Efficacy of Enrofloxacin and Enrofloxacin with Ampicillin sulbactam Combination in the Treatment of Subclinical Mastitis in Dairy Cows*

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ABSTRACT

The present study was undertaken to evaluate and compare the efficacy of Enrofloxacin and Enrofloxacin with Ampicillin sulbactam, in treatment of Subclinical mastitis (SCM). Eighteen animals with SCM, randomly divided into three treatment groups each comprised of 6 cows - Group I (control group), Group II (Enrofloxacin @ 5 mg/Kg body weight Intramsucular) and Group III (Enrofloxacin @ 5 mg/Kg body weight with ampicillin-sulbactam @ 10 mg /Kg body weight. Treatment was offered for 5 days. Somatic Cell Count(SCC) in milk was done on zero, 4th, 8th, 15th, 30th, 45th, 60th, 75th, 90th, 105th and 120th day post treatment to assess the response to the treatment.

Statistical analysis of mean SCC from 4th to 120th day post treatment within each treatment group revealed significant decrease ($P \leq 0.05$) from the zero day value. Further mean SCC in Group II and Enrofloxacin + Ampicillin Sulbactam(Group III) resulted in less than 5 Lakh/ml upto 30th day and 90th day post treatment respectively. It was concluded that Enrofloxacin with Ampicillin sulbactam is more efficacious and has a prolonged therapeutic effect over Enrofloxacin alone, in treatment of SCM in dairy cows.

Keywords : Bovine - Subclinical mastitis - Treatment - Evaluation**.**

Mastitis, an inflammation of the mammary gland, usually occurs primarily in response to intramammary bacterial infection, but also to mycoplasmal, fungal, viral or algal infections. Mechanical trauma, thermal trauma, and chemical insult may predispose the gland to intramammary infection (IMI). Occurrence of mastitis depends on the interaction of host, agent, and environmental factors. The severity of mastitis can be classified into sub-clinical and clinical form, of which subclinical mastitis is most important as far as dairy economics is considered, affecting 20 to 50 per cent of cows (Wilson *et al.*, 1997 and Pitkala *et al*., 2004). Although the loss due to SCM is difficult to quantify, it costs the average dairy farmer, more than the clinical mastitis does (Hegde, 2011).

Subclinical mastitis can be diagnosed only by laboratory examination, as there are no gross inflammatory changes in the cow, udder and milk. Though several diagnostic tests are adopted, International Dairy Federation (IDF) recommendation is based on SCC and microbiological status of the udder (Hillerton, 1999).

Treatment of SCM in dairy cows is important. Antibiotics or antibacterials are used either alone or in combination and with supportive chemotherapeutic agents (anti inflammatory drugs, vitamins, etc.,) or chemicals. In the present study efficacy of a single chemotherapeutic agent (Enrofloxacin) and a combination of them (Enrofloxacin + Ampicillin sulbactam), in treatment of SCM was evaluated.

MATERIALS AND METHODS

Eighteen dairy cows confirmed positive for SCM were divided into three groups each comprised of six animals as under :

Group I : Control Group

Group II : Enrofloxacin* @ 5 mg/Kg body weight Intramsucular s.i.d for five days.

Group III : Enrofloxacin @ 5 mg/Kg body weight Intramsucular s.i.d for five days and Ampicillin with sulbactam combination** @ 10 mg /Kg body weight Intramuscular s.i.d. for five days.

Floxidin ^{R} was obtained from M/s.Intervet India Pvt. Ltd., Pune

Ampicillin Sulbactam – was procured from M/s. Unichem Laboratories Pvt. Ltd, Mumbai.

Response to the treatment was assessed by collecting milk samples from all the eighteen cows

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under study, on zero, 4th, 8th, 15th, 30th, 45th, 60th, 75th, 90th, 105th and 120th day post treatment and subjected to determination of SCC.

Somatic Cell Count : SCC was done using Nucleocounter (Chemo Metec, Denmark). The data generated in the study was statistically analyzed by Student's 't' test and one way ANOVA at $P \leq 0.05$, by using statistical software.

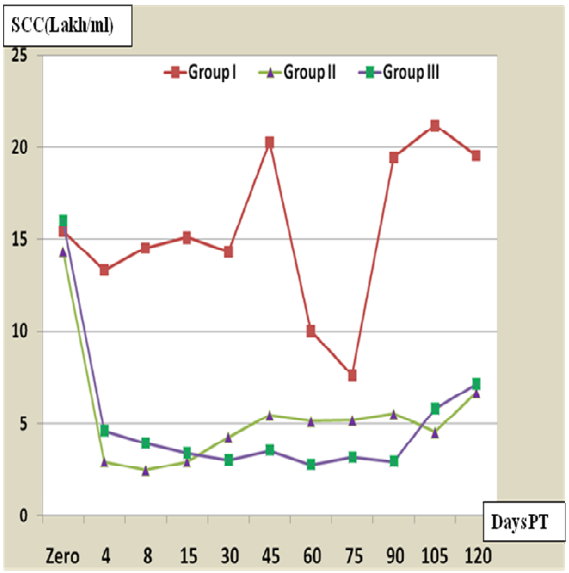
RESULTS AND DISCUSSION

In Group I (control group), the mean SCC value from zero to 120th day ranged between 7.63 Lakh/ml to 21.17 Lakh/ml with lot of variations and no definite pattern. In Group II (Enrofloxacin alone), mean SCC values decreased from a base value of 14.35 Lakh/ml (zero day) to a lowest value of 2.45 Lakh/ml on 8th day post treatment, later increased without any definite pattern and remained in the range between 2.45 Lakh/ml to 6.67 Lakh/ml. In Group III (Enrofloxacin with Ampicillin Sulbactam), mean SCC decreased from 16.00 Lakh/ml (zero day) to 2.75 Lakh/ml on 60th day post treatment and subsequently it increased without any definite pattern and on 120th day the mean SCC was 7.12 Lakh/ml.

Table I. Mean ± SE of SCC in Control group(Group I) and groups treated with Enrofloxacin(Group II) and Enrofloxacin + Ampicillin Sulbactam (Group III), in treatment of SCM in dairy cows.

Post treatment days of collection	Group I SCC (Lakh/ml)	Group II SCC (Lakh/ml)	Group III SCC (Lakh/ml)
Zero	$^{x}15.44\pm2.90^{a}$	$^{x}14.35\pm2.69^{a}$	$^{x}16.00\pm1.62^{a}$
4	$^{x}13.33\pm1.94^{a}$	$^{y}2.92\pm0.48^{b}$	$^{y}4.58\pm0.78^{b}$
8	$x14.53\pm2.54^{a}$	$^{y}2.45\pm0.39^{b}$	$^{y}3.92\pm1.12^{b}$
15	$^{x}15.09\pm2.54^{a}$	$^{y}2.92\pm0.53^{b}$	$^{y}3.39\pm0.74^{b}$
30	$^{x}14.33\pm2.77^{a}$	$^{y}4.25\pm0.61^{b}$	$^{y}3.00\pm0.63^{b}$
45	$^{x}20.27\pm8.27^{a}$	$^{y}5.44\pm0.33^{b}$	$^{y}3.52\pm0.60^{b}$
60	$^{x}10.02\pm2.95^{a}$	$^{y}5.10\pm0.22^{a}$	$^{y}2.75\pm0.45^{a}$
75	$^{x}7.63\pm3.82^{a}$	$^{y}5.17\pm0.27^{a}$	$^{y}3.16\pm0.58^{a}$
90	$^{x}19.45\pm12.07^{a}$	$^{y}5.51\pm0.40^{a}$	$^{y}2.93\pm0.39^{a}$
105	$^{x}21.17\pm14.14^{a}$	$^{y}4.51\pm0.49^{a}$	$^{y}5.79\pm0.32^{a}$
120	$^{x}19.56\pm9.96^{a}$	$^{y}6.67\pm0.44^{a}$	$^{y}7.12\pm3.24^{a}$

Fig. I. Mean SCC in Control group (Group I) and groups treated with Enrofloxacin (Group II) and Enrofloxacin + Ampicillin Sulbactam (Group III)



Statistically mean SCC from 4th to 120th day post treatment in each treatment group found significantly decreased ($P \leq 0.05$) from the base value (zero day). This indicates that both the treatments i.e., Enrofloxacin alone and Enrofloxacin + Ampicillin sulbactam, decreased SCC values significantly from 4th day to 120 days.

The SCC more than 5 Lakh/ml is considered positive for SCM (Narayana and Iya, 1954). It was observed that the Enrofloxacin group (Group II) resulted in mean SCC less than 5 Lakh/ml upto 30th day post treatment, whereas Enrodloxacin + Ampicillin Sulbactam (Group III) treatment resulted it upto 90th day post treatment. This indicated that efficacy of combination (Enrofloxacin + Ampicillin Sulbactam) was better in Group III, when compared to single antibacterial (Enrofloxacin) in Group II. This observation is in agreement with the findings of Ergun *et al.* (2000), Langoni *et al.* (2000), Hase *et al.* (2008), Peer (2008), Chetan kumar (2009) and Sekkin *et al.* (2010) who have also reported that treatment with combination of antibiotics or antibacterial agents results in better recovery in cases of SCM in dairy cows.

CONCLUSION

It can be concluded that antibacterial combination (Enrofloxacin with Ampicillin sulbactam) has a better therapeutic effect over single antibiotic (Enrofloxacin) in reducing SCC and could be a treatment of choice in treatment of subclinical mastitis in dairy cows.

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Study on Antibiogram Pattern of Bacterial Isolates from Clinical Cases of Mastitis*

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ABSTRACT

In the present study, predominant bacterial isolates recovered from 24 clinical cases of bovine mastitis were *Staphylococcus* sp. followed by *Escherichia coli*, *Streptococcus sp.* and *Klebsiella sp*. The antibiogram pattern revealed that most of the isolates were sensitive to enrofloxacin, amoxycillin followed by erythromycin, ceftriaxone, chloramphenicol, ciprofloxacin gentamicin, and lincomycin.

Key Words: antibiogram, bovine mastitis, *Staphylococcus* sp, *Escherichia coli*, *Streptococcus sp.*

Mastitis is considered as economically devastating and complex disease of dairy animals, predominantly cattle, (Zahid, 2004). A worldwide annual loss due to mastitis is about 35 billion dollars. In India dairy industry, the estimated total loss amounts to Rs. 6053.21 crores per annum in India (Dua, 2001). Multiple etiological agents have been attributed to mastitis viz; bacterial and fungal. The most important bacterial etiology involved in mastitis are *Staphylococcus aureus, Streptococcus* sp*, Escherichia coli, Corynebacterium* sp*, Proteus* sp*, Pseudomonas* sp and *Klebsiella* sp. There exists a distinct difference in incidence and the pattern of causative agents from place to place, herd to herd and even time to time. To reduce economic loss and for effective treatment of mastitis, there is a need to update the current status of the pathogens involved in mastitis and their antimicrobial sensitivity pattern for effective treatment.

