

Evaluation of Therapeutic Efficacy of Immunoglobulins in Visceral Gout affected Broiler chicks

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ABSTRACT

An experimental trial was conducted in which a total of 100 broiler chicks aged 5 days affected with visceral gout were selected from a commercial broiler poultry farm, to study the effect of immunoglobulin administration in visceral gout affected chicks. These birds were grouped randomly in to two groups namely Group I, which served as untreated control and Group II chicks were injected with immunoglobulins at the rate of 0.5 ml (4.3 g/dl of protein) by subcutaneous route on Day 5. Data with respect to body weight and mortality was collected from both groups at weekly interval for 6 weeks and assessment of biochemical parameters viz., uric acid (mg/dl), GGT and total protein (g/dl) was done at Day 7, 14, 21 and 28. Mortality observed in Group I was 24 % whereas it was 14% in Group II. Mean body weight in Group II was significantly ($P<0.05$) higher as compared to Group I on Day 14, 21, 28 and 35. Mean serum uric acid level and serum GGT level in group II was significantly ($P<0.05$) lower as compared to Group I on Day 14, 21 and 28 whereas mean total serum protein level was significantly ($P<0.05$) higher as compared to Group I. The findings showed that immunoglobulins can be successfully used as an alternative to conventional therapy against visceral gout in broiler chicks.

Key words: Visceral gout, broilers, immunoglobulin

Visceral gout is a common metabolic disorder characterized by high level of uric acid in the blood causing deposition of urates on the surfaces of various visceral organs and is responsible for a great deal of morbidity and mortality in broilers. It assumes prime economic importance in poultry industry due to increased incidence causing production loss, regular low mortality and lack of availability of specific treatment (Dhara *et al.*, 2010). Owing to lack of availability of specific treatment, several field trials have been undertaken by different workers (Upendra, 1991; Dhara *et al.*, 2010) for the treatment with variable results. Hence, the study regarding the evaluation of immunoglobulins in therapy of visceral gout in poultry was undertaken.

MATERIALS AND METHODS

A total of 100 broiler chicks aged 5 days affected with visceral gout were selected from a commercial broiler poultry farm based on reported clinical symptoms of loss of appetite, weakness, droopiness, emaciation and lameness prior to death

and post-mortem lesions revealing chalky white deposition on the serosa of liver, kidneys, heart, spleen, intestines, muscles and joint. These birds were grouped randomly in to two groups namely Group I which served as untreated control and Group II chicks were injected on Day 5 with immunoglobulin at the rate of 0.5 ml (0.02 g Protein) by subcutaneous route. Both the groups were maintained under same managerial conditions. Data with respect to body weight and mortality was collected from both groups at weekly interval for 6 weeks. Serum was separated from the blood samples collected from 6 birds at Day 7, 14, 21 and 28 and was subjected for estimation of uric acid (mg/dl), GGT (U/L) and total protein (g/dl) using automated biochemical analyzer. For immunoglobulin precipitation, approximately 60 ml of pooled serum was collected from 15 broiler birds after 3 weeks of recovery from visceral gout affected flock. Precipitation of immunoglobulins was done with PEG (Polyethylene glycol) 6000 method as per procedures described by Mahboob *et*

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al. (2002). Immunoglobulin was quantified using auto-analyzer for the total protein content.

RESULTS AND DISCUSSION

The mortality per cent reported in the present study was 24 % (12) and 14 % (7) for Group I and Group II respectively. Mortality during 8-14 days and 15-21 days of age was comparatively low in Group II compared to Group I indicating that passive administration of immunoglobulin reduced mortality in visceral gout affected broiler chicks. The mortality observed in visceral gout can be attributed to kidney dysfunction (Feizi *et al.*, 2011; Singh *et al.*, 2013) and dehydration (Crespo and Shivaprasad, 2012).

Mean \pm SE of body weight in group II was significantly ($P < 0.05$) higher as compared to Group I on Day 14, 21, 28 and 35 (Table). Thus, the mean body weight in immunoglobulin treated visceral gout affected broiler chicks was statistically higher indicating that passive administration of immunoglobulin caused increase in body weight in visceral gout affected broiler chicks. The reduced body weight in visceral gout affected broiler chicks can be attributed to loss of appetite, dullness and unthriftiness as rightly reported by Guo *et al.* (2005) and Jana *et al.* (2009).

Table: Mean \pm S.E. of Body Weight and Biochemical parameters of broiler chicks with or without immunoglobulin therapy.

Days Group	Parameters	7	14	21	28
I	Body Weight (g)	162 \pm 4 ^a	395 \pm 8 ^a	790 \pm 20 ^a	1000 \pm 15 ^a
II		163 \pm 3 ^a	410 \pm 5 ^b	910 \pm 15 ^b	1250 \pm 20 ^b
I	Uric acid (mg/dl)	3.60 \pm 0.32 ^a	7.90 \pm 0.19 ^a	7.00 \pm 0.20 ^a	6.57 \pm 0.13 ^a
II		3.58 \pm 0.13 ^a	6.50 \pm 0.20 ^b	5.81 \pm 0.15 ^b	5.97 \pm 0.22 ^b
I	Total protein (g/dl)	3.32 \pm 0.04 ^a	3.51 \pm 0.04 ^a	4.37 \pm 0.04 ^a	4.54 \pm 0.04 ^a
II		3.47 \pm 0.04 ^b	3.64 \pm 0.04 ^b	4.71 \pm 0.04 ^b	5.71 \pm 0.04 ^b
I	GGT level (U/L)	8.64 \pm 0.06 ^a	16.56 \pm 0.07 ^a	16.62 \pm 0.14 ^a	16.62 \pm 0.09 ^a
II		8.71 \pm 0.05 ^a	9.74 \pm 0.15 ^b	15.26 \pm 0.16 ^b	14.44 \pm 0.11 ^b

I :untreated control, II: Immunoglobulin treated group

Values with different superscript differ significantly ($P < 0.05$).

There was no statistically significant difference ($P > 0.05$) in Mean \pm SE of uric acid levels between Group I and Group II on Day 7 however, the mean uric acid gradually decreased from Day 14, 21 and 28 and there was a statistically significant ($P < 0.05$) difference compared to Group I (Table). This indicated that the mean uric acid level in visceral gout affected broiler chicks was significantly decreased by passive injection of immunoglobulin. Birds are uricotelic and lack uricase enzyme so impairment of kidney functions in visceral gout outbreaks results in hyperuricaemia (Prathap Kumar *et al.*, 2008), and uric acid itself is not toxic but precipitated crystals can cause severe mechanical damage to tissues of kidneys, heart, lungs and intestine (Eldaghayes *et al.*, 2010) in visceral gout affected birds.

Total serum protein values were higher ($P < 0.05$) in Group II as compared to Group I and this upward trend was observed on Day 7, 14, 21 and 28 (Table). Similar findings were also reported by earlier workers (Rao *et al.*, 1993; Feizi *et al.*, 2012). The increase in total serum protein in visceral gout affected chicks may be attributed to dehydration which is a precipitating factor for gout (Silva *et al.*, 2007).

Mean \pm SE of GGT level between Group I and Group II showed no statistically significant difference on day 7. However, mean GGT level in Group II was significantly ($P < 0.05$) lower as compared to Group I on Day 14, 21 and 28 (Table). Thus indicating that the mean GGT level in Immunoglobulin treated visceral gout affected broiler chicks was statistically lower. Deposition of urates in kidney and liver were observed in gout affected birds (Feizi *et al.*, 2011) and liver had congestion and haemorrhages with occasional urate deposition in visceral gout affected birds (Jana *et al.*, 2009) and GGT level is considered as indicator of liver damage in experimentally induced liver damage in birds (Lumeij, 1988).

The antiviral antibodies are capable of binding to intact viral proteins, including structural components of viral particles and also viral proteins present at the surface of infected cells, by being able to bind to conformationally complex

structures. Antiviral antibodies have the unique ability to neutralize the infectivity of viral particles present in the circulation or at mucosal surfaces, a function that cannot be performed by T cells. T cells cannot recognize intact viral proteins and therefore play no role in directly neutralizing whole viral particles. (Zajac and Harrington, 2008).

From the results of present study it was concluded that immunoglobulins administered to Group II might have neutralized the infectivity of the viral particles responsible for causing gout in birds, thus reducing the severity of gout. The reduced severity of disease resulted in reduced mortality in broiler chicks, caused statistically significant higher mean body weight and a statistically significant decrease in mean uric acid levels and mean serum GGT level and a statistically significant increase in total serum protein level in Group II as compared to Group I. So, immunoglobulins can be successfully used as a promising candidate, as an alternative to conventional therapy against visceral gout in broiler chicks.

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Effect of Feeding on Birth Weight and Neonatal Mortality in Lambs*

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ABSTRACT

The present study was undertaken to study the birth weight and neonatal mortality of lambs born under two different feeding strategies. Sixty adult NARI Suwana ewes of same age (1.5 to 2.0 Years) were divided randomly in to two groups. Group I ewes (n=30) were fed with balanced sheep feed and group II were (n=30) allowed for only grazing in the field and not fed any balanced feed throughout the experiment. Further, ewes in both the groups were artificially inseminated at appropriate time during estrus. Following lambing birth weight and neonatal mortality were recorded. The mean birth weight of lambs born as singleton under scientific feeding and pasture grazing was recorded as 2.6 ± 0.36 and 1.92 ± 0.18 Kg, respectively. Similarly, the mean birth weight of lambs born as multiples under scientific feeding and pasture grazing was recorded as 1.73 ± 0.1 and 1.09 ± 0.05 Kg, respectively. The neonatal mortalities observed for multiple births under scientific feeding and pasture grazing were 18.51% and 38.09%, respectively. In conclusion, all the lambs born as singleton survived in both the groups and the incidence of neonatal mortality of lambs born as multiples on pasture group was significantly higher (38.09%) than the ewes maintained on balanced feed (18.51%).

Key words: Birth Weight, Mortality, Ewes, Scientific feeding and Pasture grazing

Birth weight is a significant predictor for later health outcomes (Gardener *et al.*, 2007). It is well documented that low birth weight negatively affects lambs survival (Fogarty *et al.*, 2000). Trace minerals (Hostetler *et al.*, 2003; Gardner *et al.*, 2007) and energy (Stafford *et al.*, 2007) affect both fetal growth and lamb birth weight. Live weight of lambs at birth depending on the number of the new born can fluctuate in a wide range from 2.50 to 7.00 kg (Gardener *et al.*, 2007).

Mortality in lambs is complex problem which may result from a variety of climatic, nutritional, management, infectious, genetic and other factors (Khan *et al.*, 2006). Ahmed *et al* (2010) reported that, the mortality rate in singletons, twins and triplet lambs were 43.90%, 34.15% and 21.95% respectively in Sokoto breed of sheep during cool and hot seasons. Mortality in lambs that weigh 2.5 to 3.0 kg was 8.54% whilst 10.98% lambs of weight 3.5 to 4.0 kg died. Mortality amongst lambs of weight 4.5 to 5.0 kg was 17.07%. (Ahmed *et al* 2010). However, increased litter size at birth usually leads to increased lamb mortality. Lamb birth has long been considered a major risk factor for lamb viability

and survival (Gama *et al.*, 1991; Christley *et al.*, 2003), extreme birth weights being highly related to substantial increases in lamb mortality (Christley *et al.*, 2003; Casellas *et al.*, 2007). Therefore, the present study was undertaken to assess the birth weight and neonatal mortality of lambs maintained under two different systems of feeding systems.

MATERIALS AND METHODS

Sixty adult NARI Suwana ewes of same age group were randomly divided in to two groups. All the ewes were vaccinated against Foot and Mouth, Enterotoxemia, Pestes des petits ruminants and Black Quarter diseases and also dewormed once in three months using broad spectrum anthelmintics. Ewes in group I (n=30) received approximately 250g of balanced sheep feed, comprising of yellow maize, soya been meal, wheat bran, salt and mineral mixture daily as per the recommendations described by Brown, (1994). In addition to the concentrate feed ewes were fed with *ad lib* ragi straw and water. Ewes in group II (n=30) did not receive any concentrate feed or mineral mixture and were allowed to graze in the field for a period of 10 hrs daily and these ewes were maintained under pasture grazing throughout the period of study.

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Further, ewes in both the groups identified to be in estrus were inseminated (AI) using freshly collected and diluted ram semen of the same breed. All the inseminations were carried out at approximately 12 hrs after the identification of estrus and ewes were artificially inseminated only once. Those ewes not returning to estrus were subjected to pregnancy diagnosis between 30 to 35 days of post AI using ultra sound scanner. Following lambing, the neonatal mortality and frequency of single/ multiple births in ewes were recorded. Multiple births were further categorized as twins, triplets and quadruplets. Two way ANOVA was used to study the effect of system of feeding and multiple births on mean birth weight and Chi square test to study the effect of multiple births on neonatal mortality.

RESULTS AND DISCUSSION

The mean birth weight and viability status of newborn lambs delivered from ewes maintained with either a balanced sheep feed or under pasture grazing are presented in Table. The birth weight of lambs which were delivered as a single, in ewes fed with a balanced feed was significantly higher ($p \leq 0.05$) when compared to the lambs born as singleton to ewes maintained on pasture grazing. The mean birth weights of lambs born as singleton under scientific feeding and pasture grazing were recorded as 2.6 ± 0.36 and 1.92 ± 0.18 kg, respectively. Further, the mean birth weights of lambs born as multiple (twins, triplets or quadruplets) was also significantly higher in the group of ewes maintained with a balanced sheep feed as compared to the mean birth weight of multiple births observed in pasture grazed ewes (1.73 ± 0.1 Vs 1.09 ± 0.05).

Table: Birth weight and neonatal mortality of lambs born to ewes maintained under two different systems of feeding strategies

System of Feeding	Mean Birth Weight(Kg)		Neonatal Mortality (%)		
	n=30	Single	Multiple	Single	Multiple
Scientific feeding		2.60 ± 0.36 (6) ^{aA}	1.73 ± 0.10 (54) ^{bA}	0 (0/6)	18.51 (10/54) ^A
Pasture grazing		1.92 ± 0.18 (9) ^{aB}	1.09 ± 0.05 (42) ^{bB}	0 (0/9)	38.09 (16/42) ^B

The values with different small letter superscripts in rows differs significantly at $p = 0.05\%$. The values with capital superscripts in columns differs significantly at $p = 0.05\%$.

All the lambs born as singleton survived in both the groups of ewes, The incidence of neonatal mortality of lambs born as multiples to ewes on pasture grazing was as high as 38.09% and this incidence was nearly two times higher than the incidence recorded in animals maintained on a balanced feed (18.52%).

In the present study, the birth weight of the lambs as well as their viability appeared to be significantly influenced by the system of feeding on which the ewes were maintained. The body weight of the lambs delivered as a single in ewes maintained with a balanced feed was nearly 700 grams higher than the body weight of lambs which were delivered as single under pasture grazing. Interestingly, every lamb born as a single, survived irrespective of the type of feeding. On the other hand, the mean body weight of lambs born as multiples to ewes fed with a balanced feed was recorded as 1.73 ± 0.1 kg and this was almost 800 grams lower than the body weight of lambs born as singles to ewes maintained under balanced feeding. Interestingly, the mortality rate of lambs born as multiples in the pasture fed group was nearly 2 times higher than the ewes fed with a balanced diet. A similar trend was also noticed in the mean birth weight of lambs born as multiples to ewes maintained on pasture grazing (Table). Further, while all the lambs born as single survived in both the groups, nearly 38.00% of the lambs born as multiples to ewes maintained on pasture grazing failed to survive beyond 48 h. This incidence of neonatal mortality was almost 2 times higher than those recorded in ewes maintained on balanced feed. It was further observed that, the chances of the lamb surviving were almost zero when the birth weight of the lamb was less than 1300 g. The results of the present study revealed that, NARI Suwarna ewes is indeed a very high prolific breed but the survivability of the lambs is greatly influenced by the type of feeding on which the pregnant ewes are maintained. Ewes maintained on a balanced feed appear to deliver lambs of a higher body weight and lambs have a better chance of survivability than ewes maintained under pasture grazing. It is reasonable to recommend that, NARI Suwarna ewes should receive some sort of balanced

feed in addition to the pasture grazing to improve the viability of the neonate. Body weight, therefore, is a significant predictor for later health outcomes. Fogarty *et al.*, (2000) documented that the low body weight negatively affected the lamb survival, deficiency of trace minerals and energy (Hostetler *et al.*, 2003; Stafford *et al.*, 2007) affected both fetal growth and lamb body weight.

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Mineral Profiles in NARI Suwarna Ewes Maintained Under Two Different Systems of Feeding*

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ABSTRACT

The present study was under taken to assess the blood serum mineral profile in NARI Suwarna ewes maintained under two different systems of feeding strategies. Fifty adult NARI Suwarna ewes of same age were divided randomly in to two groups. Group I (n=25) were fed with balanced sheep feed and group II (n=25) were allowed for only grazing in the field. The mean serum concentrations of Calcium (Ca), Phosphorus (P), Glucose (G), Zinc (Zn), Copper (Cu) and Iron (Fe) in ewes maintained under scientific feeding and pasture grazing were 10.31±0.3 and 9.36±0.4mg/dl, 6.38±0.29 and 6.03±0.29 mg/dl, 62.42±2.49 and 53.2±2.69 mg/dl, 0.9±0.07 and 0.9±0.08 ppm, 1.11±0.09 and 1.14±0.12 ppm, and 1.64±0.15 and 1.57±0.14 ppm respectively. The mean serum calcium and glucose levels were significantly higher in ewes maintained under scientific feeding as compared to those maintained under pasture grazing.

Key Words: Minerals, Ewes, Scientific feeding, Pasture grazing

Studies have demonstrated interaction between nutrition and reproduction in sheep (Griffiths *et al.*, 2007) but few studies have also reported the lack of a clear nutrition-reproduction interaction (Smith and Akinbamijo, 2000). Minerals such as phosphorous (P), calcium (Ca), copper (Cu), and zinc (Zn) are involved in governing successful reproductive processes (Wilde, 2006).

Results of research on mineral concentration and interrelationship in soil, forage and blood serum of sheep in Mexico have shown that there are imbalances of minerals, with excesses of P (Phosphorus) and Fe (Iron) and deficiency of Cu (Copper) in sheep. These imbalances were attributed to deficiencies of Cu and excesses of Fe in the soil and in forages (Chan *et al.*, 1998).

Pradhan *et al.* (1991) reported that calcium and inorganic phosphorous levels were 6.16±0.19 mg/100ml, 6.55±0.23 mg/100ml in post partum anestrus ewes and 10.76±0.22 mg/ml and 5.96±0.24 mg / 100ml in cycling ewes, respectively. Radostits *et al.* (1994) also observed the normal range of values for biochemical parameters in sheep and reported figures of 11.5 – 13.0 mg/dl, 4.0-7.0 mg/dl and 30-65 mg/dl and for calcium, phosphorous and glucose, respectively.

The mean blood glucose, calcium and phosphorous levels were 77.56±4.39 mg/dl, 11.11±0.86 mg/dl and 4.23±0.37 mg/dl in rainy season; 72.91±4.37 mg/dl, 7.19±0.45 mg/dl and 4.19±0.24 mg/dl in winter season and 92.13±6.68 mg/dl, 9.05±0.40 mg/dl and 5.47±0.30 mg/dl in summer season, respectively, in Merino Nilagiri crossbred sheep (Ramprabhu and Dhanapalan.1998). Highly significant difference in the calcium concentration was observed between rainy and winter and summer seasons.

Ravindra Reddy *et al.* (2010) reported that in Nellur Weaner lambs under semi intensive management system, the biochemical values were 34.68±3.68 mg/dl, 9.36±0.63 mg/dl and 4.50±0.30 mg/dl for blood glucose, calcium and phosphorous, respectively. Sejian *et al.* (2010) reported that glucose values were 52.08±2.43, 47.80±1.61, and 44.19±1.91 and 42.99±2.52 g/dl in control, thermal stress, combined stress and nutritional stress animals, respectively in Malpura ewes of Rajasthan. Therefore, the present study was undertaken to study the blood serum mineral profile in NARI Suwarna ewes maintained under two different systems of feeding.

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MATERIALS & METHOD

Fifty adult NARI Suwarna ewes of same age were randomly divided into two groups. All the ewes were vaccinated against Foot and Mouth, Enterotoxemia, Peste des petits ruminants and Black Quarter diseases and also dewormed once in three months using broad spectrum anthelmintics. Ewes in group I (n=25) received approximately 250g of balanced sheep feed for a period of 6 months. The feed comprised of Yellow maize, Soya bean meal, Wheat bran, salt and mineral mixture and it was designed to have energy 45.00%, crude protein 15.00%, crude fiber 35.50%, salt 2.00% and mineral mixture 2.00% as per the recommendations described by Brown, (1994). In addition to the concentrate feed, ewes were fed with *ad lib* ragi straw and water. Ewes in group II (n=25) did not receive any concentrate feed or mineral mixture and were allowed to graze in the field for a period of 10 hrs daily and these ewes were maintained under pasture grazing throughout the period of study (6 months). Further, blood samples were obtained to determine the concentrations of serum calcium, phosphorus, glucose, zinc, copper and iron. The serum samples were obtained from all the ewes maintained under controlled conditions and pasture grazed ewes. Serum samples were analysed for the concentration of calcium, phosphorus and glucose using automated blood biochemical analyser (Trivitron Labmate biochemical analyser), as per the techniques described by Kaneko (1997). Zinc (Zn), Copper (Cu) and Iron (Fe) were analyzed by using atomic absorption spectroscopy (210VGP, AAS, Buck scientific) as per the procedure described by Fernandez and Kahn (1971). The concentrations of serum biochemical parameters were studied in relation to the type of feeding.

Students' t' test was used to study the significant differences of serum mineral profiles in ewes maintained under scientific system of feeding and pasture grazing.

RESULTS AND DISCUSSION

The serum concentration of calcium, phosphorus and glucose in NARI Suwarna ewes maintained under two different systems of feeding management have been presented in Table. The concentration of serum calcium was significantly higher (10.31 ± 0.3 mg/dl) in ewes maintained with a balanced feed as compared to those maintained under pasture grazing (9.36 ± 0.4 mg/dl).

The mean serum phosphorus concentrations in animals maintained with a balanced feed was recorded as 6.38 ± 0.29 mg/dl in the group of ewes maintained with a balanced feed. Its concentration in the serum of animals under pasture grazing was recorded as 6.03 ± 0.29 mg/dl, the difference being non significant. The serum glucose concentration was significantly ($p < 0.01$) higher in ewes fed with a balanced diet as compared to those maintained under pasture grazing (62.42 ± 2.49 Vs 53.2 ± 2.69 mg/dl).

The mean serum concentrations of Zn, Cu and Fe in ewes fed with a balanced diet were recorded as 0.9 ± 0.07 , 1.11 ± 0.09 and 1.64 ± 0.15 ppm, respectively, in ewes fed with a balanced diet. The corresponding mean serum concentrations of Zn, Cu and Fe in animals maintained under pasture grazing were recorded as 0.9 ± 0.08 , 1.14 ± 0.12 and 1.57 ± 0.14 ppm respectively. The concentration of Zinc, Copper and Iron in the serum samples did not differ significantly between the two groups of ewes.

A majority of the sheep and goat production systems in India are based on pasture

Table: Mean serum concentration of Calcium, Phosphorus, Glucose, Zinc, Copper and Iron in NARI Suwarna ewes maintained under two different systems of feeding strategies

Feeding strategy	No. of animals	Calcium (mg/dl)	Phosphorus (mg/dl)	Glucose (mg/dl)	Zn (ppm)	Cu (ppm)	Fe (ppm)
Scientific feeding	25	10.31±0.3^a	6.38±0.29	62.42±2.49^a	0.9±0.07	1.11±0.09	1.64±0.15
Pasture grazing	25	9.36±0.4^b	6.03±0.29	53.2±2.69^b	0.9±0.08	1.14±0.12	1.57±0.14

The mean values in the column with different superscript differ significantly at $p = 0.05\%$

grazing and do not provide any mineral supplementation. The use of commercial premixture or mineral blocks is also a practice that is usually not performed. Further, trace elements inclusion is also rarely taken in to the account so that their contribution is null and void, coupled with deficiency of elements such as copper and selenium in some regions, where the animals are grazed (Gupta *et al.* 2005). Researchers on mineral concentrations and inter relationships in soil, forage and blood serum of sheep have shown that there are imbalances of minerals with excess of Phosphorus and Iron in sheep with copper deficiency and excess of iron in soil and forages (Ramana *et al.*, 2001). Several studies have also demonstrated interaction between nutrition and reproduction in sheep. For example, flushing on minerals improved production and reproduction parameters (Fernández *et al.*, 2004; Almeida *et al.*, 2007; Griffiths *et al.*, 2007). Minerals such as phosphorus, calcium, iodine magnesium, copper selenium and zinc are involved in governing successful reproductive processes (Wilde, 2006).

It was observed that among the various macro and micro elements investigated, the mean concentrations of calcium and glucose were significantly lower in the serum of pasture grazed animals as compared to those maintained with a balanced feed (Table). The low concentrations of glucose recorded in the serum of pasture grazed animals suggested a gross energy deficiency.

Schillo (1992) stated that energy restriction suppresses the increase in LH that is necessary for ovarian follicles in the preovulatory stage. For this reason, flushing is generally recognized as a significant regulator of reproduction and has been accomplished either by allowing animals to graze on lush nutritious pasture or by feeding energy rich supplements (Webb and Mamabolo, 2004). Further, more energy supplementation has been reported to positively influenced the body condition, fertility and ovulation rate in ewes on pure nutritional status (Chowdhury *et al.*, 2002; Karikari and Balsu, 2009). Rhind and Schanbache (1991) stated that energy supplementation could also increase the

secretion of insulin increased the sensitivity to pituitary gonadotrophins and enhance the number of growing follicles. El-shahat, and Abo-el matty (2010) reported that energy supplementation improved the size of the preovulatory follicles and ovulation date in ewes. It is therefore, possible that, the low reproductive efficiency of ewes maintained under pasture grazing is perhaps more related to energy deficiency as reflected by the hypoglycaemic status and this could adversely affect the reproductive efficiency.

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Acute Oral Toxicity Profile of *Ficus Benghalensis* Leaves in Rats

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ABSTRACT

The plant, *Ficus benghalensis* popularly known as Banyan is used in India for a variety of ailments since ancient times. Charaka had prescribed, the aqueous extract of leaf buds of Nyagrodha (*Ficus benghalensis*) for checking diarrhea (Khare, 2004). While the latest scientific investigations provide evidence to the anti-ulcerogenic (Mayank Kulshreshtha *et al.*, 2010); analgesic and anti-pyretic properties of leaves of *Ficus benghalensis* (Sachdev Yadav *et al.*, 2011). However, the toxicity profile of the leaf extract is yet to be explored. The study was carried out to determine the preliminary oral toxicity profile of the methanolic *Ficus benghalensis* leaf extract (FbLEx) in Wistar albino rats.

The acute oral toxicity study was conducted using fixed dose procedure according to the Organization for Economic Co-operation and Development (OECD/OCDE) test guidelines on acute oral toxicity based on Acute Toxic Class Method (OECD TG 423). Rats were orally administered single dose of 50, 300, 2000 and 5000 mg/kg of FbLEx. General behavior and toxic symptoms were observed for 14 days. The effects of *Ficus benghalensis* leaf methanolic extract (FbLEx) were examined in rats for its acute toxicity. No mortality nor changes in behavior were noted after 14 days of treatment. Neither were there any clinical signs of toxicity indicating that the LD50 estimate of the extract to be greater than 5000 mg/kg body weight by oral route. However, histological examination revealed mild microscopic changes in kidneys and heart. In summary, the methanolic *Ficus benghalensis* leaf extract is found to be relatively safe toxicologically when administered orally, however, further repeated dose toxicity studies would be needed to support the long-term safe use of the leaves of *Ficus benghalensis*.

Key words: *Ficus Benghalensis*, Rats, Toxicity

Herbal medicines are being increasingly used as medicines and dietary supplements by general population. With the ever increasing use of plants for their medicinal values, there should be proper scientific documentation of their medicinal properties. This has led to necessitate the screening of plants of medicinal values and to analyze their possible role in treating various ailments; one such plant being *Ficus benghalensis*. Although these natural products provide valuable bioactive components for treating ailments, they also pose a serious threat of causing toxic symptoms when consumed inappropriately. Thus all the pharmacological investigations should be preceded by toxicological evaluation to ensure safety of the consumers who prefer plant based medicines over allopathic drugs, owing to the adverse side effects involved with the use of the latter. Hence, the present study was thus aimed at evaluating the acute toxic potential of *Ficus benghalensis* leaf extract (FbLEx).

Ficus benghalensis popularly known as “Banyan tree” in India is an evergreen tree, cultivated throughout India and revered as sacred by Hindus. It is locally known as Aalada mara. It is claimed to possess medicinal value as per ancient ayurvedic and Unani literatures and is supported by ever increasing scientific evaluation on the plant. All parts of the tree have been used in traditional medicine for treatment of variety of pathologies. The hanging roots of *Ficus benghalensis* have been reported as anti-diarrhoeal agents (Mukherjee *et al.*, 1998). The fruit extract of *Ficus benghalensis* has been documented for its anti-tumor and anti-bacterial activities (Mousa *et al.*, 1994). While the latest scientific investigations provide evidence to the anti-ulcerogenic (Mayank Kulshreshtha *et al.*, 2010); analgesic and anti-pyretic properties of leaves of *Ficus benghalensis* (Sachdev Yadav *et al.*, 2011), the toxicity profile of the leaf extract is yet to be explored. The present study was thus aimed to determine the preliminary oral toxicity profile of

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Ficus benghalensis leaf (Methanolic) extract (FbLEx) in Wistar albino rats as a prerequisite to the evaluation of its medicinal properties.

MATERIALS AND METHODS

Preparation of plant extract: The leaves of *Ficus benghalensis* were collected in September, 2012 from the premises of the Veterinary College, Hebbal, Bangalore, Karnataka State. The authentication of the plant was done by Dr. K. Shankar Rao, Retired Professor, CES, IISc, Bangalore, Karnataka, after comparing it with the collection housed at the herbarium of Center for Ecological Sciences, IISc with specimen reference no. CJS 12154. The fresh leaves of *Ficus benghalensis* collected were dried under shade for several days. After grinding into coarse powder, the dried leaves (100g) were soaked in 1000 ml of methanol and kept for 5 days with periodical shaking and stirring. After 5 days, contents were filtered through Buchner's funnel in a conical flask and it was further concentrated by rotary flash evaporator (Superfit India Ltd., Mumbai) at 39-40°C till the solvent got completely evaporated and extract settled down to bottom. The residual methanol from the extract was evaporated after keeping the extract in a Petri dish in a vacuum oven at 60°C at the pressure of 25 psi. The residues were weighed after drying. Alcoholic extraction was preferred over aqueous extract because it helps in capturing more semipolar/semi-lipophilic constituents along with the polar constituents. Further phytochemical analysis was performed using HPTLC technique.

Experimental animals: Wistar albino rats aged 9 weeks weighing 170 ± 10 g adult were procured from Sri Venkateshwara Enterprises, CPCSEA No. 237, for use in the present work. The animals were kept separately in polypropylene cages at a temperature of 25 ± 2 °c, 50-60% relative humidity, with 12 h light/dark cycle respectively and were allowed to acclimatize to the experimental conditions for 7 days before the commencement of actual studies under standard hygienic conditions. The feed and water were provided *ad libitum*. The animals were maintained as per the protocol outlined in publication of the Committee for the

Purpose of Control and Supervision of Experiments on Animals standard guidelines (CPCSEA) and the experiment was conducted with prior permission obtained from Institutional Animal Ethics Committee (IAEC) with reference No. LPM/IAEC/100/2011.

Acute oral toxicity study: Acute oral toxicity study for the methanolic leaf extract of *Ficus benghalensis* was conducted in female Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class Method (OECD 423). The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. Four doses were selected for determining LD₅₀ value with a limit dose of 5000mg/kg body weight. Acute toxicity was conducted in female rats as per OECD 2000. Five groups of rats consisting 3 female rats in each group were used for determining LD₅₀ value in female rats. The animals were fasted overnight prior to the administration of the substance. The FbLEx in graded doses were administered as a single dose to animals by gavage using a gavaging tube. The volume of administration was maintained to 2 ml/200 g through proper dilution of methanolic leaf extract of *Ficus benghalensis*.

The group details and dose administered per kg are as follows:

SL. No.	Group	No. of female rats	Dose (mg/kg)
1	Control	3	Distilled water
2	Group I	3	50
3	Group II	3	300
4	Group III	3	2000
5	Group IV	3	5000

Observation of animals: General clinical observations were made at least once a day throughout the study period of 14 days considering the period of anticipated effects after dosing. All the animals were observed for health condition like abnormal motor activity, alteration in water or food intake, writhing, straub reaction, sedation, diarrhoea, piloerection, opisthotonus, exophthalmos

and tremors at least twice daily, and also observed for morbidity and mortality.

Sacrifice of animals: All the tested groups of animals were sacrificed humanely on day 15. Detailed post mortem examination was carried out and the organs (viz., liver, spleen, kidney, heart, lung, stomach, intestine, brain and ovary) were collected for histological examination.

RESULTS AND DISCUSSION

Phytochemical analysis: The phytochemical analysis of the *Ficus bengalensis* methanolic leaf extract performed using HPTLC revealed the presence of anthracenes, flavonoids, glycosides, bitter principles and coumarins.

Acute toxicity study: There were no deaths and clinical signs of toxicity in any of the test groups within 24 h after the administration of *Ficus bengalensis* methanolic leaf extract. The treated groups were kept for observation for a period of 14 days. No mortality and clinical signs of toxicity were observed in any of the tested groups in a given dose and duration. LD50 value of both *Ficus bengalensis* methanolic leaf extracts in rats were more than 5 g/kg (Table).

