs are detailed in the Table.

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Table: Least Squares Means ± SE of litter size at birth and at weaning in Pigs

Effects	n	Litter Size (numbers)	
		at Birth	at Weaning
A. Breed: Genetic Factor			
a) LWY	196	$7.678^{a} \pm 0.217$	$5.304^{a} \pm 0.160$
b) LR	369	$8.150^{b} \pm 0.142$	$6.419^{b} \pm 0.160$
c) LWYxLR	218	$8.486^{b} \pm 0.190$	$6.719^{b} \pm 0.214$
d) LR x D	161	$7.946^{a} \pm 0.214$	$5.444^{a} \pm 0.242$
B. Period: Non-Genetic Factors			
a) Period 1	207	8.496 ± 0.194	8.496 ± 0.194
b) Period 2	167	7.960 ± 0.226	7.960 ± 0.226
c) Period 3	220	7.717 ± 0.193	7.717 ± 0.193
d) Period 4	196	8.200 ± 0.212	8.200 ± 0.212
e) Period 5	154	8.71 ± 0.236	8.71 ± 0.236
C. Parity: Non-Genetic Factors			
a) Parity 1	275	7.806 ± 0.181	7.806 ± 0.181
b) Parity 2	238	8.143 ± 0.189	8.143 ± 0.189
c) Parity 3	198	8.160 ± 0.199	8.160 ± 0.199
d) Parity 4	146	8.075 ± 0.224	8.075 ± 0.224
e) Parity 5	87	8.291 ± 0.287	8.291 ± 0.287

n= Number of observations in each subclass of a factor. Mean with different superscript differ significantly at $p<0.05$.

Litter Size at Birth: The average litter size at birth in LWY, Landrace, LWY x LR and LR x D were estimated to be 7.678 ± 0.217, 8.150 ± 0.142, 8.486 ± .190 and 7.946 ± 0.214 respectively. The highest and lightest litter size at birth was observed in LWY x LR and LWY, respectively. Previous studies have reported the average litter size at birth of 6.4 to 10.60 in LWY (Singh *et al*., 1979; Mohanty and Nayak, 1986; Goonewardene *et al*., 1984; Jayarajan and Ulaganathan, 1992); 6.8 to 10.12 in LR (Kumar *et al*., 1990; Chauhan *et al*., 1993; Sharma and Singh, 1993); and 5.71 to 10.17 in LWY x LR (Sharma and Singh, 1993; Singh and Devi, 1997; Sukhades *et al*., 1979;). Therefore, the litter size at birth values obtained in the present study for various subclasses of genetic factor was well within the reported range.

A significant ($P<0.01$) effect of genetic factor on litter size at birth in pigs has been revealed by Least Squares Analysis of variance. This is in accordance with the findings of Sharma and Singh (1993), Pandey *et al.* (1996) and Singh and Devi (1997, 1997a). Landrace and LWY x LR had significantly ($p<0.05$) higher litter size at birth than that in LWY and LR x D. Although, LWY x LR had 0.336 higher litter size at birth than LR they did not differ significantly due to the similar managemental conditions followed for all the genetic groups. Similarly, Landrace differ significantly from Large White Yorkshire X Landrace and Landrace x Desi for litter size at birth. Least squares analysis of variance has found a non-significant influence of period on Litter size at birth in pigs. This is supported aptly by the findings of Mishra *et al*. (1990). However, the significant effect of period on litter size at birth has been reported by Gupta *et al*. (1982). Similarly, non-significant effect of parity on litter size at birth was also found in present investigation. This is in synch with the findings of Sukhdeo *et al*. (1979) who also reported the non significant effect of parity on litter size at birth in Large white Yorkshire, Landrace and their crosses.

Litter Size at Weaning: The average litter size at weaning of 5.304 ± 0.245, 6.419 ± 0.160, 6.719 ± 0.214 and 5.444 ± 0.242 respectively has been revealed for LWY, LR, LWY x LR and LR x D pigs. Highest and lowest litter size at weaning was found in LWY x LR and LWY, respectively. Erstwhile researchers have revealed the average litter size at weaning of 5.1 to 8.4 in LWY (Mohanty and Nayak, 1986; Goonewardene *et al*., 1984) and value more than the finding of present study has been found by Singh *et al*. (1977), Sukhdeo *et al*. (1979), Sing *et al*. (1979 a), Gupta *et al*. (1982), Goonewardene *et al*. (1984), Singh *et al*. (1979); 4.2 to 7.3 in LR (Chauhan *et al.*, 1993; Sukhdeo *et al.*, 1979) and value more than the present study has been found by Siagian *et al.* (1986), Mukhopadhyay *et al.* (1992), Sharma and Singh (1993), Singh and Devi, 1997; 6.5 to 7.7 in LWY x LR (Sukhdeo *et al*., 1979; Sharma and Singh, 1993); and 5.52 to 6.00 in LR x D pigs (Mukhopadhyay *et al*., 1992; Singh and Devi,

1997). These reports suggest the similarity in the values obtained in our investigation.

Least squares analysis of variance has revealed the significant ($P<0.01$) effect of genetic group on litter size at weaving in pigs. Large White Yorkshire x Landrace and Landrace had significantly ($p<0.05$) higher litter size at weaning than Large White Yorkshire and LR x D pigs. Although Large White Yorkshire X Landrace had 0.300 higher litter sizes at weaning than Landrace yet it did not differ significantly. This variation in the present finding for same genetic groups might be due to difference in managemental and environmental conditions. Similar reports of significant influence of genetic factor on litter size at weaning was observed in LWY, LR and their crosses (Sukhdeo *et al*., 1979); in indigenous, Large White Yorkshire and their crosses (Goonewardene *et al*., 1984); in exotic, desi pigs and their crosses (Singh *et al*., 1990; Kumar *et al*., 1990); in Landrace, Desi, Tomworth and their crosses (Mukhopadhyay *et al.*, 1992); in Desi, Landrace, Tamworth and their various mating combination groups (Sharma and Singh, 1993). Contrarily to the present finding, the non-significant effect of genetic factor on litter size at weaning has been found by Mohanty and Nayak (1986) in Local, LWY and their crosses and Chatterjee *et al.* (1988) in Desi, LWY and their crosses. Least squares analysis of variance presented a non-significant effect of period on Litter size at weaning. This is supported by the similar finding of Mishra *et al*. (1990). Contrast to this, Gupta *et al.* (1982) has reported significant effect of period on litter size at weaning. Similarly, a non-significant effect of parity on litter size at weaning has been found in this investigation by least square analysis of variance. Similar to this result, Sukhdeo *et al.* (1979) found a non-significant influence of parity on litter size at weaning in LR, LWY, Desi and their crossbreds.

Present study was undertaken to evaluate the effect of genetic and non-genetic factors on litter size of pig. Least Squares Analysis of data has revealed the significant effect of genetic factor and non-significant effect of environmental factors on litter size. This result might be attributed to the uniform housing, feeding, and other managemental conditions provided to the different groups of pig. Also, the higher litter size at birth and at weaning, when compared to the previous reports, in some pig breeds under this investigation, suggest the importance of genetic factor in improving reproductive efficiency of sows than non-genetic factors. Although, this finding is of use in pig rearing practices, Least Squares Analysis of more data with additional environmental factors will prove the importance of reducing/ eliminating non-genetic factors in enhancing the returns on investments in pig husbandry.

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