MATERIALS AND METHODS

Milk samples collected from 24 cows affected with acute mastitis presented to Veterinary College Hospital. Bangalore Veterinary College dairy farm and Teaching Veterinary Clinical Service Complex, Yehalanka, Bangalore. The pure cultures were obtained from collected samples and further isolates were subjected for identification and confirmation. The isolates were subjected to antimicrobial sensitivity test using disc diffusion method as per Cruickshank *et al*. (1975) on Mueller Hinton agar plate.

RESULTS AND DISCUSSION

Isolation and identification: The frequency of isolation of different bacterial isolates from clinical mastitis cases has been depicted in Table 1. Of the 30 isolates, 20 (66.66%) were Gram-positive and remaining 10 (33.33%) were Gram-negative. This could be due to the fact that Gram positive organisms are the main causative agents for mastitis in cows and similar observation also made by Cruz-Carillo *et al.* (2007).

Table 1. Frequency of different bacterial isolates from 24 bovine mastitis milk samples.

Organisms	Total no. of isolates	Per cent
Staphylococcus sp.	13	43.33
Streptococcus sp.	7	23.33
E. coli	8	26.66
Klebsiella sp.	2	6.66
Total	30	100

The predominant bacterial isolates recovered were *Staphylococcus* sp. (43.33%), followed by *Escherichia coli* (26.66%), *Streptococcus* sp. (23.33%) and *Klebsiella* sp. (6.66%). Many other workers have also made the similar observations Rajeev (2006), Sumathi (2005) and Cruz-Carillo, *et al.* (2007). In contrast, Rahman *et al.* (1984) reported high prevalence of *Staphylococcus aureus* followed

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by *Streptococcus* sp. Rao *et al*. (1989) reported high prevalence of *Streptococcus agalactiae* followed by *Staphylococcus aureus*. Varma *et al.* (2000) reported high prevalence of *E. coli*, followed by *Staphylococcus.* Wani and Bhat (2003) reported high prevalence of *Staphylococcus aureus* followed by *Klebsiella* species. This variation may be due to changing scenario of pathogen from time to time, from herd to herd and from one geographical area to other.

Antibiogram of isolated organisms: The antibiogram patterns of different bacterial isolates have been depicted in Table 2. In the present study, majority of *Staphylococcus* sp. isolates were sensitive to enrofloxacin, amoxycillin and chloramphenicol followed by erythromycin, ampicillin, ceftriaxone, methicillin, spectinomycin, lincomycin and streptomycin respectively. Majority of isolates were resistant to tetracycline, trimethoprim, neomycin, ciprofloxacin, gentamicin,

Table 2: The antibiogram pattern of different bacterial isolates

Organisms	*Staphylococcus* sp	*Streptococcus* sp	*E. coli*	*Klebsiella* sp
Antibiotic	**Sensitivity (%)**			
Ampicillin	76.92	100	12.5	50
Amoxycillin	92.30	100	75	50
Ceftriaxone	76.92	71.42	75	100
Ciprofloxacin	46.15	85.71	75	100
Cloxacillin	23.07	85.71	12.5	0
Chloramphenicol	92.30	28.57	87.5	50
Erythromycin	84.61	85.71	75	50
Enrofloxacin	92.30	85.71	87.5	100
Gentamicin	38.46	85.71	87.5	50
Lincomycin	61.53	85.71	50	50
Methicillin	69.23	85.71	50	100
Neomycin	46.15	71.42	100	100
Penicillin	30.76	28.57	25	100
Streptomycin	61.53	57.14	50	50
Spectinomycin	69.23	14.28	62.5	50
Sulphadiazine	23.07	14.28	62.5	50
Trimethoprim	53.84	42.85	50	50
Tetracycline	61.53	28.57	25	100

penicillin, sulphadiazine and cloxacillin. These findings are in agreement with the observations of Owens *et al.* (1997) and Mallikarjunaswamy and Murthy (1997) with only minor changes in the antimicrobial sensitivity pattern.

The *Streptococcus* sp. isolates were sensitive to ampicillin and amoxycillin. Most of the isolates were sensitive to ciprofloxacin, cloxacillin, erythromycin, enrofloxacin, gentamicin, lincomycin, methicillin, Ceftriaxone and neomycin. Most of the isolates showed resistant to streptomycin, trimithoprim, chloramphenicol, tetracycline, penicillin, spectinomycin and sulphadiazine. These findings in the present study are in accordance with the observations made by Rameshkumar and Anshusharma (2002) with only a minor variations. In contrast Shukla *et al.* (1998) found that Streptococci were most sensitive to penicillin (98.73 %). All *E. coli* isolates were sensitive to neomycin. Most of the isolates showed sensitivity to chloramphenicol, enrofloxacin, gentamicin, amoxycillin, ceftriaxone, ciprofloxacin, erythromycin, sulphadiazine and spectinomycin. Majority of isolates showed resistance to lincomycin, methicillin, streptomycin, trimethoprim, tetracycline, penicillin, ampicillin and cloxacillin. These findings are in agreement with the observations of Vijayalakshmi and Prathaban (2007) with minor variation in sensitivity pattern.

In present study only two isolates of *Klebsiella* sp. were detected. Both isolates were sensitive to ceftriaxone, ciprofloxacin, enrofloxacin, methicillin, neomycin, penicillin and tetracycline. One isolate was found to be sensitive to ampicillin, amoxicillin, chloramphenicol, erythromycin, gentamicin, lincomycin, streptomycin, spectinomycin, sulphadiazine, and trimethoprim. Both the isolates were resistant to cloxacillin. These findings are in agreement with findings of Schroder *et al.,* (2005) with only minor variations. In contrast Vijayalakshmi and Prathaban (2007) reported highest sensitivity to sulphadiazine, and trimithoprim. However, no conclusion can be drawn since only two isolates were used to study the antimicrobial sensitivity pattern in the present study.

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Surgical Management of Patent Urachus in Calves

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ABSTRACT

Five cow calves were presented with the history of dribbling of urine from the umbilicus. All the five calves were diagnosed as patent urachus based on clinical evaluation and were subjected for surgical management. The mid ventral area around the umbilicus was aseptically prepared for surgery. A mid ventral laparotomy was performed. The urachus cord was dissected, separated out from the attachment and it was severed after double ligation proximal to site of infection. The laparotomy wound was closed as per standard procedure. All the cases were given postoperative antibiotic and analgesia and wound dressing was carried out till the healing of the surgical wound. All the calves had an uneventful recovery.

Key words: calves, patent urachus, congenital condition

The most common congenital condition of the urinary bladder is patent urachus. Which is also known as pervious urachus? This condition is more commonly observed in the foals (Mc Gavin *et al*., 2001), Cow calves (Dilipkumar and Dhage, 2010) and rare in the buffalo calves (Mouli, 1988 and Sharma and Shing, 2004). In such calves, urine dribbles from the umbilicus and scalding is observed. The etiology are foetal remnant of urachus, umbilical arteries and veins failure to involude, neonatal omphalitis, umbilical abscess and congenital urethral obstruction (Mc Gavin *et al*., 2001).

This paper presents the surgical treatment of the patent urachus in five cow calves presented to the Teaching Veterinary Referral Hospital Department of Surgery and Radiology Veterinary College, Bidar.

Case history and clinical examination: Four cow calves were presented with the history of dribbling of urine from the umbilicus and one calf had tubular umbilical swelling without dribbling of urine. On clinical examination three calves showed clinically normal heart rate, respiration rate and rectal temperature. However the other two calves showed increase in heart rate, respiratory rate and temperature with umbilical area inflamed, edematous, and pain on palpation. Four calves had patent natural orifice along with dribbling of urine from the umbilicus however in one case it could pass urine only from pervious urachus opening as the natural orifice was not patent. In this calf the distension of abdomen was seen. After performing the abdominal ballottement and abdominocentesis it was confirmed as uroperitonium. All the five calves were diagnosed as patent urachus based on clinical evaluation and they were subjected for surgical treatment.

Surgical Treatment: The mid ventral area around the umbilicus was prepared aseptically. All the cases were administered preoperative antibiotic and analgesic Inj. Streptopencillin @ 10 mg per kg B. Wt. I/M and Inj. Meloxiacm @ of 0.3mg per kg B. Wt. I/M respectively. The calves were controlled in dorsal recumbency and the mid ventral laparotomy was performed under local infiltration anaesthesia with Inj. 2% Xylocaine. Three calves underwent mid ventral laparotomy operation by giving incision over the umbilicus cranial and caudal to it. The careful dissection of the umbilical contents was performed after entering into the abdomen without injuring the abdominal contents i.e. intestines and omentum. Then the urachus cord was dissected, separated out from the attachment and after double ligation with chromic catgut no.1 at bladder side it was severed. Similarly the umbilical remnants along with artery and veins were ligated with chromic catgut in a healthy area and it was severed out from the attachment. One calf which had the tubular swelling, the incision was made over it and the accumulated urine was allowed to drain out from the incision site. Then in similar fashion as explained above urachus cord was ligated and severed. In another calf which had the uroperitonium the incision was given over the

caudal mid ventral region. The complete urine was suctioned out from the abdomen by lavaging two to three times with luke warm normal saline. The ruptured portion of urachus was identified as cause for the uroperitonium and it was dissected and severed from the vertex of the bladder. The severed bladder stump was closed with chromic catgut suture of size 2-0 using purse string technique. Laparotomy wound was closed in two layers with silk and chromic catgut as per standard procedure. In first layer peritoneum and rectus abdominus muscle was sutured with overlapping suture pattern using silk no.1 and in second layer the abdominal fascia was reinforced with first layer using chromic catgut no.1 by interrupted pattern. The skin was sutured with nylon using interrupted mattress pattern in all the five claves. The calf which had urethral obstruction was cleared with delicate introduction of the teat siphon in rotatary motion through the urinary miatus. All the cases were given postoperative antibiotic and analgesia for five days and wound dressing was carried out till the healing of the abdominal wound.

RESULTS AND DISCUSSION

Three calves which had only umbilical dribbling were surgically managed by dissection of the urachus cord and severing it from the bladder after ligation. There were no complications and calves recovered without any uneventful incidences. Similar results were reported by the (Fazili *et al.,* 1998 and Dilipkumar and Dhage, 2010). In one calf which had the tubular umbilical swelling, resembling hernia was surgically treated after the confirmation by aspiration of the contents. The surgical herniorraphy treatment as reported by Dilipkumar and Dhage, (2010) was performed in this calf and it recovered without complications. The calf which had the uroperitonium, cystitis and rupture of the urachus cord was successfully treated by surgery. The biochemical estimation of the serum sample for BUN and creatinine before operation were 55mg/dl and 2mg/dl respectively, indicates the uremic status of calf. After the third day post operation the titre were reduced to 35mg/dl and1.5mg/dl. The uremic condition was reversed by giving the intravenous fluids with Inj. Dextrose noramal saline, Inj. Ringer's lactate during surgery, peritonium lavage with normal saline and post surgery as reported by Fazili *et al.,* 1998. After surgery this calf had urinary incontinence and further it was treated with the Inj. Calcium gluconate 5 ml I/V, Inj. Tonophosphon 2ml i/v and Inj. Beeplex forte having B1, B2 and B6 of 2ml I/V for four times, once in alternate days. The antibiotic for this calf was changed to Inj. Amikacin 250mg i/v to control infection daily for eight days. The calf recovered without complications.