Table. Mortality pattern in female rats in the acute oral toxicity study of FBLEx

Group	Dose (mg/kg)	No. of female rats	Mortality observed	Mortality (%)
Group I Control	Distilled Water	3	0	0
Group II Leaf extract	50	3	0	0
Group III Leaf extract	300	3	0	0
Group IV Leaf extract	2000	3	0	0
Group V Leaf extract	5000	3	0	0

Pathology: At necropsy none of the treated and control rats showed any gross pathological lesions even the rats which were treated with a dose of 5000 mg/kg. Whereas the histological examination

of various organs of female rats revealed normal architecture of all the organs except those administered a dose of 5000 mg/kg which revealed occasional thickening of mesangium, atrophy of glomeruli with normal architecture of tubules in kidney (figure I); where-as the cardiac fibers revealed inter-muscular edema, discontinuation of fibers at some places, along with loss of cross striations and more eosinophilic sarcoplasm indicating necrosis (figure II).

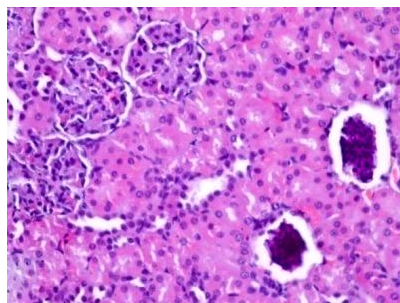


Figure I: Kidneys showing thickening of mesangium, atrophy of the glomeruli which appeared condensed and dark with disappearance of glomerular tuft in some. Mild to moderate degree of congestion was also observed.

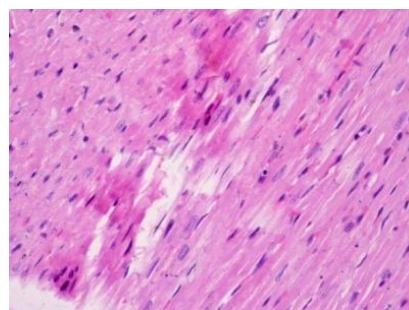


Figure II: Heart showing inter-muscular edema, discontinuation of fibers, loss of cross striations and more eosinophilic sarcoplasm indicating necrosis

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short term exposure by the oral route. It is traditionally a step in establishing a dosage regimen in other studies by providing initial information on the mode of toxic action of a substance.

The methanolic leaf extract of *Ficus bengalensis* did not reveal any mortality even at the highest dose tested (5000 mg/kg) and clinical signs of toxicity, but at microscopic level there was

presence of inter-muscular edema, discontinuation of fibers at some places, along with loss of cross striations and more eosinophilic sarcoplasm indicating necrosis in cardiac tissue at the dose of 5000mg/kg. Kidney tissue also revealed occasional thickening of mesangium and atrophy of glomeruli forming a condensed mass, thus indicating that the *Ficus bengalensis* leaf extract had tendency to cause cardiac damage and renal glomerular damage at very high doses. As per the OECD TG 423 the substances with LD 50 value greater than 5 g/kg are practically non toxic. This is further supported by Schorderet (1992), who mentioned that the chemicals with LD50 values greater than 5 g/kg of body weight are considered to be practically non toxic. Thus, the methanolic leaf extract of *Ficus bengalensis* can be classified in the category of substances with non to low toxicity.

Similar efforts were put by other workers to find out LD 50 value in the toxicity of plant or its extract in rat as a model with other species of *Ficus* such as Jayashree (2010) and Lokesh (2007) who conducted the toxicity study of on *Ficus virens* and *Ficus amplissima*, respectively in rats.

CONCLUSION

A conclusion could be drawn by the above finding that *Ficus benghalensis* methanolic leaf extract was non lethal at the dose of 5000 mg/kg. This study provides an initial data on the toxicity profile of FBLEx that should be essential for future study. Further studies may focus on chronic toxicity studies of FBLEx in order to evaluate its long-term effects.

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Comparative Efficacy of Anesthetic Protocols Used for C- Section on the Safety of Dam and Neonates*

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ABSTRACT

The safety of different anesthetic protocols for cesarean section was studied in 48 bitches with live fetuses. The anaesthetic protocols evaluated were propofol only, propofol with ketamine, isoflurane and epidural anesthesia. There was a significant drop in heart, pulse and respiratory rates following induction of anesthesia suggesting the necessity of anesthesia being monitored closely and surgery carried out with minimum dosage possible and in shortest time. On the basis of ease of induction, effect on hematological and biochemical parameters, degree of muscle relaxation, time taken for recovery from anesthesia and puppy survival rate, it was concluded that all the anesthetic protocols evaluated in the present study were equally satisfactory for cesarean section in dogs. However, in view of ease of administration, availability of drug and satisfactory puppy survival rate, propofol or combination of propofol and ketamine anesthesia appears to be the best choice for C- section in dogs.

Key words: C- section, Bitch, Anaesthesia.

In recent years the awareness among canine breeders in getting number of live fetuses and preserving the future breeding of the bitch is increasing day by day. The act of parturition perhaps is the most anxious time for the dog breeders, as the puppy survival rate and the future reproduction of the dam are influenced by events at this stage. In view of considerable economy and owners concern involved, it becomes extremely important to carry out comprehensive studies with regard to the anaesthetic protocols for C- section in bitches. It is also important to formulate guidelines on selection of anaesthetic procedures in an attempt to reduce the incidence of mortality of the dam and the neonate.

MATERIALS AND METHODS

The animals which were selected for C-section after premedication were randomly allotted to three general anesthetic protocols with each group comprising of 12 animals. In addition, another group of 12 animals premedicated were subjected to cesarean section under epidural analgesia. In Group I, The general anaesthesia was induced and maintained by administering inj. propofol initially at a dose rate of 5-10mg/kg body weight intravenously to effect as evidenced by the absence of pedal reflexes, palpebral reflexes and ventro-medial deviation of the eyeball

(third stage of anesthesia). Subsequently, Propofol was administered intermittently as and when needed to maintain general anaesthesia. In Group II, the general anaesthesia was induced and maintained using a combination of fast acting IV anaesthetic agent (Propofol) and dissociative anaesthetic agent (ketamine). The anesthetic agents propofol and ketamine were drawn into the same syringe in ratio of 1:1 (V/V) and administered intravenously to effect as judged by the signs of third stage of anaesthesia. In Group III, cesarean section was carried out under general anaesthesia using Isoflurane (FORANE[®], Abbott Laboratories Ltd., England) an inhalant anaesthetic agent. Isoflurane was administered through a face mask using a Boyle's anesthetic apparatus initially at the rate of 3-5 per cent administered until the animal attained the third stage of anesthesia. Simultaneously, oxygen was also infused at the rate of 1.5 per cent. Once the animal attained third stage of anesthesia, animals were maintained in the third stage using Isoflurane at 1 per cent concentration and oxygen at 1.5 per cent concentration. In Group IV, Epidural anesthesia was induced by injecting 2 per cent Lignocaine hydrochloride solution at the rate of 1 ml per 3 kg body weight.

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Evaluation of anesthesia, In every anesthetic protocol used, the following parameters were recorded to compare the effects of the anesthetic protocol on the dam and the neonates. The duration of surgical anesthesia and complications of anesthetic procedure observed if any

- a. Time taken for induction of general anesthesia.
- b. The ease of induction of anaesthesia and the degree of skeletal muscle relaxation.
- c. Time taken by the dam to exhibit the first sign of recovery from anesthesia after the completion of surgical procedure and discontinuation of administration of anaesthetic agent.
- d. The degree of cardiovascular and respiratory depression of the neonate with various anesthetic protocols.
- e. Percentage of viable fetuses delivered with each anesthetic protocol.

RESULTS AND DISCUSSION

The study of four different protocols namely Propofol, combination of Propofol and ketamine, Isoflurane and Epidural analgesia were evaluated for the ease of induction of skeletal muscle relaxation, time taken for recovery from anesthesia after surgery and degree of cardio vascular and respiratory depression in the neonates (Table 1). It was observed that the ease of induction of chemical restraint was best with intravenous anesthetic solution namely Propofol and Propofol plus ketamine and was most difficult with epidural analgesia as most animals struggled during this technique. Further, the skeletal muscle relaxation achieved with an epidural analgesia was also very poor. In addition, maintaining the animal in dorsal recumbency for surgery presented some difficulties and the number of persons required to restrain the animal during the course of surgery was also more with the epidural analgesia as only the posterior part of the body was under analgesia. The animals also could not be carried easily as the animal remained conscious and alert. Animals subjected to epidural analgesia also had a prolonged recovery time. A positive aspect of epidural analgesia however, was that the neonates delivered had a

minimal cardiovascular and respiratory depression and majority of the neonate born were squealing almost immediately after they were removed from the uterus. 97 per cent of the puppies delivered were live and the puppy mortality rate was about three per cent which was highly encouraging (Table 2). In human, epidural techniques produced less neonatal depression compared to general anesthesia (Rolbin *et al.*, 1994; Ratcliffe and Evans, 1993). But, potential adverse effect such as severe hypotension observed with this technique indicated that it is not perfectly safe (Boyler, 1993).

Table 1. Evaluation of different anesthetic protocols, epidural analgesia for C- section in bitches

Evaluation Parameter	Anesthetic protocols			
	Propofol	Propofol-Ketamine	Isoflurane	Epid-ural
Ease of induction of anaesthesia	Easily induced	Easily induced	Slight struggling at the time of induction	Struggling
Skeletal muscle relaxation	Good	Good	Profound	Poor
Time taken for recovery from anesthesia after surgery (minutes)	2-7 (4.18± 0.6)	5-15 (10.14± 0.2)	Almost immediate	45-130 (62.17± 5.85)
Degree of cardio vascular and respiratory depression in the neonates	Some degree	Some degree	Some degree	Little effect

The puppy survival rate with the other three anesthetic protocols namely Propofol, Propofol plus ketamine and isoflurane ranged between 92.18 to 93.42 per cent. The skeletal muscle relaxation was best with isoflurane and these animals also had the shortest recovery time. However, the animals exhibited slight struggling at the time of induction of anesthesia. Kaneko *et al.*, (1993) have also reported that isoflurane anesthesia resulted in a better puppy survival rate when used as an inhalant anesthetic for C- section in bitches. Moon *et al.*, (1998) reported that isoflurane was associated with an improved neonatal survival rate.

Table 2. Puppy survival rate of bitches subjected to C-section under different anaesthetic protocols and epidural analgesia.

Anaesthetic protocol used	No of animals	No of fetuses delivered	No of Live fetuses (%)
Propofol	12	64	59 (92.18)
Propofol-Ketamine	12	71	66 (92.95)
Isoflurane	12	76	71 (93.42)
Epidural	12	68	66 (97.05)
Total	48	279	262 (93.90)

With Propofol and Propofol plus ketamine anesthesia, although the ease of induction and skeletal muscle relaxation were satisfactory, the neonates delivered had some degree of cardio vascular and respiratory depression and in some cases took more than one hour for the neonates to start squealing, although the puppy survival rate was comparable with the puppies delivered following isoflurane anesthesia (Table 2). Similar, observations have also been made by Seliskar *et al.*, (2007).

All the four anesthetic protocols used in the present study are equally satisfactory in terms of puppy survival rate and the choice of anesthetic protocol will therefore will be dictated by factors such as the familiarity with the technique (epidural), availability of equipment to administer anesthetics (Isoflurane) and ease of the availability of anesthetic drug itself. Combination of Ketamine and Propofol are presently extensively used in human obstetrics and are easily available for

veterinary use. It seems reasonable to recommend that most cesarean sections can be carried out with confidence with either Propofol or a combination of Propofol and ketamine anesthesia. A combination of Propofol and ketamine anesthesia is probably superior to Propofol alone as Propofol when used alone needs to be administered frequently because of its short acting nature which makes it expensive when used alone.

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Efficacy of Natural and a Synthetic PGF_{2α} Analogue by Intramuscular and Intravulvosubmucosal route for inducing estrus in crossbred dairy cows*

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ABSTRACT

The present study was carried out utilizing 100 Holstein Friesian and Jersey crossbred cows aged 3-8 years with first to fifth lactation. As per the history of previous estrus and findings of rectal palpation to confirm the presence of corpus luteum; the selected cows were between 8-16 days of the estrus cycle. The investigation was planned to assess the relative efficacy of natural (Dinoprost Tromethamine) and a synthetic (Cloprostenol Sodium) PGF_{2α} at different doses either by IM or IVSM route for estrus induction and time required for onset of estrus. The estrus induction response was 100 percent in cows which received 500µg Cloprostenol Sodium by IM route and 250 µg Cloprostenol Sodium by IM or IVSM route. The response for administration of 125µg Cloprostenol Sodium was optimum (80 %) by IVSM route whereas it was poor (50 %) by IM route. The percentage of cows in estrus (estrus induction response) was 90 percent following administration of 25 and 12.5mg of Dinoprost Tromethamine by IM route whereas, it was only 60 percent with 6.25mg by IM route. The response was optimum with 70 percent in both the groups of cows which received either 12.5 or 6.25 mg of Dinoprost Tromethamine by IVSM route. The mean duration taken for the onset of estrus in responded cows after administration different doses of Cloprostenol Sodium and Dinoprost Tromethamine by IM and IVSM route did not vary significantly and majority of cows which responded were in estrus on day 3 post injections.

Key word: Prostaglandin f2 alpha, Estrus, HF cross bred cows

Inefficient estrus detection and improper timing of artificial insemination are major causes for the failure of conception which results in prolonged service period, long calving intervals and reduced lifetime production. In dairy cows, upto 40 percent of estrus periods may pass undetected inspite of normal ovarian cyclicity (King *et al.*, 1976). Consequently, controlled breeding with induction of estrus and fixed time inseminations have gained importance. Among many functions of PGF_{2α}, their ability to cause luteolysis has been most exploited for induction of estrus, treatment of unobserved estrus and subestrus in dairy cattle (Pant *et al.*, 1992). Many commercial preparations of PGF_{2α} remain costly and unaffordable by dairy farmers in many of the developing countries and therefore efforts have been made to reduce the dose in order to cut down the cost of treatment (Chauhan *et al.*, 1986; Horta *et al.*, 1986).

MATERIAL AND METHODS

The study was carried out in Holstein Friesian and Jersey crossbred cows belonging to the farmers of

10 villages of Bangalore north taluk. The animals selected for the study were 3-8 years of age (first to fifth lactation), apparently normal with good body condition and regular cyclicity. The animals included in the study had normal calving and had no history of retention of fetal membranes and uterine diseases like endometritis. All the animals were subjected for gynecological examination and the presence of corpus luteum was confirmed on ovary. As per the history collected and the findings of rectal examination to confirm the presence of developed corpus luteum; the selected animals were between 8 and 16 days of the estrous cycle.

The PGF_{2α}, utilized in the study for the induction of estrus were Dinoprost Tromethamine (Lutalyse[®], 5mg/ml, Upjohn, USA) and Cloprostenol Sodium (Vetmate[®], 263 µg/ml, Vetcare, India) which are natural and synthetic PGF_{2α}, respectively. The study included one hundred crossbred dairy cows which were randomly assigned for ten treatment groups with ten animals in each group and received

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PGF_{2α}, during their luteal phase of the cycle as follows. The IVSM injection of PGF_{2α}, was ipsilateral to the ovary bearing corpus luteum.

Cloprostenol Sodium was used by IM route with 500, 250 and 125 µg doses in group I to III and by IVSM route with 250 and 125 µg doses in group IV and V respectively. Whereas, Dinoprost Tromethamine was used by IM route with 25, 12.5 and 6.25mg doses in group VI to VIII and by IVSM route with 12.5 and 6.25mg doses in group IX and X respectively.

All the experimental animals of different groups were observed for the onset of estrus and estrus signs after the administration of PGF_{2α}. The animals reported in estrus were subjected for rectal palpation to confirm estrus.

RESULTS AND DISCUSSION

The estrus induction response following administration of Cloprostenol Sodium at normally recommended dose of 500 µg was 100 percent (Table 1). This is in agreement with the findings of Godfrey *et al.*, (1989), where as it was higher than that reported by and Horta *et al.*, (1986). The percentage of cows in estrus after administration of 250µg of Cloprostenol Sodium either by IM or IVSM route was similar (100 %) to that obtained with 500µg of Cloprostenol Sodium by IM route. The response recorded after administration of 125µg of Cloprostenol Sodium by IVSM route was 80 percent but the response obtained with the same dose by IM route was only 50 percent (Table 1).

and 125µg IVSM did not differ significantly ($P \leq 0.05$; Table 1). Similarly, Horta *et al.*, (1986) recorded no difference in percentage of cows in estrus after administration of 500µg of Cloprostenol Sodium by IM route and 250 and 125µg of Cloprostenol Sodium by IVSM route. Chauhan *et al.*, (1986) reported higher percentage of cows in estrus after administration of 250 and 125µg of Cloprostenol Sodium by IVSM route than of 500µg by IM route. Holy (1984) recorded estrus induction in 85.5 percent of cows with in 72 hr of administration of 250µg Cloprostenol Sodium by IVSM route.

Horta *et al.*, (1986) and Chauhan *et al.*, (1986) suggested administration of 125µg of Cloprostenol Sodium by IVSM route which is 1/4th of the normal dose (500µg) recommended by IM route to cause luteolysis, estrus induction and fertility in heifers and adult cows. The findings of the present study indicated that optimum estrus induction can be achieved by administration of Cloprostenol Sodium at the dose of 250 µg either by IM or IVSM route and 125µg by IVSM route in crossbred dairy cows during 8-16 days of estrus cycle.

The estrus induction response recorded was 90 percent with 25mg of Dinoprost Tromethamine by IM injection (Table 1) and it was similar to that reported by Gacche *et al.*, (2002). But it is higher than those reported by Whittier *et al.*, (1989) by using same dose and route of injection. However, the response obtained was

Table 1. Estrus induction response following administration of PGF_{2α} in cross bred dairy cows

PGF _{2α}	Cloprostenol sodium					Dinoprost tromethamine				
	Im			IVSM		IM			IVSM	
Dose	500µg	250µg	125µg	250µg	125µg	25mg	12.5mg	6.25mg	12.5mg	6.25mg
No. responded	10	10	5	10	8	9	9	6	7	7
No. not responded	0	0	5	0	2	1	1	4	3	3
% responded	100 ^a	100 ^a	50 ^b	100 ^a	80 ^a	90 ^a	90 ^b	60 ^b	70 ^{ab}	70 ^{ab}

Note: comparison was made with in drug between routes and doses

Percentage values bearing different superscripts within drug vary with each other ($P \leq 0.5$) as compared by proportion test

The percentage of estrus induction obtained following administration of Cloprostenol Sodium at 500 µg IM, 250µg either IM or IVSM

slightly less than that (100 %) recorded in treatment of subestrus cows with similar route and dose of Dinoprost Tromethamine by Tandle *et al.*, (1997).

The percentage of animals in estrus following IM injection of 12.5mg of Dinoprost Tromethamine was similar to that obtained with 25mg. Further, IVSM administration of Dinoprost Tromethamine ipsilateral to the ovary bearing corpus luteum at the rate of 12.5 and 6.25mg resulted in acceptable (70 %) estrus response. As per the proportion test applied, the percentage response obtained between these groups was not significant ($P \leq 0.05$; Table 1).

Rao and Venkataramiah (1990) reported 21 percent overt estrus signs but 71 percent luteolysis and ovulation in cows which received 5mg of Tham salt by IVSM route. Ono *et al.*, (1982) and Pawshe *et al.*, (1991) have reported estrus induction of 79, 81.75 and 80 percent with 6, 10 and 5 mg of Dinoprost Tromethamine by IVSM route. However, Krishnakumar and Subramanian (1999) have reported estrus induction in 80 and 100 percent cows which received 5 and 10mg of Dinoprost Tromethamine respectively by IVSM route.

Table 2: Onset of estrus in cows responded for PGF₂α administration (Mean±SE)

Cloprostenol sodium	IM	IVSM	Dinoprost tromethamine	IM	IVSM
500µg	60.60 ± 1.31		25mg	66.67 ± 3.35	
250µg	59.90 ± 4.17	59.90 ± 2.51	12.5mg	58.78 ± 3.35	63.28 ± 3.37
125µg	73.80 ± 1.56	62.38 ± 2.63	6.25mg	67.00 ± 4.32	65.53 ± 3.06

Note: The proportion test applied within drug between routes and doses indicated no significant difference ($P \leq 0.5$).

The results of the present investigation have revealed that IM administration of half the dose (12.5mg) of Dinoprost Tromethamine induces similar estrus response obtainable with normally recommended dose (25mg). Further, IVSM administration 12.5 or 6.25mg of Dinoprost Tromethamine ipsilateral to the ovary bearing corpus luteum resulted in acceptable estrus induction in cows during day 8-16 of the estrus cycle.

The mean duration required by the cows which responded for different doses of Cloprostenol Sodium by IM and IVSM routes did not differ significantly and it ranged from 59.90±2.51 to 73.80±1.56 hr (Table 2). Out of the animals responded, the distribution of onset of estrus (in days) differed significantly ($P \leq 0.05$) with majority of the cows showing estrus symptoms on day three post injection (Table 3).

The mean interval from IM administration of normal dose (500µg) of Cloprostenol Sodium to onset of estrus was 60.60±1.31 hr and all the animals were in estrus on day 3 post treatment. The response was quicker than 130 hr reported by Godfrey *et al* (1989) with 500 µg of Cloprostenol Sodium by IM route and is very close to 58.5hr with same dose and route reported by Colazo *et al.*, (2002).

The mean estrus response interval after IM or IVSM administration of 250µg and IVSM administration of 125µg of Cloprostenol Sodium did not differ significantly from that recorded for IM administration of 500µg of Cloprostenol Sodium. Further, almost all cows responding for these reduced doses were also in estrus on day 3

Table 3: Estrus distribution (days) in responded cows following PGF₂α administration.

Route	Cloprostenol sodium					Dinoprost tromethamine				
	Im	IVSM	Im	IVSM	Im	IVSM	Im	IVSM	Im	IVSM
Dose	500µg	250µg	500µg	250µg	500µg	25mg	12.5mg	6.25mg	12.5mg	6.25mg
< 2 days				1 (10)			1 (11.11)			
2-3 days	10 (100) ^a	9 (90) ^b	2 (40) ^c	9 (90) ^b	8 (100) ^b	8 (80)	8 (88.89) ^{ad}	4 (66.67) ^b	7 (100) ^c	6 (85.71) ^{cd}
> 3 days		1(10)	3(60)			1(10)		2(33.00)		1(14.29)
Total	10	10	5	10	8	9	9	6	7	7

Figures in parenthesis indicate percentage of the total animals responded

Note: Comparison was made with in drug between routes and doses

Percentage values bearing different superscripts within drug vary with each other ($P \leq 0.5$) as compared by proportion test

post treatment similar to that observed with 500 μ g by IM route. This is in agreement with Horta *et al.*, (1986) who also did not find significant difference in the mean duration from treatment to estrus with different doses and routes of administration of Cloprostenol Sodium. In contrast Colazo *et al.*, (2002) recorded significantly shorter interval from treatment to estrus in cows which received 500 μ g of Cloprostenol Sodium by IM route compared to that of 250 and 125 μ g by the same route.

The cows which responded for 125 μ g by IM route required 73.80 \pm 1.56 hr for onset of estrus and only 40 percent of the responded animals were in estrus on day three and the remaining 60 percent showed estrus symptoms after day 3 post treatment. This is in close agreement with Colazo *et al.*, (2002) who have reported 78.00hr mean interval from injection of 125 μ g of Cloprostenol Sodium by IM route to onset of estrus.

As indicated by the results of the present investigation, the interval from administration of lower dose of Cloprostenol Sodium i.e 250 and 125 μ g either by IM or ivsm route was similar to that obtained with standard dose (500 μ g) by IM route. Further, majority of the animals (except 125 μ g IM route) which received reduced dose of Cloprostenol Sodium were in estrus on day 3 after treatment. This will help to follow fixed AI protocol similar to that which is in practice with standard dose (500 μ g) by IM route, after further detail studies.

The mean interval for the onset of estrus in cows which received 25, 12.5 and 6.25mg of Dinoprost Tromethamine by IM route and 12.5 and 6.25mg by IVSM route did not differ significantly and ranged from 58.78 \pm 3.24 and 67.00 \pm 4.32 hr (Table 2). Further, out of the animals responded for different routes, majority of them were in estrus on day 3 post treatment (Table 3).

The average interval from IM administration of normally recommended dose (25mg) of Dinoprost Tromethamine to onset of estrus was 66.67 \pm 3.35 hr and 80 percent of the responded animals were in estrus on day 3 after treatment. The interval for estrus response recorded, closely agrees with that of Tandle *et al.*,

(1997) who used same dose and route. But, it was more than that reported by Ansari and Kumaresan (2001) who also used same dose and route. However, the interval to estrus was less than that reported by Lauderdale *et al.*, (1972) with 30mg of Dinoprost Tromethamine by IM route and whittier *et al.*, (1989) with 25mg of Dinoprost Tromethamine by IM route.

The interval from administration to estrus with 12.5 and 6.25mg Dinoprost Tromethamine by IM and IVSM route did not differ from that of 25mg by IM route. Further, majority of the animals were in estrus on day 3 after treatment which was similar to that recorded in group receiving normally recommended dose (25mg) by IM route. Krishnakumar and Subramanian (1999) have reported no difference in estrus induction interval in cows receiving either 5 mg or 10 mg of Dinoprost Tromethamine by IVSM route.

The findings of the present investigation relating to interval from treatment to occurrence of estrus and distribution of estrus in responded animals with reduced dose namely, 12.5 and 6.25mg of Dinoprost Tromethamine by IM or IVSM route indicated no difference from that obtained with standard dose of Dinoprost Tromethamine (25mg) by IM route.

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Evaluation of Salpingectomy as Technique for Birth Control in Dogs*

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ABSTRACT

Six adult female Mongrel dogs presented to Teaching Veterinary Clinical Complex, Veterinary College, Bidar, for the purpose of birth control formed the material for this study. Salpingectomy after tubal ligation was performed through right flank approach. No complications were observed during exteriorization, identification, ligation or cutting of the oviduct. The Mean \pm SE duration required to perform salpingectomy after tubal ligation was 25.5 ± 1.23 minutes. All animals recovered uneventfully after surgery. The animals were kept under observation for a period up to one and a half years post salpingectomy. All animals showed behavioural signs of heat and mating. None of the animals became pregnant or showed signs of pseudocyesis, during the period of observation. The present study showed that salpingectomy with tubal ligation can be carried out routinely in female dogs without any complications.

Key words: - salpingectomy, female dogs, birth control.

Pet overpopulation is a problem of enormous proportion in the world. Globally millions of dogs are rendered shelter less, killed in accidents, die of diseases which could be prevented by vaccination or treated by prompt medical attention. Unwanted stray dogs and uncontrolled pets are primary ecological and social menace. Dog bites are a serious public health concern. Besides injuries and adverse psychological impacts, dog bites can be complicated by infection including rabies, which has the highest case fatality rate of all infectious diseases. The female dogs are still widely perceived as the primary source of reproductive control. As a result, the development of contraceptive for control of birth in dogs has been primarily directed towards the female of the species. The most common reason for neutering dogs is to prevent unwanted pregnancies. Though spaying as a method of rendering bitches sterile has been recorded by several practitioners, the reasons cited against neutering bitches include; obesity (Joshua, 1965), perivulvar dermatitis (Jackson, 1984), vaginitis (Olson *et al.*, 1986), behavioural changes (Joshua, 1965), urinary incontinence (Joshua, 1965, BSAVA congress, 1975 and Jackson, 1984) and increased risk of complication during surgery and anaesthesia (Chalifoux *et al.*, 1981). Hence, other techniques which can prevent or decrease the problems of ovariohysterectomy are needed to be explored.

Not much literature is available on the method of sterilization in bitch sterile by means of

ligating and bisecting the fallopian tubes (Salpingectomy) which is a proven method of sterilization in women. It has become imperative to resort to this technique of birth control, without affecting the confirmation and at the same time preserving all the feminine characters (Saxena, 1966). Saxena (1966) had just ligated the junction between the ovary and uterine horn and had not cut it. Hence, the present study was undertaken with the objective to identify the fallopian tube and evaluate the feasibility of salpingectomy after tubal ligation, for birth control in female dogs.

MATERIALS AND METHODS

Six adult mongrel female dogs presented to Teaching Veterinary Clinical Complex, Veterinary College, Bidar, for the purpose of birth control formed the material for this study. All the animals were prepared for right flank surgery in standard protocol. Preoperatively a single dose of Benzathine penicillin G @ 20,000 IU per kg intramuscularly was administered. The animals were maintained on a single bolus of midazolam – ketamine anaesthesia (midazolam @ 0.4 mg/kg i/v and ketamine @ 10 mg/kg i/v). The female reproductive tract was approached through the right flank, after incising the skin (average length 1.5 to 2 cm) and bluntly dissecting the abdominal muscles and peritoneum. The ovary was identified visually and exteriorized to the site of incision. The oviduct / fallopian tube which is narrow, tortuous, tubular structure running along the ovarian surface within

the mesosalpinx was identified, separated and ligated with silk (Plate 1) at two places at about two centimetre apart. The portion in between the two ligations was cut (Plate 2) with scissor. The ovary was placed back in the abdomen. Similar procedure was repeated on the contralateral ovary. The abdominal muscles and skin were closed according to the standard procedure (Slatter, 1985). Analgin was administered @ 40 mg/kg intramuscular one dose only. No antibiotic was administered postoperatively. Post operatively the wound was dressed surgically until healing. Skin sutures were removed on 8th post operative day.

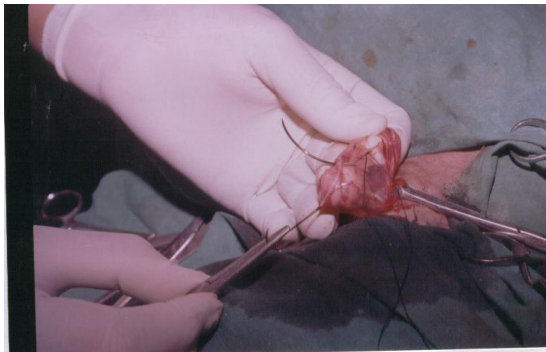


Plate 1: Identification, separation and ligation of the oviduct

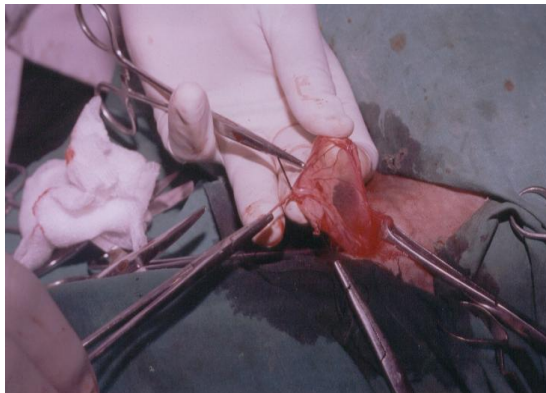


Plate 2: Salpingectomy in between the two ligatures

The animals were kept under close observation post operatively for any complications up to a period of one and a half years during this study. The animals were evaluated for infection, auto mutilation, wound dehiscence and wound healing, and were observed for behavioural signs of heat, mating, pregnancy, and further any related developments.

RESULTS AND DISCUSSIONS

The objective of the present study was to evaluate the feasibility of salpingectomy after tubal ligation for birth control in bitches. The right flank prepared for surgery was easier to exteriorize the ovaries and resulted in negligible complications postoperatively. An average length of incision ranging between 1.5 to 2 cm was sufficient to exteriorize both the ovaries through the right flank approach for conduct of salpingectomy procedure in all the animals. However, the earlier workers (Shuttleworth and Smith, 2000) had performed ovariohysterectomy with a larger incision of 3 to 4 cm. No complications were seen during the surgery, either with regard to the exteriorizing of ovaries or identification and ligation of fallopian tube or its ligation. No bleeding was noticed during or after ligating the fallopian tube. The skin sutures were removed by 8th post operative day. All the animals recovered uneventfully.

All animals were observed for behavioural signs of heat, mating, pregnancy and any other developments. Post operatively the animals came into heat only once or twice during the study period of one and a half year. During proestral period, which varied from seven to nine days, bleeding, swelling of vulval lips, aggression and non acceptance of male was observed in all female dogs. During the estral period, which lasted from six to ten days, acceptance of male, courtship behaviour and the “tie” (Plate 3) was also seen in all bitches. Upon mating by intact dogs no pregnancy was observed in any of the bitches during the study period. The signs of pregnancy, pseudopregnancy or ectopic pregnancy were not noticed.



Plate 3: Copulatory Locking / tie in salpingectomized bitch during mating

Primiparous bitches, in which the mammary gland was not developed before estrus, remained without development even after a period of two months after mating (Plate 4). Pluriparous bitches, in which the mammary gland was already developed even before estrus, remained flaccid even after two months of mating (Plate 5). No signs or symptoms of pyometra were noticed after the gestation period in any of the operated bitches. Signs of urinary incontinence were not observed either in any of the operated bitches. The results of salpingectomy after tubal ligation confirmed the findings of Saxena (1966) and Greenwood (1992) who reported that ligation of fallopian tube maintains the hormonal balance and helps in maintaining the normal characters in bitch.



Plate 4 : Primiparous salpingectomized bitch after two months of mating – No development of mammary gland



Plate 5 : Pluriparous salpingectomized bitch after two months of mating – flaccid Mammary gland.

The above results indicate that salpingectomy is an effective means of birth control in bitches and can be routinely performed without complications within a short duration. There were no references on the present approach of ligating and cutting of the fallopian tube in dogs. The study was the first of its kind.

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Microbial Quality of Pasteurised Milk Samples Collected from Hyderabad Karnataka Region

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ABSTRACT

In the present research work, 150 pasteurised milk samples collected from the retail shops and milk parlours from Hyderabad Karnataka region of Karnataka state, India, were analyzed for the microbial quality; total viable count and isolation and confirmation of *Staphylococcus*, *E. coli*, *Bacillus*, *Salmonella*, *Listeria* and *Clostridium* by selective plating, microscopic examination and biochemical characterization. As per Food Safety and Standards (FSS) regulations 2011, of the samples analyzed, 29 (19.33%) samples exceeded the limit of 30,000 CFU/g of total viable count. Thirteen (8.66%), 16 (10.66%), 11 (7.33%) and only 2 (1.33%) samples were positive for *Staphylococcus*, *E. coli*, *Bacillus* and *Salmonella* respectively. None of the samples were positive for *Listeria* and *Clostridium* spp. The number of samples that exceeded the limit set by Food Safety and Standards (FSS) regulations 2011 for *Staphylococcus*, *E. coli* and *Salmonella* respectively were 8 (5.33%), 9 (6%) and 2 (1.33%). The findings in this study specifies that significant percentage of pasteurized milk samples were exceeding standards set by FSSA with respect to total viable count and significant number of samples were positive for *Staphylococcus*, *E. coli*, and *Bacillus* which could be because of poor quality of milk procured or improper pasteurisation and post pasteurisation contamination during packaging and transportation.

Key words: Milk; Microbial Quality; Total Viable Count; Selective Plating; FSS Regulations.

Milk is a highly nutritious food and is being consumed by human beings since time immemorial. At the same time it serves as a good medium for the growth of many microorganisms such as *Salmonella*, *Bacillus*, *Staphylococcus*, *E. coli* and other Coliforms (Torkar and Teger, 2008) which can cause food-borne illness and are a threat to consumer's health. Milk from healthy cows/buffaloes contains relatively few bacteria (10^2 – 10^3 cfu/ml); however, the bacterial load may increase up to 100 fold or more once it is stored for some time at room temperature. Hence, milk is pasteurized with high temperature short time system (HTST) which extends the shelf life of the milk up to seven days at room temperature (Meunier and Sandra, 2002).

There are country-specific legal standards for the upper limit of microflora in pasteurized milk. In India, the Food Safety and Standard Act (FSSA) has been enacted w.e.f. 5th August, 2011 (FSS regulations, 2011) which specifies the standards for all foods including the pasteurized milk.

The microbial quality of milk and milk products is very important with regards to public health significance. As the consumer awareness is increasing the demand for pasteurized milk is also

getting increased. However, worldwide there are many reports of food poisoning cases, caused by different food borne pathogens, because of consumption of pasteurized milk (Bryan 1983, Ryan *et al.*, 1987 and Silva *et al.*, 2010). Post-pasteurization contamination has been found to contribute most of the bacteria in milk that are capable of growth and subsequent food poisoning (Maxcy, 1967 and Muir, 1996).

Hence the present research work was undertaken to check the microbial quality of the pasteurized milk collected from milk parlours and retail shops from Hyderabad Karnataka region with the following objectives;

1. To collect the pasteurized milk samples from milk parlours and retail outlets in Hyderabad-Karnataka region of the Karnataka state, India.
2. To check the microbial quality of these samples by estimation of total viable count and isolation and confirmation of the common food borne pathogens - *Staphylococcus*, *E. coli*, *Bacillus*, *Salmonella*, *Listeria* and *Clostridium*
3. To check whether the estimated microbiological criteria are within the limits set by FSS regulations, India.

MATERIALS AND METHODS

Sample collection: One hundred and fifty (150) pasteurised milk samples were collected from different retail outlets and milk parlours of various towns and districts of Hyderabad Karnataka region, Karnataka state, India. Approximately 200 ml of milk sample was collected, packed in box embedded with ice packs and transported to the lab. The samples were processed within 24 hr. after bringing to the laboratory.

Sample Preparations: The milk samples were mixed gently and thoroughly in the sachet and 10 ml of each sample was taken and mixed with 90 ml normal saline under sterile conditions. Ten fold dilutions of the mixed volume up to 10^{-5} in normal saline were made using sterile pipettes (Fawole and Oso, 2001).

Microbial analysis:

Media: All the selective media used were purchased from Himedia®

Total viable count: The total viable count (TVC) was determined by standard pour plate method (Scott Sutton, 2011). Dilutions of 10^{-5} were prepared. Dilutions of each sample were inoculated in duplicate in to the total viable count agar medium just before solidification of the agar. On solidification of agar, the plates were incubated at 37°C for 24 hr. After 24 hours of incubation the colonies were counted using colony counter. The result was calculated using following formula;

$$N = \sum c / (n1 + 0.1 \times n2) \times d$$

Where

$\sum c$: Sum of colonies counted on all the dishes retained.

$n1$: Number of dishes retained in the first dilution.

$n2$: Number of dishes retained in the second dilution.

d : Dilution factor corresponding to the first dilution.

The total viable counts were expressed as CFU/g.

Isolation and enumeration of specific bacteria:

The specific bacteria; *Staphylococcus*, *E. coli*, *Bacillus*, *Salmonella*, *Listeria* and *Clostridium*, which cause food poisoning were isolated by using

selective media. The specific bacteria were identified and confirmed by the colony characters on the selective media, microscopic examination (Beveridge, 2001) and biochemical characterization. The samples which were positive for the aforesaid bacteria were diluted up to 10^{-3} and inoculated on to the selective media and the specific counts were enumerated. The spread or pour plate method was employed for the inoculation with 0.1 ml inoculum.

***Staphylococcus spp.*:** Baird Parker agar was used for isolation and enumeration of *Staphylococcus* spp. The plates were incubated aerobically at 37°C for 24-48 hr.

***Salmonella spp.*:** Each of the samples (25 g) were homogenised and pre-enriched in 225 ml buffered peptone water at 37°C for 24-48 hr. One ml of culture was transferred to 10 mL of selenite cystine broth (selective enrichment medium) and incubated at 44°C for 18 hr. Selective plating was done on Brilliant Green Agar incubated aerobically at 43°C for 24 hr.

***E. coli*:** The samples were enriched in MacConkey broth and incubated at 44°C for 24 hr. Then selective plating was done on MacConkey agar and incubated at 44°C for 24 hours. The *E. Coli* was further confirmed by inoculating the colonies from MacConkey agar on to Eosin Methylene Blue (EMB) agar.

***Bacillus spp.*:** The samples were inoculated into trypticase-soy-polymixin enrichment broth and incubated at 37°C for 18 hours. An inoculum from this broth was inoculated on selective agar-polymixin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA) and incubated at 37°C for 24 hours.

***Listeria spp.* :** The samples were enriched in two steps using Fraser Broths I and II and incubated at 30°C for 24 hours. The selective plating was done on Polymixin B Acriflavin Lithium Chloride Ceftazidime Ascolin and Mannitol (PALCAM) agar and incubated at 30°C for 48 hours.

***Clostridium spp.*:** The samples were enriched in Robertson Cooked Meat Medium incubated at 44°C for 24 hours. The selective plating was done on Sulphate Polymixin Sulphadiazine Agar and

incubated at 37°C for 24 hours in anaerobic conditions using anaerobic jar.

RESULTS AND DISCUSSION

Total viable count: Of the 150 pasteurized milk samples analyzed, 29 (19.33%) samples exceeded the limit of 30,000 CFU/g total viable count, the limit set by Food Safety and Standards (FSS) Regulations, India, 2011 (**Table**).

Table: Microbiological quality of the milk samples

Sample/ Parameter		Pasteurized milk
No. of Samples analyzed		150
<i>Staphylococcus</i>	*	13(8.66%)
	**	8(5.33%)
<i>E. coli</i>	*	16(10.66%)
	**	09 (6%)
<i>Bacillus</i>	*	11 (7.33%)
	**	Standard not available
<i>Salmonella</i>	*	2 (1.33%)
	**	2 (1.33%)
<i>Listeria</i>	*	--
	**	--
<i>Clostridium</i>	*	--
	**	--
No. of samples that exceeded TVC limit		29 (19.33%)
Average TVC in the samples exceeding the limit		$5.9 \times 10^4 \pm 0.0024$

* no. Of samples positive

** no. of samples exceeding limit

TVC = Total viable count

Isolation and enumeration of specific bacteria:

The bacteria responsible for food poisoning; *Staphylococcus*, *E. coli*, *Bacillus*, *Salmonella*, *Listeria* and *Clostridium*, were isolated and enumerated by using selective media. The bacteria were identified and confirmed by the colony characters on the selective media, microscopic examination (Beveridge 2001) and biochemical characterization.

The result is presented in the **Table**. Of the 150 pasteurized milk samples analysed only two (1.33%) samples were positive for *Salmonella*. However, 16 (10.66%) samples were positive for *E. Coli*, 11 (7.33%) samples were positive for *Bacillus* and 13 (8.66%) samples were for *Staphylococcus*. None of the samples were positive for *Listeria* and *Clostridium* spp.

The Prevention of Food Adulteration (PFA) act merged under FSSA specifies the maximum upper limit of microflora in pasteurized milk as 30,000 cfu/g. Further this act specifies the limits for Coliform as absent/0.1 g, For *E. coli*, *Staphylococcus aureus*, anaerobic count (*Clostridium*), *Listeria monocytogenes* as absent per gram for each and for *Salmonella* as absent per 25 g of pasteurized milk. There is no specification for *Bacillus* spp (FSS regulations 2011 and PFA act 1954).

The findings of this study indicate that significant number (29) of pasteurized milk samples were exceeding the limit of 30,000 cfu/g total viable count. The Average TVC in the samples exceeding the limit was found to be $5.9 \times 10^4 \pm 0.0024$. Significant number of samples were positive and exceeded the limit for the food poisoning causing bacteria - *Staphylococcus*, *Salmonella*, *E. Coli*. Though there are no set standards for *Bacillus* spp in pasteurized milk, 11 number of samples were positive for *Bacillus* spp is again a significant number. This study shows that the microbial quality of pasteurized milk marketed in Hyderabad Karnataka region is of poor quality.

The spoilage of processed milk is primarily due to bacterial activity, presence and activity of post-pasteurization contaminations (Eneroth *et al.*, 2000; Silva *et al.*, 2010) and types and activity of pasteurization resistant micro-organisms which are the main limiting factors in extending the shelf life of high temperature short time (HTST) pasteurized milk (Fromm and Boor, 2004). In addition other factors which limit the shelf life of refrigerated pasteurized milk include the time and temperature of pasteurization (Monika and Poonam, 2013), temperature maintenance during the transportation of milk, storage temperature of milk after pasteurization (Rankin, 2002) and faulty packaging practices. Other factors which will affect rate of deterioration of pasteurized milk include temperature, agitation and dissolved oxygen (Allen and Joseph 1985). Anderson and Stone (1955) also remarked that pasteurized milk might be contaminated due to poor bacteriological quality of milk and inadequate plant cleanliness.

Many literature are available from various countries and different states of India which report the microbial quality of pasteurized milk. Outbreaks of salmonellosis and listeriosis in pasteurized milk linked to post-pasteurization contamination have been reported in Brazil Bryan (1983) and Jervis (1988). Ubeyratne *et al.* (2014) reported that the mean total viable count and coliform count at various stages after pasteurization as 2.75×10^5 and 9.8×10^1 cfu/ml, respectively, in milk just after pasteurisation, 4.1×10^5 and 2.69×10^5 cfu/ml, respectively, in milk pasteurised and stored in vats and 4.81×10^5 and 1.007×10^3 cfu/ml, respectively, in milk pasteurised and commercially available in Srilanka. Saha and Ara (2012) reported that the total viable count of pasteurized milk samples in Bangladesh ranged from 54200 to 68400 cfu/ml.

Srujana *et al.* (2011) reported that around 18.1% pasteurized milk samples collected from Warangal district (Andra Pradesh) were of poor microbial quality. The average TVC of the pasteurized milk in India ranged from $3.43 + 0.17$ to $4.82 + 0.05$ log cfu/ml total viable count (Monika and Poonam, 2013).

Favourable finding of our study was that no sample was positive for *Clostridium* and *Listeria monocytogenes*. Among various microorganisms, psychrotrophic (*Listeria*) bacteria are by far the most important factor influencing pasteurized milk quality (Sorhaug and Stepaniak, 1997). These organisms are remarkable for their rapid growth and short generation multiplication under low temperatures; in raw milk or in pasteurized milk under refrigeration.

CONCLUSION

The result obtained in this study signifies the requirement of evaluation of various critical control points at different stages of processing of milk till it reaches the consumers so that the stage of contamination can be found out and control measures could be employed at respective stage to improve the microbial quality of the pasteurized milk and hence the consumers be protected from milk poisoning.

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Dietary Supplementation of Monensin Sodium Enhances Milk Yield in Early Lactating Buffaloes

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ABSTRACT

An experiment was conducted to assess the dietary supplementation of monensin sodium on dry matter intake, milk yield and milk composition in early lactating buffalo. Twelve apparently healthy buffaloes in their 2nd week of lactation were selected and divided into two equal groups as control and treatment. Control group received the standard ration comprising concentrate, green fodder and dry fodder whereas the treatment group was supplemented with monensin sodium @ 200mg/head/day in the standard ration up to 12th week of lactation. The dry matter intake, milk yield and milk compositions were recorded at weekly interval during the experimental period. The average dry matter intakes (kg/d) was 10.31±0.01 and 10.02±0.05 in control and treatment groups respectively indicating a significant (p<0.05) decrease in DMI of about 2.89 per cent in monensin supplemented group. The average milk yield (kg/d) in control and monensin supplemented buffaloes was 7.17±0.10 and 7.76±0.24. Monensin sodium supplemented buffaloes yielded about 8.22 per cent more milk than the control. Monensin supplemented buffaloes showed significantly lower fat % than the control group (4.89±0.13 v/s 5.41±0.13). There was no significant difference in milk lactose, SNF, protein, total solids and total ash percentage between the two groups.

Key words: Buffalo, Monensin, DMI, Milk yield. Milk composition

Buffaloes contributed 47.8 million metric tons of milk amounting to 55 per cent of total milk produced in India. Though India is the largest milk producer of milk in the world, the national average milk yield of buffalo is 4.6 kg/day which is just half of the global average per buffalo. The average low milk yield per buffalo in India is mainly due to the poor nutritional status of animals. One of the strategies to improve the production potential of buffalo is mainly by improving the feeding regimes, digestibility and feed utilization. Monensin is the carboxylic polyether ionophore produced by a naturally occurring strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1967) which is commonly used to enhance feed utilization and improve the milk production of dairy animals in western countries. It has several modes of action i.e. modifying the proportion of rumen volatile fatty acid production, altering the feed intake, rumen gas production, rumen fill and passage rate and modifying the digestibility of feed. Monensin changes the ratio of volatile fatty

acids production in the rumen towards increased molar percentage of propionic acid and decreased molar percentages of butyric and acetic acid production (Richardson *et al.*, 1976). This increased rumen propionic acid leads to improved gluconeogenesis resulting in increased availability of glucose to the animals for maintenance and milk production (Schelling, 1984). However, there are only few scientific reports on the effects of monensin sodium supplementation in lactating buffaloes and hence this experiment was planned to assess the influence of dietary supplementation of monensin sodium on DMI, milk yield and milk composition in early lactating buffaloes.

MATERIALS AND METHOD

Twelve apparently healthy early lactating cross breed buffaloes in their 2nd week of lactation, having average body weight of 350-400kg were selected from organized private dairy farm in Benchincholli village of Bidar District and were divided randomly in to two equal groups. The

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buffaloes of group-I were fed with standard ration consisting of concentrate feed, Napier and Jowar stovers as per Paul *et al.*, (2002), which served as control. The buffaloes of group-II were supplemented with monensin sodium @200mg/head/day in addition to the standard ration as that of group-I, which served as treatment group. Before the start of the experiment, the group-II buffaloes were kept under adaptation period for monensin one week i.e 3rd week of lactation. During this period monensin was supplemented at 50mg per day for first two days, 100 mg per day on 3rd and 4th day of adaptation and 150 mg per day on 5th, 6th and 7th day of adaptation period. Finally, the buffaloes of treatment group were supplemented with 200 milligram per head per day throughout the experimental period i.e up to 12th week of lactation. Dry matter intake, milk yield and milk composition of both the control and treatment groups were recorded at weekly interval

Dry matter intake of buffaloes was estimated by taking known quantity of feed sample in pre-weighed moisture cups and these cups were placed in hot air oven at 100±2°C for 8-12 h and the dry matter content of feed was estimated by using formula.

$$DM = \frac{\text{Weight of the sample after drying}}{\text{Weight of sample taken}} \times 100$$

Then the dry matter intake was calculated by deducting the residue dry matter from offered feed dry matter

Milk yield of both morning and evening milking of individual buffaloes was recorded in kilograms using a standard weighing balance and the values were pooled to express the daily milk yield. A 50ml of representative pooled morning and evening milk sample was collected and stored by adding formalin @ 1drop/100ml milk and kept at +4 °C until analyzed for the milk fat, total solids, solid not fat (SNF), total protein, lactose content using ultrasonic milk analyzer (**Lactoscan**[®], Milkotronic Company). Total ash content of pooled samples was also analysed (AOAC, 1995). The means of DMI, milk yield and milk composition of control and treatment groups were

statistically compared using student 't'-test as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The average DMI and milk yield of control and treatment groups on 2nd week of lactation i.e before the adaptation period is given in Table 1 and there was no significant difference in these parameters between the control and treatment groups.

Table 1: DMI, milk yield and milk composition of control and treatment group before adaptation period (Mean±SE)

Parameter	Control group	Treatment group
Dry Matter Intake (Kg/d)	2.95±0.01	2.94±0.02
Milk Yield (Kg/d)	6.69±0.37	6.60±0.40
Milk Fat (%)	5.68±0.20	5.67±0.21
Solid Not Fat (%)	9.48±0.28	9.44±0.25
Milk Protein (%)	3.83±0.10	3.80±0.08
Milk Lactose (%)	5.47±0.09	5.43±0.15
Total Solids (%)	15.17±0.39	15.11±0.20
Total Ash (%)	0.83±0.02	0.81±0.02

The week wise mean DMI of control and treatment groups from 4th week to 12th week of lactation indicated that the DMI of treatment group was significantly lower ($P \leq 0.05$) compared to that of control group throughout the experimental period. The mean DMI of entire experimental period (10.31±0.01 vs 10.02±0.05 Kg/d) was significantly ($P \leq 0.05$) lower by 2.89 per cent (0.29kg) in monensin supplemented group as compared to control group. These findings are in agreement with the results of Singh and Mohoni (1999) and Duffield *et al.* (2008), who have reported the decreased DMI in monensin supplemented cows. The mechanism of action of monensin in reducing the DMI might be mediated through increased propionate supply to meet the increased glucose demand by the mammary gland during lactation (Gandra *et al.*, 2010). Oba and Allen (2003) have proposed that the greater demand for glucose increases gluconeogenesis and reduces propionate oxidation in the liver, improving the blood glucose level that could have an effect on satiety centre in hypothalamus causing reduced

feed intake. However, Khodamoradi *et al.* (2013) in cows and Helal and Lasheen (2008) in Egyptian buffaloes have observed no significant difference in the dry matter intake in response to dietary supplementation with monensin. These discrepancies might be related to difference in the productive status of animals, stage of lactation and also the composition of the diet.

Table 2: Average DMI, milk yield and milk composition of control and monensin supplemented early lactating buffaloes (Mean±SE)

Parameters	Control	Treatment
Dry matter intake (kg/d)	10.31±0.01 ^a	10.02±0.05 ^b
Milk yield (kg/d)	7.17±0.10 ^a	7.76±0.24 ^b
Milk Fat (%)	5.41±0.13 ^a	4.89±0.13 ^b
Solid Not Fat (%)	9.77±0.08	9.67±0.13
Milk Protein (%)	3.46±0.03	3.39±0.04
Milk Lactose (%)	4.66±0.05	4.69±0.04
Total Solids (%)	14.84±0.13	14.68±0.18
Total Ash (%)	0.73±0.02	0.74±0.02

Means with different superscript in each row differ significantly with other ($P \leq 0.05$)

The week wise mean milk yield (Kg/d) of control and treatment groups revealed no significant difference from 4th to 7th week of lactation and from 8th to 12th week of lactation monensin supplemented buffaloes showed significantly ($P \leq 0.05$) higher milk yield than that of control group. The average milk yield was significantly higher ($P \leq 0.05$) in treatment group 7.76±0.24Kg/d as compared to control group (7.17±0.10Kg/d). On an average, monensin sodium supplemented lactating buffaloes yielded 8.22 per cent (7.17 v/s 7.76 kg/d) more milk than the control group during the experimental period. Results of this experiment are similar to the findings of Ipharraguerre and Clark. (2003) and Gandra *et al.* (2010) who have reported increased milk yield in different breeds of dairy cows upon dietary supplementation of monensin. However, Van *et al.* (2001) and Lamba *et al.* (2013) had observed no significant difference in the milk yield in monensin supplemented cows.

The mean fat percentage throughout the study was significantly ($P \leq 0.05$) lower in monensin supplemented buffaloes than those of control group, by 9.61 per cent (Table 2). These results are in agreement with the results of previous study of McGuffey *et al.* (2001), Broderick (2004) and Duffield *et al.* (2008). Acetate and butyrate are the major lipogenic precursors for milk fat synthesis. The milk-fat depressing effect of monensin might be attributed to the reduced ruminal production of acetate and butyrate, which might result in a shortage of lipogenic precursors for the synthesis of fatty acids in the lactating mammary gland. Alternatively, data from *in vitro* experiments by Bauman and Griinari (2001) indicated that monensin sodium may inhibit ruminal biohydrogenation of long chain fatty acids, which in turn might enhance the supply of trans-10, cis-12 CLA to the mammary gland. Increased availability of this trans- fatty acid in the mammary gland, which appears to be a potent inhibitor of the *de novo* synthesis of fatty acids might be part of the mechanism responsible for the reduced milk fat output of monensin supplemented lactating animals. In contrast, Van *et al.* (2001), Da Silva *et al.* (2007) had observed no significance difference in the milk fat percentage in monensin fed cows as compared to control cows.

The average milk lactose content was not significantly ($P \leq 0.05$) different from that of control group (Table 2). Abo El-Nor *et al.* (2007) and Gandra *et al.* (2010) reported increased in milk lactose percentage in cattle, in response to monensin supplementation.

The average SNF, protein, total solids and total ash content throughout the experimental period showed no significant ($P \leq 0.05$) difference in control and treatment groups. These results of milk protein content was consistent with the reports of previous studies of Fatahnia *et al.* (2010) and Gandra *et al.* (2010) in dairy cows and contrary to these results, Abo El-Nor *et al.* (2007), Lamba *et al.* (2013) reported increase in milk protein content in cows. However, Broderick (2004) observed decrease in milk protein content when monensin was supplemented to lactating cows. This inconsistency in results with milk protein

percentage in response to monensin supplementation might be because of difference in the productive status of experimental animals, nutritional status of animals and mainly the quality and composition of the ration fed to animals. Helal and Lasheen. (2008) reported no significant difference in SNF, total solids and total ash when monensin was supplemented @ 400mg/day to Egyptian buffaloes.

CONCLUSION

From this experiment it could be concluded that the dietary supplementation of monensin sodium @200mg/day to early lactating buffaloes significantly ($P \leq 0.05$) decreased the DMI by 2.89% or 0.29Kg/d, yielded an average of 8.22% more milk and reduced the milk fat percentage. No significant difference was observed for other components of milk like SNF, lactose, protein and total ash by monensin sodium supplementation.

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Study of Genetic Variation of Kappa Casein Gene in Local and Exotic Breeds of Cattle*

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ABSTRACT

The kappa casein (k-CN) is genetically polymorphic and is controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance. The present research work was undertaken to analyze the genetic variation of kappa casein gene and their association with milk quality traits in Jersey, Hallikar and Malnad Gidda breeds of cattle by PCR-RFLP. A forward (JK5) and a reverse (JK3) primers were used to amplify 350 bp fragment of kappa casein gene and found no variation in size of amplified product either within or between the breeds. The 350 bp PCR amplified product was digested using the enzyme *Hinf*I that resulted in three allelic patterns. The Bovine k-casein AA genotype has two restriction sites for *Hinf*I at 350 bp amplicon yielding two major fragments of 134 bp and 132 bp and a minor fragment of 84 bp. BB genotype had only one restriction site resulting in two fragments of sizes 266 bp and 84 bp. The AB genotype had fragments of 266, 134, 132 and 84 bp. The association between the kappa casein gene and milk composition was found to be non significant in Jersey.

Key words: Kappa Casein, Hallikar, Malnad Gidda, Jersey, PCR, RFLP

Milk protein is one of the important components of milk that determines its quality. Bovine milk protein are divided into four groups, and the most important one are Alpha-lactoglobulin, Beta-lactoglobulin and whole casein. Whole casein constitutes a mixture of four proteins *viz*, Alpha Casein S₁, Alpha Casein S₂, Beta casein and Kappa casein (Swaisgood, 1992), which are genetically polymorphic and are controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance (Aschaffenburg and Drewy, 1957). Kappa casein gene was considered as genetic marker for altering the milk composition and whose variants had significant effect on milk composition (Bovenhuis *et al.*, 1992).

Though extensive work has been done on milk protein gene in exotic breeds of cattle, such studies are very limited in indigenous cattle though they are traditionally believed to possess better milk quality. With this background, the present research work was undertaken to study and analyze the genetic variation of kappa casein gene and their association with milk quality traits in Jersey, Hallikar and Malnad Gidda breeds of cattle by PCR-RFLP.

MATERIALS AND METHODS

Blood and milk samples were collected from 25 Malnad Gidda cows from village of Shivamogga district, 25 Hallikar cows from villages of Tumkur

district and 10 Jersey cows from Semen station, Karnataka Milk Federation, Kakol, Bangalore rural district. Genomic DNA was isolated from venous blood by high salt method as described by Miller *et al.* (1988). A forward primer JK5 (5'ATCATTATGGCCATCCACCAAAG 3') and a reverse primer JK3 (5'GCCATTTCGCCTTCTCTGTAACAGA3') which were the flanking region of Kappa casein gene locus in exon IV and part of intron IV were used to amplify 350 bp fragment of kappa casein gene (Medrano and Aguilar-Cordova, 1990).

All the reactions were carried out in 0.2 ml reaction tubes. Just before setting of the reaction, a master mix was prepared by using 10X PCR buffer (500 mM KCl, 100 mM Tris.HCl, pH 8.3) (2 µl), 2.0 mM MgCl₂ (1 µl), 200 µM dNTP's (1.6 µl), 1.0 unit of Taq DNA polymerase (0.33 µl), 25 picomole of each primer (1 µl) and Filtered Milli Quartz (FMQ) water (12.07 µl). Each reaction mix consisted of 19 µl of master mix and one µl (100 ng) of template DNA and placed in the thermal cycler block.

An initial denaturation was done at 94°C for two minutes and subsequent denaturation, primer annealing and primer extension were carried out at 94°C, 58.2° and 72°C each for one minute, respectively. The number of cycles were kept constant at 34. After the last cycle, a final extension was carried

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out at 72°C for ten minutes and the samples were then cooled down to 15°C until retrieved.

Restriction enzyme digestion of the PCR product of kappa-Casein gene: A 20 µl of digestion mixture, in 0.5 ml micro centrifuge tube consisted of 15 µL of the PCR product, 2 µl (1X) of recommended buffer, 2 µl of FMQ and six units of (0.6µl) of restriction enzyme. The digestion mixture was mixed thoroughly in vortex mixture and incubated at optimum temperature of the enzyme *Hinf I* for three and half hour in dry bath. The digested product was run on two percent gel along with the standard DNA molecular marker of 100 bp at 50 volts for three hours and observed under UV transilluminator. The Agarose gel photographs were taken by using gel documentation instrument

RESULTS & DISCUSSION

The high salt DNA extraction method of Miller *et al* (1988) was followed for the isolation of DNA, which yielded good quality DNA. The OD ranged between 1.7 and 1.9 and the bright streaks on gel electrophoresis.

PCR amplification of kappa casein gene: Annealing temperature in the present study was lower than 60°C that was used by Medrano and Aguillar-Cordova (1990) for amplification of exon IV of kappa casein gene in *Bos taurus* while it was almost similar (58.4°C) to the one used in *Bubalis bubalis* (Darshan raj, 2006). The variation observed in the optimal annealing temperature may possibly due to the varying laboratory conditions.

The amplified product was approximately of 350 bp in length with no variation in size either within or between the breed studied (Figure.1). The sizes of the amplification products were identical in all the cattle breeds studied suggesting that this region is conserved. Medrano and Aguillar-Cordova (1990) and Darshan Raj (2006) also had obtained amplified product of similar size in *Bos taurus* and *Bubalus bubalis* species respectively suggesting the conservation of kappa casein gene between *Bos taurus*, *Bubalus bubalis* and *Bos indicus* species.

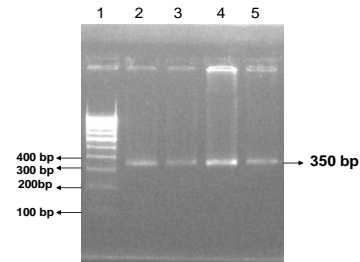


Fig 1. PCR amplified products of 350 bp, kappa casein gene.

Lane 1 - molecular marker (100bp).
Lane 2 - Malnad gidda
Lane 3- Jersey
Lane 4 & 5- Hallikar

Genotyping genetic variants of kappa casein gene using RFLP technique: The 350 bp kappa casein gene amplified product was digested with enzyme *Hinf I*. This resulted in three different allelic patterns. The first allelic pattern with two major fragments of 134 bp and 132 bp and a minor fragment of 84 bp, was classified as AA genotype. Second allelic pattern with a two fragments of sizes 266 bp and 84 bp was classified as BB type and third pattern with four fragments of 266, 134, 132 and 84bp was classified as AB genotype (Figure 2). The present findings were in agreement with earlier reports (Medrano and Aguillar-Cordova, 1990; Darshan raj, 2006)

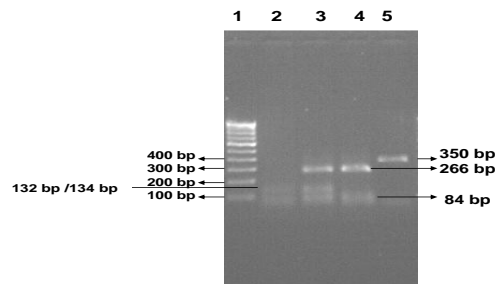


Fig. 2. Genotypic variants of kappa casein gene variants

Lane 1- Molecular marker
Lane 2- Genotype AA
Lane 3-Genotype AB
Lane 4-Genotype BB
Lane 5-Uncut 350 bp amplified product

The gene and genotype frequency of milk protein (kappa casein) gene for target breeds (Malnad Gidda, Hallikar and Jersey) were calculated and is presented in Table. The observed

gene frequency of 0.98 and 0.96 for kappa casein variant A in Malnad Gidda and Hallikar observed in the present study was in agreement with the reports of Kemenes *et al* (1999) who observed almost similar frequencies of A allele in few other *Bos indicus* breeds, viz; Gyr (0.93), Guzera (0.92) and Nelore (0.90). Almost similar A allele frequency was also reported by Murphy and Downey (1969) in Irish Kerry breed.

Table. Genotype and gene frequency of Kappa casein gene in Hallikar, Malnad Gidda and Malnad Jersey.

Breed	Genotype frequency			Gene frequency		Expected genotype frequency		
	AA	AB	BB	A	B	AA	AB	BB
Hallikar	0.92	0.08	Nil	0.96	0.04	0.9216	0.0768	0.0016
Malnad Gidda	0.96	0.04	Nil	0.98	0.02	0.9604	0.0392	0.0004
Jersey	0.40	0.10	0.50	0.98	0.02	0.2025	0.496	0.3025

In the present study B allele was predominantly found in Jersey (*Bos taurus*) animals and was in very low frequency in Malnad Gidda and Hallikar (*Bos indicus* breeds) where allele A was predominant. Similar finding was also reported by Malik *et al* (2000) who observed the predominance of A allele in Sahiwal cattle and B allele in cross breeds. The predominance of B alleles in Jersey was also reported by MacLean *et al.* (1984).

The genotype frequency was 0.92, 0.96 for AA and 0.08, 0.04 for AB in Hallikar and Malnad Gidda breeds respectively. Complete absence of BB genotype was observed in the present study in Malnad Gidda and Hallikar cattle. Similar result of absence of BB genotype was also reported by Mitra *et al* (1998) in Sahiwal cattle and Nili ravi, Murrah and Egyptian buffalo breeds.

In contrast to the present study, Burzynska and Topczewski, (1995) genotyped exon IV of the k-casein gene of *Bison bonosus* (European bison) and found only BB variants with allele A being totally absent. They concluded that homozygosity could be due to the loss of genetic variability in the small and isolated population studied and same results were also observed by Darshan Raj, (2006)

in *Bubalus bubalis* species, who recorded only BB variants with the complete absence of allele A.

Association of kappa casein gene and milk composition: In Malnad Gidda and Hallikar cattle the AA genotype was found in almost all the animals, except in one and two animals respectively. Due to the less number of genotype AB and total absence of BB genotype so it was not possible to determine the association between milk traits with these genotypes in these breeds. Jersey animals exhibited all the three genotypes, however no association could be established which could be due to the fact that only a small number of Jersey animals were studied.

CONCLUSION

The PCR amplified product obtained in the present study was approximately of 350 bp in length with no variation in size either within or between the breeds studied indicating that this region is conserved in all the cattle breeds. The presence of B allele predominantly in high milk yielding cattle breed such as Jersey and higher frequency of A allele in low producing *Bos indicus* cattle gives some indication of association of B allele with high milk production. However, further, extensive study is needed to confirm this by involving larger number of samples and more breeds.

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Nutritive Evaluation of Sorghum Stover (*Sorghum bicolor*) Cultivars in Khillari Cows

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ABSTRACT

Among various sorghum stover cultivars grown in northern part of Karnataka, five varieties viz. DSV-5, DSV-4, M-35-1, CSV-216R and CSV-22 were chosen for evaluation of palatability, digestibility and nutritive value in Khillari cows. Four Khillari cows were selected for the study and fed chopped sorghum varieties to the same group with a gap of two weeks. The CP and total ash content of stovers ranged from 11.5 to 13.4 and 9.62 to 13.0 % respectively. Significantly higher ($P>0.01$) nutrient intake was noticed in DSV-4 variety. Similarly, significantly higher ($P<0.05$) digestibility of CP and CF were observed in DSV-4 and CSV-216R cultivars. DCP and TDN content were highest in DSV-4 (7.73 and 59.6%) and CSV-12R (6.97 and 60.9%), cultivars respectively. The results indicated that the DSV-4 and CSV-216R sorghum stover cultivars were better in terms of palatability, nutrient digestibility and nutritive value when compared to other varieties.