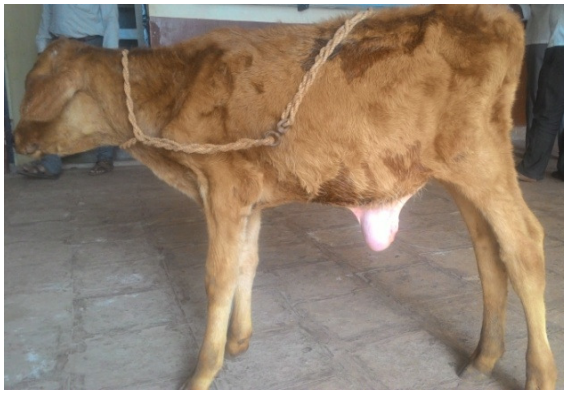

Fig.1 Calf with patent urachus

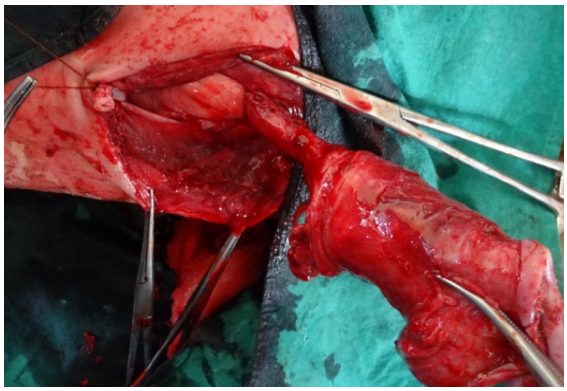

Fig. 2 Photograph showing the resected umbilical artery after ligation and the tubular urachus cord insitu with the umbilicus

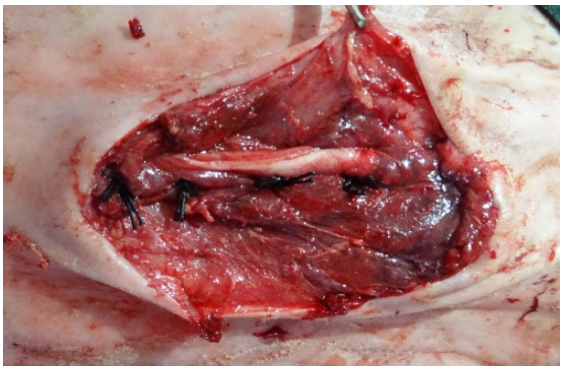

Fig.3 Photograph showing the laparotomy wound sutured with over lapping pattern using silk

CONCLUSION

The calves presented with patent urachus may be a simple case that can be managed by surgical treatment. However the cases like uroperitonium along with urinary obstruction needs thorough therapeutic approach along with surgery in saving the life of the calves.

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Prevalence of Subclinical Mastitis in Goats in and Around Bidar*

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ABSTRACT

Mastitis constitutes one of the important and expensive diseases of dairy industry. Subclinical mastitis (SCM) is difficult to detect due to the absence of any visible indications in the mammary gland and in milk. Two hundred goats under study were grouped based on the parity and stage of lactation. Based on estimation of somatic cell count (SCC), Electrical conductivity (EC) N-acetyl-β-D-glucosaminidase (NAGase) activity and Liquid Phase Blocking ELISA (LPB ELISA), prevalence of SCM was determined for each parity and stage of lactation. It was observed that overall prevalence of SCM based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively. The parity wise per cent prevalence of SCM in goats was highest during seventh parity compared to primiparous goats. Lactation stage wise prevalence was highest during early and mid lactation and least in late lactation.

Key words: Goats, Prevalence, Subclinical mastitis**.**

Goats play an important role in the nutrition and income of people worldwide, as these animals serve primarily as source of meat, but also provide milk and skin (Hansen and Perry, 1994). Goat milk differs from cow milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values. Mastitis, the inflammation of udder, constitutes one of the economically important diseases of dairy industry. Subclinical mastitis (SCM) is difficult to detect due to the absence of any visible indications in the mammary gland and in milk (Mohammadian, 2011). SCM is important due to the fact that it is 15 to 40 times more prevalent and long duration. The condition is difficult to detect, adversely affects milk quality, production and constitutes a reservoir of microorganisms that can infect other animals due to its contagious nature (Schultz *et al.* 1978). The present study was taken up to determine the overall, parity wise and lactation stage wise prevalence of subclinical mastitis in goats in and around Bidar.

MATERIALS AND METHODS

Two hundred apparently healthy goats belonging to various villages in and around Bidar, Karnataka formed the source of animals for the study. Goats were grouped based on the parity and lactation stage. Prevalence of SCM was determined based on estimation of Somatic Cell Count (SCC), Electrical Conductivity (EC), and N-acetyl-β-D-glucosaminidase (NAGase) activity and Liquid Phase Blocking ELISA (LPB ELISA) in milk. SCC was estimated by direct microscopic SCC method and a value more than 5.00 lakh/ml of milk was taken as criteria to declare the milk / animal as subclinically mastitic / infected (Contreras *et al.*, 1996). EC was determined by using ECO Testr EC High (M/s Eutech Instruments, Singapore) and a value more than 6.8 mS/cm was considered as positive for subclinical mastitis (Chen *et al.*, 2008). NAGase activity was estimated as per the method described by Kitchen and Middleton (1976) and value of more than 14.04 µmoles/min/ml was considered as positive for SCM. LPB ELISA was estimated as per the method described by Suryanarayana (2012) and OD value of less than 0.55 was considered as positive for SCM.

RESULTS AND DISCUSSION

The overall, parity wise and lactation stage wise percentage prevalence of subclinical mastitis in goats are presented in Table 1, 2 and 3.

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Overall Prevalence of SCM: In the present study the overall prevalence of SCM in goats based on SCC, EC, NAGase activity and LPB ELISA was 57.50, 52.50, 46.00 and 35.00 per cent respectively (Table 1). This observation of overall prevalence is in corroboration with the observations made by earlier workers namely Leitner *et al.* (2004); Diaz *et al.* (2011); Islam *et al.* (2012) and Zhao *et al.* (2015) who have reported prevalence of SCM in goats to be 52.00, 48.60, 44.59 and 45.82 per cent respectively based on different diagnostic test namely; cultural test, SCC, CMT, White side test and Surf field mastitis test.

Table 1. Percentage prevalence of subclinical mastitis in goats as detected by different diagnostic tests

Screening test (n=200)	No. of animals positive	No. of animals negative	Per cent prevalence
SCC	115	85	57.50
EC	105	95	52.50
NAGase	92	108	46.00
LPB ELISA	70	130	35.00

Earlier workers namely Manju *et al.* (2012) and Prabavathy (2013) have recorded a higher overall prevalence of SCM than the current observation and ranged from 72.50 to 96.81 per cent. Further, overall prevalence of SCM in goats as observed by Kostelic *et al.* (2009); Gebrewahid *et al.* (2012) and Mishra *et al.* (2013) have recorded lower prevalence than the current observation and ranged from 9.70 to 28.14 per cent.

Wide variation in the percentage prevalence of SCM observed by previous workers could be due to the difference in the managemental condition and different diagnostic tests employed (Mishra *et al,,* 2014) and could also be due to breed of the animal, immune response of animals and climatic condition (Gebrewahid *et al.,* 2012). Per cent prevalence of SCM varies significantly with variation or interplay of the risk factors. Further these studies have been conducted in different geographical areas and that can be another reason for recording different percentage prevalence of SCM by some of the earlier workers (Islam *et al.,* 2012). High prevalence of SCM observed by earlier workers could be due to presence of many subclinical carriers, poor milking hygiene and less awareness of subclinical mastitis cases. Due to the poor management, the infected goats including clinical and subclinical mastitis were usually not separated from the healthy goats, and this contaminative environment and equipment would cause a new infection might be greatly contributed to high prevalence or any other deficiency in the management as opined by Zhao *et al.* (2015). Low prevalence of SCM observed by earlier workers may be due to adoption of better managerial practices at the farms, improved techniques of husbandry and inculcation of awareness among dairymen for timely and appropriate treatment of animals and good hygienic management (Tiwari *et al.,* 2000).

Parity wise prevalence of SCM: The parity wise per cent prevalence of SCM based on all the four diagnostic tests, was found to be highest during seventh parity and least prevalence was noticed during first and second parity. Further the per cent prevalence of SCM in goats was found to be increasing trend from second to seventh parity (Table 2). This observation of parity wise prevalence is in corroboration with the observations made by earlier workers namely Sanchez *et al.* (1999) and Kostelic *et al.* (2009) who have reported prevalence increase with the number of lactation. Increase in the prevalence of SCM with increase in number of parity could be associated with gradual loss of immune response in the body of the animal with increase in number of parity, which makes it susceptible to infection and may also be associated with inefficient sphincters. Similar observations were made by Sudhan *et al.* (2005). The increase in prevalence can be explained as a consequence of the long-term mechanical irritation of the teat, the streak canal and the entire udder, due to nursing goat kids and milking (Kostelic *et al.,* 2009).

Table 2. Parity wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

Parity	% Prevalence			
	SCC	EC	NAGase activity	LPB ELISA
I	14.71	26.47	8.82	5.88
II	12.90	9.68	6.45	12.90
III	47.06	35.29	26.47	29.41
IV	76.67	66.67	63.33	23.33
V	93.33	83.33	76.67	53.33
VI	92.59	81.48	85.19	66.67
VII	100.00	100.00	92.86	92.86

Lactation stage wise prevalence of SCM: In the current study the lactation stage wise per cent prevalence of SCM based on all the four diagnostic tests was found to be highest during early and mid lactation and least in late lactation (Table 3). Variation in the prevalence of SCM could be due to immune response of animals and climatic condition (Bachaya *et al.*, 2005). It is strongly suggested that increased prevalence is associated with intramammary infection during this period. Similar opinion was expressed by Leitner *et al.* (2004).