Key words: Digestibility, Khillari cows, Nutrient density, Sorghum straw cultivars

Sorghum stover is the major cereal crop residue for ruminant feeding in dry land agriculture which has greater economic significance in farming system particularly in northern part of Karnataka. It is cultivated in *kharif* and *rabi* seasons in India and it becomes major bulk feed resource for livestock but the palatability and nutritive value vary with the variety. It has poor feeding value because of low nitrogen and high fibre content which limits intake (Ramachandra, 2009). The differences in nutritive value of sorghum stover occur due to genotype, environmental factors, location and season (Badve et al. 1993). Among various varieties available, choosing the right variety which has more feeding and nutritive value to increase the intake. Hence, five commonly grown genotypes of sorghum stovers were chosen for evaluation in khillari cows for their palatability, nutrient digestibility and nutritive value.

MATERIALS AND METHODS

Five sorghum stover varieties viz. DSV-5, DSV-4, M-35-1, CSV-216R and CSV-22 were procured from Sorghum Scheme, Regional Agriculture Research Station, Bijapur. Sorghum varieties were chopped to a length of around 3-4 cms and fed *ad libitum* as a sole source of feed to Khillari cows ($n=4$, 246-252kg B.W., 3 yrs of age) without any

supplementary feed. The duration of the feeding trial for each variety was four weeks which comprised one week adjustment period, two weeks observation period and last week as collection period. Each variety was evaluated in five separate experiments on the same Khillari cows with a gap of two week for each variety. Sorghum straws and dung samples collected during each trial were subjected for proximate analysis (AOAC, 1995). The data was subjected for statistical analysis (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

The chemical composition of five sorghum variety stovers is given in Table 1. The CP content of sorghum varieties ranged from 11.5 to 13.4% whereas TA content ranged from 9.62 to 13.0 which were far higher than the values reported by Sanjay (2007) and Venkatesh et al (2008). The level of the CP in all varieties were well within the minimum level of protein recommended (6-8%) for diets of ruminants to maintain normal intake and digestibility (Van Soest, 1994). The average intake of nutrients from five sorghum varieties by Khillari cows is given in Table 2. Significantly higher ($P>0.01$) DM intake (4.8 kg/d) was noticed in DSV-4 variety followed by CSV-22, CSV-216R, M-35-1 and DSV-5 cultivars. The DM intake on

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percent body weight was significantly higher ($P>0.01$) in DSV-4 variety (1.91% of B.wt.) than other varieties (ranged from 1.15 to 1.43% of B.wt.). Waghmare et al. (1987) reported DM intake of 2.0 per cent of body weight in HFXGir bulls when fed with M-35-1 sorghum stover variety whereas Reddy et al. (1993) observed 2.39, 3.09 and 3.21 per cent DM intake in Nellore rams fed with local, SPV 351 and SPV 475 sorghum straw varieties, respectively. The intake of stover was increased (1.7 to 1.85 % of b.wt.) when buffaloes supplemented with GNC. The CP intake was significantly higher ($P<0.05$) in DSV-4 cultivar fed group even though the CP was higher in M-35-1 variety (13.4%) which was due to higher intake of DM from the DSV-4 variety. Significantly higher ($P<0.05$) intake of all other nutrients (OM, CF, EE, NFE and TA) in DSV-4 variety than other varieties were again due to higher intake of DM both on per cent body weight and kg per day. It was an indication of higher palatability associated with DSV-4 sorghum stover variety in Khillari cows. The nutrient digestibility of five sorghum varieties evaluated in khillari cows is presented in Table 3. Significantly higher ($P<0.05$) digestibility of CP and CF were observed in varieties DSV-4 and CSV-216R which were higher than the values reported in study on bullocks (Patel et al., 2007). Even though the digestibility of DM, OM, EE and NFE were not statistically significant among the varieties, but numerically higher digestibility of these nutrients were noticed in DSV-4 variety. On contrary, Waghmare et al. (1987) noticed significantly higher ($P>0.01$) OM and NFE digestibility in HFXGir bulls received M-35-1 sorghum stover variety. The digestibility of all nutrients reported in this study, however, were far higher than the values reported in the studies fed sorghum stover to buffaloes and Gir bullocks (Ramachandra et al. 2002; Patel et al. 2007). DCP and TDN content were 7.73 and 59.6; 6.97 and 60.9 % in DSV-4 and CSV-12R varieties, respectively. However, no significant difference was observed in DCP and TDN content among the varieties. The DCP and TDN of the sorghum stover varieties evaluated in this study were ranged from 6.15 to 7.73 and 52.49 to 60.90 % respectively which were

higher than the values (1.66 & 2.29 and 50.6 & 49.30%) reported in buffaloes (Natarajan et al. 1994; Ramachandra et al. 2002) and Gir bullocks (2.35 and 50%) respectively. Within the voluntary DM intake limit of all sorghum stover varieties in khillari cows, all five varieties could supply more than the maintenance requirement of DCP (170g/d) and only DSV-4, CSV-216R and CSV-22 varieties could meet the maintenance requirement of TDN (2.0 kg/d) as recommended by ICAR (2013) except DSV-5 and M-35-1 varieties where they supplied around 0.3 to 0.5kg short of TDN. This indicated that these two varieties DSV-5 and M-35-1 require the supplementary feed to meet the maintenance requirement.

Table 1. Chemical composition (% on DMB) of five sorghum varieties.

Particular	Sorghum varieties				
	DSV-5	DSV-4	M-35-1	CSV-216R	CSV-22
Dry matter	97.64	97.53	97.89	97.82	97.19
Organic matter	89.84	87.00	89.18	90.09	90.38
Crude protein	12.85	12.45	13.40	11.51	11.56
Crude fiber	40.58	39.14	40.10	40.17	39.82
Ether extract	1.63	1.83	1.73	1.60	1.85
Nitrogen free extract	34.78	33.59	33.96	36.81	37.14
Total ash	10.16	13.00	10.82	9.91	9.62

Table 2. Average intake of nutrients (kg/d) from five sorghum varieties in Khillari cows

Particular	Sorghum varieties					SEM
	DSV-5	DSV-4	M-35-1	CSV-216R	CSV-22	
DMI**	2.87 ^a	4.80 ^b	3.22 ^a	3.51 ^a	3.59 ^a	0.254
100kg B.wt.**	1.15 ^a	1.91 ^b	1.28 ^a	1.40 ^a	1.43 ^a	0.106
OMI**	2.58 ^a	4.18 ^b	2.87 ^a	3.16 ^a	3.24 ^a	0.228
100kg B.wt.**	1.03 ^a	1.66 ^b	1.14 ^a	1.26 ^a	1.29 ^a	0.095
CPI**	0.37 ^a	0.60 ^b	0.43 ^a	0.40 ^a	0.41 ^a	0.031
100kg B.wt.**	0.15 ^a	0.24 ^b	0.17 ^a	0.16 ^a	0.17 ^a	0.013
CFI**	1.17 ^a	1.88 ^b	1.29 ^a	1.41 ^a	1.43 ^a	0.102
100kg B.wt.**	0.47 ^a	0.75 ^b	0.51 ^a	0.56 ^a	0.57 ^a	0.043
EEI**	0.04 ^a	0.08 ^c	0.05 ^{ab}	0.05 ^{ab}	0.06 ^b	0.004
100kg B.wt.**	0.02 ^a	0.03 ^c	0.02 ^{ab}	0.02 ^{ab}	0.02 ^b	0.002
NFEI**	1.00 ^a	1.61 ^c	1.09 ^{ab}	1.29 ^b	1.33 ^{bc}	0.089
100kg B.wt.**	0.40 ^a	0.64 ^c	0.44 ^{ab}	0.51 ^b	0.53 ^{bc}	0.037
TAI**	0.29 ^a	0.62 ^b	0.35 ^a	0.35 ^a	0.34 ^a	0.027
100kg B.wt.**	0.12 ^a	0.25 ^b	0.14 ^a	0.14 ^a	0.14 ^a	0.011

** $P<0.01$, Means bearing different superscripts differ statistically

Table 3. Nutrient digestibility (%) and nutrient density (%) of five sorghum varieties in Khillari cows.

Particular	Sorghum varieties					SEM
	DSV-5	DSV-4	M-35-1	CSV-216R	CSV-22	
Nutrient digestibility						
DM	56.17	70.32	58.38	67.48	67.14	0.917
OM	61.55	73.35	61.12	71.44	70.61	0.529
CP*	54.81 ^{abc}	62.06 ^c	46.53 ^a	60.54 ^{bc}	53.17 ^{abc}	0.529
CF*	66.36 ^{abc}	68.54 ^b	60.75 ^a	72.51 ^c	69.49 ^{bc}	0.207
EE	61.19	73.38	62.47	72.83	71.56	1.570
NFE	48.76	66.73	58.08	60.97	63.81	0.854
Nutrient density						
DCP	7.04	7.73	6.23	6.97	6.15	0.067
TDN	52.94	59.6	52.49	60.9	60.26	0.304

* $P < 0.05$, Means bearing different superscripts differ statistically

CONCLUSION

Even though all five varieties meet maintenance requirement of DCP, DSV-5 and M-35-1 varieties failed to meet the maintenance requirement of TDN. However, from the results of the study, it can be concluded that even though all the five sorghum cultivars had almost similar chemical composition, the DSV-4 and CSV-216R sorghum varieties were found to be better in terms of nutrient digestibility, density and the palatability when compared to other varieties.

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Effect of Feeding Sorghum Stover and Sorghum Stover Silage on the Performance of Lactating Crossbred Cows

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ABSTRACT

An experiment was carried out for a period of ten weeks including two weeks of adjustment period. Eight crossbred (Deoni x Holstein Friesian or Jersey) cows in mid lactation were divided into two groups of four cows in each group based on comparable milk yield, body weight, number of lactations completed and days in lactation. The cows in group I received sorghum stover dry (SstD) as main roughage in the diet and cows in group II sorghum stover silage (SstS) in period I followed by switching over the roughage component in Period II. The mean DMI (kg/d) for SstS and SstD groups were 13.47 ± 0.74 and 11.77 ± 0.78 respectively. The difference between the two groups in DMI from maize stover was significant ($P < 0.0001$) but not with other components of the diet. The intake of OM, and CP ($P < 0.0001$) differed between the two groups. There was no significant difference between the two groups in digestibility of any nutrient

The average daily milk yields and 4 % FCM yield for SstS and SstD groups were 12.46 ± 0.31 and 11.55 ± 0.08 ; 14.15 ± 0.06 and 12.30 ± 0.03 kg respectively. The difference between the two groups was statistically significant for milk yield ($P < 0.007$) and 4 % FCM yield ($P < 0.005$). The mean total solids, fat content, protein, lactose, SNF and ash in milk for SstS and SstD groups were 13.92 ± 0.43 and 13.55 ± 0.12 ; 4.91 ± 0.12 and 4.46 ± 0.30 ; 3.35 ± 0.01 and 3.00 ± 0.03 ; 4.87 ± 0.11 and 4.46 ± 0.06 ; 9.05 ± 0.32 and 9.09 ± 0.06 ; and 0.02 , 0.64 ± 0.01 , respectively. The difference between the two groups in milk fat ($P = 0.0093$), protein ($P < 0.081$) and lactose ($P < 0.084$) were statistically significant. The results indicated that the conservation of sorghum stover in the form of silage is a better option than in the conventional dry form

Key words: Crossbred cows, sorghum, stover, composition, milk yield

Sorghum (*Sorghum bicolor* (L.) Moench) is an important food, fodder and also bio-fuel crop of the world and is considered as King of millets. Karnataka is one of the leading states in sorghum cultivation next to Maharashtra, with a total annual production of 1.82 million tons of grain and 3.73 million tons of stover (Anon, 2009).

For ruminant livestock in India, crop residues continue to remain as staple fodder. Among the crop residues sorghum stover is the most preferred fodder. However, its utilization in ruminant feeding is not complete. A considerable quantity (70%) of dried stover is left over and wasted (Anon, 2009).

Drying of sorghum fodder after harvesting grains is the traditional practice in India. Although

the advantage of drying of crop residues for prolonged storage is undisputable, it is also true that the succulent crop residues, soon after grain harvest are relished better than those that are completely dried. For example, dry matter intake and digestibility of succulent rice straw following grain harvest was reported to be higher compared to dry rice straw (Sharif, 1984, Krishnamoorthy *et al.*, 2006, Santos *et al.*, 2010), Dried sorghum stover is not relished better by ruminants and the suggested methods such as sprinkling of water, supplementation with urea, molasses and mineral mixture etc., to improve their palatability are not well accepted by the farmers. However, it is the common observation that succulent stover, soon after grain harvest is better relished by cattle and

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buffaloes (FAO, 2000b). Therefore, it is hypothesized that the conservation of sorghum stover by making silage when it is in the succulent (plant having thick, juicy leaves or stem) form can be more effective way of utilization for feeding.

Radotra and Upadhyay (2005) conducted an experiment to see the effect of ensiled sorghum stover on the performance of lactation cows. The crossbred cows (8) of mid lactation yielding 6 to 7 kg/day divided into two equal groups were offered either sorghum stover (SS) or ensiled sorghum stovers (ESS) *ad lib* as basal roughage, supplemented with 30 kg green berseem and one kg concentrate mixture for 90 days. The DM and DCP intake were comparable though TDN intake was higher ($P < 0.05$) in ESS as compared to SS group. The digestibility of most of the nutrient was comparable in two groups except that the CP and total carbohydrates which was higher ($P < 0.05$) in ESS as compared to SS fed group. Both milk and FCM were higher ($P < 0.05$) in ESS than SS fed animals. It was shown that nutrient utilization and milk production efficiency was better in cows fed ensiled sorghum stover as compared to stover feeding.

Evaluation of the sorghum stovers as well as silage was carried out by Singh *et al.* (1993) under this; two experiments were carried out on sorghum stover conservation. In one experiment, sorghum varieties (CSH-9, CSH-5, MP Chari and PC6) after removal of matured grain were chopped and ensiled in glass silos with (0.5%) and without urea. The silages were evaluated after 50 days storage. Grain type (CSH-9 and CSH-5 had higher moisture, soluble sugar and CP than the fodder varieties (MP Chari and PC-6), pH of the silages ranged from 3.03 to 4.54 per cent. To study the effect of feeding sorghum stover silage versus dried sorghum stover on dry matter intake, nutrient digestibility, milk production and milk composition.

MATERIALS AND METHODS

An experiment was conducted for a period of ten weeks with adjustment period of two weeks to study the effect of feeding dry stover and stover silage on DMI, milk yield and milk composition in lactating cows at KVK, Hittnalli Farm, Bijapur, UAS - Dharwad.

Cultivation and preparation of fodder: Sorghum (DSV-4) was sown in two acres by line sowing method as per the recommended package of practice (Anon, 2008). The stover was harvested after the maturity at 105 days, the ear heads were removed manually. One half of the harvested stover was bundled and transported for ensiling while other half was left in the field for sun drying. The green stover collected for ensiling was chaffed and jaggery @ 20 kg/ton of fodder as is, was dissolved in water and sprayed on the chaffed fodder to a length of one cm in a chaff cutter (HIMCO C-5, Industrial Estate, Clutterbuckganj, Bareilly, UP, India) and filled into a concrete circular silo measuring 15' depth x 5' dia. This sequence was repeated until complete filling of sorghum stover for ensiling. The sun dried stover was chaffed and stored for use in the feeding trial.

Animals and feeding: Eight crossbred (Deoni X Holstein Friesian or Jersey) cows in mid lactation were divided into two groups of four cows in each group based on comparable milk yield, body weight, number of lactations completed and days in lactation. The cows in Group I received SstS as main roughage in the diet and cows in Group II SstD in period I followed by switching over the roughage component in Period II. Each period is lasted for four weeks with an adjustment period of two week before trial.

The diet of experimental cows comprised on *ad lib* of each SstS or SstD and compound feed mixture (CFM) at the rate of 55 per cent of their milk yield, to fulfill their nutrient requirements as per ARC (1980). The leftover of SstS and SstD offered were weighed on the next day morning to obtain the estimate of intake. The CFM was fed at the time of milking at 7.00 am and 2.30 pm in two portions. The cows were allowed to have free access to water at 9.30 am and 2.00 pm.

Chemical analysis: The samples of SdoS and SstS were analyzed for DM, CP, EE and total ash according to AOAC (1990). The NDF, ADF and lignin were determined as per the method described by Van Soest *et al.* (1991).

Dry matter intake: Daily intake of SstS, SstD and CFM were recorded samples of feed and fodder

offered were collected weekly once for the determination of dry matter by drying samples at 65°C to a constant weight. Using DM value and the mean daily intake of feed and fodder, DMI was calculated.

Milk yield and composition: The cows were hand milked twice daily at 7.00 am and 2.30 pm. Morning and evening milk yield of individual cows were weighed and recorded daily by Avery spring balance (clock type). Once in a week about 100 ml of milk samples were collected soon after milking in the morning and the successive evening while recording yield and kept refrigerated until further analysis. Milk samples were analyzed for total solid (TS), lactometer reading (LR), milk fat (Gerber's Method), milk protein (Kjeldhal method), total ash and solids not fat (SNF) according to AOAC (1990). The lactose was calculated by subtracting sum of fat, protein and ash from total solids.

Statistical Analysis: The data were analyzed using Graph pad Prism4 for windows (2004) Software for rumen *in vitro* gas production (RIVGP) kinetics analysis and SPSS for Windows (2008) Statistics 17.0 Ink, Software for feeding trial.

RESULTS AND DISCUSSION

Yield of sorghum grains and fodder: The grain and stover yield obtained during the experiment was 3.23 and 4.56 tons/ha respectively. This was well above the national average of 1.17 tons/ha of sorghum grain production (ICRISAT, 2009). The ratio of grain to stover yield recorded in the study was 1:1.41. Scheiere *et al.* (2004) reported that the ratio of grain to stover yield was 1:1.29, the ratio obtained in the present was higher in stover yield, and this might be because of improved varieties which have been bred for higher grain and stover yield.

Chemical composition: The chemical composition, ME content and RIVGP characteristics of sorghum stover silage and sorghum stover dry differed (Table). A slightly lower ADF (42.74 vs 43.55) and lignin (7.21 vs 6.83), and higher EE (1.97 vs 2.53) in SstS against SstD, is perhaps suggestive of a satisfactory fermentation in pilot scale ensilage. Incomplete elimination of air from the silos, leads to oxidative reactions resulting in heat production and damage. This loss can be as high as 25 per cent

(Morrison, 1957 and McDonald, 1981). In silage subjected to heat damage, ADF and lignin content dramatically increases subject to heat change. The increased ADF and NDF contents observed in SstD in the present study are in consistent with the previous observations, where ensiled mature sorghum stover had greater N content and lower NDF, and ADF contents when compared to the mature sorghum stover, but these values (NDF and ADF) were higher in SstS than the normal silage (Van Soest, 1994, Arias, *et al.*, 2003, and Tang, *et al.*, 2008). A satisfactory fermentation of sorghum stover is also evident from low pH of this silage

Table: Chemical composition (% DM), mean daily nutrient intake (kg/day) and mean values of milk yield (kg/d), 4% FCM yield (kg/d), lactometer reading, milk composition (%) and component yield (kg/d) in crossbred cows fed with sorghum stover silage (SstS), sorghum stover dry (SstD) and compounded feed mixture (CFM) used in the feeding trial.

Constituent	SstS	SstD	CFM/SEM	p
DM	91.37 ± 0.48	91.14 ± 0.24	97.11±0.00	-
OM	91.49± 0.29	92.42± 0.84	89.65±0.00	-
CP	3.82 ± 0.31	1.81 ± 0.15	24.25±0.08	-
NDF	65.71 ± 1.78	67.88 ± 1.30	48.14±0.95	-
ADF	42.74 ± 1.52	43.55 ± 1.04	17.78±0.15	-
EE	1.97 ± 0.12	2.53 ± 0.25	2.61±0.00	-
TA	8.51 ± 0.22	7.58 ± 0.96	10.35±0.00	-
Lignin	7.21 ± 0.76	6.83± 0.30	6.76±0.51	-
Dry matter Intake (kg/d)				
CFM	3.65 ±0.33	3.65 ±0.33	-	1.000
SstS	6.51 ±0.26 ^a	-	-	0.0021
SstD	-	5.45 ±0.43 ^b	-	
Total DMI	10.16 ±0.25	9.10±0.34	-	0.0512
DMI% of BW	2.56±0.33	2.33±0.22	-	0.0853
OM	8.92±0.94	8.84±0.92	-	0.5621
DOM	5.25±0.31	5.03±0.23	-	0.1102
CP	1.09±0.15 ^a	0.97±0.15 ^b	-	0.0010
NDF	6.20±1.12	5.90±1.02	-	0.5147
ADF	4.58±0.50	4.06±0.27	-	0.6658
Milk yield and milk constituents				
			SEM	
Milk yield	8.82 ±1.81	8.01 ± 1.79	2.715	0.107
4% FCMyield	9.33 ±1.99 ^a	7.80 ± 1.67 ^b	1.388	0.009
Lactometer reading	29.48 ± 0.84	29.39 ± 0.96	1.35	0.855
Total solids	12.24 ± 0.71	11.90 ± 0.66	0.511	0.149
Yield	1.07 ± 0.20 ^a	0.95±0.21 ^b	0.248	0.022
Fat	4.26 ± 0.34 ^a	3.84 ± 0.34 ^b	0.120	0.009
Yield	0.39 ± 0.09	0.31 ± 0.07	0.010	0.175
Protein	3.05 ± 0.23	3.01 ± 0.07	0.032	0.181
Yield	0.26 ± 0.04	0.24 ± 0.04	0.002	0.259
SNF	7.98 ± 0.56	8.07 ± 0.63	0.310	1.150
Yield	0.34 ± 0.04	0.31 ± 0.03	0.112	0.201

Note: Means bearing different superscripts row wise differ significantly.

The mean intake (kg/day) in SstS and SstD groups with respect to OM, CP, NDF and ADF were 8.92 ± 0.94 , 8.84 ± 0.92 ; 1.09 ± 0.15 , 0.97 ± 0.15 ; 6.230 ± 1.12 , 5.90 ± 1.02 and 4.58 ± 0.50 , 4.06 ± 0.27 respectively. The intake of OM ($P=0.5621$) and CP ($P < 0.0010$) differed between the two groups.

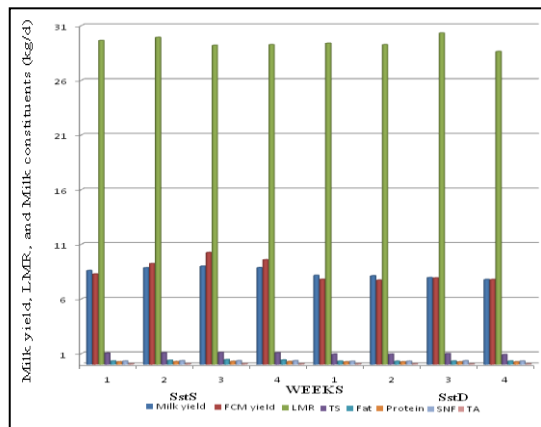
Intake: The dry matter intake of CFM for the two groups was similar (Table). The DMI was significantly higher for SstS group than for SstD group resulting in an increase in CP intake. Although the nutrient composition of SstS and SstD were similar (Table), DM intake from SstS was significantly higher than from SstD (6.51 vs 5.45).

Van Soest (1994) reported that forage intake and digestibility are influenced differently by different constituents. While the DM intake has a negative correlation with NDF content, DM digestibility is negatively influenced by the ADF and lignin. In this study, the NDF and ADF content of SstS and SstD were similar (65.71 vs 67.88 and 42.74 vs 43.55) with lignin being 7.21 and 6.83 respectively. Therefore, the difference in DM intake between SstS and SstD cannot be explained by the NDF content of these two roughages. Further, digestibility of DM and all nutrients except OM and CP were similar for the two groups although the ADF content of SstD was substantially higher than that of SstS. One possible reason for not observing the correlation trend between fiber fractions and the DM intake and digestibility similar to that reported in the literature (Van Soest, 1994) is that the sorghum stover in the form of silage or dry being less than 50 per cent of the total DM intake, other components of the diet might have masked this difference. Higher DM intake in SstS group resulted in higher DOMD and CP intake, that are largely responsible for driving productivity functions such as milk yield, milk component yield and body weight gain.

Milk yield and milk composition: The average milk yield in crossbred cows before and feeding trial with sorghum stover silage (SstS) and sorghum stover dry (SstD) is presented graphically in figure. The mean daily milk yield and 4% FCM for the groups is given in Table. The average daily

milk yields and 4% FCM yield for SstS and SstD groups were 8.82 ± 1.81 and 9.33 ± 1.99 ; 8.01 ± 1.79 and 7.80 ± 1.67 kg respectively. The difference between the two groups was statistically non significant for milk yield ($P < 0.107$) but significant for 4% FCM yield ($P < 0.009$).

Fig. Average weekly milk, FCM, LMR and milk compositions yields (kg/d) in crossbred cows before and during feeding trial with sorghum stover silage (SstS) and sorghum stover dry (SstD)



The average lactometer reading, mean total solids (%), fat content (%), mean protein (%), mean milk SNF (%) and mean milk ash (%) for SstS and SstD groups were 29.48 ± 0.84 , 29.39 ± 0.96 ; 12.24 ± 0.71 , 11.90 ± 0.66 ; 4.26 ± 0.34 , 3.84 ± 0.34 ; 3.05 ± 0.23 , 3.01 ± 0.07 ; 7.98 ± 0.56 , 8.07 ± 0.63 ; 0.70 ± 0.06 , and 0.71 ± 0.06 ; respectively. The difference between the two groups was not significant except the fat per cent which was statistically significant ($P = 0.009$).

The average TS yield for the two groups for both the periods are presented in Table. The mean yields (kg/d) of total solids, fat, protein, SNF and ash in milk for SstS and SstD groups were 1.07 ± 0.20 , 0.95 ± 0.21 ; 0.39 ± 0.09 , 0.31 ± 0.07 , 0.26 ± 0.04 , 0.24 ± 0.04 ; 0.34 ± 0.04 , 0.31 ± 0.03 and 0.06 ± 0.013 , 0.06 ± 0.012 respectively. The yields were non-significant between the two groups in all the constituents except for yield it was highly significant ($P = 0.009$).

The FCM yield in SstS group was significantly higher than in SstD group (9.33 vs 7.80 kg, Table). Fat, protein and lactose content of milk were also higher in SstS than in SstD. However the daily yields of milk constituents were similar for

the two groups except for total solids which were influenced by the SstS feeding. In conformity with higher intake of DOMD in SstS group leading to higher level of milk production. Thus, an increase in milk yield and total solids in milk, together with an increase in DM intake in cows fed with SstS suggests that SstS is of higher nutritional value than the SstD. Further, the milk yield response to change of roughage source (SstS versus SstD) is quick and can be seen as early as on the first day of change.

Radotra and Upadhyay (2005) also reported significantly higher milk yields (both 4% FCM and Milk yields) and milk compositions in ensiled sorghum stover (ESS) when compared to the sorghum stover (SS). Hargreaves *et al.* (1984) reported that the DMI of corn silage was higher (20 kg/d) when compared to stalklage silage (16.9 kg/d). Further the milk compositions were all within normal ranges and did not differ significantly between corn silage and stalklage, except that the CP content in milk tended to be higher in corn silage. Gebrehawariat *et al.* (2010) reported higher milk CP content in animals fed with normal silage than in those fed with stover silage, which might be due to slightly higher protein intake in these groups. This shows that the stover silage when conserved at low level moisture can be nutritionally acceptable. Further, the SstS prepared in the present experiment can be comparable with the normal silage. However, the increased DMI might be attributed to earlier stage of lactation of animal. The DMI and milk yield and milk compositions as reported in trial, indicates that the SstS and SdoS can be comparable, and that the stover soon after harvest can easily be made into good silage. This is in correlation of the earlier works of Singh *et al.* (1993) and Radotra and Upadhyay (2005) in sorghum and Rahaman *et al.* (2003), Mohamed (1997), Iwama (1999) and Keys and Smith (1982a) in maize. A contradictory result to above findings decreased DMI in maize stover silage was reported in steers by Keys and Smith (1982b) and Mader and Britton (1995). Morrison (1957) reported a reduction of 10.6 per cent milk yield in stover silage fed animals when compared to corn silage fed group.

Morrison (1957) compared corn stover silage and corn-fodder in dairy cows. Sorghum stover soon after harvesting could be ensiled to get good silage which was more palatable than stover or dry fodder. The silage could be fed to dairy cattle as well as beef cattle with required supplementation of energy and protein. In silage feeding, N utilization is negatively affected because of high solubility of N (Bareeba and McClure, 1993). In this study, N balance data revealed that N excretion in faeces and urine was slightly higher in SstS compare to SstD (Table).

Hamilton and Rusk (1927) and Colenbrander *et al.* (1971 a & b) reported that conservation of maize stover in the form of silage can be an option, since the both sorghum and maize behave in the similar pattern in their physical form and structure conservation of sorghum stover in the form of silage can also be best option (Singh *et al.*, 1993). Seanger *et al.* (1982) reported that the dairy cows fed with corn stover dry lost body weight up to 20.7 kg in a 70 day feeding trial. It was also reported that intake of stover was limited by fill and crude protein and concluded that storage of green stover in the form of silage is the better option to preserve nutrients (Gaafar *et al.*, 2010).

Since fodder shortage for 2 to 3 months prior to monsoon is a recurring problem, popularization of sorghum stover ensilage among farmers can have an impact on fodder resource management at the macro-level. Therefore, the feasibility of adoption of ensiling technique in farmer's doorstep was explored.

CONCLUSION

The sorghum stover harvested soon after physiological maturity /grain harvest can be converted into silage of acceptable quality. A higher DMI, higher milk production and better milk quality can be achieved by feeding SstS as compared to SstD. Therefore, conservation of sorghum stover as silage is a better option than in the conventional dry form. This can have a significant impact on resource utilization specially in mitigating fodder shortage during lean season.

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Housing Management Practices of Deoni Cattle in Bidar District*

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ABSTRACT

Bidar district of Karnataka is a major part of the breeding tract of the Deoni breed of cattle. The study was carried out by documenting the housing management practices of 200 respondents, spread over 10 villages in two talukas of Bidar district. Most of the animals were found to be in good body condition inspite of lack of any sort of housing (46 per cent). Young calves and breeding bulls were provided greater care and protection; in many instances by housing them within the farmers' residences. Most of the cattle sheds (54.6 per cent) were of the *kutch* type, with a thatch roof. The mean roof height at the highest and lowest points was found to be 2.17 and 1.60 m, respectively. The most common material used for supporting the roof was wood with 83.3 per cent of the farmers using it. Stone walls (93.9 per cent) and unplastered (87.8 per cent) wall surfaces were most common in the *pucca* houses. Most of the farmers (86.1 per cent) did not construct basement in the cattle shed. The overall average area of livestock sheds of respondents having constructed sheds was found to be 11.08 sq. m. In view of the prevailing climatic conditions, low incidence of diseases and good body condition of the animals, this pattern was found to be viable. However, the absence of a feed manger, or inadequate width and height of the feed manger, in most instances led to excessive loss of fodder by way of trampling and wastage. The absence of basement in the livestock shed was also not conducive to hygienic rearing of cattle.

Key words: Deoni cattle, Bidar, Karnataka, Housing

Bidar district has an area of 5448 square kilometres and lies between 17°35' and 18°25' North latitude and 76°42' and 77°39' East longitude. The climate of Bidar district is characterized by general dryness throughout the year, except during the southwest monsoon. The summer season is from the middle of February to the first week of June. This is followed by the southwest monsoon season, which continues till the end of September. The months of October and November constitute the post-monsoon or retreating monsoon season. The winter season is from December to middle of February and the temperature begins to decrease from the end of November. With the withdrawal of southwest monsoon in the first week of October, there is slight increase in day temperature but night temperature decreases steadily. After October, both day and night temperatures decrease progressively. December is the coldest month with mean daily maximum temperature of 27.3°C and mean daily minimum of 16.4°C. From the middle of February,

both day and night temperatures begin to rise rapidly. May is the hottest month with mean daily maximum temperature of 38.8°C, and mean daily minimum of 25.9°C. The annual normal rainfall for the district (based on 1941-1990 data) is 886 mm (Zilla Panchayat, 2012).

The Deoni is a very important dual-purpose breed of cattle of the Marathwada region of Maharashtra state and adjoining parts of Karnataka and Andhra Pradesh. It is also known as the Surti, Dongarpati or Dongri (which means "of the hills"). There are varying accounts as to its origin. Some believe it to have developed from Gir cattle 280-300 years back as a result of migration of nomadic tribes like the Rabaris, Bharwads, Charans, Maldars and Ahirs from Gujarat to the southern parts of the country during periods of fodder scarcity, leading to an admixture of Gir blood with local herds (Vishwagou, 2012). The Deoni is the only established cattle breed found in Bidar district and it plays a vital role in the cropping practices of

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farmers of the region. This study was undertaken to document the housing management practices of Deoni cattle in Bidar district.

MATERIALS AND METHODS

The present study was carried out in Bidar district of Karnataka. A multistage stratified sampling procedure was followed to select the talukas, villages and respondents. In the first stage of selection, out of the five talukas in Bidar district, Aurad and Bhalki talukas were selected for the study based on the larger population of Deoni cattle in these two talukas as per the 2007 livestock census and consultations with officials of the Animal Husbandry department regarding the availability of purebred Deoni cattle. In the second stage of selection, ten villages were selected for the detailed survey, 3 from Aurad taluka and 7 from Bhalki taluka, based on the strength of Deoni cattle as per the 2007 livestock census, and giving due consideration to factors like availability of purebred Deoni cattle and road connectivity. Most villages having pure Deoni cattle were situated in close proximity to the Manjra river. In the third stage of selection, 20 Deoni cattle owners were selected at random from each village for study of the housing management practices of Deoni cattle, thus giving a total of 200 respondents.

A structured schedule was developed for collection of information on the housing management practices of Deoni cattle in consultation with staff of the Department of Animal Husbandry and Veterinary Services, Government of Karnataka. The schedule was pre-tested under existing field conditions before finalization. The respondents were interviewed personally for the collection of desired information, with questions being asked in the language in which they were most fluent. Dimensions of the livestock houses were measured using a metal tape. The housing practices documented included - location and type of housing; roof material, height, supports and ventilation; wall material, surfacing, basement, floor material; drainage type; shed dimensions; method of restraint; feed manger, type and dimensions; watering equipment and shed cleaning frequency.

Data from the schedules was compiled and analysed using the data analysis tools in Microsoft Excel 2007 software. Summary statistics were calculated to identify the predominant management practices of Deoni cattle.

RESULTS AND DISCUSSION

The housing management practices followed by Deoni cattle farmers are presented in Table. Most of the farmers (54 per cent) reared cattle in loose houses, whereas the remaining did not have any permanent housing for their cattle. Such cattle were tied outside the house on the road side, and occasionally provided shelter with a plastic sheet during the rainy season. Most of the animals were found to be in good body condition in spite of lack of any sort of housing (46 per cent). A similar figure was reported by Singh *et al.* (2002) in Deoni cattle of Maharashtra. However, in contrast, a larger number of farmers (24.5 per cent) had *pucca* houses as compared to Maharashtra (3.0 per cent). In view of the fact that the average number of rainy days per year in Bidar was 52, and that the lowest mean daily minimum temperature was 16.4°C, the open housing system practised by a large number of farmers, with slight protection during rainy months, was found to be viable.

Table: Housing management practices of Deoni cattle (%)

Parameter	N	Aurad	Bhalki	Overall
Type				
Open	92	43.3	47.1	46.0
Loose	108	56.7	52.9	54.0
Closed	0	0.0	0.0	0.0
Location				
Adjacent (lean-to)	64	55.9	60.8	59.3
Within compound	42	44.1	36.5	38.9
At farm	2	0.0	2.7	1.9
Structure				
<i>Kutcha</i>	59	58.8	52.7	54.6
<i>Pucca</i>	49	41.2	47.3	45.4
Roof material				
Thatch	59	58.8	52.7	54.6
Stone	5	5.9	4.1	4.6
GI sheet	43	35.3	41.9	39.8
Asbestos sheet	1	0.0	1.4	0.9

Roof height (m)				
Highest point	108	2.14± 0.03	2.19± 0.03	2.17± 0.02
Lowest point	108	1.54± 0.03	1.62± 0.03	1.60± 0.02
Roof supports				
Wood	90	85.3	82.4	83.3
Steel	15	11.8	14.9	13.9
Stone	3	2.9	2.7	2.8
Ventilators				
None	41	85.7	82.9	83.7
Wall	8	14.3	17.1	16.3
Ridge	0	0.0	0.0	0.0
Wall				
Stone	46	100.0	91.4	93.9
Brick	2	0.0	5.7	4.1
Wood	1	0.0	2.9	2.0
Wall surfacing				
Unplastered	43	85.7	88.6	87.8
Plastered	6	14.3	11.4	12.2
Basement				
Yes	15	11.8	14.9	13.9
No	93	88.2	85.1	86.1
Floor				
Mud	40	41.2	35.1	37.0
Dung	58	50.0	55.4	53.7
Stone	6	5.9	5.4	5.6
Brick	0	0.0	0.0	0.0
Cemented	4	2.9	4.1	3.7
Drain				
Mud	88	85.3	79.7	81.5
Stone	18	14.7	17.6	16.7
Cement	2	0.0	2.7	1.9
Shed area (sq. m.)	108	10.90± 0.87	11.16± 0.54	11.08± 0.46
Restraint				
Ground peg	157	88.3	74.3	78.5
Wooden log	25	6.7	15.0	12.5
Cement pole	18	5.0	10.7	9.0
Feed manger				
None	58	31.7	27.9	29.0
Wooden log	76	41.7	36.4	38.0
Stone	53	23.3	27.9	26.5
Cement pipe	1	0.0	0.7	0.5
Constructed	12	3.3	7.1	6.0
Watering				
Bucket	177	91.7	87.1	88.5
Basin	21	8.3	11.4	10.5
Constructed tank	2	0.0	1.4	1.0

Shed cleaning				
Once a day	26	15.0	12.1	13.0
Twice a day	174	85.0	87.9	87.0

Young calves and breeding bulls were provided greater care and protection; in many instances by housing them within the farmers' residences. None of the respondents had a closed housing with a constructed barn. The livestock housing was found to be of the 'lean-to' type, adjacent to the farmer's residence, in 59.3 per cent of the respondents who had a constructed cattle shed. Of the remaining, 38.9 per cent had cattle sheds located within the compound of their houses in the village, and only 1.9 per cent had cattle sheds located in their fields.

Most of the cattle sheds (54.6 per cent) were of the *kutch* type, with a thatch roof. The most commonly used thatch material was pigeon pea straw. A plastic sheet was commonly used between layers of thatch to protect cattle during the rainy season. The usage of other roof materials like galvanized iron, stone and asbestos cement sheets was 39.8, 4.6 and 0.9 per cent, respectively. The roof height at the highest point was found to be 2.17 m, whereas that at the lowest point was found to be 1.60 m. The most common material used for supporting the roof was wood with 83.3 per cent of the farmers using it, whereas steel and stone columns were used by 13.9 and 2.8 per cent, respectively. Among the farmers who had *pucca* houses, most (83.7 per cent) did not make any provision for ventilators. Only 16.3 per cent had openings for ventilators in the wall, while none of the cattle sheds had ridge ventilation. Many other workers have reported the use of simple, thatched housing for Hallikar (Singh *et al.*, 2008), Khillar (Gokhale *et al.*, 2008) and draught cattle in Tamil Nadu (Akila and Chander, 2010). However, the lack of ventilators in a majority of the *pucca* sheds and the very low shed height was not desirable. The farmers justified these practices by claiming that they reduced the cost of construction and added greater stability to the structure. Further, they stated that the animals were housed in the shed only from sunset to sunrise, and spent their remaining time either tied in the compound or in the fields.

Stone walls (93.9 per cent) and unplastered (87.8 per cent) wall surfaces were most common in the *pucca* houses. Most of the farmers (86.1 per cent) did not construct basement in the cattle shed. Instead, they used a raised mud flooring of about 5-8 cm height to keep the shed floor dry. The incidence of use of dung, mud, stone and cemented flooring material was found to be 53.7, 37.0, 5.6 and 3.7 per cent, respectively. Though these factors were not conducive to hygienic rearing of cattle, the animals did not commonly suffer from parasitic diarrhoea or mastitis, possibly due to their greater innate immunity and the fact that the shed got adequate time to dry. However, there were cases of calves suffering from naval ill and respiratory infections. Rough cement flooring was not preferred, even though it was supported under the Mahatma Gandhi National Rural Employment Guarantee Act (MNREGA) scheme, as cattle were found to be uncomfortable and developed hair loss and bed sores on weight bearing parts. Drainage in the form of a mud channel was the most common (81.5 per cent) type. Stone drains and cemented drains were also found in 16.7 and 1.9 per cent of the livestock houses, respectively. The most common method of restraint of the cattle was found to be steel pegs in the ground, with 78.5 per cent of the farmers using it. Wooden logs and cement poles were also used by 12.5 and 9.0 per cent of the farmers, respectively.

The overall average shed area of livestock sheds of respondents having constructed sheds was found to be 11.08 sq. m, with Bhalki taluka having a higher average area of 11.16 sq. m as compared to Aurad taluka with 10.90 sq. m. In most cases, the size of the livestock shed was just enough for a cow, her calf and two bullocks to stand in. The limited shed size led to a very narrow feed manger (30.13 cm) and poor hygiene. In some instances, the livestock shed was also used to store agricultural equipment and crop residues. Many of the respondents (29.0 per cent) did not have any type of feed manger. Wooden logs, stone slabs, cement pipes and constructed feed mangers were seen with 38.0, 26.5, 0.5 and 6.0 per cent of the farmers, respectively. These factors, coupled with a

low feed manger height (13.12 cm) led to great losses of fodder by way of trampling and wastage.

Most of the cattle (88.5 per cent) were given water using a bucket. Only 10.5 per cent of the respondents used a basin made of steel or cement, and 1.0 per cent had a constructed water tank for watering the cattle. A majority of the respondents (87.0 per cent) were found to clean the livestock shed or tying area twice a day, whereas the rest did it once a day. The shed cleaning frequency and watering practices were found to be adequate.

From the present study, it was concluded that loose housing of the 'lean-to' type with *kutchra* shed, thatch roof, wooden supports, no ventilators, stone walls with unplastered surfaces and mud floor was the predominant design; though a large number of farmers did not provide any type of permanent housing. In view of the prevailing climatic conditions, low incidence of diseases and body condition of the animals, this pattern was found to be viable. However, the absence of a feed manger, or inadequate width and height of the feed manger, in most instances led to excessive loss of fodder by way of trampling and wastage. The absence of basement in the livestock shed was also not conducive to hygienic rearing of cattle.

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Production Performance of Deoni Cows in their Breeding Tract*

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ABSTRACT

Deoni cattle are uniquely suited to the agro-climatic conditions and cropping pattern followed by farmers in their breeding tract. The study was carried out by recording the performance of 82 Deoni cows in 10 villages spread over two talukas of Bidar district. The lactation milk yield, milk fat and milk SNF were estimated using the Test Interval Method (Interpolation Method) approved by the International Committee for Animal Recording. The mean lactation milk yield, peak yield, lactation length, milk fat and milk SNF were found to 881.35 kg, 4.19 kg, 253.66 days, 4.25 per cent and 9.02 per cent, respectively. The vast range in the performance traits indicated great potential for genetic improvement of the breed by using selective breeding techniques.

Key words: Deoni cattle, Karnataka, Performance, Milk yield, Lactation length

The livestock sector plays an important role in the socio-economic development of rural households in India. Livestock also reduce the seasonality in livelihood patterns and act as a buffer in times of crop failure (Bairwa *et al.*, 2013). Even today, draught bullocks are the predominant source of mobile power on about 60 per cent of cultivated area, consisting of about 85 million hectares (Karanjkar and Patil, 2008). The National Cattle Breeding Policy mandates the development of important indigenous dual-purpose breeds of cattle through selective breeding in their home tracts.

Sirohi and Saxena (2012) assessed the regional priorities for sustainable milk production in Karnataka and recommended the upgrading of non-descript stock with native cattle breeds like Deoni, which have a much higher production potential. Molecular genetic studies on targeted genes in relation to production, thermo-tolerance and disease resistance traits of Deoni cattle indicated that these animals have unique characteristics of adaptability with moderate production performance and disease resistance. Genetic characterization has also shown that there is a wide scope for genetic improvement of this native breed through selection based on quantitative trait loci information in combination with performance data (Das *et al.*, 2012).

A review of the available literature on the production performance of Deoni cattle revealed that most of these studies have been carried out in Government farms under controlled conditions. Very few studies have recorded the production performance of Deoni cattle under rural conditions of India, and there are no such reports from Bidar district, which is the only district of Karnataka having significant number of Deoni cattle. Given the priority to developing indigenous animal genetic resources in our country and the indispensable nature of field level data in the formulation of a breeding strategy for the improvement of the breed, this study was undertaken to record the production performance of Deoni cows under village conditions in their breeding tract.

MATERIALS AND METHODS

Sampling design: The present study was carried out in Bidar district of Karnataka. A multistage stratified sampling procedure was followed to select the talukas, villages and respondents. In the first stage of selection, out of the five talukas in Bidar district, Aurad and Bhalki talukas were selected for the study based on the larger population of Deoni cattle in these two regions as

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per the 2007 livestock census and consultations with officials of the Animal Husbandry department regarding the availability of purebred Deoni cattle. In the second stage of selection, ten villages were selected for the detailed survey, 3 from Aurad taluka and 7 from Bhalki taluka, based on the strength of Deoni cattle as per the 2007 livestock census, and giving due consideration to factors like availability of purebred Deoni cattle and road connectivity. In the third stage of selection, 20 Deoni cattle owners were selected at random from each village for recording the performance data of Deoni cows.

Data collection: The performance data of Deoni cows was recorded over a period of 17 months from December 2012 to April 2014. The selected farmers were given a calendar to note the dates of important events like estrus, natural service, artificial insemination, abortion, drying off and calving. This information was collected from them at monthly intervals. Incomplete data arising due to sale or death of animal was discarded for the purpose of performance recording. The various parameters studied were – lactation milk yield, peak yield, lactation length and milk composition.

The lactation milk yield was estimated using the Test Interval Method (Interpolation Method) approved by the International Committee for Animal Recording (2012). The lactation milk yield (MY), in kilograms, was calculated as per the following formula:

$$MY = I_d M_1 + \frac{I_1 (M_1 + M_2)}{2} + \frac{I_2 (M_2 + M_3)}{2} + \dots + \frac{I_{n-1} (M_{n-1} + M_n)}{2} + I_n M_n$$

where,

M_1, M_2, M_n are the weights, in kilograms, given to one decimal place, of the milk yielded in the 24 hours of the recording day,

I_1, I_2, I_n are the intervals, in days, between recording dates,

I_d is the interval, in days, between the lactation period start date and the first recording date, and

I_n is the interval, in days, between the last recording date and the end of the lactation period.

Approximately 100 ml of milk sample was collected in a sample bottle in proportion of milk yielded in the night and on the morning of recording for evaluation of milk constituents at monthly intervals. The milk sample was subjected to analysis of milk fat and SNF content using an electronic milk tester. The milk fat and SNF per cent were calculated as per the formula laid down by the International Committee for Animal Recording, which was similar to the procedure given for milk yield. Data was analysed using the data analysis tools in Microsoft Excel 2007 software. Summary statistics were calculated to identify the mean performance of various parameters of Deoni cattle.

RESULTS AND DISCUSSION

The production performance of Deoni cows and the range of various production parameters are presented in Table.

Table: Production Performance of Deoni cows

Parameter	N	Mean ± SE	Range
Lactation milk yield (kg.)	82	881.35±37.64	685 – 1187
Peak yield (kg)	88	4.19±0.10	3.1 – 10.8
Lactation length (days)	82	253.66±2.29	167 – 335
Milk fat (%)	82	4.25±0.11	4.0 – 4.6
Milk SNF (%)	82	9.02±0.06	8.6 – 9.3

Lactation milk yield: The mean lactation milk yield of Deoni cows, as calculated by the Test Interval Method specified by the International Committee for Animal Recording was found to be 881.35 kg. This was considerably lower than the reports of other workers – 1364 kg (Dasgupta, 1945), 1135 kg (Sastry and Thomas, 2005) and 1100 kg (Nagarcenkar *et al.*, 2012). However, it compared favourably with the reports of – 943 kg (Deshpande and Singh, 1977a), 868 lit. (Singh *et al.*, 2002), 779 kg (Das *et al.*, 2011), 911 kg (Das *et al.*, 2012), 820 kg (Basak *et al.*, 2013) and 911 kg (Kuralkar *et al.*, 2014). Birthal *et al.* (1999) estimated that at the existing level of technology and prevailing organisation of production, the current mean yield of Deoni cattle is about three-

fourths of the attainable yield of 880 kgs per lactation. In contrast, some scientists have reported very low lactation milk yield of Deoni cows – 518 kg (Thombre *et al.*, 2001), 239 kg (Chakravarthi *et al.*, 2002) and 544 kg (Kumar *et al.*, 2006).

The better performance of Deoni cows in villages as compared to some institutional farms could be explained by the fact that the herd sizes of Deoni cows in the villages was very small, not exceeding 2 cows in most cases, and it would be possible for the farmer to pay individual attention to the feeding and care of the cattle. Joshi and Singh (2005) suggested that the expected genetic improvement in well-defined dual-purpose cattle breeds through selective breeding would be 1 to 1.5 per cent per annum in herds at organized farms and 8 to 10 per cent per annum in farmers' herds. The vast range in the lactation milk yield from 685 to 1187 kg suggests high variation amongst the population with regard to this trait. Similar findings have been reported by Sastry and Thomas (2005) and the National Bureau of Animal Genetic Resources (2008). This indicates that there is great potential for genetic improvement of the breed using selective breeding techniques.

Peak yield: The mean peak yield of Deoni cows was found to be 4.19 kg, with a range of 3.1 to 10.8 kg. This was lower than the findings of Deshpande and Singh (1977a) and Kakde *et al.* (1980) who reported figures of 4.48 and 5.53-6.28 kg, respectively. However, it was well above the figure of 2.28 kg reported by Chakravarthi *et al.* (2002). The highest recorded peak yield of 10.8 kg in the present study indicates huge scope for improvement in the population. When seen in conjunction with the low lactation milk yield and short service period, it could be inferred that the cattle show low persistency in lactation due to inadequate nutrition during the milking phase.

Lactation length: The mean lactation length of the Deoni cow was found to be 253.66 days, with a range of 167 to 335 days. This was much lower than the figure of 293 days reported by Deshpande and Singh (1977b), but comparable to the value of 246 days reported by Kuralkar *et al.* (2014). However, various other scientists reported much

lower figures of – 149 days (Chakravarthi *et al.*, 2002), 186 days (Kumar *et al.*, 2006), 187 days (Das *et al.*, 2011), 206 days (Das *et al.*, 2012) and 195 days (Basak *et al.*, 2013). All these reports were of Deoni cows being reared outside their breeding tract, suggesting that the agro-climatic conditions of these areas may not have been suitable for the breed.

Milk composition: The mean milk fat content, as calculated by the Test Interval Method specified by the International Committee for Animal Recording was found to be 4.25 per cent, with a range of 4.0 to 4.6 per cent. Similar findings have been reported by Sontakke *et al.* (1978), Singh *et al.* (2002), National Bureau of Animal Genetic Resources (2008) and Kuralkar *et al.* (2014). This compares favourably with the milk of crossbred cattle, and can aid in obtaining better price. The mean SNF content was found to be 9.02 per cent, with a range of 8.6 to 9.3 per cent. This was lower than the value of 9.76 reported by Sontakke *et al.* (1978), but higher than the value of 8.43 reported by Kuralkar *et al.* (2014.)

From the present study, it was concluded that the production performance of Deoni cattle in their breeding tract was better than that in institutional farms. Further, the vast range in the performance traits indicates great potential for genetic improvement of the breed by using selective breeding techniques.

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Analysis of the Constraints to Deoni Cattle Production in Karnataka*

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ABSTRACT

The Deoni is the only viable dual-purpose breed of cattle in Karnataka. The study was carried out by evincing the responses of 200 respondents, spread over 10 villages in two talukas of Bidar district, to a structured schedule. The major categories of constraints to Deoni cattle production were ranked using the Garrett's Ranking technique and the detailed list of constraints were subjected to Likert's Scale analysis. The major constraint categories, in descending order of importance were issues relating to breeding, general problems, feeding, breed characteristics, marketing and health care with respective Garrett's scores of 72.52, 62.52, 60.97, 37.31, 34.77 and 33.93. Most of the constraints within the category of breed characteristics were found to be of minor importance, with only short lactation length being graded as moderate. Non-availability of good quality bulls, general disinterest in livestock rearing, and the high costs of natural service, crop residues and labour were the other major constraints to Deoni cattle production

Key words: Deoni cattle, Karnataka, Constraints

The Deoni is a medium-sized dual-purpose indigenous cattle breed which is predominantly found in Bidar district of Karnataka and Latur district of Maharashtra. This breed is also distributed in small numbers in the neighbouring districts of Gulbarga in Karnataka; Parbhani, Nanded and Osmanabad districts of Maharashtra, and some parts of Medak district of Andhra Pradesh (Das *et al.*, 2011). Maniam (1938) reported that Deoni cows were the best milkers in the Nizam's dominions. Deoni cattle have also previously been classified as a milch breed (Sastri and Thomas, 2005). The population of Deoni cattle in 1998 was estimated to be 1,18,945, with breedable females, breeding bulls and bullocks constituting 33.6, 1.5 and 41.1 per cent, respectively (Singh *et al.*, 2002). In view of the farmers' preference for low-input, organic farming, the suitability of Deoni cattle to the local agro-climatic conditions, and their ability to withstand drought, there is an urgent need for the genetic improvement of the breed and enhancement of its productivity. *In situ* conservation of this precious genetic resource is vital as the Deoni is the only viable dual-purpose cattle breed of Karnataka and

has immense potential for the development of high milk yielding crossbreds like the *Holdeo*. This study was undertaken to identify the major constraints to Deoni cattle production in Karnataka.

MATERIALS AND METHODS

Sampling design: The present study was carried out in Bidar district of Karnataka. A multistage stratified sampling procedure was followed to select the talukas, villages and respondents. In the first stage of selection, out of the five talukas in Bidar district, Aurad and Bhalki talukas were selected for the study based on the larger population of Deoni cattle in these two regions as per the 2007 livestock census and consultations with officials of the Animal Husbandry department regarding the availability of purebred Deoni cattle. In the second stage of selection, ten villages were selected for the detailed survey, 3 from Aurad taluka and 7 from Bhalki taluka, based on the strength of Deoni cattle as per the 2007 livestock census, and giving due consideration to factors like availability of purebred Deoni cattle and road connectivity. In the third stage of selection, 20 Deoni cattle owners were selected at random from

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each village for identification of the constraints faced by Deoni cattle farmers, thus giving a total of 200 respondents.

Data collection: A structured schedule was developed for collection of information on the constraints faced in Deoni cattle production. The schedule was designed in consultation with staff of the Department of Animal Husbandry and Veterinary Services, Government of Karnataka, who had served in the study area for many years. The schedule was then pre-tested under existing field conditions before finalization. The respondents were interviewed personally for the collection of desired information, with questions being asked in the language in which they were most fluent.

Garrett’s Ranking technique: The six major categories of constraints faced by the sample respondents were prioritized by using Garrett’s ranking technique (Garrett and Woodworth, 1971). The respondents were asked to assign ranks from 1 to 6 to the six major categories of constraints - breed characteristics, breeding, feeding, health care, marketing and general; in the order of importance, with rank 1 for the most important constraint and rank 6 for the least important constraint. In the next stage, rank assigned to each constraint was converted into per cent position using the following formula:

$$\text{Per cent position} = \frac{100 (R_{ij} - 0.5)}{N_j}$$

where,

R_{ij} is the rank given for the ith variable by the jth respondent, and

N_j is the number of variables ranked for the jth respondent.

The per cent position was converted into scores by referring to the Garrett’s tables. The scores for each constraint were summed over the number of respondents who ranked that factor. In this way, total scores were arrived at for each of the six constraints and mean scores were calculated by dividing the total score by the number of respondents who gave ranks. The final overall ranking of the six major constraints was done by assigning rank 1 to the constraint with the highest mean score, and so on.

Likert’s Scale technique: The other constraints enlisted within the major categories were analysed

using the Likert’s Scale technique (Likert, 1932). The respondents were asked to reply to each item within the list of constraints and grade them as either major, moderate, minor or nil, depending on their importance. These responses were assigned numerical values, with major, moderate, minor and nil being assigned scores of 3, 2, 1 and 0, respectively. The frequency analysis of responses for each item was carried out and modal value calculated to grade the constraint. The total score for each constraint was arrived at by summing the numerical values of all the respondents for that constraint. The final ranking of each constraint within its major category was done by assigning rank 1 to the constraint with the highest score, and so on.

RESULTS AND DISCUSSION

The mean Garrett’s scores and ranks of the major categories of constraints in Deoni cattle production are presented in Table 1. The distribution of responses, modal values and ranks of constraints in Deoni cattle production as per the Likert Scale analysis are presented in Table 2.

Table 1: Mean Garrett’s scores and ranks of the major categories of constraints in Deoni cattle production

Constraint	Garrett’s score		Rank
	Total	Mean	
Breed characteristics	7461	37.31	4
Breeding	14503	72.52	1
Feeding	12193	60.97	3
Health care	6785	33.93	6
Marketing	6954	34.77	5
General	12504	62.52	2

Table 2: Distribution of responses, modal values and ranks of the constraints in Deoni cattle production (as per Likert’s Scale technique)

Constraint	Responses (%)				Rank
	Major	Moderate	Minor	Nil	
Breed characteristics					
Calf mortality	0.0	4.0	84.5	11.5	5
Delayed maturity	3.5	6.5	66.0	24.0	7
Reproductive problems	5.5	8.5	79.0	7.0	4
Low milk yield	12.5	15.5	53.5	18.5	2
Short lactation length	10.5	41.0	34.5	14.0	1
Low milk fat	2.0	13.0	59.5	25.5	6
Susceptibility to disease	9.0	18.5	57.0	15.5	3

Breeding					
Ambiguity regarding breed definition	24.0	38.5	27.0	10.5	5
Non-availability of good quality bulls	44.5	32.0	14.0	9.5	3
High prices for natural service	42.5	33.5	20.5	3.5	1
Spread of reproductive diseases via NS	3.0	13.5	67.0	16.5	7
Distant AI facilities	30.5	39.0	10.5	20.0	4
Higher repeat breeding with AI	35.5	41.5	23.0	0.0	2
Difficulty in detecting estrus	9.5	31.0	40.5	19.0	6
Feeding					
Poor community grazing lands	13.5	14.0	5.5	67.0	6
High cost of fodder production	32.0	43.5	14.0	10.5	3
Shortage of green fodder	15.5	30.5	36.5	17.5	5
High cost of crop residues	47.5	27.0	13.5	12.0	1
High cost of concentrate feeds	23.0	36.5	25.5	15.0	4
Acute fodder shortage during drought	32.5	44.0	16.0	7.5	2
Health care					
Distant veterinary facilities	27.5	34.0	12.0	26.5	2
High cost of treatment	23.5	39.5	23.0	14.0	1
Inadequate vaccination	18.0	20.5	40.5	21.0	4
Spread of infectious diseases	20.5	25.0	37.0	17.5	3
Marketing					
Low price for milk	18.5	25.5	35.0	21.0	1
Inaccurate milk pricing	11.0	18.0	38.5	32.5	3
Infrequent milk payment	6.5	11.5	27.5	54.5	4
Cheating in livestock markets	15.0	22.5	49.5	13.0	2

General					
Disinterest in livestock rearing	34.0	24.5	28.5	13.0	1
High labour costs	39.5	21.0	9.0	30.5	2
Inadequate training	10.5	19.0	30.5	40.0	4
Difficulty in obtaining finance	26.0	14.5	38.0	21.5	3

Note: Modal values are in bold font.

The most important constraint to Deoni cattle production was found to be breeding management, with an overall rank of 1 and mean Garrett score of 72.52. This was primarily due to the fact that Government veterinary institutions were not having adequate stock of Deoni semen, thus leaving the farmers at the mercy of private bull owners. Most of the respondents felt that the non-availability of good quality bulls and the high prices for natural service were major constraints. This was also evident from the fact that ambiguity regarding the definition of Deoni breed was felt to be a moderate constraint. Artificial insemination in Deoni cattle was not viewed favourably by the respondents and many of them identified higher repeat breeding with AI and distant AI facilities as moderate constraints. Difficulty in detecting estrus was also felt to be a minor constraint by the respondents. None of the other workers who studied constraints faced by Deoni cattle farmers (Mande and Thombre, 2009; Thombre *et al.*, 2010) have reported similar findings. This could be due to the fact that they have conducted their studies in adjacent districts of Maharashtra state, which have better quality Deoni bulls.

Another important category of constraints was found to be the general problems associated with Deoni cattle rearing, with an overall rank of 2 and mean Garrett score of 62.52. High labour costs and disinterest in livestock rearing were identified as major constraints, while difficulty in obtaining finance was identified as a minor constraint. The high labour costs were an important factor for medium and large farmers, who were dependent on hired labour to rear their cattle. A general disinterest towards livestock rearing and shift towards short-duration cash crops was also evident. Most of the farmers did not feel that lack of training

was a constraint to Deoni cattle production. Similar findings have been reported by Thombre *et al.* (2010) and Kathiravan and Selvam (2011).

Problems in the feeding of Deoni cattle was another important constraint, with an overall rank of 3 and mean Garrett score of 60.97. The high cost of crop residues was identified as a major constraint, while high cost of fodder production, high cost of concentrate feeds and acute fodder shortage during drought were found to be moderate constraints. Shortage of green fodder was a minor constraint, while poor quality of community grazing lands was not felt to be a constraint to Deoni cattle production. The shortage of crop residues was primarily due to the shift in the *kharif* season cropping pattern from a combination of hybrid *jowar* and pigeon pea to cash crops, especially soyabean. Many of the farmers were reluctant to spare agricultural land for fodder production, and preferred to buy sorghum stover and other crop residues in case of fodder shortage. This situation was further exacerbated during periods of drought. Very few of the respondents were dependent on community grazing lands for the feed requirements of their cattle. Even landless agricultural labourers grazed their cattle on the farms where they were working. Other workers (Mande and Thombre, 2009; Thombre *et al.*, 2010; Kathiravan and Selvam, 2011; Kurup, 2001, Rathod *et al.*, 2012) have also made similar observations regarding the high prices of concentrate feed and fodder.

The breed characteristics of Deoni cattle had an overall rank of 4 and mean Garrett score of 37.31. Among the breed characteristics, short lactation length was found to be a moderate constraint, while all other factors like calf mortality, delayed maturity, reproductive problems, low milk yield, low milk fat, and susceptibility to disease were minor constraints. These indicate that farmers were generally satisfied with the performance of Deoni cattle in terms of production, reproduction and disease resistance. As most farmers reared Deoni cows for multiple benefits like milk, calf and manure, and the milk of Deoni cows was preferred for domestic consumption, it is evident that the breed is integrated into their farming system, and

performs well within the available conditions. In contrast, Patil *et al.* (2009) and Kathiravan and Selvam (2011) reported that the low productivity of *desi* cows was the most important constraint faced by livestock farmers.

The marketing problems in Deoni cattle had an overall rank of 5 and mean Garrett score of 34.77. As none of the villages surveyed had any cooperative milk collection centre, most of the milk produced was consumed by the farmers themselves, and frequency of milk payment was not a constraint. However, low price for milk and inaccurate milk pricing were found to be minor constraints. Cheating in livestock markets was also a minor constraint. Farmers were found to frequent nearby cattle markets to buy and sell year-old calves, under-producing cows, bullocks and replacement bulls. Similar findings have been reported by Mande and Thombre (2009), Thombre *et al.* (2010), Kathiravan and Selvam (2011) and Rathod *et al.* (2012). In contrast, Patil *et al.* (2009) reported delay in milk payments to be a major constraint.

The problems in health care management of Deoni cattle were ranked last and had an overall mean Garrett score of 33.93. This could be due to the innate resistance of Deoni cattle to prevailing diseases, thus causing the respondents to identify spread of infectious diseases and inadequate vaccination as minor constraints. Distant veterinary facilities were identified as a moderate constraint. This could be due to the fact that five of the selected villages had Government veterinary institutions, and another four were located within 5 km of Government veterinary institutions. Given the shortage of supply of drugs in veterinary hospitals, high cost of veterinary treatment was also found to be a moderate constraint. Similar findings were reported by Patil *et al.* (2009), Thombre *et al.* (2010), Kathiravan and Selvam (2011) and Kurup (2001).

From the present study, it was concluded that Deoni cattle were well-suited for the agro-climatic conditions and cropping practices followed in the region, with low lactation length being a moderate constraint. Non-availability of good

quality bulls, general disinterest in livestock rearing, and the high costs of natural service, crop residues and labour were the other major constraints to Deoni cattle production.

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A Study on the Credibility of Information Sources Perceived by Dairy Farmers of Punjab

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ABSTRACT

Dairy farming is a large and dynamic segment of agricultural economy of India. Various new technologies are generated day by day by researchers in the field of dairying. Importance of improved technology for dairy farmers to a large extent depends on the effective sources of information and channels to which they are generally exposed directly or indirectly. The present study was conducted to know the credibility of different information sources, viz. mass media sources, institutional sources, non-institutional sources and extension activities by the dairy farmers of Punjab for perceiving knowledge about animal husbandry practices. For this purpose, 250 dairy farmers were randomly selected to obtain information through pre-structured schedule. The study revealed that Internet, Veterinary university, family members and *pashu palan mela* were playing important role in transfer of dairy technologies among dairy farmers and found to be highly credible sources among different categories of information sources. It is important to note that scientists of the university were perceived as much the credible source of information by dairy farmers.

Keywords: Information Sources, Credibility, Utilization, Animal Husbandry Practices

In today's world there is explosion of information in every field including agriculture and allied sectors like dairying. Growth of nation and rural person is possible if such information is efficiently transferred to target group. Transfer of new technologies to the markets is essential for the farmers to access better information. However an information gap exists between the various sources of agricultural information and their potential recipients (Nair, 2006). Often there is emphasis on information gap based on social, economic and cultural characteristics of the recipients. Other factors accounting for the existing information gap include language, literacy, education, physical location, economic and socio-cultural factors (Wessler and Brinkman, 2003). Almost every aspect of dairy farming requires a substantial exchange of information, communication, knowledge and skill transfer (McNamara, 2008). Information may come to farmers from various sources. They may be personal, impersonal, institutional/non-institutional, localite-cosmopolite or mass-media in nature. The appropriateness of these sources varies from enterprise to enterprise, situation to situation and from time to time. Further, the credibility of information sources also varies with respect to their competency and trustworthiness. Hence, it becomes quite important

to channelize the right information at the right time through the right channel, for which knowledge of different information sources are consulted and used by farmers. So it is very much necessary to identify different information sources and channels of animal husbandry information available to the dairy farmers and to locate the most credible sources and channels so as to develop a suitable communication strategy.

MATERIALS AND METHODS

The study was conducted in the State of Punjab, India. For the study, 250 dairy farmers with four or more than four dairy animals were randomly selected from the farmers visiting to veterinary clinic of GADVASU attending various training programmes, animal welfare camps, *pashu palan mela* (animal fair). The respondents were categorized as small (4-15 animals), medium (16-45 animals) and large (>45 animals) on the basis of herd size. The credibility of different information sources measured through response categories as highly credible, credible and not credible giving score 2 for highly credible, 1 for credible and 0 for non-credible. The data was collected through personal interview on the basis of structured interview schedule. The data was statistically analyzed with the help of mean score, percentage, Z test and ANOVA.

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RESULTS AND DISCUSSION

Socio-economic Characteristics of Respondents:

Personal profile of the respondents in Table 1 showed that most of the dairy farmers (39.60%) belonged to middle age group, i.e. age of 31-40 years. Among them maximum (55.76%) were large farmers and minimum (31.62%) were small farmers. Dwivedi (1993) also reported that majority of livestock owners (58.12%) belonged to middle age group. Majority (27.00%) of dairy farmers had education level up to higher secondary and only 4.80% farmers had their education up to graduation. Maximum (15.38%) large farmers and minimum (1.71%) small farmers were educated up to graduation. These results were in line with the findings of Rajak (2005) and Sharma (2004). Most of the dairy farmers (54.40%) lived in joint family. Majority (45.60%) of dairy farmers was having medium family size. Major portion (59.20%) of the dairy farmers were having dairy as their main occupation while 40.80% of dairy farmers were having dairy as their secondary occupation.