Table 3. Lactation wise prevalence of subclinical mastitis in goats as detected by different diagnostic tests

Lactation stage	% Prevalence			
	SCC	EC	NAGase activity	LPB ELISA
Early	63.75	57.50	56.25	41.25
Mid	64.00	58.67	46.67	34.67
Late	35.56	33.33	26.67	24.44

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Serum Enzymatic Alterations Like Ast, Alt and Alp in Ochratoxicosis Amelioration with Dae and Vitamin-E in Broiler Chickens

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ABSTRACT

The present study was conducted to study the important serum biochemical alterations in ochratoxicosis followed by amelioration effect of DAE and Vitamin-E in broiler chickens. The toxigenic strain of *Aspergillus parasiticus*, NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture were used for the present study. A total number of 320 birds were divided in to 40 each in the Group I to VIII, Group-I being the control. Number of treatments were 8 and selection of birds used for base line data were 06 and the duration of the experiment was 35 days. Powdered ochratoxins were incorporated in to the feed at the rate of 1 ppm, vitamin-E was fed to the birds at the rate of 80 mg / kg feed and DAE at the rate of 2000 mg / kg feed. Birds from each group were sacrificed on day 7, 14, 21, 28 and 35 and the essential samples were collected and sera samples were obtained by taking 5 ml of blood from each bird. Sera samples were used for biochemical studies which included serum Enzymes viz. AST, ALT and ALP were analyzed. Different levels of test serum samples for test serum enzymatic parameters were studied.

Key words: DAE, OA, ALT, AST, ALP

Ochratoxins are nephro toxic, hepato toxic, carcinogenic, immunotoxic and teratogenic mycotoxins. Exposure to low concentration of ochrotoxin caused structural and functional changes in kidney and liver of birds, thus releasing the enzymes either in higher concentration or in lower consentration. The present study has being attempted to assess the efficacy of DAE a toxin binder to ameliorating the toxic effect of ocrotoxin and Vitamin – E an anti oxidant.

MATERIALS AND METHODS

The present research work was carried out at the Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru-560024 to study the ameliorating effect of diatamaceous earth (DAE) and immunomodullatory effects of Vitamin E in the experimentally induced ocharatoxicosis in broilers.

Fungal Culture: The toxigenic strain of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture maintained at the Department of Veterinary Pathology, Veterinary College, KVAFSU Bangaluru were used in the study. Ochratoxin was produced on the broken wheat using *Aspergillus ochraceus* NRRL 3174 as outlined by Trenk *et al.* (1971) with minor modifications. Ochratoxin was quantified using the Thin Layer Chromatography (TLC) at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institution (TANUVAS), Namakkal-367002.

Experimental Information: The experiment was carried out for 5 weeks using 320 birds kept in battery cages. Trial consisted of 8 treatments with 40 birds in each treatment. 6 birds were used for generating base line data

Experimental Birds and Rearing System: A total of 320 day old commercial broiler chicks were procured from a reputed commercial hatchery. The chicks were weighed individually and reared in battery brooder fitted with electrical bulbs on raised wire mesh floor under optimum condition of brooding and management.

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Experimental Feed: Commercial broiler starter (0 – 3 weeks) and finisher feed (4 – 5 weeks) were produced from Department of Poultry Science, KVAFSU, Bengaluru Nutrient composition of the basal diet is given below. The feed samples were screened for mycotoxin content prior to use in feed. Powdered ochratoxin culture material was incorporated into the feed at the rate of 1 ppm. Vitamin E (VE) (Tocopherol Acetate absorbed on precipitated silicon dioxide from M/s Mercks Pvt. Ltd., Goa) was mixed with the feed at the rate of 80 mg/kg feed. Mycotoxin ameliorating agent Diatomaceous Earth (DAE) obtained from M/s Agripower, Australia was incorporated in feed at the rate of 2000 mg / kg feed).

Biochemical Reagents: Biochemical Reagent Kits viz. AST, ALT and ALP were produced from Swemed Diagnostics, Bangaluru.

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

Table 1: Experimental Design

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

Sequential Pathology: Six experimental birds from each group were sacrificed on day 7, 14, 21, 28 and 35 to study the biochemical parameters and morphological changes in target organs.

Biochemical Parameters: During each sacrifice 5 ml of blood was collected from six birds of each group at weekly interval in a dry test tube and serum was separated after eight hours (Calnek *et al.*, 1992). They were analysed for serum enzyme levels viz. AST, ALT and ALP using Lab Met clinical analyzer as per the procedure described in the manual by using reagent kits supplied by M/s Swemed Diagnostics Ltd., Bengaluru.

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

RESULTS AND DISCUSSION

Serum Enzymes

Serum Alanine Aminotransferase: The results of mean serum alanine aminotransferase (ALT) value in birds with different dietary treatments are presented in Table 2. The mean serum ALT values of Group I to VIII birds were 13.96, 14.84, 13.11, 12.81, 32.30, 18.42, 21.86 and 13.61 IU/L, respectively at the end of 5th week. The mean serum ALT values of Group I to IV were similar during 1st to 5th week of experiment. However, toxin fed birds (Group V) showed a significant ($P<0.05$)increase in mean serum ALT values during 1st to 5th week of age. The mean serum ALT values of toxin fed birds showed a significant ($P<0.05$) increase, however the birds supplemented with diatomaceous earth, Vitamin E and both (Group VI to VIII) showed a significant ($P<0.05$) decrease as compared to only toxin fed birds throughout the period of experiment.

Serum Alanine Aminotransferase: In the present investigation, the Group V birds showed an increased level of ALT in toxin fed birds. However, supplementation of herbal oil, toxin binder and both together showed a marginal increase in Serum ALT levels as compared to control birds (Group I). Increase in ALT values in birds fed with ochratoxin could be attributed to the hepatic damage caused by ochratoxin. These findings were supported by Mohiuddin *et al*., (1993) and El-Bagury *et al*., (1997). Supplementation of herbal oil, toxin binder

and both combined together to the birds fed with toxin in the diet reduced the above enzyme level in broiler chickens indicating their beneficial effect in ameliorating ochratoxicosis.

the period of experiment. The mean serum AST showed a significant ($P<0.05$) increase from 1st to 5th week in birds fed with toxin in comparison with untreated control birds (Group I). The mean serum

Table-2: Mean (± SE) values of serum ALT (IU/L) in broiler chicks fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/ Weeks	1	2	3	4	5
I	16.59±3.27 ^{ab}	6.86±0.60 ^{a}	8.52±078 ^{a}	14.23±1.74^{a}	13.96±3.69^{a}
II	13.95±1.43 ^{a}	7.57±1.47 ^{a}	18.06±0.78^{bd}	14.19±2.12^{a}	14.84±3.57^{a}
III	15.61±1.70 ^{ab}	1.62±0.79^{bd}	13.01±1.52 ^{ad}	14.43±3.52^{a}	13.11±5.66^{a}
IV	10.88±0.83 ^{a}	10.21±0.15 ^{ad}	18.88±2.65^{bd}	14.06±0.87^{a}	12.81±3.25^{a}
V	21.42±0.75 ^{b}	18.08±0.81^{c}	34.69±1.09 ^{c}	40.87±0.55^{b}	32.3±0.64^{b}
VI	15.55±0.75 ^{ab}	13.23±0.66^{bd}	24.06±1.56^{bc}	24.43±3.17^{ac}	18.42±1.35^{a}
VII	16.93±0.73 ^{ab}	15.03±0.66^{bc}	28.67±2.87^{ce}	30.68±3.70^{bc}	21.86±1.67^{ab}
VIII	13.49±0.35 ^{a}	12.18±0.71^{bd}	23.75±1.38^{be}	18.34±0.40^{a}	13.61±0.28^{a}

Mean values bearing common superscript within columns did not differ significantly ($P\geq0.05$)

Serum Aspartate Aminotransferase: The weekly mean serum aspartate aminotransferase (AST) of different groups are presented in Table 4. The mean serum AST values of Group I to VIII birds were 269.10, 227.50, 255.50, 230.00, 276.20, 246.70, 255.60 and 239.10 IU/L, respectively at the end of 5th week. The mean serum AST values of Group I to IV were similar and did not differ significantly ($P>0.05$) with Groups VI, VII and VIII throughout AST values of Group VI to VIII showed a slight decrease, but statistically non-significant as compared to only toxin fed birds (Group V).

Serum Asparate Aminotransferase: The serum AST level showed a significant decrease in the toxin fed (Group V) and against untreated controls. The increase of AST level in the present study, which could be due to leakage of enzyme contributing to liver damage, as opined by Kumar

Table-3: Mean (± SE) values of serum AST (IU/L) in broiler chicks fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/ Weeks	1	2	3	4	5
I	229.00±11.28^{a}	204.00±1.33^{ab}	245.00±20.32^{abd}	206.40±6.68^{ab}	269.10±5.83^{ab}
II	212.90±10.73^{ab}	207.20±5.13^{ab}	211.80±16.45^{abce}	221.10±7.74^{a}	227.50±10.27^{a}
III	216.10±4.85^{ab}	206.40±7.33^{ab}	200.20±4.66^{abc}	217.90±8.77^{ab}	255.50±15.87^{ab}
IV	19.3.800±10.20^{b}	182.40±4.66 ^{a}	189.00±7.67^{c}	230.50±10.94^{a}	230.00±2.85^{a}
V	234.30±2.92^{a}	241.30±1.86^{b}	268.20±1.43^{df}	260.30±2.09^{b}	276.20±3.67^{b}
VI	225.30±1.42^{a}	214.20±4.17 ^{ab}	249.40±1.36^{bf}	240.40±1.98^{a}	246.70±13.33^{ab}
VII	230.60±0.75^{a}	225.40±3.63 ^{ab}	258.40±0.60^{ef}	243.90±4.97^{a}	255.60±8.24^{ab}
VIII	221.50±2.52^{ab}	210.40±3.05 ^{ab}	242.60±6.54^{b}	233.40±1.83^{a}	239.10±7.59^{ab}

Mean values bearing common superscript within columns did not differ significantly ($P\geq0.05$)

Table-4: Mean (± SE) values of serum ALP (IU/L) in broiler chicks fed with ochratoxin, diatomaceous earth, vitamin E and their combination

Groups/ Weeks	1	2	3	4	5
I	2058±349.70^{ac}	2073±55.21^{a}	2741±265.00^{a}	2044±153.30^{a}	2098±142.00^{a}
II	2289±413.80^{a}	2316±250.70^{a}	2311±149.90^{a}	1877±105.30^{a}	2042±131.20^{a}
III	2268±130.20^{c}	2331±69.75^{a}	2477±419.70^{a}	2066±201.20^{a}	2771±310.10^{ab}
IV	2437±114.20^{ad}	2258±183.10^{a}	2504±521.50^{a}	2407±310.50^{a}	2613±355.80^{a}
V	4718±223.30^{b}	4252±93.83^{b}	4268±51.68^{b}	3648±339.10^{b}	3644±184.20^{b}
VI	3726±62.93^{be}	3952±20.11^{b}	3001±161.9^{ab}	2198±294.30^{a}	2149±216.80^{a}
VII	3953±40.67^{be}	4014±72.12^{b}	3299±350.8^{ab}	2650±238.50^{ab}	2388±219.10^{a}
VIII	3317±98.68^{de}	3859±26.56^{b}	2555±54.89^{a}	2195±289.00^{a}	2058±163.10^{a}

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

et al., (2003). However, supplementation of herbal oil and toxin binder with toxin in the diet numerically reduced the above said enzymes indicating their protective role in ameliorating ochratoxicosis, Mohiuddin *et al.*, (1993), Raju and Devegowda (2000) and Shivappa (2005) reported lower serum enzyme values in the toxin binders, treated groups indicating the protective role of toxin binder during ochratoxicosis. Similarly, Shanker *et al.*, (2007) reported the protective role of herbal extract during ochratoxicosis. Further, supplementation of both herbal oil and toxin binder together with herbal oil marginally decreased the enzymes in the serum than, when supplemented alone and thus indicating that they were effective counteracting the toxin when supplemented together.

Serum Alkaline Phosphatase: The weekly mean serum alkaline phosphatase (ALP) values are presented in Table 4. The mean serum ALP values of Group I to VIII birds were 2098.00, 2042.00, 2771.00, 2613.00, 3644.00, 2149.00, 2388.00 and 2058.00 IU/L, respectively at the end of 5th week. The mean serum ALP values of Group I to IV were similar, but the toxin fed birds (Group V) showed a significant (P < 0.05) increase in mean serum ALP values during 1st to 5th week of age. The mean ALP values of toxin fed birds showed a significant increase (P<0.05) as compared to control birds during the entire period of study. However, the birds of Group VI to VIII showed a significant decrease (P<0.05) as compared to Group V birds on all days of observation.

Serum Alkaline Phosphatase: The present study revealed that Group V birds showed an increase in serum ALP level as compared to control birds, and whereas birds of Group VI, VII and VIII showed a marginal increase in ALP level in their serum respectively as against control birds (Group I). The increased level of this enzyme could be correlated to the degenerative changes noticed in the live leading to seepage of enzyme into serum and this was supported by similar findings of Raina *et al.*, (1991).

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Assessment of Serum Urea Levels in Ochratoxicosis Ameliorating Effect with DAE and Vitamin-E in Broiler Chickens

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ABSTRACT

The present study was conducted to study the serum urea levels in ochratoxicosis besides amelioration effect of DAE and Vitamin-E in broiler chickens. The toxigenic strain of *Aspergillus parasiticus*, NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture were used for the present study. A total number of 320 birds were divided in to 40 each in the Group I to VIII, Group-I being the control. Number of treatments were 8 and selection of birds used for base line data were 06 and the duration of the experiment was 35 days. Powdered ochratoxins were incorporated in to the feed at the rate of 1 ppm, vitamin-E was fed to the birds at the rate of 80 mg / kg feed and DAE at the rate of 2000 mg / kg feed. Birds from each group were sacrificed on day 7, 14, 21, 28 and 35 and the essential samples were collected and sera samples were obtained by taking 5 ml of blood from each bird. Sera samples were used for biochemical studies which included serum biochemical parameters namely serum urea levels were analyzed. Different levels of test serum urea parameters were studied.

Key Words: DAE, OA, *Aspergillus fumigatus* / ochraceus, serum urea

Ochrotoxins are nephro toxic, hepato toxic, carcinogenic, immunotoxic and teratogenic mycotoxins Exposure to low concentration of ochrotoxin caused structural and functional changes in kidney and liver of birds, thus altering the levels of above said biochemical parameters. The present study has been attempted to assess the efficacy of DAE a toxin binder to ameliorate the toxic effect of ocrotoxin and Vitamin – E, an anti-oxidant.

MATERIAL AND METHODS

The present research work was carried out at the Department of Veterinary Pathology, Veterinary College, Hebbal, Bangaluru-560024 to study the ameliorating effect of diatamaceous earth (DAE) and immunomodulatory effects of Vitamin E in the experimentally induced ochratoxicosis in broilers.

Fungal Culture: The toxigenic strain of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture maintained at the Department of Veterinary Pathology, Veterinary College, KVAFSU Bangaluru were used in the study. Ochratoxin was produced on the broken wheat using *Aspergillus ochraceus* NRRL 3174 as outlined by Trenk *et al.* (1971) with minor modifications. Ochratoxin was quantified using the Thin Layer Chromatography (TLC) at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institution (TANUVAS), Namakkal-367002.

Experimental Information: The experiment was carried out for 5 weeks using 320 birds kept in battery cages. Trial consisted of 8 treatments with 40 birds in each treatment. 6 birds were used for generating base line data

Experimental Birds and Rearing System: A total of 320 day old commercial broiler chicks were procured from a reputed commercial hatchery. The chicks were weighed individually and reared in battery brooder fitted with electrical bulbs on raised wire mesh floor under optimum condition of brooding and management.

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Experimental Feed: Commercial broiler starter (0 – 3 weeks) and finisher feed (4 – 5 weeks) were procured from Department of Poultry Science, KVAFSU, Bengaluru Nutrient composition of the basal diet is given below. The feed samples were screened for mycotoxin content prior to use in feed.

Ochratoxin Feed: Powdered ochratoxin culture material was incorporated into the feed at the rate of 1 ppm. Vitamin E (VE) (Tocopherol Acetate absorbed on precipitated silicon dioxide from M/s Mercks Pvt. Ltd., Goa) was mixed with the feed at the rate of 80 mg/kg feed. Mycotoxin ameliorating agent Diatomaceous Earth (DAE) obtained from M/S Agripower, Australia was incorporated in feed at the rate of 2000 mg / kg feed).

Biochemical Reagents: Biochemical Reagent Kits *viz*. urea is processed from M/s Swemed Diagnostics, Bangaluru.

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

Table 1: Experimental Design

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

Sequential Pathology: Six experimental birds from each group were sacrificed on day 7, 14, 21, 28 and 35 to study the biochemical parameters and morphological changes in target organs.

Biochemical Parameters: During each sacrifice 5 ml of blood was collected from six birds of each group at weekly interval in a dry test tube and serum was separated after eight hours (Calnek *et al.*, 1992). They were analysed for serum urea using Lab Met clinical analyzer as per the procedure described in the manual by using reagent kits supplied by Swemed Diagnostics Ltd., Bangaluru.

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

RESULTS AND DISCUSSION

Serum Urea: The weekly mean serum urea levels in different groups have been presented in Table 2. The mean serum urea levels for Group I to VIII were 16.77 ± 2.76, 16.67 ± 0.69, 15.73 ± 1.1, 32.37±7.09, 26.27±2.57, 22.12±0.99, 18.40±2.56 and 18.17±0.6 mg / dl respectively at 35 days of age. The mean serum urea levels of birds in Group I to III did not alter significantly during the entire period of the experiment. A significant ($P < 0.05$) increase in the mean serum urea levels in Group IV toxin fed birds was observed as compared with control (Group I) birds from day 7 till the end of the experiment, where as in Group V significant increase in mean serum urea levels were observed on day 7 of the experiment, but numerical increase was seen from second week onwards till the end of the experiment. However, the birds of Group VI and VII showed numerical decrease in mean serum urea levels as compared to that of toxin fed birds (Group IV) till day 28 of the experiment and significant decrease was observed on day 35 of the experiment. The birds in Group VIII showed numerical decrease as compared to Group V throughout the period of experiment. However,

there was no significant difference with that of control group.

the rise of serum urea values by its protective effect in case of ochratoxicosis.

Table 2: Mean (±SE) values of serum Urea(mg/dl) in broiler chicks fed with ochratoxin (OA), Diatomaceous earth (DAE) Vitamin E and their combination.

Groups/ Weeks	1	2	3	4	5
I	16.52±1.53a	21.12±1.46a	20.77±2.22ab	17.61±1.28a	16.77±2.76ab
II	17.96±1.52ab	21.32±1.39a	19.54±3.94a	24.14±0.54ab	16.67±0.69ab
III	18.90±1.47ab	21.82±2.87a	19.77±3.20a	21.81±1.73ab	15.73±1.10a
IV	26.94±4.72c	37.34±2.34c	31.44±0.56c	35.44±0.99b	32.37±7.09c
V	26.27±0.44c	27.87±6.42ab	24.94±1.66abc	32.04±5.38ab	26.27±2.57bc
VI	25.37±1.85bc	31.47±2.14bc	28.07±0.94bc	29.67±5.25ab	22.12±0.99ab
VII	26.12±0.96bc	27.57±1.64ab	21.60±0.26ab	27.77±11.33ab	18.40±2.56ab
VIII	22.32±2.5abc	24.57±0.96ab	23.17±2.40ab	26.30±3.50ab	18.17±0.60ab

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

Serum Urea: It is evident from the present study that the significant increase in serum urea levels was seen in the toxin fed birds (Group IV and V) as compared to control birds (Group I, II and III) on all the days of observation which was in accordance with the earlier reports reported by Sakhare *et al*., (2007), Sawarkar *et al*., (2011); Manafi and Bagheri (2011); Sajid Umar *et al*., (2012); Hassan *et al*., (2012) and Indresh and Umakantha (2013). The increased levels of urea could be due to inflammatory or degenerative changes in the kidney. Nephrotoxicity is assumed to be due to interference with transport function in collecting tubular cells together with diffused impairment of proximal tubular function as it is evident from the observations of microscopic changes in the kidneys of the present study. The same also been supported by the significant increase in relative weight of kidney in toxin fed birds. However, decrease in serum urea levels as seen in birds supplemented with DAE along with toxin (Group VI, VII and VIII) as compared to toxin fed birds (Group IV and V) indicates that supplementation of DAE has helped in restoring

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Seroepidemiological Aspects of Infectious Bovine Rhinotracheitis in the Buffaloes of North-Eastern Region of Karnataka*

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ABSTRACT

A total of 370 serum samples were collected from the four districts of North-Eastern region of Karnataka *viz.*, Bidar, Kalaburgi,Vijaypura and Yadgir districts, and serum samples were then subjected to indirect ELISA for screening of infectious bovine rhinotracheitis virus(IBR).The overall prevalence of IBR in this region was found to be 36.49% in buffaloes. The seroprevalence of IBR in buffaloes aged more than five years was high (27.57%) when compared to buffaloes aged less than five years (8.92%). Buffaloes with the history of abortion had 9.19% seropositivity for IBR. District-wise seroprevalence was more in Bidar compared to other districts. Bidar had 51.09% seropositivity followed by Kalaburgi (43.48%), Vijaypura(35.87%) and lowest in Yadgir district (15.96%). The seroprevalence of IBR was more in buffaloes of organized sector, when compared to unorganized sector. The higher seroprevalence in organized sector may be attributed to overcrowding and poor management practices.

Key words: Seroprevalence, seropositivity, buffalo

Respiratory and reproductive disorders in dairy animals due to various etiological agents had lead to severe economic losses in India and the losses are mainly due to abortions in pregnant buffaloes and decreased milk production. Among the major infectious agents causing these disorders, bovine herpes virus-1(BoHV-1) causing infectious bovine rhinotracheitis was studied in detail.