Credibility of Information Sources as Perceived by the Dairy Farmers:

The data presented in Table 2 revealed that mean credibility score of information sources perceived by dairy farmers. Among mass media sources the mean score (1.39±0.04) of credibility for 'Internet' was found to be maximum by dairy farmers. Significant difference has been observed in between small, medium and large dairy farmers in relation to their credibility for Internet. The mean level (1.28±0.03) of credibility for 'Veterinary university' was found to be higher among all institutional sources as compared to other sources. This is attributed to high intensity of influence and specialization of scientist of university in the specific subject. Our findings are similar in line with Singh (1998) and Sharma *et al* (2008). A significant difference has been found among small, medium and large dairy farmers with respect to their credibility for Veterinary university and *Krishi Vigyan Kendra* (Farm Science Center). Among all non-institutional sources, the mean score (0.83±0.04) for credibility of 'family members' was

Table 1. Personal Profile of the Respondent

Socio-personal characteristics		Category-wise number (N) of farmers			Total (N=250)
		Small (n=117)	Medium (n=81)	Large (n=52)	
Age (years)	≤30	50 (42.73)	34 (42.00)	10 (19.23)	94 (37.60)
	31-40	37 (31.62)	33 (40.74)	29 (55.76)	99 (39.60)
	>40	30 (25.64)	14 (17.28)	13 (25.00)	57 (22.80)
	Mean±SE	35.37±1.15	32.40±0.98	36.21±1.00	34.58±0.66
Education	Illiterates	30 (25.64)	16 (19.75)	6 (11.53)	52 (20.80)
	Primary	15 (12.82)	7 (8.64)	3 (5.77)	25 (10.00)
	Middle	23 (19.65)	10 (12.34)	5 (9.62)	38 (15.20)
	High school	17 (14.52)	24 (29.62)	14 (26.92)	55 (22.00)
	Higher secondary	30 (25.64)	22 (27.16)	16 (30.75)	68 (27.00)
	Graduation	2 (1.709)	2 (2.47)	8 (15.38)	12 (4.80)
Family type	Nuclear	51 (43.59)	33 (40.74)	30 (57.69)	114 (45.60)
	Joint	66 (56.41)	48 (59.26)	22 (42.31)	136 (54.40)
Family size	Small (≤4)	19 (16.24)	11 (13.58)	14 (26.92)	44 (17.60)
	Medium (5-8)	53 (45.30)	37 (45.68)	24 (46.15)	114 (45.60)
	Large (>8)	45 (38.46)	33 (40.74)	14 (26.92)	92 (36.80)
	Mean±SE	7.96±0.32	9.09±0.45	7.42±0.49	8.21±0.24
Occupation	Dairy as main occupation	65 (55.56)	47 (58.02)	36 (69.23)	14 (59.20)
	Dairy as secondary occupation	52 (44.44)	34 (41.98)	16 (30.77)	102 (40.80)

high than other source which might be due to large faith and trust of dairy farmers with their family. Gunawardana and Sharma (2006) and Hai *et al* (2003) also reported similar findings. Among all extension activities the mean score (1.57 ± 0.02) for credibility of 'pashu palan mela' was high by dairy farmers. This might be because it is most popular in Punjab, since it is organized twice in a year in all regional stations of Punjab. Singh (1998) also reported the same.

Internet, veterinary university, family members and pashu palan mela by the dairy farmers of Punjab. Credibility for non-institutional sources was found to be higher by small farmers as compared to medium and large farmers. Credibility for Internet was found to be higher by large farmers followed by medium and small farmers.

Table 2. Mean (\pm SE) level of credibility for different information sources perceived by dairy farmers

Information sources		Small	Medium	Large	Total	F-ratio
Mass media	Radio	0.50 \pm 0.04	0.48 \pm 0.05	0.42 \pm 0.05	0.48 \pm 0.02	0.98
	TV	1.05 \pm 0.06	1.20 \pm 0.06	1.15 \pm 0.05	1.12 \pm 0.03	1.53
	Newspapers	1.09 \pm 0.09	1.02 \pm 0.09	0.93 \pm 0.10	1.03 \pm 0.05	1.13
	Magazines	0.19 \pm 0.04	0.34 \pm 0.03	0.36 \pm 0.03	0.28 \pm 0.01	0.87
	Internet	1.14 \pm 0.06	1.47 \pm 0.07	1.54 \pm 0.06	1.39 \pm 0.04	3.54*
Institutional sources	Veterinary university	1.41 \pm 0.05	1.26 \pm 0.05	1.06 \pm 0.06	1.28 \pm 0.03	4.90*
	Krishi Vigyan Kendra	1.26 \pm 0.06	1.06 \pm 0.06	1.00 \pm 0.07	1.13 \pm 0.03	3.19*
	Dairy Development Department	0.87 \pm 0.09	0.98 \pm 0.10	0.87 \pm 0.13	0.91 \pm 0.06	0.78
	Dairy Co-operatives	1.18 \pm 0.04	1.24 \pm 0.05	1.38 \pm 0.05	1.25 \pm 0.03	1.28
Non-institutional sources	Friends	0.84 \pm 0.09	0.73 \pm 0.11	0.64 \pm 0.13	0.76 \pm 0.06	2.07
	Relatives	0.96 \pm 0.17	0.77 \pm 0.19	0.66 \pm 0.21	0.79 \pm 0.11	1.57
	Neighbors	0.84 \pm 0.14	0.76 \pm 0.17	0.69 \pm 0.24	0.78 \pm 0.10	0.78
	Family members	0.89 \pm 0.06	0.83 \pm 0.08	0.73 \pm 0.07	0.83 \pm 0.04	1.89
Extension activities	Training	0.75 \pm 0.10	1.01 \pm 0.09	1.30 \pm 0.10	0.97 \pm 0.05	1.18
	Livestock shows	1.20 \pm 0.08	1.32 \pm 0.10	1.30 \pm 0.11	1.26 \pm 0.06	1.23
	Animal Welfare Camps	1.33 \pm 0.10	1.36 \pm 0.11	1.51 \pm 0.10	1.38 \pm 0.06	2.09
	Pashu Palan Mela	1.51 \pm 0.04	1.59 \pm 0.03	1.65 \pm 0.03	1.57 \pm 0.02	1.98
	Lectures	1.37 \pm 0.09	1.38 \pm 0.10	1.52 \pm 0.11	1.41 \pm 0.06	1.19

CONCLUSION

Based on the findings it is concluded that the overall credibility score for veterinary university was found to be higher by dairy farmers of Punjab followed by Internet and KVK. Among different categories of information sources, viz. mass media, institutional, non-institutional and extension activities maximum credibility was observed for

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A Study on the Toxin Binding Efficacy of High Grade Bentonite in Broiler Chicken Diets*

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ABSTRACT

A study to evaluate the ameliorating effect of high grade bentonite on broilers induced with OchratoxinA (OA), T-2 toxin (T-2) and their combinations was carried out. Three hundred and thirty six day-old Cobb broiler chicks were obtained from a commercial hatchery and dietary levels of Ochratoxin (1.0 ppm), T-2 toxin (2 ppm) and High-grade Bentonite at 1.00 percent were tested in a completely randomized design manner. At 5 wk, six birds from each treatment were sacrificed and liver, kidney, gizzard, pancreas, spleen, bursa of Fabricius and thymus were extracted and weighed. The serum samples were analyzed for total proteins, uric acid, serum albumin, serum globulin and the activities of gamma glutamyltransferase (GGT), alanine amino transferase (ALT). Supplementation of 1.0 per cent High grade Bentonite to OA, and OA plus T-2 toxin containing diets did not show significant improvement in body weight at all ages, but with T-2 alone fed group showed significant ($P < 0.05$) improvement in body weight till fourth week of age. However, high grade bentonite supplementation at 1% did not improve cumulative feed consumption, feed conversion ratio, mortality percentage, relative weights of lymphoid organs, serum biochemistry, serum proteins as compared to toxin fed group. It is concluded that high grade bentonite was not effective in counteracting the effect of toxins in broiler chicken diets.

Keywords: Ochratoxin, T-2 toxin, High grade bentonite, Broilers.

It is estimated that as much as 25 percent of the world's cereals are contaminated with known mycotoxins while a higher percentage could be contaminated with mycotoxins as yet unidentified. No region of the world escapes from these silent killers and their negative impact on animal productivity and human health is enormous.

Fungi belonging to genera *Aspergillus*, *Fusarium* and *Penicillia* mainly produce these mycotoxins. Warmer climatic zones tend to succumb to aflatoxins and fumonisins, while cooler areas with higher moisture are subjected to ochratoxin, T-2 toxin, zearalenone, vomitoxin, etc.

Among Aflatoxins (AF), AFB₁ is the most potent hepatotoxin having carcinogenic and severe performance depressive effects on poultry. Aflatoxin ingestion leads to aflatoxicosis, which is a chronic or acute condition characterized by growth reduction, bruising, decreased feed consumption and feed conversion efficiency, immunosuppression and mortality. The major adverse effects of aflatoxin lie in its residual toxic

effect in food products. The AF contamination of feedstuffs has been reported to range from 10 to 1500 ppb in commonly used ingredients and 34 to 115 ppb in mixed feed samples in developing countries (Devegowda and Aravind, 2003).

Many methods such as use of microbial inactivation, irradiation, mold inhibitors, fermentation, physical separation, thermal inactivation, ammoniation, ozone degradation and sequestering agents have been tried for decontamination and remediation of contaminated feedstuffs. Unfortunately, most of these measures are costly, time consuming and only partially effective. The practical implementation and standardization of these approaches were found to be difficult.

One of the most encouraging approaches is the addition of non-nutritive adsorbent materials to contaminated feed in order to selectively bind the mycotoxin during the digestive process and make it harmless to the feed. The major advantages of adsorbents include low cost, safety and the ease

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with which they can be added to animal feeds (Anjum *et.al.*, 2011). Layered aluminosilicates such as bentonite are already available commercially for the detoxification of AF contaminated feedstuffs.

Ashafeed, a natural raw material produced from high grade bentonite is claimed to possess high adsorption capacity due to high surface area. However, ability of bentonite to bind mycotoxins depends on p^H , molecular arrangements and its geographic region of origin (Diaz *et.al.*, 2009). Because the binding spectra of these substances are quite broad and nonspecific, nutritional components such as vitamins may also be removed.

Hence, the present study was conducted to evaluate the ability of graded levels of High-grade bentonite (ASHAFEED) in countering the adverse effect of ochratoxin, T-2 toxin and combined ochratoxin and T-2 toxin in broilers.

MATERIALS AND METHODS

Experimental Animals and Design: A total of 336-day-old commercial broiler chicks (Vencobb), were procured from a local hatchery, divided at random into 24 replicates of 14 each having equal number of males and females and three such replicates were allotted to one of 8 dietary treatments. Dietary levels of ochratoxin (1.0 ppm), T-2 toxin (2 ppm) with or without High-grade bentonite at 1.00 percent were tested in a completely randomized design manner, forming a total of 8 dietary treatments each with three replicates. Each replicate group of chicks housed in an independent pen, conventional deep litter house. A basal diet (T_0) was used to formulate seven other experimental diets to contain ochratoxin (T_2), T-2 toxin (T_3) or ochratoxin + T-2 Toxin (T_6) or by supplementation of 1% HGB to the diet T_0 . T_2 , T_3 & T_6 to contribute dietary treatment of T_1 , T_4 , T_5 and T_7 respectively. Chicks in all the replicates were reared up to five weeks of age under uniform standard conditions throughout the study. Brooding was done till three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided continuous light throughout the study.

Toxin Production: OA and T-2 were produced using pure culture of *Aspergillusochraceus* MTCC 4643 and *Fusariumsporotrichoides* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India) grown on Czapek's agar and oatmeal agar, respectively. Then OA and T-2 toxin were extracted as described by (Rukmini and Bhat, 1978; Romer, 1978) and quantified by thin layer chromatography (TLC) as described by AOAC (1995).

Diet formulation: Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-3 wks) (2895 kcal/kg ME and 20.84% CP) and finisher (4-5 wks) (2994 kcal/kg ME & 18.58% CP) feed as per BIS standards. Chicks were provided *ad libitum* feed and water throughout the study. To this basal diet, required quantities of contaminated culture materials were added to attain dietary levels of 1.0 ppm of ochratoxin and 2.0 ppm of T-2 toxin to each of the toxin containing diets, High-grade bentonite at 1.00% was added to prepare the different experimental diets. Compounded experimental diets were analyzed for mycotoxin content by TLC method. Feeding of test diets commenced at first day of age and continued till the termination of experiment at five weeks of age. Chicks were vaccinated against Newcastle Disease (ND) on 7th day using F1 strain and against Infectious Bursal Disease (IBD) on 14th day using intermediate strain (Ventri's Biologicals, Bangalore, India).

Data Collection: Body weight and feed intake were recorded weekly. At the end of the trial, blood was collected in non-heparinized tubes from six birds in each treatment (3 males and 3 females) by puncturing the brachial vein during 5th week of age. Serum was separated after 8 to 10 hours as per the standard procedures of Calnek *et.al.*, (1992) and was stored at $-20^{\circ}C$ for subsequent analysis. The individual serum samples were analyzed for total protein, serum albumin, uric acid and the activities of gamma glutamyltransferase (GGT) and alanine amino transferase (ALT) using automatic analyzer (BoehringerMannheim Hitachi 704 automatic analyzer, Japan). The three birds were sacrificed from each replicates as per the standard

procedures and after due permission from the Institutional Animal Ethics committee and liver, kidney, gizzard, spleen, bursa of Fabricius and thymus were extracted and weighed. The weights were adjusted to one kg live weight and the treatment means were calculated.

Statistical Analysis: The experimental data were subjected to one way ANOVA test by using the General Linear Model procedure of Statistical Analysis System (SAS, 2006). Duncan multiple range test was employed for comparison of the means (Duncan, 1955).

RESULTS AND DISCUSSION

Body weight: Compared with control, body weights were significantly ($P < 0.05$) lower beginning with first week for OA and OA plus T-2

addition of 1.0 per cent HGB to diet containing OA, T-2 or OA plus T-2 showed no significant improvement in body weights when compared to their respective unsupplemented groups. The inability of HGB to protect chicks from growth depressing effects of OA, T-2 toxin alone and in combination could be attributed to the inability of HGB to effectively access binding sites within the inter layer of the clay. Further, it has been shown that a variety of functional property of clay i.e. cation exchange capacity, surface density, surface properties are critical for the immobilization of diverse ligands. The results agree with previous reports of Devi *et.al.*, (2000) where Bentonite failed to ameliorate the adverse effects of OA on body weights in young chicks.

Table 1: Effect of high grade bentonite (HGB) on body weight (g) of broiler chickens (Mean ± SE)

Treat	Experimental Diets		Week				
	Mycotoxin	HGB (1%)	I	II	III	IV	V
T0	-	-	135.20±0.34 ^a	314.±2.17 ^{ab}	615.6±2.22 ^a	1013±1.94 ^a	1320±4.73 ^a
T1	-	+	136.40±2.71 ^a	320.4±1.50 ^a	617.9±3.56 ^a	1008±4.27 ^a	1327±6.00 ^a
T2	OA	-	80.40±1.10 ^{bc}	111.9±3.28 ^c	231.5±3.54 ^c	446.4±3.72 ^c	562.2±4.15 ^c
T3	T-2	-	131.0±1.36 ^a	304.3±0.74 ^b	582.9±4.24 ^b	981.3±5.22 ^b	1282±3.69 ^b
T4	OA	+	86.23±1.44 ^b	118.9±2.96 ^c	237.1±3.53 ^c	432.5±3.44 ^c	610±5.10 ^c
T5	T-2	+	133.10±2.54 ^a	306.5±1.48 ^b	608.7±2.45 ^a	996.6±6.05 ^{ab}	1290±6.17 ^b
T6	OA +T-2	-	75.37±1.87 ^c	98.33±3.66 ^d	193.6±2.25 ^d	321.5±2.28 ^d	491.2±2.28 ^d
T7	OA +T-2	+	78.23±0.61 ^{bc}	97.47±2.02 ^d	194.2±2.06 ^d	318.6±2.87 ^d	493±3.60 ^d

OA = Ochratoxin (1 ppm); T-2 = T-2 toxin (2 ppm)

Means within each column bearing common superscripts do not differ significantly ($P < 0.05$)

treatments, during third week for T-2 treatment and this reduction in body weights continued throughout the study. The lowest body weight was seen in group fed with combination of OA and T-2 toxin, whereas highest body weight was recorded in control diet. Feeding OA and T-2 toxin in combination to chicks caused a significantly ($P < 0.05$) lower reduction of body weight gain than either of the toxins alone during the study which is consistent with earlier reports (Manegar *et.al.*, 2010 and Mujahid *et.al.*, 2012). The addition of 1.0 per cent HGB to control diet (T₁) did not show any significant effect on body weight gain as compared to unsupplemented control diet (T₀). The

Feed conversion ratio: Ochratoxin and T-2 toxin alone and in combination showed significant ($P < 0.05$) reduction in feed efficiency values as compared to the control in all age groups. The poorest feed efficiency was seen in group fed with combination of OA and T-2 toxin, whereas highest feed efficiency value was recorded in control diet. Poor feed conversion efficiency noted with OA, T-2 and with OA+T-2 combination at all ages seems to have been mediated through decreased nutrient utilization, reduced amino acid, dry matter digestibility and energy utilization in broilers (Wafaa *et.al.*, 2013). The results of present study confirmed the observations made by several

workers (Mujahid *et.al.*, 2012 and Santin *et.al.*, 2003) for OA and T-2 toxin (Diaz *et.al.*, 2009). Addition of 1.0 per cent HGB to control diet showed no significant improvement in feed conversion ratio as compared to control group. Supplementation of 1.0 per cent HGB to diets containing OA, T-2 toxin alone and OA plus T-2 combination showed no significant improvement in feed conversion ratio values as compared to their respective toxin groups. However, the FCR values of T-2 and 1.0 percent HGB combinations are comparable with that of control diet. The results of present finding on effect of Bentonite on feed efficiency during aflatoxicosis are comparable to earlier observations of Devi *et.al.*, (2000).

Organ weights: The weight of liver, kidney and gizzard was significantly ($P<0.05$) increased in the groups receiving Ochratoxin A or T-2 toxin alone or in combination, whereas the weight of pancreas was not significantly different among treatments. These findings agree with the results of Verma *et.al.*, (2004) and Awad *et.al.*, (2005). The supplementation of 1.0 per cent HGB to control diet and to diets containing OA and T-2 toxin alone or in combination did not bring about any reduction in weights of liver, kidney, pancreas and gizzard as compared to their respective toxin control groups and groups fed with control diet.

Weights of lymphoid organs: Compared with those of control group, the relative weight of thymus was significantly ($P<0.05$) decreased in the

Table 2: Effect of high grade bentonite (HGB) on organ weights (g/ kg live wt.) of broiler chickens (Mean \pm SE)

Treat	Mycotoxin	HGB (1%)	Liver	Kidney	Pancreas	Gizzard	Spleen	Bursa	Thymus
T0	-	-	27.0 \pm 0.57 ^c	8.0 \pm 0.56 ^c	4.66 \pm 0.33	23.33 \pm 0.33 ^d	1.33 \pm 0.16	2.0 \pm 0.28 ^a	3.66 \pm 0.33 ^a
T1	-	+	27.33 \pm 0.33 ^{bc}	8.0 \pm 0.58 ^c	4.33 \pm 0.33	23.0 \pm 0.57 ^c	1.66 \pm 0.16	1.17 \pm 0.28 ^a	3.66 \pm 0.33 ^a
T2	OA	-	30.67 \pm 0.66 ^a	11.33 \pm 0.66 ^{ab}	3.66 \pm 0.33	24.67 \pm 0.88 ^{ab}	1.83 \pm 0.16	1.16 \pm 0.16 ^{ab}	2.16 \pm 0.44 ^b
T3	T-2	-	28.0 \pm 0.33 ^{ab}	10.0 \pm 0.57 ^{ab}	4.66 \pm 0.33	26.0 \pm 0.57 ^{abc}	1.83 \pm 0.16	1.33 \pm 0.16 ^{ab}	1.83 \pm 0.16 ^b
T4	OA	+	28.67 \pm 0.88 ^a	10.33 \pm 0.33 ^{ab}	3.66 \pm 0.33	24.67 \pm 0.33 ^{ab}	1.66 \pm 0.16	1.17 \pm 0.28 ^{ab}	2.0 \pm 0.28 ^b
T5	T-2	+	27.67 \pm 0.33 ^{abc}	9.33 \pm 0.33 ^{bc}	4.33 \pm 0.33	25.33 \pm 0.57 ^{bcd}	1.33 \pm 0.16	1.66 \pm 0.16 ^{ab}	2.66 \pm 0.16 ^{ab}
T6	OA +T-2	-	30.67 \pm 0.66 ^a	11.17 \pm 0.16 ^{ab}	3.33 \pm 0.33	27.0 \pm 1.0 ^{ab}	1.83 \pm 0.16	0.833 \pm 0.16 ^b	1.83 \pm 0.16 ^b
T7	OA +T-2	+	30.67 \pm 0.66 ^a	11.33 \pm 0.16 ^a	3.33 \pm 0.33	27.17 \pm 0.83 ^a	1.83 \pm 0.16	0.833 \pm 0.16 ^b	1.83 \pm 0.16 ^b

OA = Ochratoxin (1 ppm); T-2 = T-2 toxin (2 ppm)

Means within each column bearing common superscripts do not differ significantly ($P<0.05$)

Table 3: Effect of high grade bentonite on levels of serum biochemical parameters of broilers (Mean \pm SE)

Treat	Mycotoxin	HGB (1%)	Serum protein (g %)	Serum Albumin (g %)	Uric acid (μ g/dl)	GGT (IU/L)	ALT (IU/L)
T0	-	-	2.93 \pm 0.13 ^{ab}	1.25 \pm 0.08 ^a	675.6 \pm 12.72 ^d	9.4 \pm 0.69 ^c	29.25 \pm 1.2 ^a
T1	-	+	3.04 \pm 0.24 ^a	1.46 \pm 0.12 ^a	677.4 \pm 15.54 ^d	9.8 \pm 0.40 ^{bc}	29.78 \pm 1.79 ^a
T2	OA	-	1.88 \pm 0.02 ^{cd}	0.76 \pm 0.01 ^b	1712.0 \pm 11.38 ^a	15.9 \pm 1.5 ^{ab}	22.4 \pm 1.27 ^b
T3	T-2	-	2.60 \pm 0.06 ^{bc}	1.17 \pm 0.06 ^a	1507.0 \pm 5.28 ^b	13.93 \pm 1.8 ^{abc}	25.5 \pm 1.3 ^{ab}
T4	OA	+	1.95 \pm 0.01 ^{cd}	0.78 \pm 0.03 ^b	1705.0 \pm 8.35 ^a	15.23 \pm 0.83 ^{abc}	23.0 \pm 0.41 ^b
T5	T-2	+	2.67 \pm 0.14 ^{ab}	1.25 \pm 0.03 ^a	1058.0 \pm 32.67 ^c	13.9 \pm 0.11 ^{abc}	26.5 \pm 0.72 ^{ab}
T6	OA +T-2	-	1.84 \pm 0.03 ^d	0.78 \pm 0.01 ^b	1664.0 \pm 58.69 ^{ab}	16.43 \pm 1.8 ^a	21.7 \pm 1.19 ^b
T7	OA +T-2	+	1.79 \pm 0.008 ^d	0.78 \pm 0.01 ^b	1640.0 \pm 67.96 ^{ab}	16.77 \pm 1.45 ^a	21.43 \pm 0.43 ^b

OA = Ochratoxin (1 ppm); T-2 = T-2 toxin (2 ppm)

Means within each column bearing common superscripts do not differ significantly ($P<0.05$)

groups receiving Ochratoxin A or T-2 toxin alone or in combination, whereas relative weights of bursa and spleen were not altered. The findings of the study were comparable to the reports of Xue *et.al.*, (2010) and Jayaramu *et.al.*, (2012). Addition of 1.0 per cent HGB to control, OA, T-2 and OA plus T-2 containing diets showed no significant improvement in lymphoid organ weights as compared with their respective toxin groups.

Serum biochemistry: A significant ($P<0.05$) reduction in serum total protein, albumin and increase in serum uric acid level was observed in OA alone and OA plus T-2 toxin combination fed groups. In T-2 toxin alone treated group, serum uric acid values significantly ($P<0.05$) reduced from control values at fifth week of age. The highest depression of serum total protein, albumin and highest increased levels of serum uric acid was recorded in the group fed OA plus T-2 toxin combination, followed by OA and T-2 toxin alone fed groups, and it might be due to the impairment of protein synthesis by inhibiting m-RNA transportation by binding to DNA (Mujahid *et.al.*, 2012, Awad *et.al.*, 2005 and Manafi, 2011). The inclusion of 1.0 per cent HGB in control and diets containing OA, T-2 toxin alone or OA plus T-2 toxin diet did not improve the serum biochemical values.

Serum enzyme activity: Compared to the controls, serum GGT activity was significantly ($P<0.05$) increased by OA and OA plus T-2 toxin combination as compared to control group, but the level of GGT was not altered by T-2 toxin alone treatment. Highest level of serum GGT activity was recorded in OA plus T-2 combination group followed by OA and T-2 alone fed groups and lowest level of activity was observed in group fed control diet. The lowest level of serum ALT activity was seen in OA plus T-2 combination group followed by OA and T-2 fed alone groups and the highest level of serum ALT activity was recorded in control diet. The increased serum GGT levels noted could be due to the sequelae of hepatocyte degeneration and subsequent leakage of enzymes into the circulation. The results of altered levels of serum GGT and serum ALT agree with reports of (Eriksen and Pettersson, 2004 and

Manafi *et.al.*, 2009). The addition of 1.0 per cent to the control and diets containing OA, T-2 and OA plus T-2 toxin combination resulted in no significant improvement in GGT activity indicating no protective effect of HGB on adverse effects of mycotoxins. Similarly, inclusion of HGB to diets containing OA, T-2 and OA plus T-2 combination showed a marginal but non-significant improvement in serum ALT level.

CONCLUSION

The results of the present study revealed that supplementation of high grade bentonite at 1% of diet was not effective in reducing the toxicity resulting from either OA, T-2 toxin alone or a combination of OA and T-2 toxin. Hence, further research is essential for preventing economic losses in poultry production.

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Prevalence of Cryptorchidism and its forms in Sheep Breeds of Karnataka, India

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ABSTRACT

A study was conducted to ascertain the prevalence of Cryptorchidism and its forms in sheep breeds of Karnataka. The data on Cryptorchidism and horn pattern of Mandya, Hassan, Bellary, Kenguri and Nondescript breeds of sheep were collected from shepherds' herds in the home tract of the breeds. The proportion of cryptorchid testes retention at abdominal, inguinal and subcutaneous regions was studied in Hassan and Mandya sheep by Abattoir study and ultrasonography scanning. Cryptorchidism prevalence was highest in Mandya sheep (18 %), moderate in Hassan and nondescript (4-5 %), and low in Bellary and Kenguri sheep (2.5 %). Bilateral cryptorchidism was significantly higher in Mandya and Hassan sheep. Polled condition was found to be significantly associated with Cryptorchidism in all the breeds studied. Cryptorchid testes retention was significantly higher in abdominal region compared to inguinal and subcutaneous region in both Mandya and Hassan breeds indicating involvement of defect in first phase of testicular descent in these breeds.

Key words: Mandya Sheep, Cryptorchidism, Polled and Ultrasound

Cryptorchidism is the failure of one or both testes to be positioned in the scrotum at the time normal for a species and is the most common genetic defect of the male genital system. Occurrence of Cryptorchidism in sheep leads to economic loss and decreased selection potential. The knowledge of prevalence of cryptorchidism in different breeds helps to study its economic impact and position of testicular retention helps to narrow down the search for the causative genetic defect in the physiological process of testicular descent. The information on prevalence of cryptorchidism in sheep breeds of India in general and sheep breeds of Karnataka in particular is scanty. Among the sheep breeds of Karnataka, Mandya breed is said to have high rate of cryptorchidism and found to be the major cause for decline in its population (Bhatia and Arora, 2005). Hence, the present study is undertaken to study the incidence of cryptorchidism and its forms in various sheep breeds of Karnataka, India.

MATERIALS AND METHODS

The prevalence of cryptorchidism was studied in the sheep breeds of Karnataka viz., Mandya, Hassan, Bellary and Kenguri. The data was obtained from the shandies and farmer's flocks in their respective breeding tract. The sheep of six months old and above age were considered for study. The sheep scrotal region was palpated and examined for presence of testicles. The males with no palpable testicles in scrotum were further

examined for the scars on the scrotum to rule out castration. Cryptorchid animals were classified as bilateral and unilateral cryptorchids. The unilateral cryptorchids were further classified as right and left cryptorchid based on the non descent of right and left testicles into the scrotum, respectively.

A total of 325 Mandya, 863 Hassan, 538 Kenguri, 897 Bellary and 650 nondescript male sheep were physically examined and screened for cryptorchid condition. The minimum sample size for the reliable estimation of prevalence of this condition was calculated based on the formula given by Daniel (1999).

$$n = \frac{NZ^2P(1-P)}{d^2(N-1) + z^2P(1-P)}$$

where n = sample size

N = Population size

P = Expected prevalence or proportion

Z = z statistic for a level of confidence

d = precision (if precision is 5%, d = 0.05)

The proportion of different positions of retained testes was studied based on slaughter house survey data and ultrasound scanning. From slaughter house survey data, 24 and 46 cases of bilateral cryptorchid condition was detected in Mandya and Hassan sheep, respectively. The position of testis between kidney and internal inguinal ring was considered as abdominal, between internal inguinal and external inguinal ring

(within inguinal canal) was considered as inguinal and testes retained in subcutaneous region at the entrance of scrotal sac was considered as subcutaneous.

The ultrasonography was performed on 10 and 15 bilateral cryptorchid sheep belonging to Hassan and Mandya sheep respectively. The cryptorchid sheep were starved over night. The wool around sub lumbar area, right and left flank, and caudo-ventral region was shaved to facilitate ultrasound scanning. A portable, B-mode, real time scanner fitted with a 7.5 MHz, linear array transducer was used. After applying acoustic coupling gel, the transducer was held parallel to the long axis of the animal for longitudinal images and perpendicular to the spine for transverse images.

GENMOD procedure of SAS. The significance level of 0.05 was considered for the study.

RESULTS AND DISCUSSION

The Daniel (1999) method of sample size estimation for the study of prevalence at the assumed precision of 0.05, with the maximum expected prevalence of 20 % was 246 animals. Hence, sample size of more than 246 male sheep and about one percent of the population was considered for all the breeds under study. The precision values of the prevalence study for Mandya, Hassan, Kenguri, Bellary and nondescript sheep were 0.043, 0.026, 0.034, 0.026 and 0.031, respectively. The sample size studied, precision of the study and prevalence of cryptorchidism of sheep breeds of Karnataka is given in Table 1.

Table 1: Various forms of cryptorchidism and polled condition in different sheep breeds of Karnataka

Breed	Precision of study	Cryptorchid % (No.)	Bilateral Cryptorchid	Unilateral Cryptorchid	Right Cryptorchid	Left Cryptorchid	Polled Normal	Polled Cryptorchid
Mandya	0.043	18.15 ^a (59)	94.92 ^{aA} (56)	05.08 ^{aB} (3)	66.67 ^A (2)	33.33 ^B (1)	83.83 ^{aA} (223)	94.92 ^B (56)
Hassan	0.026	4.98 ^b (43)	81.40 ^{aA} (35)	18.60 ^{aB} (8)	62.50 ^A (5)	37.50 ^B (3)	25.98 ^{bA} (213)	88.37 ^B (38)
Kenguri	0.034	2.42 ^c (13)	46.14 ^b (6)	53.85 ^b (7)	57.14 ^A (4)	42.86 ^B (3)	12.00 ^{cA} (63)	84.62 ^B (11)
Bellary	0.026	2.79 ^c (25)	44.00 ^b (11)	56.00 ^b (14)	64.29 ^A (9)	35.71 ^B (5)	07.00 ^{cA} (61)	84.00 ^B (21)
ND	0.031	5.38 ^b (35)	65.71 ^{cA} (23)	34.29 ^{cB} (12)	58.33 ^A (7)	41.67 ^B (5)	20.65 ^{bA} (127)	85.71 ^B (30)

Values are presented as percentage (number).

Values with different uppercase superscripts between paired columns differ significantly at $\alpha = 0.05$

Values with different lower case superscripts within a column differ significantly at $\alpha = 0.05$

The animals were positioned in lateral recumbancy and the kidneys were imaged from the uppermost or the dependent side. After locating the kidneys, the testes were searched from the caudal border of the kidneys to downwards. The area around the scrotal neck was examined followed by the inguinal region before moving cranially to the area surrounding the bladder and bladder neck.

Statistical analysis for differences in prevalence, unilateral and bilateral cryptorchidism, right and left cryptorchid, and association of polled condition with cryptorchid among different breeds were studied by Wald chi-square analysis using

The present study revealed that the Mandya sheep had a cryptorchid prevalence of 18.15 per cent under field conditions which is similar to the finding of Govindaiah (2006). Among the genetic groups studied, the prevalence was highest among Mandya sheep followed by Hassan and local nondescript sheep. Further, Kenguri and Bellary sheep had lesser prevalence of cryptorchidism at around 2.5 per cent. The differences in the cryptorchid prevalence rate between breeds was found to correspond with the differences in the sheep breeds based on discriminant function analysis of morphometric traits as studied by Yadav *et al.* (2013). Thus the

differences in morphological measurements among breeds might be one of the factor responsible for differences in prevalence rates of cryptorchidism in sheep breeds studied. Mandya sheep, which has a typical morphology of a compact body with a typical reversed U-shape wedge from the rear (Acharya, 1982), needs to be evaluated for its possible role in retention of testes and thus one of the causative factors for higher prevalence of cryptorchidism in the breed.