Diagnosis of BoHV-1 infection is always challenging because of its subclinical nature, immune evasion strategies and most importantly the latency, it establishes in the host. World Health Organization (WHO) suggests indirect ELISA, virus isolation and real time PCR as approved tests for diagnosis of BoHV-1, but routinely for screening of BoHV-1, indirect ELISA is recommended (Nandi *et al*., 2007).In India, IBR was first reported by Mehrotra *et al*. (1976). Extensive studies have been carried out on seroprevalence of BoHV-1 in different parts of India *i.e.,* South India (Renukaradhya *et al.,*1996); Jhansi (Nandi *et al*.,2007); Punjab (Aradhana *et al*.,2004); Karnataka (Koppad *et al*.,2007); West Bengal (Ganguly *et al.,*2008); Odisha (Priyaranjan *et al*.,2014); Gujarat(Lata *et al*.,2008) etc.

There was not much information available on the seroprevalence of IBR in buffaloes of North-Eastern region of Karnataka. Hence , the present study was undertaken.

MATERIALS AND METHODS

Serum samples from various districts of North-Eastern region of Karnataka were collected *viz.,* Bidar, Kalaburgi, Vijaypura and Yadgir districts from both organized and unorganized sectors. Information of the individual animal was collected through questionnaires. Total of 370 serum samples were collected and among these, 200 serum samples were from organized sector and 170 serum samples from unorganized sector. In the similar manner, 104 serum samples were from buffaloes of less than five years age and 266 serum samples were from buffaloes more than five years of age

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collected. Among these, 68 buffaloes had the history of abortion. Then these serum samples were subjected to indirect ELISA at the Institute of Animal Health and Veterinary Biologicals (IAH&VB), Kalaburgi using IDEXX Trachitest serum screening kit (Switzerland) specially used for screening of IBR. Protocol was performed as per manufacturer's guidelines. After performing the above protocol, the antigen coated ELISA plate was made to run through the ELISA reader, to know the optical density values(O.D).Based on the optical density values the results of individual samples were read as positive and negative.

Statistical analysis was done as per Snedecor and Cochran methods (1989) using Graphpad prism trial version software.

RESULTS AND DISCUSSION

The overall prevalence of IBR in buffaloes of North-Eastern region of Karnataka was 36.49%, which was in close conformity with the findings of Anita *et al.*(2013), where in, they found the prevalence of IBR around 36.02% in the cattle herds. Similarly 31 % seroprevalence was found by Chinchkar *et al.* (2002) and 38.78% by Pandita and Srivastava (1993) in the buffalo farms. In contrast to these findings, higher seroprevalence of 62.30% was observed by Suribabu *et al.*(1984) , 52.50% by Renukaradhya *et al.* (1996) and 55.60%. by Tongaonkar *et al.* (1986). However lowest seroprevalence was observed in the buffaloes by Moussa *et al.*(1990) of 15.40%, Afshar and Tadjbakhche(1970) of 6.80% in water buffaloes, Aruna and Suribabu(1992) in A.P. found 21.10% seroprevalence of IBR in buffaloes.

The total prevalence of IBR was highest (51.09%) in the Bidar district followed by Kalaburgi (43.48%),Vijapura (35.87%) and lowest (15.96%) in Yadgir district. There was significant difference between the total positive and negative values of IBR of Vijaypura and Yadgir districts ($p\leq0.05$ and $p\leq0.01$ respectively) whereas, the total positive and negative values of Bidar and Kalaburgi districts differed non-significantly ($p>0.05$). The IBR positive samples of Bidar district were significantly higher ($p\leq0.01$) when compared to Vijaypura district. Similarlly the positive samples of Vijaypura,Bidar and Kalaburgi districts were significantly($p\leq0.05$) higher than positive samples of Yadgir district, where as the positive samples of Kalaburgi-Vijaypura and Bidar- Kalaburgi differed non-significantly ($p>0.05$). The prevalence of IBR was more in Bidar as compared to other districts which may be attributed to more number of samples drawn from organized sector compared to other districts. Poor managemental practices might have resulted in the higher prevalence of IBR, which was in conformity with the findings of Rashmi and Sharad (2010) and Chandranaik *et al.,* (2014).

There was significant($p\leq0.05$) difference between less than 5 years aged positive and negative samples of Vijaypura, Bidar, Kalaburgi and Yadgir districts , where in, the total negative samples of Vijaypura, Bidar, Kalaburgi and Yadgir districts were higher compared to positive samples. The positive samples of less than 5 years age group of different districts differed non-significantly. Similarly the negative samples of less than 5 years age group of different districts differed non-significantly($p>0.05$), which was in conformity with the findings of Rajesh *et al.*(2007).

There was significant difference between more than 5 years aged positive and negative samples of Vijaypura district ($p\leq0.01$) and similarly the significant difference ($p\leq0.05$) was observed between the positive and negative values of Kalaburgi and Yadgir districts, where in the negative values were higher than positive values, however the positive and negative values of Bidar district differed non-significantly ($p>0.05$). The positive values of more than 5 years age of Bidar and Kalaburgi districts were higher significantly ($p\leq0.05$) than the positive values of Yadgir district, where as the positive values of Vijaypura district differed non-significantly from positive values of other districts($p>0.05$).Similarly the positive values of Bidar and Kalaburgi district differed non-significantly($p>0.05$), which was in conformity with the findings of Amitkumar *et al.*(2014), Anita *et al.*(2013) and Dora *et al.*(2013).These authors were of the opinion that the prevalence of IBR increased with age. Aruna and Suribabu(1992) also reported higher prevalence in aged ones.

Table 1: District wise prevalence of IBR in the North-Eastern region of Karnataka

Name of district	Total samples	Total Prevalence (%) Positive	Total prevalence (%) Negative	Positive (%) Less than 5 yrs	Negative (%) Less than 5yrs	Positive (%) More than 5 yrs	Negative (%) More than 5yrs	Positive (%) (Abortion)	Negative (%) (Abortion)
Vijaypura	92	33^{xa} (35.87%)	59^{*} (64.13%)	11 (11.96%)	81^{*} (88.04%)	22 (23.91%)	70** (76.09%)	9 (09.78%)	83^{*} (90.22%)
Bidar	92	47^{xy} (51.09%)	45^{a} (48.91%)	8 (08.70%)	84^{*} (91.30%)	39^{x} (42.39%)	53^{a} (57.61%)	10 (10.87%)	82^{*} (89.13%)
Kalburgi	92	40^{c} (43.48%)	52^{x} (56.52%)	10 (10.87%)	82^{*} (89.13%)	30^{a} (32.61%)	62^{*} (67.39%)	8 (08.70%)	84^{*} (91.30%)
Yadgir	94	15^{byd} (15.96%)	79^{**by} (84.04%)	4 (04.26%)	90^{*} (95.74%)	11^{yb} (11.70%)	83^{*b} (88.30%)	7 (07.45%)	87^{*} (92.55%)
Cumulative total	370	135 (36.49%)	235 (63.51%)	33 (8.92%)	337 (91.08%)	102 (27.57%)	268 (72.43%)	34 (9.19%)	336 (90.81%)

Note: The values in the parenthesis indicate percentage of prevalence.

1) " * "Super scripted numbers describe Significant at 5% level of significance (between samples)($p \leq 0.05$)
2) " ** "Superscripted numbers describe Significant at 1% level of significance (between samples) ($p \leq 0.01$)
3) " x " and" y" superscripted numbers indicate significance difference at 5% level of significance (between districts)($p \leq 0.05$)
4) "a " and "b" superscripted numbers indicate significance difference at 5% level of significance (between districts)($p \leq 0.05$)
5) "c " and "d" superscripted numbers indicate significance difference at 5% level of significance(between districts)($p \leq 0.05$).

There was significant difference ($p \leq 0.05$) between total positive and negative samples of Vijaypura, Bidar, Kalaburgi and Yadgir districts with history of abortion, where in negative samples of Vijaypura, Bidar, Kalaburgi and Yadgir with history of abortion were higher significantly($p \leq 0.05$) than positive samples with history of abortion. The variation between different districts of positive samples with history of abortion was statistically non significant ($p > 0.05$). Similarly the variation between the different districts of negative samples with history of abortion was statistically non-significant($p > 0.05$). Higher prevalence in Bidar district may be attributed to more samples drawn from organized sector, higher density of herds increased the potential transmission of virus from herd to herd through frequent visits by animal handlers, wind borne aerosols and contact between animals while grazing, which was in conformity with the findings of Van Schaik *et al*.(1998) and Van Wuijckhuise *et al*. (1998).

Transmission of BoHV-1 is also possible by artificial insemination, where in the semen of single ejaculate may be inseminated to thousands of females with clinical consequences as stated by Smits *et al*.(2000). Higher prevalence in Bidar district may be attributed to more samples from organized sector, poor managemental practices and farms having more reproductive problems compared to the other districts. The present findings were in conformity with the findings of Miller (1991).

CONCLUSION

In the present study, seroprevalance of IBR in the buffaloes of north-Eastern Karnataka region was studied. Seroprevalance was more in organized sector, when compared to unorganized sector, which may be attributed to overcrowding and poor managemental practices.

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Isolation and Characterization of Lytic Bacteriophages of *Escherichia Coli* and their Therapeutic Application.

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ABSTRACT

Escherichia coli is an important bacterial pathogen of gastroenteritis revealing multidrug resistance and has zoonotic implication. In an approach towards alternatives to antibiotics, lytic bacteriophages were isolated against *Escherichia coli* from sewage effluent using double agar overlay method. The bacteriophages *viz.* ϕEC1,ϕEC2,ϕEC3,ϕEC4 and ϕEC5 isolated and characterized microbiologically revealed host range of 80-90% individually and 100% collectively within the genus. Biophysical characterization of the phages showed that they were stable at 16°C, 37°C, 42°C and pH 4, 7 and 9 for a period of 4hrs, supporting their therapeutic application. Electron microscopy of the ϕEC1 showed icosahedral head (54.92nm), contractile tail (26.41nm) and belonging to the family *Myoviridae* of the order *Caudovirales*. Further, molecular characterization of ϕEC1 revealed 28.8kb nucleic acid and digested by restriction endonucleases i.e., *EcoR* I, *Bam H* I and *Hae* III. The therapeutic application of the isolated phage cocktail was ascertained in Swiss albino mice models by infecting the control and treatment groups with $3x10^8$ cfu/ml of the *E.coli* intramuscularly and orally. Following challenge the treatment group administered with $3x10^9$ pfu/ml of phage mixture showed significant decrease in number of colony forming units of bacteria *in vivo*.

Key words: Bacteriophages, *Escherichia coli*, Characterization, Therapeutic application in mice.