Bilateral cryptorchidism was significantly higher than unilateral cryptorchidism in Mandya, Hassan and nondescript sheep, whereas no such relation was observed in Kenguri and Bellary sheep breeds. Variable proportions of bilateral to unilateral cryptorchidism have been reported in various breeds of sheep. From the present study and earlier literature, it is observed that bilateral to unilateral cryptorchid ratio is more in the breeds with higher prevalence of cryptorchidism and the ratio decreases with prevalence.

study, scur were considered as horned. The proportion of animals with polled condition was highest in Mandya breed (83.83 %), followed by Hassan (25.98 %) and nondescript sheep (20.65 %). Majority of Bellary and Kenguri sheep were horned with only 7.0 per cent and 12.0 per cent of males being polled. The proportion of polled animals among cryptorchids was significantly more compared to the polled among normal males in all the breeds under study.

Position of retained testes was studied in Mandya and Hassan sheep by ultrasonography and slaughter survey methods. Slaughter data revealed significantly more testicular retention in abdominal region compared to inguinal and sub cutaneous region in both Mandya and Hassan breeds (Table 2). Similar higher abdominal retention of testes in sheep has been reported by Lianas and Deligiannis (2002) and Smith *et al.* (2012) in abattoir studies.

Table 2: Proportion of different positions of testicular retention in cryptorchid sheep of Mandya and Hassan breeds

		Abdominal	Inguinal	Sub- cutaneous	Total
Mandya	Ultrasound	90.00 ^A (27)	10.00 ^B (3)	0 ^C (0)	30
	Slaughter house	91.66 ^A (22)	8.34 ^B (2)	0 ^C (0)	24
	Total	90.74 ^{A a} (49)	9.26 ^{B a} (5)	0 ^{C a} (0)	54
Hassan	Ultrasound	75.00 ^A (15)	20.00 ^B (4)	5.00 ^C (1)	20
	Slaughter house	71.74 ^A (33)	21.74 ^B (10)	6.52 ^C (3)	46
	Total	72.73 ^{A b} (48)	21.21 ^{B b} (14)	6.06 ^{C b} (4)	66

Values are presented as percentage (number).

Values with different uppercase superscripts within rows differ significantly.

Values of breed total of a column with different lowercase superscript differ significantly

Among unilateral cryptorchids, the proportion of right cryptorchids was significantly higher than left cryptorchids in all the sheep breeds studied. Similar observations in sheep have been reported by Lianas and Deligiannis (2002) and Smith *et al.* (2012), but the reason for preferential right testicular retention in sheep is not known.

Depending on the horn or polled phenotype, sheep studied were categorized into horned, scur horned, and polled. In the present

Transabdominal real time ultrasonography scanner fitted with a 7.5 MHz, linear array transducer was found to be effective in identifying the position of testicular retention in sheep. The ultrasonography method of detecting the position of testes was found to be equally effective as the one based on slaughter data, and hence can be effectively used in diagnosis of cryptorchids in farms. The significantly higher abdominal retention of testes observed in Mandya breed as compared to

Hassan breed may indicate occurrence of more abdominal retention in breeds with higher prevalence of cryptorchidism. Further, defects in transabdominal phase of testicular descent may be the prominent cause for cryptorchidism in both Mandya and Hassan breeds of sheep.

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Effect of Lactose Hydrolysis and Packaging Materials on Storage Studies of Lactose Hydrolysed Peda*

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ABSTRACT

Peda is a popular indigenous product, traditionally prepared by blending with khoa and sugar and is rich source of energy and protein. Numerous varieties of peda are manufactured and are available in market. In this investigation an attempt was made to develop Lactose Hydrolysed Peda to meet lactose-intolerant consumer who are unable to digest lactose due to absence of enzyme lactase. Initially whole milk was hydrolysed to 25, 50, 75 and 100 % lactose hydrolysis and khoa was prepared. Among the different levels of lactose hydrolysed khoa the best selected and comparable with control was 25% lactose hydrolysed khoa. The 25% lactose hydrolysed khoa was utilized for the preparation of lactose hydrolysed peda and were packaged in Parchment paper/LDPE and Metalized polyester. They were subjected for storage studies at ambient temperature (30°C). To assess the effect of storage condition, they were analysed for Free Fatty Acid, Peroxide and Overall acceptability. From the studies it was evident that lactose hydrolysed peda packaged in metalized polyester had the storage stability of 3 weeks at ambient temperature.

Keywords: Lactose, Lactose Hydrolysis, Khoa, Peda

India stands first in milk production comprising nearly to 140 MT, Only 46% of milk produced is consumed as liquid milk. Milk is a wholesome food which provides excellent support towards human nutrition and contains balanced quantities of required nutrients such as protein, fat, minerals, carbohydrate and vitamins. Lactose is an important carbohydrate and milk sugar present in milk. Lactose intolerance is a preceding problem faced by humans from decades. The clinical entity called lactose intolerance is always related to "Low lactase activity". Lactose Intolerance is a health condition in which body is incapable of digesting lactose due to deficiency of enzyme Lactase (β -galactosidase) If the amount of lactose ingested exceeds hydrolytic capacity of available intestinal lactase, a portion of lactose remains undigested and is transported in to large intestine.

On reaching large bowel, the undigested lactose is degraded by bacterial enzymes, leading to osmolality of intestinal fluid and thus water from the tissues in to the intestines and generates organic acids, carbon di oxide and hydrogen. These products together with large amount of water drawn in to intestine are responsible for various symptoms such as cramps, diarrhoea, abdominal pain, loss of appetite, nausea, vomiting, heart burn to head ache (Fonden *etal* 2000).

The quantity and production of peda exceeds any other indigenous milk based sweets utilizing khoa as a raw material. Peda is a popular indigenous product, traditionally prepared by blending with khoa and sugar and is rich source of energy and protein.

The main focus of research was to develop method for preparation of lactose free product for lactose intolerance individuals. Such products can be prepared from either physical removal of lactose such as membrane technology or by hydrolysis of lactose in to its corresponding monosaccharides glucose and galactose. Thereby the lactose intolerant individuals can consume milk and milk products instead of ceasing milk consumption. With this proposal an investigation was conducted with the following objectives:

- To optimise the process for the development of Lactose Hydrolysed milk and Khoa
- To develop the process for Lactose Hydrolysed Peda
- To study the shelf-stability of standardized products using selected packaging materials.

*Article is part of M.Sc Thesis submitted by the first author

MATERIALS AND METHODS

Cow milk was procured from students Experimental Dairy Plant (SEDP) Dairy Science College, Hebbal, Bangalore.

Enzyme: Lactozym 3000L, type HP-G manufactured by Novo Nordisk, Denmark was used for hydrolysing lactose present in cow milk.

Enzyme Concentration : The enzyme concentration of 0.3, 0.5 and 1.0 ml/l was standardized.

Incubation Temperature : The effect of incubation time-temperature on degree of lactose hydrolysis in milk was studied. In this set of experiments, 3 levels of temperatures 20, 30, 40 °C were studied. The Percentage of lactose hydrolysis were studied every 30 min up to maximum duration of 5h.

Degree of Lactose Hydrolysis: Lactose contents of milk and the residual lactose after hydrolysis were determined calorimetrically by the method of Nickerson *et al* (1976).

Process optimization for preparation of lactose hydrolysed peda : Milk sugar was hydrolysed to 25, 50, 75 and 100% using enzyme, @ 1 ml/ L at 40°C and incubating for 30, 60, 180 and 300 minutes respectively. Lactose hydrolysed milk of all four levels was used to prepare lactose Hydrolysed khoa. The best suited khoa was selected for the preparation of Lactose Hydrolysed peda

The Chemical analysis: It was determined as per ISI: SP 18 (Part XI 1981). FFA was determined as per the procedure of Ramamurthy, 1983 respectively. Peroxide value was estimated as per the procedure outlined in IS: 3508 (1966).

Sensory Analysis: The sensory analysis was carried out by 9-point hedonic scale the sample was served to a panel of judges to adjudge the quality of product.

Storage studies: The best selected Lactose Hydrolysed Peda was packaged in Parchment Paper (20x20cm) / LDPE (23x17cm) and Metalized Polyester (23x12.5cm) for four weeks at room temperature, at a regular interval of three days the samples were subjected to sensory and chemical analysis.

Statistical Analysis: The results (average of three trails) were statistically analysed as per the procedure of Sundarraj *et al.*, 1972 by testing for significance using CRD (completely randomized design) and randomized block for one way and two-way analysis.

Flowchart for Preparation of Lactose Hydrolysed Peda: Whole milk → Standardization (3.5%FAT & 8.5%SNF) → Pasteurization (72°C /15 s) → Cooling (40°C) → Addition of enzyme (LACTASE 1ML /L) → Incubation (40°C) → 25% (30 MIN)/ 50% (60 MIN)/ 75% (180 MIN)/ 100% (30 MIN) → Lactose Hydrolysed Milk → (OPEN PAN EVAPORATION WITH CONTINUOUS STIRRING & SCRAPPING) → Lactose Hydrolysed Khoa (25%) → Addition of sugar @ 10% → Kneading (50° C) → Cooling (30° C) → Moulding → Packaging (Metalized Polyester) → **Lactose Hydrolysed Peda**

RESULTS AND DISCUSSION

The effect of incubation time-temperature on degree of lactose hydrolysis in milk was studied. In this set of experiments 3 levels of temperatures 20, 30, 40 °C were studied. The percentage of lactose hydrolysis were studied every 30 min up to maximum duration of 5h. From the result obtained it was noted that both temperature and duration of incubation had significant effect on degree of lactose hydrolysis. Lactose hydrolysis levels of 25, 50, 75 and 100% levels were attained at a constant enzyme concentration of 1ml / litre at a temperature of 40 ° C incubated for 30, 60, 180 and 300 minutes respectively.

The Lactose hydrolysed milk of all 4 levels was used for khoa preparation. It was evident that Lactose Hydrolysis had significant effect on chemical composition, physico-chemical characteristics and sensory attributes of khoa. Among the different levels of lactose hydrolysed khoa the best selected and comparable with control was 25% lactose hydrolysed khoa. The 25% lactose hydrolysed khoa was utilized for the preparation of lactose hydrolysed peda and were packaged in Parchment paper/LDPE and Metalized polyester. They were subjected for storage studies at ambient temperature (30°C). To assess the effect of storage

condition, they were analysed for Free Fatty Acid, Peroxide and Overall acceptability. From the studies it was evident that lactose hydrolysed peda packaged in metalized polyester had the storage stability of 3 weeks at ambient temperature.

Effect of type of Packaging Material on FFA(% oleic acid) content of lactose hydrolysed peda during storage: It is evident from Table1 that the FFA content of lactose hydrolysed peda were 0.18 in both the packaging material viz Parchment paper/LDPE and Metalized Polyester at ambient temperature on first day of storage. The FFA increased progressively during the successive weeks of storage. The FFA content packaged in Parchment paper/ LDPE were 0.38 and 0.58% oleic acid for first and second week of storage and later on in third week the product was discarded. Similarly the FFA content of peda packaged in Metalized Polyester had storage stability of 3 weeks with 0.30,0.49 and 0.67 % oleic acid during first, second and third week of storage. The stastical analysis confirmed that FFA content significantly increased and type of packaging material had significant effect on FFA content of peda during Storage.

Table-1 Effect of type of packaging material on FFA (% oleic acid) content of lactose hydrolysed peda during storage

Storage period (Weeks)	FFA% oleic acid	
	Parchment paper/ LDPE	Metalized Polyester
0	0.18	0.18
1	0.38	0.30
2	0.58	0.49
3	----	0.67
4	----	----

CD Packaging material-0.008, CD Time 0.005

The FFA as expressed in terms of % oleic acid increased significantly during storage at ambient temperature irrespective of packaging material .However, the extent of increase in FFA content was slower when packed in metalized polyester than Parchment paper/ LDPE which had storage stability of 3 and 2 weeks respectively. The lower FFA content in lactose hydrolysed peda is mainly due to inhibition of growth of yeast and

molds by intermediary compounds of maillard reaction especially HMF, which have been shown to have antibiotic activities against *Bacillus subtilis*, *Lactobacillus* and *Staphylococcus* strains(Sahni and kumar,1988). Similar result was observed by Prakash and Sharma (1984a), Balasubramanya (1988) and SharanaGouda (1996) for lactose hydrolysed khoa and kalakand respectively.

Effect of Packaging Material on Peroxide content (miliequivalents of O₂/kg fat) of lactose hydrolysed peda during storage: As it could be observed from Table 2 that peroxide content of 25% lactose hydrolysed peda packaged in two packaging material viz Parchment paper/LDPE and Metalized Polyester were zero on the initial day of storage. The peroxide content of peda packaged in Parchment paper/LDPE was 1.45 and 2.72 mili equivalents of oxygen/kg of fat during first and second week of storage at ambient temperature as against 1.37,1.93 and 2.99 for peda packaged in metalized polyester during first, second and third week of storage respectively. There is significant increase in peroxide value and type of packaging material had significant effect on per oxide content of lactose hydrolysed peda during storage.

Table-2 Effect of packaging material on Peroxide content (miliequivalents of O₂/kg fat) of lactose hydrolysed peda during storage

Storage period (Weeks)	Peroxide content (miliequivalents of O ₂ /kg fat)	
	LDPE/parchment paper	Metalized Polyester
0	0.00	0.00
1	1.46	1.37
2	2.72	1.93
3	----	2.99
4	----	----

CD Packaging material-0.37, CD Time 0.23

The increase in peroxide value was comparatively slow in case of metalized polyester, this could be attributed to fact that metalized polyester has lower oxygen and water transmission rate than PE that prevents rancidity and oxidized flavours apart from inhibiting the absorption of foreign odours and discolouration (Goyal and

Rajorhia, 1991) apart from this the browning intermediates, HMF leads to the formation of naturally occurring anti-oxidants (Sahni and Kumar ,1998).

Effect on type of Packaging Material on overall acceptability of lactose hydrolysed peda during storage: The results on effect on type of packaging material on overall acceptability of lactose hydrolysed peda during storage are depicted in Table 3. The overall acceptability of lactose hydrolysed peda decreases as the period of storage increases. On the initial day of storage the score was 8.26 later on the scores decreased in subsequent weeks for LDPE/ Parchment paper packaged peda from 7.16 to 7.00 during second week indicating the storage stability of two weeks after which the product was unacceptable. Whereas, for peda packaged in metalized polyester. The scores awarded were 8.20, 8.00 and 7.90 during first, second and third week of storage respectively, indicating storage stability of 3 weeks. The result also reveals that there was significant difference in overall acceptability between various storage periods.

Table-3 Effect on type of packaging material on overall acceptability of lactose hydrolysed peda during storage

Storage period (weeks)	Overall acceptability	
	LDPE /parchment paper	Metalized Polyester
0	8.26	8.26
1	7.16	8.20
2	7.00	8.00
3	----	7.90
4	----	----

CD Packaging material-0.04

CD Time 0.02

The overall acceptability scores for lactose hydrolysed peda reveals that the acceptability decreases significantly as storage progress. Peda packaged in metalized polyester had better acceptability which resulted in storage stability of 3 weeks without any textural changes or alteration in flavour profile. This could be due to HMF and lower water activity of product due to generation of extra molecule of glucose and

galactose upon lactose hydrolysis which has more water binding property. The result was in close agreement with Prakash and Sharma (1984a) and prajapati *etal* (1986).

CONCLUSIONS

Lactose Hydrolysis is a health issue faced by millions of people around the world. Addressing this problem is required and a challenge to technologists. Hydrolysis of milk sugar resolve this and results in many benefits. The resultant Lactose Hydrolysed Khoa opens a wide option to be used in many of dairy products such as Peda, Burfi, Gulab Jamun etc. as a base material for preparation and also supplements Lactose Intolerant Individuals. Lactose hydrolysed peda offers a wide opportunity for the producers and consumers specially lactose intolerant individuals. By utilizing lactose hydrolysed khoa, for peda preparation the addition of sugar can also be reduced from 30 % to 10% as the hydrolytic end products glucose and galactose contributed to the natural sweetness this in turn help to overcome hypoglycaemia.

Apart from this, the adoption of standard packaging materials and techniques both regard to testing and usage would go long way to improve productivity and keeping quality of the product. This in turn shoots up the national and international trade that can contribute to stabilize economy of the nation.

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Development of a *Rabadi*-like Wheat Based Fermented Milk Beverage

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ABSTRACT

Rabadi is an indigenous natural lactic acid cereal based fermented beverage most popular in few regions of India (Haryana, Punjab & Rajasthan). The technology of manufacturing of acceptable beverage involves; mixing of 2% 24hr germinated wheat flour in skim milk, heating to 90°C/15 min, cooling to 37°C, inoculating with NCDC-263 yoghurt culture (2%), followed by incubating at 37°C/4-5hr till curd attain final titratable acidity of about 1% lactic acid. The curd thus obtained is mixed with pasteurized stabilizers solution (0.15, 0.6% of CMC & Pectin respectively) in the ratio of 2.5:1, salt (0.7%) and spices (roasted cumin and black pepper at 0.1% each), then blended and pasteurized (80°C/15 sec) followed by hot filling in pre-sterilized glass bottles (200ml), immediately cooling to 4°C and storing at 6±1°C. The optimized product contained 9.81, 5.73, 2.54, 0.28, 1.38, and 0.76% of total solids, carbohydrates, protein, fat, ash and lactic acid, respectively. The microbiological quality of the optimized product was excellent (Coliforms and yeast & molds counts were nil). The type (24hr germinated wheat flour) and level of wheat flour (at 2%) exhibited highly significant effect on overall acceptability of the beverage

Key words:- *Rabadi*, Milk, Wheat flour and Yoghurt culture

Cereals are staple foods and are important sources of nutrients in both developed and developing countries. Cereals contribute approximately 50% calories, and providing proteins (Cereal proteins contribute the major protein source for much of the world population), dietary fibers, minerals and vitamins required for human health. Cereals can be used as either alone or incorporating fruits, vegetables, legumes, and/or milk solids. Milk, curd, butter milk, whey addition has complementary and supplementary effect on cereal proteins. Among the cereals, wheat represents the most important staple food for human beings. Wheat has unique nutritional and functional advantages. Wheat contains 13.20 – 13.34% total and 3.4 – 3.54% soluble dietary fiber (Table 1). Consumption of foods containing dietary fibers is believed to play a major role in preventing or reducing some of the diseases such as constipation (Gastro intestinal disorders), hypertension, hypercholesterolemia, diabetes and colon cancer (Ahmet Ayar *et al.*, 2005).

Fermentation is one of the simplest methods of food preservation. The fermented wheat based milk products are extremely popular in many Balkan, Mediterranean, Middle East countries and

Indian sub-continent. They have different names in different areas such as Kishk in Syria, Palestine, Jordan, Lebanon and Egypt; Kushuk in Iran and Iraq; Tarhana in Turkey, Trahanas in Greece and *Rabadi* in India. For the manufacture of these products generally butter milk or whey or curd and wheat flour/parboiled cracked wheat are used. In addition, tomato paste, red peppers, onions, turnips, garlic and other vegetables and spices may be added along with salt with various combinations and percentages.

Table 1. Physico-chemical composition of wheat

Source: Suhasini *et al.*, (1995)

Constituents	Value	Constituents	Value
1000-Kernel weight (g)	51.00–51.17	Ascorbic acid (mg %)	0.67
1000-Kernel volume (ml)	35.50–35.66	Thiamine (mg %)	0.27
Density (g/ml)	1.44–1.45	Riboflavin (mg %)	0.07
Hardness(Kg/cm ²)	13.10–16.00	Niacin (mg %)	2.62
Proteins (%Nx5.77)	12.40–13.10	Iron (mg %)	7.25
Crude fat (%)	1.86–1.87	Copper (mg %)	0.69
Starch (%)	72.0073.90	Zinc (mg %)	2.57
Dietary fiber (%)	13.20–13.34 3.40 – 3.54	Manganese(mg %)	4.26
		Calcium (mg %)	93.40
		Phosphorus (mg %)	355.40

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Rabadi is one such product that is prepared and consumed in few regions of India (Haryana, Punjab and Rajasthan) by mixing the surplus butter milk with cereal flour, fermenting the mixture at elevated temperatures (35 – 40°C) during summer months for 4 – 5hr, cooking the fermented mass and diluting the gelatinized mass with water. Salt and spices are added before serving. The quality of *Rabadi* varies considerably as the manufacturing procedures practiced at homes are not standardized which results in low yield, poor quality and wide varying composition in the final product. Moreover, traditional method is not suitable for large-scale production and there is no reported work on wheat based *Rabadi* and method of manufacturing it on commercial scale. Therefore, this project was undertaken to develop a technology for the manufacture of *Rabadi*-like beverage by utilizing wheat and skim milk solids in highly uniform, safe and organoleptically acceptable form.

MATERIALS AND METHODS

Skim milk: Fresh skim milk of 0.1 to 0.2% fat was collected. Chemical composition of mixed skim milk was analyzed as per the BIS procedure described in IS:1479, Part-II (1964) is shown in Table 2.

Table 2. Proximate chemical composition of skim milk

Constituents	Range		Average
	Minimum	Maximum	
Total Solids (%)	8.47	9.10	8.73
Fat (%)	0.10	0.30	0.20
Protein (%)	3.32	3.64	3.46
Ash (%)	0.75	0.81	0.78
Acidity (%LA)	0.144	0.153	0.15

Note: All values in above table are average of three replicates.

Table 3. Proximate chemical composition of raw, 24hr and 48hr germinated wheat flour

Constituents	RWF			24hr GWF			48hr GWF		
	Range		Avg.	Range		Avg.	Range		Avg.
	Min	Max		Min	Max		Min	Max	
Moisture (%)	9.08	9.54	9.35	6.13	6.70	6.42	6.02	6.21	6.12
Fat (%)	1.89	1.95	1.92	1.90	1.87	1.89	1.66	1.85	1.76
Protein (%)	11.85	12.0	11.93	11.83	11.76	11.80	11.69	11.50	11.61
Ash (%)	1.46	1.51	1.48	1.40	1.41	1.41	1.39	1.41	1.40
Carbohydrate (%) (By difference)	75.72	75.0	75.32	78.74	78.26	78.48	79.24	79.03	79.11

Note: All values in above table are average of three trials.
RWF → Raw Wheat Flour,
GWF → Germinated Wheat Flour

Wheat flour: Fresh and clean wheat grains (Var. WH 343) were procured from the local market, Karnal town. Raw wheat flour: Wheat grains were cleaned, washed and dried at 50°C. The dried grains were ground and sieved. Germinated wheat flour (24, 48, 72hr): Wheat grains were cleaned, washed, steeped at 25°C for 17hr (Wheat and water 1:2 ratio), and germinated at 25°C for 24, 48 and 72hr, respectively in a wet muslin cloth. Germinated grains were dried in oven at 50°C for 24hr. The rootlets were removed by rubbing. Dried germinated grains were ground and sieved. The wheat flour thus obtained was kept in an airtight container. The chemical composition of wheat flour used is shown in Table 3.

Starter culture: NCDC-167 (mesophilic mixed dahi culture) and NCDC-263 yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were procured from National Collection of Dairy Cultures, Dairy Microbiology Division, NDRI, Karnal.

Stabilizers: Pectin pure (Poly - D - Galacturonic Acid Methyl Ester) was procured from Central Drug House Private Lt., New Delhi. Carboxy Methyl Cellulose (CMC) low viscosity was procured from Titan Biotech Ltd., Bhiwadi-301019 (Raj.).

Salt and spices: Salt, cumin, and black pepper: Fresh commercially available salt (NaCl) AR grade procured from Titan Biotech Ltd., Bhiwadi-301019 (Raj.). Cumin and Black Pepper were purchased from a reputed dealer, Karnal. Cumin and black pepper were roasted, ground, sieved and stored in airtight containers separately.

Analysis methods:-

Sensory evaluation of beverage:- Sensory evaluations of different formulations were carried out by a panel of trained judges using 9 point Hedonic scale (Peryam and Pilgrim, 1957). The best formulation was selected on the basis of sensory evaluation.

Statistical analysis:- The data obtained through experiments were subjected to statistical analysis to arrive at valid and meaningful inferences. The data obtained in this investigation were subjected to CRD, RBD. The ANOVA thus obtained was utilized to calculate the CD (critical difference) by using formula as below:

$$C.D = \sqrt{\frac{2xE_{mss}}{n}} \times t_{\text{value},df}$$

Product analysis:- The formulated product was analyzed for chemical composition and microbiological analysis as per standard procedure.

Total solids:- Total solids content was estimated by gravimetric method as per the BIS method described in IS: SP: 18, Part-XI (1981).

Fat:- Fat content in terms of percentages was determined by Mojonnier method as per the BIS method described in IS: SP: 18, Part-XI (1981).

Protein:- The protein content was estimated by Micro Kjeldahl method of AOAC (1995).

Ash:- The Ash content was estimated by standard Method as per the BIS method described in IS: SP: 18, Part-XI (1981).

Titrateable Acidity and pH:- Titrateable acidity was determined using the BIS procedure of IS: 1479, Part-I (1960) and pH was determined by using pH meter.

Standard Plate count:- Standard plate counts in the beverage were determined on standard plate count agar (Marshall, 1993). Counts were made after 48hr of incubation at 37°C and reported as Standard plate count per ml of the sample.

Coliform count:- A coliform count was estimated by employing violet red bile agar (Marshall, 1993). Counts were made after 48hr of incubation at 37°C. Dark red colonies (at least 5mm diameter) showing the presence of coliform were counted.

Yeast and Molds count:- Yeast and Molds counts were estimated by employing on Yeast and Mold agar (Marshall, 1993). Counts were made after 3 – 5 days of incubation at 30°C and reported as Yeast and Molds per ml of the sample.

Method of manufacture: The technology of manufacturing of acceptable beverage involves; mixing of 2% 24hr germinated wheat flour in skim milk, heating to 90°C/15 min, cooling to 37°C, inoculating with NCDC-263 yoghurt culture (2%), followed by incubating at 37°C/4-5hr, till curd attain final titrateable acidity of about 1% lactic acid. The curd thus obtained is mixed with pasteurized stabilizers solution (0.15, 0.6% of CMC & Pectin respectively) in the ratio of 2.5:1, salt (0.7%) and spices (roasted cumin & black pepper at 0.1% each), then blended and pasteurized (80°C/15sec) followed by hot filling in pre-sterilized glass bottles (200ml), immediately cooling to 4°C and storing at 6±1°C.

RESULTS AND DISCUSSION

Preliminary studies revealed that *Rabadi*-like beverage prepared with raw wheat flour was not liked by panelists much. Hence, germination to 24, 48 and 72hr were attempted. Germination up to 72hr resulted in highly undesirable products. The flour from 24 and 48hr germinated wheat was evaluated along with control (raw wheat flour). Beverage made with three different types of flours (raw, 24hr and 48hr) at four different levels i.e. 2, 3, 4 and 5 percent. The products were subjected to sensory evaluation and the results are presented and discussed below.

The average overall acceptability score varied from 6.30 to 6.94 for the beverage with different types of wheat flour. The beverage prepared with 24hr germinated wheat flour (6.94) was significantly ($p < 0.05$) superior in overall acceptable followed by raw wheat flour (6.50) and 48hr germinated wheat flour.

The overall acceptability score varied from 6.42 to 6.98 for the beverages prepared with different levels of wheat flour. The beverage prepared with 2%

wheat flour (6.98) was significantly superior to those prepared with 3, 4 and 5% (p<0.05) wheat flour (Table 4).

Table 4. Average overall acceptability score of Rabadi - like wheat based fermented milk beverage from different types, levels of wheat flour and skim milk

Type of wheat flour	Levels of wheat flour				Overall Mean
	2%	3%	4%	5%	
RWF	6.99	6.45	6.25	6.40	6.50 ^{ab}
24hr GWF	7.17	6.90	6.85	6.82	6.94 ^a
48hr GWF	6.96	6.23	6.18	6.05	6.30 ^c
Overall Mean	6.98 ^a	6.50 ^b	6.43 ^c	6.42 ^c	

Note: All values in above table are average of three trials. Superscripts with similar alphabet are not significant from each other when read column wise. C.D= 0.20 (type of wheat flour) and C.D= 0.18 (level of wheat flour) at 5% level of significance. RWF → Raw Wheat Flour GWF → Germinated Wheat Flour

The finally developed wheat based Rabadi-like fermented beverage contained 9.81% total solids, 2.54% protein, 0.28% fat, 1.36% ash and 0.76% lactic acid. The corresponding values on dry matter basis are reported in table 5. The microbiological quality of the optimized product was excellent (standard plate count was 1.5 x 10⁷ cfu per ml, coliform and yeast & mold counts were nil) (Table 5).

Table 5. Optimized beverage analysis results

Sl. No.	Chemical Analysis		
	Parameters	Value (%)	Value (Dry matter basis)
1	Water	90.19	-
2	Fat	0.28	2.85
3	TS	9.81	100.00
4	Protein	2.54	25.89
5	Ash	1.36	13.86
6	Carbohydrate (By difference)	5.63	57.39
7	Acidity (% Lactic Acid)	0.76	7.74
Sl. No.	Microbiological Analysis		
	Parameters	Value	-
1	Standard plate count (cfu/ml)	1.5x10 ⁷	-
2	Coliform (cfu/ml)	Nil	-
3	Yeast and Mold (cfu/ml)	Nil	-

During preliminary trials two sources of milk solids namely skim milk, sour butter milk were tried to assess the suitability of source of milk solids to be used. Since traditional Rabadi is prepared using home-made sour butter milk obtained during churning of dahi. It was observed that the skim milk based beverage scored more for all attributes in comparison to butter milk based beverage (Table 6). The butter milk based Rabadi lack desirable flavour, had floury taste and viscous, which was not liked much by panelists. Taking into consideration the industrial production of beverage, skim milk was selected as base for preparation of Rabadi-like beverage. Further experiments were therefore carried out using skim milk as source of milk solids.

Table 6. Comparison between Butter milk (BM) and Skim milk (SM) based beverage (Average sensory scores)

Suhasini and Malleshi (1995) investigated that germination of wheat for 48hr is suitable to produce nutrient dense health foods because of significantly lowered viscosity (dietary bulk). However, in present investigation germination up to

Sensory Attributes	BM beverage	SM beverage
Flavour	6.50	7.25
Colour and Appearance	7.00	7.50
Consistency	6.75	7.00
Sedimentation	6.50	7.00
Overall Acceptability	6.70	7.20
Acidity (% LA)	0.57	0.65

Note: All values are mean of three replications.

24hr produced most acceptable product. The germination of wheat flour imparted malty flavour to product and it could be one of the possible reason improved flavour score. Incorporation of germinated flour significantly improved the overall acceptability of the Rabadi-like wheat based fermented milk beverage.

Yoghurt starter culture (NCDC-263), being thermophilic bacteria grows at higher incubation temperature, produced acid at a faster rate and the curd was firm. A number of literature published regarding the development of cereal milk

based fermented traditional products including kishk, tarhana, trahana, indicate the positive role of thermophilic microflora most notably yoghurt bacteria on improving the overall acceptability of these products (Ibanoglu *et al.* 1995, Tamine *et al.* 1997).

Major antinutrients present in considerable amounts in wheat such as phytic acid, myoinositol 1, 2, 3, 4, 5, 6-hexakis (Dihydrogen phosphate) are known to limit the nutritive value of food such as may adversely affect the mineral bioavailability (iron, zinc, calcium and magnesium), carbohydrate and protein digestibility (Ahmet *et al.*, 2005). Fermentation is one of the potential processing methods; that is known to reduce the level of phytic acid and improve the digestibility of protein as well as starch of various food grains (Gupta *et al.*, 1992). Dhankher and Chauhan (1987) found that temperature of processing has little influence on degradation of phytic acid and poyphenol content in cereal grains and phytic acid appears to be susceptible to degradation by fermentation. Dhankher and Chauhan (1987) observed that protein and starch digestibility increased significantly by fermenting a mixture of pearl millet flour-butter milk at 45°C for 9hr. Gupta *et al.* (1992) studied that the level of phytic acid was significantly decreased in mixture of raw as well as autoclaved wheat flour with butter milk after fermentation at 30, 35 and 40°C for 6, 12, 18, 24 and 48hr and also observed that overall improvement in nutritional quality of *Rabadi* was more pronounced when fermentation was carried out.

Effect of processing methods such as cooking, frying, roasting, fermentation, germination, soaking (steeping) on carbohydrate digestibility (*in vitro*) of cereals (rice, wheat, sorghum, pearl millet) was studied. Significant improvement in digestibility was observed after germination, fermentation, soaking and cooking (Mudambi & Rajgopal, 1983). Suhasini *et al.* (1995) found that the gelatinization temperature (GT) values were 76, 78 and 80°C for native, 72hr germinated and 120 hr germinated wheat, respectively. Chiang & Johnson (1977) have pointed out that gelatinization of starch

significantly affects the characteristics and quality of food, elasticity and softness, digestibility, palatability, texture and volume.

Fermented milks at low pH are prone to sedimentation of milk protein and whey separation, reducing their consumer appeal. Sedimentation and wheying-off can be reduced by selecting suitable processing parameters – heat treatment and time-temperature combination, pH adjustment and introduction of suitable stabilizers at desired level (Barnes, 1992). Sexena (2005) found that pectin at 0.2 to 0.4% was found most suitable to provide stability to the direct acidified lassi-like beverage, where as CMC at 0.05 to 0.25% levels offered maximum stability at pH 4.0 to 4.4. It has also been found that up to 0.3% pectin and 0.15% CMC provided best stabilizing effect and sensory properties. When cereal is used along with fermented milks the tendency of sedimentation increases further, so there is need of stabilizers. The most acceptable beverage was obtained by adding 0.15% CMC in combination with 0.6% pectin.

Gupta *et al.* (1992a) reported the protein and fat content in barley flour *Rabadi* in the range of 21.0 to 24.2% and 0.32 – 0.40%, respectively.

CONCLUSIONS

The type (24hr germinated wheat flour) and level of wheat flour (wheat flour at 2%) exhibited highly significant effect on overall acceptability of the beverage. The microbiological quality of the optimized product was excellent (Coliforms and yeast & molds counts were nil). The current investigation resulted in standardization of technological variables for nutritious, safe and organoleptically acceptable *Rabadi*-like wheat based fermented milk beverage by utilizing wheat solids, skim milk solids, salt and spices. The simple technology can help in diversifying the product profile to functional foods. Most of the pro-biotic fermented foods developed are based on dairy products and much less work is available on cereals which constitute the staple diet in developing nations (Sangeeta *et al.*, 2004). It could be concluded that functional foods based on cereals is a challenging perspective.