One of the major health concerns of buffaloes in India is calf mortality which is as high as 70% to 73% due to greater susceptibility of water buffalo to gastroenteric pathogens and poor management (Borriello *et al.,* 2012 and Shivarudrappa *et al.,* 2013). Among the different pathogens of gastroenteritis, *Escherichia coli* contributes to 35% of the disease (Borriello *et al* 2012). In order to combat these pathogens antibiotics played an indispensable role. However, the selective pressures on the bacteria lead to antibiotic resistant mutants (Aarestrup *et al.,* 2008). Moreover, the increased antibiotic resistance is viewed seriously by World Health Organization (WHO 2014). Hence in order to alleviate the pressing concern an attempt was made for alternatives to antibiotics. One such alternative is the application of bacteriophages. In this view lytic bacteriophages were isolated, characterized and observed for therapeutic application against *Escherichia coli* in Swiss albino mice.

MATERIALS AND METHODS

Bacterial strains and prophages: The *Escherichia coli (*ATCC 25922) was used as a standard culture along with the *Escherichia coli,* isolated from the diarrhoeic samples for the purpose of the isolation of the lytic bacteriophages. In order to use these organisms as host, presence of prophages was observed using the DNA damaging antimicrobial agent mitomycin as described by Miller, (1998). The host strain bacterial cultures were aliquoted into one ml volumes in sterile test tubes and Mitomycin C was added to a final concentration of 5µg/ml and incubated for 3h at 37°C. Then 20µl of chloroform was added to controls to lyse bacteria and both drug induced and control tubes were centrifuged and the supernatant was collected and subjected to double agar overlay to observe the exclusion of prophages.

Bacteriophage isolation: For the purpose of isolation of bacteriophages by large scale screening, sewage samples were obtained from the places in and around buffalo farms where there was a possibility of obtaining sewage having more organic matter. The collected sewage samples were centrifuged at 10,000rpm for 10min and then the supernatant was filtered using 0.45µ filters. To this equal volume of SM buffer (100mM NaCl, 8mM $MgSO_4$ and 1M Tris HCl pH7.5) and *Escherichia coli* ($1.5x10^8$CFU/ml) were added and incubated in orbital shaker incubator at 37°C for 24hrs. After incubation, the suspension was centrifuged at 10,000rpm for 10min and filtered through 0.45µ filters. This filtrate was used to estimate the phage population by using double agar overlay method using 20% bottom nutrient agar. Then the top agar

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was prepared using 0.5ml of the filtrate, one millilitre of the host culture ($0.5x10^8$ cfu/ml) and 1.5ml of SM buffer and incubated at 37°C for 20min. To this suspension, 2.5ml of 20x nutrient agar was added to make 10x agar and layered on bottom agar. After solidification, the plates were incubated at 37°C for 24hrs and observed for the formation of clear plaques.

Purification of Bacteriophages and microbiological characterization: From the pool of bacteriophages that were obtained on primary isolation, single plaques revealing clear plaque morphology and wide lytic zone were obtained using sterile toothpick, then inoculated into 2ml of nutrient broth having $0.5x10^8$ cfu/ml of host culture and incubated at 37°C for 24hrs in orbital shaker incubator. Later it was centrifuged and the supernatant was subjected to double agar overlay as described and the same was repeated thrice sequentially in order to obtain single lytic bacteriophage.

Among the isolated bacteriophages, five lytic phages were obtained. The host range was observed using spot assay as described by Santos *et al* (2011). These bacteriophages were multiplied further and stocks were prepared.

Biophysical characterization: The obtained bacteriophages at multiplicity of infection (MOI) one, were subjected to temperatures 16°C, 37°C, 42°C and pH 3, 7, 9 for a period of 4 hours by changing the temperature of incubation and pH of SM buffer, respectively and the decrease in bacteriophages count was observed using double agar overlay method.

Nucleic acid isolation and characterization: Bacteriophage stocks were prepared by using MOI one of phage and organisms to yield complete lysis. To the completely lysed plates one millilitre of SM buffer pH7.5 was added and then incubated for one hour at 4°C, then the supernatant was scrapped with sterile spatula and stored at -20°C for 24hrs. Later, it was centrifuged at 10,000rpm for 10min and the supernatant was used for the nucleic acid isolation as described by Santos *et al* (2011). Later the nucleic acid type was observed by conducting RNase and DNase digestion. Further the ϕST1 nucleic acid was subjected to restriction endonuclease digestion using *Eco*RI, *BamH*I and *Hae*III enzymes by following manufacturer's instructions.

Morphological characterization: Transmission electron microscopy of ϕEC1 was carried out at Central Instrumentation cell, TANUVAS, Chennai.

***In vivo* lytic activity of the bacteriophages:** Swiss Albino Mice, aged 40days, were selected and grouped into Control, Infected and Treatment groups. Each group has six mice, the control group were normal mice, the infected group received $3x10^8$ cfu/ml of organisms whereas the treatment group received both the organism ($3x10^8$ cfu/ml) and endotoxin free (Proteospin endotoxin removal kit, Norgenebiotek) lytic phage cocktail ($3x10^9$ pfu/ml) by oral and intramuscular route. During the experiment the body weights of the mice were recorded and the therapeutic effect of the bacteriophages was estimated by enumerating the number of microorganisms in faecal matter.

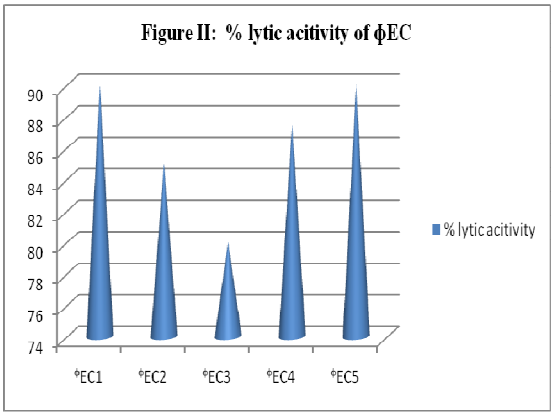
RESULTS AND DISCUSSION

The *Escherichia coli* isolates used for cultivation of bacteriophages was initially assessed for the presence of prophages using DNA damaging substance Mitmycin C in comparison with chloroform. The bacterial isolates that did not contain prophages were selected for further use. The exclusion of temperate bacteriophages from the host bacterial strains is to avoid the transduction, as the prophages are responsible for transfer of genetic material (Merabishivili *et al* 2009).

As many as 200 lytic phages were obtained from 25 different sewage water places using double agar over lay method. Form the 200 lytic phages obtained, five candidate bacteriophages (ϕEC1,ϕEC2, ϕEC3, ϕEC4 and ϕEC5) were selected for further studies based on the degree of lytic activity (estimated on the basis of the visual clarity and diameter of plaques). The diameter of the clear plaques was found to be 1 to 2mm (Figure I). According to the plaque size diameter, the bacteriophages belong to Family *Myoviridae* as the phages of this family produce plaques of 1mm diameter (Kesik-Szeloch *et al* 2013). Moreover the lytic activity of the phages belong to *Myoviridae* is higher compared to other two families of the order *Caudovirales*. Further, the host range of lytic phages yielded 80-90% individually (figure II) and 100% collectively. The collective use of lytic bacteriophages for therapeutic application was suggested by many workers (Smith and Huggins 1983, Smith *et al* 1987, Atterburry *et al* 2007 and Merabishvili *et al* 2009). However, it is necessary to have a phage cocktail that could able to lyse more than 30 *Escherichia coli* isolates so as to sear the majority of EPEC and ETEC strains worldwide (Robins-Browne, 1987; Goodridge *et al*., 2003).

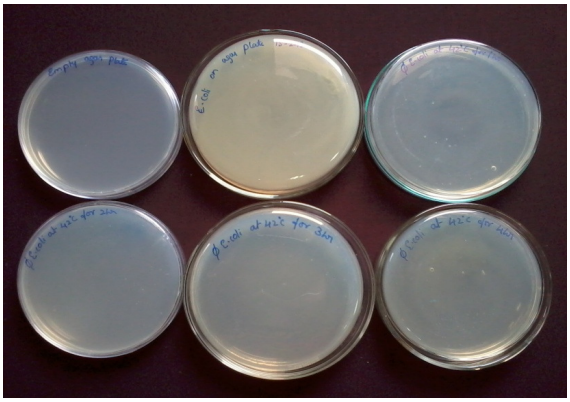
Figure I: Isolation of lytic bacteriophages of *Escherichia coli*

1. ϕEC at 10^{-6} dilution revealed complete lysis of the bacteria.



In industrialized countries, bacteriophage therapy is undergoing a renaissance (Anonymus 2004). Several studies proved the use of lytic bacteriophages in therapeutics (Miedzybrodzki *et al.,* 2007, Vinodkumar *et al.,* 2008, Merabishivili *et al.,* 2009, Vinodkumar *et al.,* 2010 and Santos *et al.,* 2011). The candidate strains used for therapeutic application were selected using biophysical characterization. In this perspective, the five bacteriophages were subjected to different temperatures 16°C, 37°C and 42°C (Figure III) and pH 4, 7 and 9 for a period of 4hrs. Over these detrimental pH and temperatures the lytic phages were found stable and revealed complete lysis at MOI one. This study supported the therapeutic use of lytic bacteriophages and there by the phage cocktail was prepared at $3x10^9$pfu/ml concentration for future use.

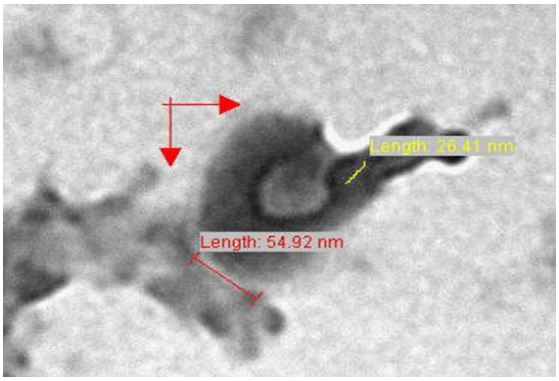
Figure III: Temperature sensitivity of ϕEC1



Legend	Top layer	Plate 1:negative control
		Plate 2: Positive control
		Plate 3: ϕEC1 at 42°C for one hour
	Bottom layer	Plate 1 : ϕEC1 at 42°C for two hours
		Plate 2: ϕEC1 at 42°C for three hours
		Plate 3: ϕEC1 at 42°C for four hours

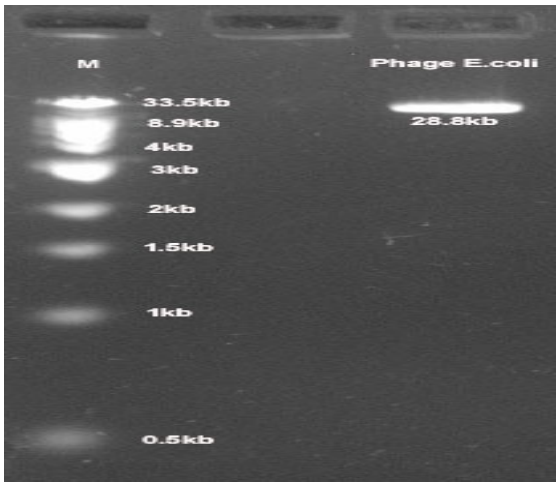
The microbiological study of ϕEC1 revealed an icosohedral head with 54.92nm in diameter and a contractile tail of 26.41nm in length (Figure IV). The electron microscopic analysis showed that ϕEC1 belongs to family *Myoviridae* and the order *Caudovirales*. It was also observed that the morphology of coliphages belong to the family *Myoviridae* (Goordridge *et al.,* 2003) and the phages that infect *Enterobacteriaceae* also belong to the family *Myoviridae* (ICTV 2014). Atterbury *et al* (2007) observed that the phages of family *Myoviridae* can produce more number of phage particles than that of family *Siphoviridae*.