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Anterior Amelia in a Female Calf of Hallikar Breed of Karnataka – A Case Report

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ABSTRACT

The present case report describes about a condition known as Anterior Amelia in a female calf of Hallikar breed of Karnataka where in the calf had no forelimbs. Amelia, a congenital anomaly where in there is total absence of limbs in the young ones. Though prognosis of such anomalies is often poor, the technique of applying prosthetic limbs to chest and fixing of wheel cart could be followed, however in most such instances the owners prefer to sell the young ones as it is an extra burden to them in raising and the practical difficulty in bearing weight of young ones by the prosthetic limbs as they grow

Key words: Amelia, Hallikar, Karnataka, Prosthetic limbs

The total absence of limbs – AMELIA, was a rare congenital malformation diagnosed in domestic animal newborns (Lapointe *et.al.*, 2000 and Vermunt *et.al.*, 2000). Malformations of the extremities or parts of them were varied in their manifestations, ranging from absence of a single structure to partial or complete absence of the limbs (Lallo *et al.*, 2001)

Hemimelia was a congenital abnormality characterized by partial or complete absence of a portion of the normal structures in an extremity (Corbera *et.al.*, 2002). Amelia was a rare congenital anomaly in tetrapods with absence of one or more limbs. Brachia indicates agenesis of both thoracic limbs. Agenesis of both hind limbs is called apodia. Absence of one limb is called monobrachia or monopodia (Leipold, 1997 and Szczerbal *et.al.*, 2006). Etiologies of limb deformities in animals are hereditary factors, environmental factors, or a combination of both. Reports indicate that limb defects are due to chromosomal aberrations in domestic animals (Morey and Higgins, 1990). The present case was very unusual and alerts the scientists to investigate the possible cause for such condition.

CASE HISTORY AND OBSERVATION

A Hallikar cow, a native breed of Karnataka state aged two and half years in its first lactation delivered to a female calf normally without any complications. The new born female calf born was with total absence of fore limbs (Fig. Anterior and Lateral view). The calf was active and alert with

normal flexion and extension of the hind limbs, normal birth weight and normal defecation and urination. The calf was able to move and stand with support by the attender. The calf tries to stand and move with the support of hind limbs but used to fall because of no support. Based on the physical appearance of the calf, the condition was diagnosed as total Amelia of thoracic limbs.

Breeding history of the cow was not known, according to the owner the cow was left for grazing with other cows and bulls in a group. The cow might have got impregnated with the bull during the grazing and the owner was unaware until the time of parturition. According to owner this was the first of its kind seen in his entire life.

DISCUSSION

Amelia, a congenital defect usually diagnosed in domestic animals after parturition. Though, most of the calves born with congenital defects will not survive, but, conditions like Amelia of either fore limbs or hind limbs with normal digestive tract and other systems of the body will survive for long time. Most of the times the calves with congenital defects are sent to slaughter or euthanized due to their inability to survive or owner some time feel it is an extra burden on his part to maintain such calves with defects.

In the present case, the author suggested the technique of application of prosthetic limbs which could be attached to the chest or Fabrication of wheel cart which could be fixed to the chest region.



Fig: Anterior Amelia in female Hallikar calf

The calf can have normal grazing, water and even can move in a herd for grazing when placed on wheel cart or fixed with prosthetic limbs. The practical difficulty is that as the calf grows bigger the fixing of the prosthetic limbs or wheel cart becomes difficult and may not with stand the weight of the growing calf.

The prognosis of such cases will be very poor since, the availability of the facilities are limited at field level and the support from the owner some time may not be available. In the present case the author suggested the technique but because of technical and practical difficulties involved in doing so, the idea was dropped.

The same technique could be adopted in small breeds like Pungnur breed and Malnad Gidda breed of Karnataka because the adult body weight of these breeds are low compared to other breeds.

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Successful Management of *Sorghum Bicolor* Poisoning in a Malnad Gidda Cow – A Case Report.

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ABSTRACT

A 5 year old Malnad gidda cow, under the jurisdiction of the Veterinary dispensary Hulikere, Chickmagalore district was presented with a clear history of ingestion of young secondary shoots of jowar (*Sorghum bicolor*). On clinical examination of the case revealed dyspnoea, shivering and brick red conjunctival mucous membrane. Treatment with sodium thiosulphate @660 mg/kg body weight in the affected case along with supportive fluid therapy, B complex and Dexamethazone yielded an uneventful recovery after 3 days.

(Key words: Malnad gidda, *Sorghum bicolor*, HCN, Sodium thiosulphate)

The present case paper highlights successful therapeutic management of HCN poisoning in a Malnad Gidda cow. A 5 year Malnad Gidda cow in a village, which comes under jurisdiction of Veterinary Dispensary, Hulikere, Tq. Kadur, Dist. Chickmagalore, State: Karnataka with a clear history of ingestion of large quantity of secondary shoots of *Sorghum bicolor* (Fig.:1). An observation made on sternal recumbent animal (Fig: 2) revealed restlessness, dyspnoea, slight salivation, shivering of whole body, brick red colored conjunctival mucus membrane (Fig: 3) with normal rectal temperature.



Fig. 1: Wilted secondary shoots of *Sorghum bicolor* in the field where animal was left for grazing



Fig. 2: Animal in sternal recumbency



Fig. 3: Brick red colour conjunctival mucus membrane

As there was clear cut history of ingestion of *Sorghum bicolor* and suggestive clinical manifestation like shivering, brick red mucous membrane, a tentative diagnosis of HCN poisoning was made and the animal was infused with sodium thiosulphate along with dextrose (5%) intravenously at the dose rate of 660mg/kg body weight with and one more dose was repeated after six hours. Supportively, normal saline (NS) 5 bottles intravenously, Inj. Dexamethasone – 5ml intravenously, B-complex preparations intramuscularly were administered. Animal showed a transient recovery and stood up from the sternal recumbency. The above supportive therapy was continued for three days. The animal recovered after third day of treatment. Many treatments were described by Radostits *et al.* (2006) among these sodium nitrite and sodium thiosulphate combination is the standard primary treatment. However sodium nitrite was not accessible to the remote village the only sodium thiosulphate (supplied by the

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department of Animal Husbandry and Veterinary Services, Government of Karnataka) was available. Hence, only sodium thiosulphate was used at larger dose.



Fig. 4: After treatment animal in standing position

Cyanide poisoning (HCN) or prussic acid poisoning or hydrocyanic acid poisoning occurs when livestock consume plant parts from specific forage plants, trees, and weed species that contain cyanogenic glycosides. Cyanogenic glycosides are plant-specific and at least 55 cyanogenic glycosides have been identified so far (Knight and Walter 2001). The glucosides content will be more when the plants are wilted (Radostits *et al.* 2006).

Accidental access to immature *Sorghum bicolor* plants which contains cyanogenic glycoside, is known to produce histotoxic anoxia followed by death in untreated cases. When cyanogenic plants are ingested and chewed, their cell walls and membranes are ruptured, releasing β -glycosidase enzymes that mix with the cyanogenic glycosides. The enzymatic reaction results in the removal of the sugar component, which in turn releases free hydrogen cyanide (HCN), which is extremely toxic to livestock especially ruminants. HCN is absorbed into the bloodstream very quickly and inhibits animal's ability to deliver oxygen to tissue for cellular respiration. Some HCN is naturally detoxified by the body, converting it to thiocyanate, which is then excreted in the urine (Knight and Walter 2001). However, toxicity will occur when the rate of HCN production exceeds the rate of detoxification. The minimum lethal blood

concentration level for HCN is approximately 3.0 $\mu\text{g/mL}$ or less (*The Merck Veterinary Manual*).

Ruminants, such as cattle and sheep, are especially susceptible because release of HCN results from ruminal microbial fermentation (Vough and Cassel 2006). While HCN is also toxic to non-ruminants, such as horses and swine, hydrochloric acid in the non-ruminant's stomach reacts with HCN resulting in some production of formic acid and ammonium chloride, both of which are less toxic than HCN.

Lohit *et al.* (2014) reported rubber tree leaves (*Hevea brasiliensis*) toxicity in Malnad gidda cattle by using picric acid paper spot test. The authors also stated that deaths were reported within 24 -48 hrs after the ingestion of the plant leaves in this breed. But in the present case, early identification of causal factor helped in successful recovery of animal.

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Congenital Ventral Abdominal Hernia in a Jersey Crossbred Calf: A Case Report.

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ABSTRACT

A six days old Jersey crossbred female calf was presented to the Department of Surgery and Radiology with a complaint of soft swelling behind the umbilical region since birth. Clinical examination revealed the hernial ring and reducible hernial contents. Herniorrhaphy was performed which led to uneventful recovery.

Key words - Hernia, Ventral, congenital

In animals ventral hernia occurs due to any trauma such as a kick, blow, horn thrust or falling on blunt objects and rupture of pre pubic tendon (Frank, 1981) and it can occur at any part of the abdominal wall other than a natural orifice (Tyagi and Jith singh, 2008). Primary ventral hernias are abdominal wall hernias that occur spontaneously and are not associated with a fascial scar or related to trauma. Ventral or lateral hernia is commonly seen along the costal arch, high or low in the flank, between the last few ribs or in the ventral abdominal wall (Berge and Westhues, 1966).

A 6 days old female Jersey crossbred calf was presented to the Department of Surgery and Radiology with a complaint of soft swelling behind the umbilical region since birth (Fig 1.). Animal was active and was taking milk normally. Clinical examination revealed a soft huge swelling behind the umbilical region. The animal was restrained in dorsal recumbancy and the hernial ring was palpated after pushing the contents into the abdomen. Hernial ring measured about 8cm and the hernial contents were reducible.



Fig 1. A huge hernial mass noticed at the ventral abdomen in a female Jersey crossbred calf.

Ventral abdominal area was prepared aseptically. Pre-operatively antibiotic ceftriaxone (Intacef, Intas Pharmaceuticals Ltd, Ahmedabad) 500mg was given intramuscularly. Local analgesia was produced by infiltrating the 2 per cent lignocaine Hcl around the hernial ring. An elliptical skin incision was made directly on the hernia swelling (Venugopalan, 2009) The sub-cutaneous tissues were dissected gently to expose the hernial ring. Examination of the hernial contents revealed intestinal loops (Fig 2.) and they were reduced back into the abdomen after washing with sterile normal saline. Hernial ring was debrided and closed by cruciate suture pattern (Fig 3.) using Polypropylene no.1(Trulene, Sutures India Pvt Ltd, Bangalore). Subcutaneous tissue was apposed with chromic catgut no.1(Trugut, Sutures India Pvt. Ltd, Bangalore) by simple interrupted pattern and finally the skin was closed by horizontal mattress pattern using nylon(Trulon, Sutures India Pvt. Ltd, Bangalore). Wound was dressed and bandaged. Post-operatively, Meloxicam(Melonex, Intas pharmaceuticals Ltd, Ahmedabad) 2ml was given intramuscularly. Antibiotic therapy was continued for another 6 days. On the 10th postoperative day sutures were removed and the animal recovered uneventfully.

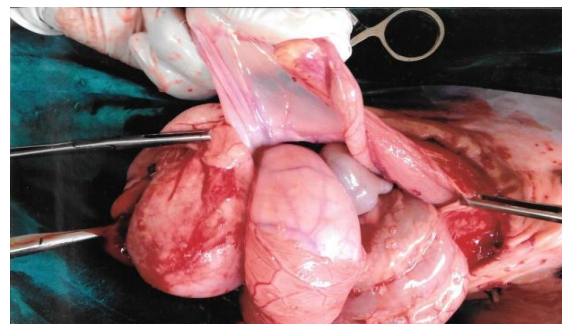


Fig 2. Intra-operative photograph showing intestinal loops as hernial content.



Fig 3. Photograph showing closure of the hernial ring by cruciate suture pattern

Ko *et al.*, (1990) quoted that the cause of congenital ventral hernia and other congenital anomalies in the calf was probably genetic in origin due to the close relationship between the dam and the sire and the negative history for such various tetragenic factors. Saradamma *et al.*, (2000) stated that faulty closure of the abdominal opening in the prenatal life resulted in the protrusion of parts of the abdominal viscera with its serous sac. In the present case, exact cause of the hernia was not known and it might be due to the congenital defect in the abdominal wall. Vidya Sagar *et al.*, (2010) mentioned that successful recovery of ventral hernia cases might depend on the early presentation to the veterinarian and its reducible nature. In the present case the animal was presented early and there was no adhesions of the hernial contents. Jawre *et al.*, (2009) reported a case on ventral abdominal hernia in a male calf and stated that there were adhesions between the loops of intestine and urinary bladder. Singhal *et al.*, (2012) used nylon mesh for hernioplasty for the repair of congenital ventral abdominal hernia in a buffalo calf. Simon *et al.*, (2013) and Thangadurai and Vijayakumar (2013) reported that hernial ring was closed with non absorbable suturing material by overlapping suture technique.

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Clinico-Epidemiological Studies of Hepatic Disorders in Dogs in Tirupati *

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ABSTRACT

A total of 55 dogs were suspected for hepatic disorders when presented to Clinics and out of them 22 were confirmed for hepatic disorders and were included for the study by comparing the clinical symptomatology and haemato-biochemical findings. The prevalence of the hepatic disorders was found to be 1.93 per cent out of total cases presented to Clinics and 40 per cent out of suspected cases. Breed wise prevalence showed highest prevalence in German shepherd and Mongrel breeds. Age wise prevalence revealed higher rate in the age group of 4 to 6 years and the average age at which the hepatic disorders occurred was 4.8 yrs. Prevalence in relation to sex depicted higher prevalence in females. Anorexia / inappetance and depression to lethargy were the most common clinical symptoms revealed by dogs with hepatic disorders. Apart from these, other clinical symptoms observed were anaemia, fever, emaciation, vomition, icterus, ascites, diarrhea, polydipsia and melena.

Key words: Hepatic disorders, Prevalence, Anaemia, Dog, Icterus

Hepatic disorders in dogs are encountered in all age groups accounting for 3 per cent of all disease (Hardy, 1983). They can occur due to direct damage to the liver by toxins, infectious agents as well as metabolic, immune-mediated and neoplastic problems (Cornelius, 1992). The present article describes the prevalence of hepatic disorders in dogs in Tirupati and breed, age and the sex wise distribution of hepatic disorders among dogs.

Among 1138 dogs presented to the Medicine Ward of Teaching Veterinary Clinical Complex and College Clinic, College of Veterinary Science, Tirupati (for the period of one year) 55 dogs with clinical signs suggestive of hepatic disorders were included in the study and were considered as suspected cases of hepatic disorders. The clinical signs suggestive of hepatic disorders like appetite, amaemia, jaundice, ascites etc were observed and recorded. Blood and serum samples were collected from suspected cases for laboratory tests. The hepatic disorders were confirmed on the basis of clinical signs and haemato-biochemical findings. The data obtained was analyzed for the rate of prevalence and breed, age and sex wise distribution of hepatic disorders.

Prevalence of Hepatic Disorders: The total number of dogs presented at the two hospitals during the study period was 1138. Among them 55 dogs were suspected for hepatic disorders as they showed few or all of the symptoms of hepatic disorders like anorexia / inappetance, loss of body weight, jaundice, ascites, vomiting, diarrhea, dullness and lethargy. Out of 55 suspected, 22 dogs were confirmed to be affected with hepatic disorders after observing the altered haemato-biochemical findings.

The prevalence rate of hepatic disorders in the present study was 1.93% (22/1138) with relation to total cases and 40% (22/55) out of suspected cases. This is slightly less compared to the observations of Chandlin (1968) and Vijayakumar *et al.*, (2003), who have reported 3.00% and 3.01% prevalence respectively.

Prevalence in relation to breed: German shepherd and Mongrel breeds showed higher rate of prevalence (36.36% and 22.72% respectively) among the various breeds of dogs affected with hepatic disorders. This is in concurrence with Rutgers *et al.*, (1993) and Vijayakumar *et al.*, (2003), who also reported higher prevalence in German shepherd. However, it was also noticed that German shepherd dogs were over represented (18/55) in the present study.

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Prevalence in relation to age: The highest prevalence rate (27.27%) was recorded in 4 to 6 years age group and the average age of occurrence of hepatic disorders was 4.8 years. Similar finding was reported by Anderson and Sevelius (1991) and Speeti *et al.*, (1996) and Vijayakumar *et al.*, (2003) recorded almost similar observations in which the average age of the dogs affected with hepatic disorders was 5.5, 5.1 and 4.8 years respectively.

Prevalence in relation to sex: Sex wise prevalence revealed higher prevalence in females (59.09%) as compared to males (40.09%). Previous studies also reported a higher prevalence rate in females (Rutgers and Haywood, 1988; Kirpensteijn *et al.*, 1993 and Vijayakumar *et al.*, 2003). The gender variation could be due to the involvement of various genes in the pathogenesis of the diseases as observed by Anderson and Sevelius (1991).

Clinical signs: Anorexia / inappetance and depression to lethargy were the most common clinical symptoms occurred in dogs with hepatic disorders which were accounting for 81.81 per cent and 63.63 per cent respectively. Apart from these other clinical symptoms observed were anaemia (61.18%), fever (31.81%), emaciation (22.72%), vomition (18.18%), icterus and ascites (18.18% each), diarrhea and polydipsia 913.63% each) and melena (9.09%). Varshney and Hoque (2002) reported common clinical findings in canine hepatopathies like nausea / vomition, jaundice, mild anaemia, abdominal distension, constipation, diarrhea, head pressing, apparent blindness, convulsion, apparent blindness, pyrexia, melena, hypotension and coma. Rothuizen and Meyer (2000) reported reduced appetite or anorexia as non-specific clinical sign encountered in liver diseases. Sevelius (1995) observed decreased appetite to anorexia and depression to lethargy, the most common complaints in chronic cholangiohepatitis. Clinical signs of chronic

hepatitis in early stage include depression, anorexia, weight loss, vomiting and diarrhea and in advanced condition, icterus, ascites, polyuria and polydipsia as well as neurological signs are the common clinical symptoms as described by Rutgers and Haywood (1988).

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Studies on the Prevalence of Mastitis and Antibiotic Sensitivity Pattern of Bacterial Isolates in Buffaloes of Tumkur and Chitradurga Districts of Karnataka.

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ABSTRACT

A study was designed to determine the prevalence of mastitis in buffaloes and susceptibility of bacterial isolates to various antibiotics. Milk samples collected from buffaloes were subjected to screening by using California Mastitis Test. The samples found positive were cultured on culture media such as Blood agar, MacConkey's sugar, Staphylococcus medium and Nutrient agar. The isolated organisms were identified and subjected to 15 commonly used antibiotics for determination of antibiotic sensitivity. Two hundred and sixty six milk samples from buffaloes were tested for mastitis out of which 195 (73.31%) were found positive. Coagulase Negative Staphylococcus was the most frequently isolated pathogen (52.7%) followed by *Streptococcus spp.* (11%), Coagulase Positive Staphylococcus (9.54%), *Bacillus spp.* (9.13%), *E. Coli* (7.88%), *Enterobacter spp.* (4.15%), *Proteus spp.* (3.32%), *Klebsiella spp.* (0.83%) and *Pseudomonas spp.* (0.83%). Bacterial isolates were highly sensitive to Chloramphenicol (81.33%) and least sensitive to Penicillin-G (21.58 %)

Key words: Antibiogram, buffalo, California mastitis test, staphylococcus

A present study was designed to determine prevalence of mastitis by California Mastitis Test in buffaloes and to isolate the bacterial organisms from mastitis positive milk samples and to determine antibiotic sensitivity of the isolates to the commonly used antibiotics.

A total of 266 milk samples of buffaloes maintained by individual farmers from Tumkur and Chitradurga districts of Karnataka were aseptically collected to determine the prevalence of mastitis based on California Mastitis Test (Muhammad *et al.*, 1995). Positive milk samples were cultured on blood agar, Macconkey's agar, *Staphylococcus* medium and nutrient agar. The inoculated plates were incubated aerobically at 37°C for 24-48 hours. The bacterial isolates were identified on the basis of their cultural, biochemical and morphological characteristics (Hargital *et al.*, 1992). All the bacterial isolates were tested *in vitro* for their sensitivity to 15 different antibiotics commonly used in veterinary practices. These included amikacin, amoxicillin/sulbactam, ampicillin, cefotaxime, cefoxitin, ceftriaxone, ceftriaxone/sulbactam, chloramphenicol, cotrimoxazole, enrofloxacin, gentamicin, methicillin, oxacillin, penicillin-G and streptomycin

antibiotic. Single colony of isolates were inoculated into BHI broth (1 ml) and incubated for six hrs at 37°C until there was visible growth. The bacterial suspension was plated on to MHA plates with sterile cotton swab. Antibacterial discs were laid on the dried inoculums with the help of a dispenser. The plates were incubated at 37 °C for 24 hrs. Sterile disc was also used as control. The zone of inhibition was estimated using antibacterial clearance zone measuring scale in mm.

Out of 266 milk samples from buffaloes 195 were found positive for mastitis. The overall prevalence of mastitis was 73.31%. The month wise prevalence of mastitis ranged from 58.18 to 91.3%. Month-wise occurrence was 58.18%, 73.3%, 75%, 91.3%, 77.5% and 64% in January, February, March, April, May and June month respectively. The highest occurrence seen in April month (91.3%) and lowest seen in January month (58.18%). These findings are in agreement with the observations of Tuteja *et al.* (2003) and Gowardhan *et al.* (2008) who reported that the occurrence of mastitis in buffaloes ranged from 72-80%. However, Kavitha *et al.* (2009) and Guha *et al.* (2012) reported a lesser prevalence of

24.21 and 42.50% mastitis cases in milking buffaloes respectively.

Coagulase negative *Staphylococcus* was the most frequently isolated pathogen (52.7%) followed by *Streptococcus* spp. (11%), Coagulase positive *Staphylococcus* (9.54%), *Bacillus* spp. (9.13%), *E. coli* (7.88%), *Enterobacter* spp. (4.15%), *Proteus* spp. (3.32%), *Klebsiella* spp. (0.83%) and *Pseudomonas* spp. (0.83%). Similar results were reported by Memon *et al.* (1999) who observed that major pathogenic organisms in mastitis were *Staphylococcus* (38%) followed by *Streptococcus agalactiae* (13%), *E. coli* (11%) and *Klebsiella pneumoniae* (11%). Bhalerao *et al.*, (2000) noted that major pathogenic organisms were *Staphylococcus* (54.55%) followed by the *Streptococci* (36.36%) *E. coli* (4.55%) and *Klebsiella* (2.27%).

In the present study the pattern of sensitivity of 241 isolates to different antibacterials in decreasing order was chloramphenicol (81.33%), co-trimoxazole (79.26%), gentamicin (73.44%), oxacillin (70.95%), amikacin (64.32%), enrofloxacin (58.1%), ceftriaxone (51.45%), cefotaxime (39.42%), streptomycin (38.59%), cefoxitin (37.34%), ceftriaxone/sulbactam (36.1%), amoxicillin/sulbactam (29.05%), ampicillin (27.8%), methicillin (25.73%) and penicillin-G (21.58%). The antibiotic sensitivity of different drugs used against different isolates obtained during the study period showed that bacterial isolates were highly sensitive to Chloramphenicol (81.33%) and least sensitive to Penicillin-G (21.58%). The findings of present study were in agreement with the findings of Rasetti (1993) who reported *Staphylococcus* sp. was 100% sensitive to Cotrimaxazole. The present findings are not in agreement with the findings of Rashid (2001) who reported different sensitivity pattern for each isolate. The variation in the present study in the sensitivity pattern could be attributed to the variation in sensitivity of different isolates in different geographical locations and resistance to commonly used antibacterials.

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Certain Biochemical Studies on the Uterine Flushings of Normal and Endometritis Cows*

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ABSTRACT

The biochemical parameters viz., pH, alkaline phosphatase (ALP) and total protein in the uterine flushings of estrus cows with endometritis (n=72) and normal ones (n=12) were studied. The pH, ALP and total protein in the uterine flushings were significantly ($p > 0.01$) higher in endometritis cows and can be used to predict subclinical endometritis.

Key words: crossbred cows, uterine flushings, pH, alkaline phosphatase, total protein, endometritis.

Endometritis due to uterine infection (Agarwal et al., 2002) and secondary inflammation following coitus, artificial insemination and parturition (Roberts et al., 1970) causes infertility in cows. Higher pH (Boitor et al., 1980), increased enzymatic activity (Denisenko et al., 1981) and increased total protein (Williamson et al., 1983) in the secretion of endometritis cows are reported. The present investigation was aimed to evaluate the pH, alkaline phosphatase activity and total protein concentration in uterine flushings of healthy and endometritis cows

Uterine flushings from 12 healthy and 72 endometritis crossbred cows were collected aseptically during estrus by infusing 20ml of sterile normal saline using ultraviolet sterilized artificial insemination sheath. The uterine flushing was collected after gentle massaging of the uterus and transferred into sterile test tubes. To remove the tissue debris, the uterine flushing was centrifuged at 5000rpm for 15 minutes and the supernatant fluid was used for estimations. The pH of uterine flushing was recorded using a digital pH meter (Systronic, Model-3335). The activity of alkaline phosphatase (Kind and King, 1954) and total protein (Lowry et al., 1951) were estimated by an autoanalyser (Merck's selector-2) using standard kit. The data was analysed according to Snedecor and Cochran (1980) and the group means were (Merck's and the group means were compared by least square significance difference test (LSDT) as per Steel and Torrie (1981).

pH: The hydrogen ion concentration of uterine flushings was significantly ($P \leq 0.05$) higher in endometritis cows (7.88 ± 0.11) than in healthy cows (7.18 ± 0.08 ; Table), which agrees with the report of Boitor et al., (1980). In repeat breeding cows with endometritis due to infection the metabolites of bacteria and inflammatory exudates may alter the pH of uterine fluid to alkaline side resulting in failure of conception due to death of spermatozoa (Raghavan et al., 1971; Singla et al., 1991)

Table. Certain biochemical attributes of uterine flushings from healthy and endometritis crossbred cows

Parameter(s)	Healthy cows (n=12)	Endometritis cows (n=72)
pH	7.18±0.08	7.88±0.08**
Alkaline phosphatase (U/L)	236.33±15.00	343.20±66.90*
Total protein (g/dl)	0.395±0.03	0.679±0.06**

* $P \leq 0.05$; ** $P \leq 0.01$.

Alkaline phosphatase: The activity of alkaline phosphatase was significantly ($P \leq 0.05$) higher in the uterine flushing of endometritis cows (343.33 ± 66.90 U/L) as compared to healthy cows (236.33 ± 15.00 U/L; Table). This is in agreement with the reports of Ahmed and Salaby (1993), Rao (1995) and Boos et al., (1988). Increased enzymatic activity in tissues, body fluids and secretions have been reported in many pathological condition including inflammation (Denisenko et al., 1981; Ismail et al., 1985).

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Total protein: The total protein concentration in the uterine flushings was significantly ($P < 0.01$) higher in cows with endometritis (0.679 ± 0.06 g/dl) than in healthy cows (0.395 ± 0.03 g/dl; Table). This is in agreement with the report of Rao (1995), in crossbred cows suffering with endometritis. The increased protein concentration in uterine flushings of endometritis affected cows might be due to increased levels of secretory proteins, cellular debris and tissue damage (Williamson et al., 1983).

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Ultrasound Diagnosis of Cryptorchidism in Sheep

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ABSTRACT

Fifteen bilateral Cryptorchid Lambs of Mandya breed maintained at Livestock Research and Information Centre (Mandya Sheep) were subjected to ultrasonography . Using real time B-mode ultrasonography , abdominally located cryptorchid testis were precisely located based on texture and echogenicity . Ten bilateral cryptorchid Sheep of Hassan breed from slaughter house were also subjected to ultrasonography before slaughter and physical position of retained testis in slaughtered carcasses were recorded. Testicular retention was found to be significantly more in abdominal region compared to inguinal and sub cutaneous region in both the breeds of Sheep. Various aspect of cryptorchidism in Sheep and efficacy of ultrasonography in detecting the cryptorchidism in sheep is discussed in this article .

Key Words : Cryptorchidism, Sheep, Ultrasonography,

Cryptorchidism is failure of one or both testis to be positioned in the scrotum at the time normal for a species. Infertility and various forms of testicular cancer are the major consequences of cryptorchidism. In animal breeding, the occurrence of cryptorchidism leads to economic loss and decreased selection potential of male breeding stock. The knowledge of the anatomical position of the retained testis helps for surgical removal of the retained testis and also to narrow down the search for the causative defect in the physiological process of testicular descent and thus the genes responsible in the process.

Diagnostic ultrasonography has become an important component of small animal theriogenology since its introduction to practice in 1978. Based on the position of testis retention along the normal route of testicular descent, the cryptorchid testis are classified as abdominal, inguinal and subcutaneous testis. Hence the present work was undertaken to evaluate ultrasonography as a diagnostic aid in identifying the position of testicular retention in sheep.

Fifteen bilateral cryptorchid lambs of Mandya breed maintained at Livestock Research and Information Centre (Mandya Sheep) were subjected to ultrasonography of the abdomen. Further, ten bilateral cryptorchid sheep of Hassan breed from slaughter house were subjected to

ultrasonography before slaughter and physical position of retained testis in slaughtered carcasses were recorded. (Fig-1)



Fig-1 Position of retained testis caudally attached to the kidneys in slaughtered carcasses

The cryptorchidism in sheep was ascertained through physical palpation. The cryptorchid sheep were separated and fasted overnight. The wool around sub lumbar area, right and left flank, and caudo-ventral region was clipped to facilitate ultrasound scanning.

The ultrasonography was performed with animal in dorsal or lateral recumbancy. A portable, B-mode, real time scanner fitted with a 7.5 MHz, linear array transducer was used. After applying acoustic coupling gel, the transducer was held parallel to the long axis of the animal for longitudinal images and perpendicular to the spine for transverse images. The animals were positioned

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in lateral recumbancy and the kidneys were imaged from the uppermost or the dependent side. After locating the kidneys, the testis were searched from the caudal border of the kidneys to downwards. The area around the scrotal neck was examined followed by the inguinal region before moving cranially to the area surrounding the bladder and bladder neck.

The cryptorchid testis was found with uniform texture with echogenicity similar to the spleen with thin centrally located mediastinum testis evident as a thin hyperechoic line. The epididymis (head, body, tail) was found less echoic than the testis. The ductus deferens was difficult to visualize. The spermatic cord adjacent to the head of the epididymis had obvious, tortuous, small diameter veins.

Transabdominalreal time ultrasonography scanner fitted with a 7.5 MHz, linear array transducer was found to be effective in identifying the position of testicular retention in sheep. The area between the caudal pole of the ipsilateral kidney and the cranial aspect of the bladder were found to be the common anatomical position for intra abdominal testicular retention. In majority of the cases, retained testis were positioned at intra abdominal region, posterior to the kidneys (Fig-2). In cases of cryptorchidtestis on the left side, the best position of the transducer for identifying the retained testis was just ventral to the sublumbar musculature caudal to the last rib and for the right retained testis, the best position for identifying the retained testis was between the 11th and 12th intercostal space.



Fig-2: Sonographic images of retainedtestis positioned at intra abdominal region, posterior to the kidneys.

Location of undescended testis differs greatly among species. For cats, dogs and horses, 50, 92 and 47-60 per cent of retained testis were in the abdominal cavity (Amann and Veeramachaneni, 2006). For humans, abdominal retention was less

common (~8%) and almost 90 per cent of undescended human testis were subcutaneous, just outside the external inguinal ring or near the neck of the scrotum (Barthold and Gonzales, 2003). In pigs, subcutaneous retention was predominant (McPhee and Buckley, 1984). In cattle, abdominal retention was observed in about 34 per cent of the cases (St Jean *et al.*, 1992). Smith *et al.* (2012) in an abattoir based study on sheep in England reported that 72.3 per cent of all the affected testicles were retained in abdomen. In the present study testicular retention was found to be significantly more in abdominal region compared to inguinal and sub cutaneous region in both the breeds of sheep. The proportion of retained testis was 90:10:0 and 75:20:5 per cent in abdominal, inguinal and sub cutaneous regions of Mandya and Hassan breeds of sheep respectively.

The retained testis can be positioned anywhere between the ipsilateralkidneyand the scrotum. A systematic evaluation of the region from the caudal renal pole to the inguinal canal can identify an oval, homogenously echogenic structure with a mildly hyper-echoic border representing the parietal and visceral tunics. The epididymis can be seen usually distinctly less echoic than the testicular parenchyma, as in the scrotal testis. The cryptorchidtestis will maintain the anatomic structure, the mediastinum testis (a hyperechoic slash), and normal testicular echogenicity despite being reduced in size as compared to a scrotal testis.

The position of all retained testis recorded by ultrasonography matched with the physical position observed in slaughtered carcasses, thus indicating the high specificity of the method in detecting. Using ultrasonography we were able to identify the position of 45 of the 50 retained testis, accounting to 90% sensitivity of the technique in identifying the position of retained testis. Even though, Scott (2012) demonstrated the utility of abdominalultrasonography as an adjunct to clinical examination in small ruminants, its utility for cryptorchid diagnosis in sheep has not been reported earlier. Schambourget *al.* (2006) reported that trans-abdominal ultrasonography as a reliable and a safe technique to diagnose cryptorchidism

precisely in horses of all ages, sizes and temperaments. Hecht *et al.* (2004) demonstrated the utility of transabdominal ultrasonography in identifying the intra abdominal retained testis with torsion in dog.

The ultrasonography method of detecting the position of testis was found to be equally effective as the one based on slaughter data, and hence can be effectively used in diagnosis of cryptorchids in farms. The significantly higher abdominal retention of testis observed in Mandya and Hassan breeds. Transabdominal phase of testicular descent may be the prominent cause for cryptorchidism in both Mandya and Hassan breeds of sheep.

Eventhough the use of ultrasound as a tool in small animal reproduction in the approach to clinical reproduction (infertility, urogenital disorders and paediatrics) is well documented, the application in identifying cryptorchid testis in small ruminants is yet to be utilised to its full potential .A systematic evaluation of the region from the caudal renal pole to the inguinal canal can identify distinguished oval, homogenously echogenic structure with a mildly hyperechoic border. The cryptorchid testis will maintain the anatomic structure, the mediastinum testis (a hyperechoic slash), and normal testicular echogenicity despite being reduced in size as compared to a scrotal testis. Application of ultrasonography can be effectively exploited in identifying cryptorchid testis in Small ruminants.

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