Figure IV: Electron micrograph of ϕEC1



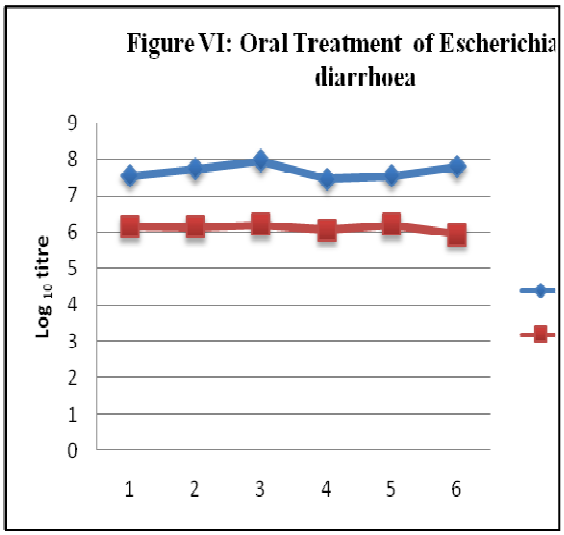
The nucleic acid of ϕEC1 was found to have 28.8kb in size (figure V) and digested by *EcoR* I, *BamH* I and *Hae* III. However, the number of bands obtained is less which indicated that the bacteriophages are resistant to the endonucleases released by the bacteria, which is in agreement with Kęsik-Szeloch *et al* 2013. Furthermore, the molecular characters of ϕEC1 supported that the phage belong to family *Myoviridae* and also thrown light over the quest for endolysins.

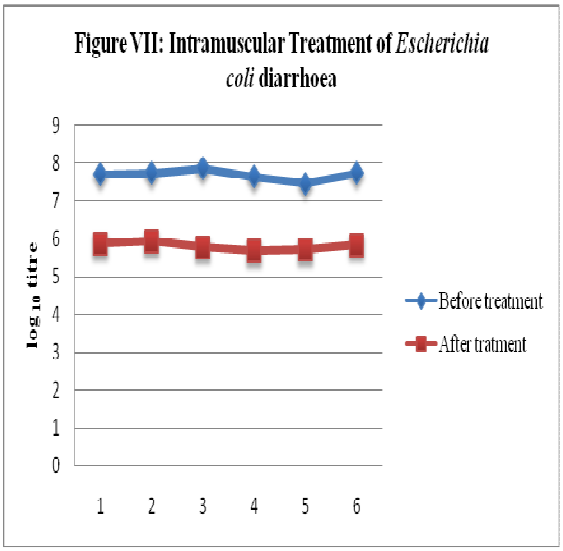
Figure V: Nucleic acid of ϕST1

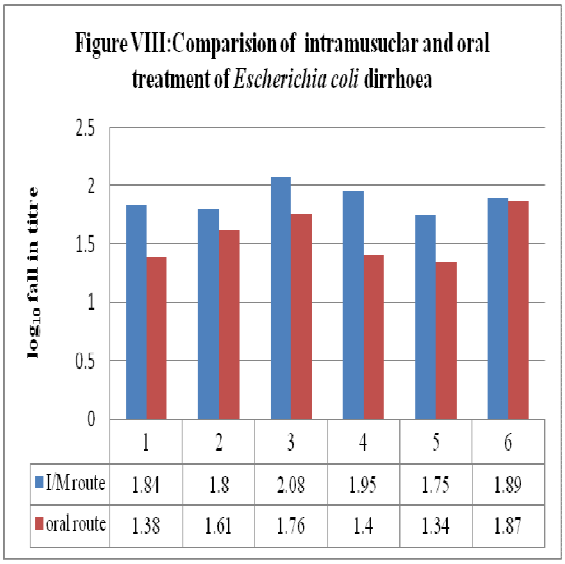


Calf mortality in buffaloes can reduce profit up to 38% as a result of shortage in milk and meat supplies (Khan and Khan, 1996). In the first month of age, diarrhoea accounts for 3.6 to 6.3 percent mortality (Khan and Khan, 1991) and overall mortality of 7.1 and 11 percent (Khan and Khan, 1996) in cow and buffalo neonates, respectively. Furthermore, the water buffalo calves are more frequently affected by gastroenteritis than bovine calves with mortality rates as high as 70% in water buffalo species is 50% in bovines (Fagiolo *et al* 2005; Foster and Smith 2009). Among the pathogens responsible for diarrhoea *Escherichia coli* shares 35% incidence (Borriello *et al* 2012). In order to combat these infections the use of antibiotics is playing a crucial role, however, the antibiotic resistance in *Escherichia coli* increased from 7.2% during 1950s to 63.6% during 2000s (Tadesse *et al* 2012). Such an increase in antibiotic resistance was viewed very seriously by WHO and many other health agencies. In view of this, therapeutic effect of lytic bacteriophages over *Escherichia coli* was studied on Swiss Albino Mice.

In this study the infected mice received *E.coli* ($3x10^8$ cfu/ml) and found significant increase in the number of bacteria from control to infected mice. The treatment group received *E.coli* ($3x10^8$ cfu/ml) along with a page cocktail ($3x10^9$ pfu/ml) intramuscularly and orally revealed a decrease in the titre of *E.coli* in treatment group. However, in the treatment group that received the phage cocktail orally (figure VI) had significantly reduced the number of *Escherichia coli* at p=0.004 where as treatment with intramuscular route (figure VII) had shown significance at $p< 0.0001$. These findings indicated that the treatment of diarrhoeic mice with bacteriophage cocktail has successfully reduced the organisms count (figure VIII) more effectively in the intramuscular route than oral route of phage application. Similarity in results was also stated by Smith and Huggins 1982 in which case single intramuscular dose of anti K1 phage was more effective than multiple intramuscular doses of tetracycline, ampicillin, chloramphenicol or trimethoprim plus sulphafuraxole in curing mice. Furthermore, it was also observed that, there is no complete decline in the number of *Escherichia coli* in the treatment group. This was supported by Chennoufi *et al* 2004 who reported mice exposed to an oral four phage cocktail did not experience a decline of their commensal *Escherichia coli*. Likewise human volunteers orally exposed to phage T4 maintained their commnesal *Escherichia coli* population (Bruttin and Brussow 2005). Further it is also observed that the intramuscular phage treatment could able to protect mice form diarrhoea than oral route of infection which is also in tune with Smith and Huggins (1982), Bull *et al* (2002) who reported the intramuscular phage protected against intra cerebral pathogen challenge.







	1	2	3	4	5	6
I/M route	1.81	1.8	2.08	1.95	1.75	1.89
oral route	1.38	1.61	1.76	1.4	1.34	1.87

These results invite the use of lytic bacteriophages as an alternative to antibiotics. However it necessitates the use of several lytic bacteriophages at least able to lyse 30 different O serotypes in order to reduce the *Escherichia coli* pathogenesis (Brussow 2005).

Further the phage cocktail can also be applied over meat and meat products to reduce contamination. Though a bit neglected in the past research, in the present era of antibiotic resistance, bacteriophages are the promising choices as antimicrobials and bio-preservatives.

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Efficacy of Silymarin as Hepato-Protectant in Dogs with Hepatic Disorders*

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ABSTRACT

Liver disorders are encountered in dogs of all age groups accounting for three per cent of all diseases. In this study six dogs with hepatic disorders were taken for therapeutic trial. Six apparently healthy dogs were kept as control group. Affected dogs were treated with Silymarin and supportive therapy. In the dogs of therapy group, mild cases showed almost complete recovery in clinical symptoms of appetite and general activity but in moderate cases appetite markedly improved by 7th day they recovered from other symptoms like fever and vomition, hydration status also improved whereas in severe cases clinical symptoms did not show much improvement by 7th day after therapy. By 7th day of therapeutic trial this group dogs showed significant rise in Hb and PCV and reduction in TLC and neutrophils. The confirmed three anaemic dogs included in this group also showed marked improvement in Hb and PCV. Elevated serum ALT and ALP were found to be significantly ($P<0.01$) reduced by 7th day of trial. Hypoproteinaemia and hypoalbuminaemia also improved by 7th day of therapy, which was true for ascitic dogs of this group. Reduced glucose level showed significant ($P<0.01$) rise after therapy and the high bilirubin in icteric dog showed significant ($P<0.05$) reduction. The therapeutic regimen showed good efficacy in treating hepatic disorders in dogs.

Key words: Hepatic disorders, Silymarin, ALT, Hepato-protectant, Efficacy

Liver disorders are encountered in dogs of all age groups accounting for three per cent of all diseases (Hardy, 1983). As the cellular guardian of gastrointestinal tract, the liver is pivotal in its regulation, digestion and metabolism. Liver plays a central role in diverse array of processes, including metabolism, detoxification, storage of vitamins and trace minerals and immunogenic surveillance. Liver disease can occur due to direct damage to the liver by toxins, infectious agents as well as metabolic, immune mediated and neoplastic problems (Cornelius and Bjorling, 1992). Several drugs have been advocated for the treatment of hepatic disorders. From ancient days, the herbal drugs are known for their vital role in improving the regenerative capacity of liver. Silymarin is one such drug which has been in use since many years. Silymarin inhibits the effect of the toxic substances and regulates the functions of hepatocyte (Displaces *et al.*, 1975).

MATERIAL AND METHODS

Dogs presented to the clinics with few or all showing the symptoms of hepatic disorders such as anorexia/inappetance, loss of body weight, jaundice, ascites, vomition, diarrhoea, dullness and lethargy without history of any premedication were selected for this study. The cases were subjected to cinical examination and haemato- biochemical tests. Out of confirmed cases of hepatic disorders, six dogs were taken for efficacy/ therapeutic studies. Six apparently healthy dogs were kept as control.

The dogs of therapy group were arbitrarily divided into mild, moderate and severe cases based on clinical symptomatology for efficacy study. Two afebrile, unvaccinated (DHPPI + L) dogs presented to Hospital showed inappetance and dullness since three days, had normal condition and hair coat which were considered as mild cases. Anorexia, fever (103.2oF) with high pulse rate (180/min) and rapid respiratory rate (in one dog), occasional vomiting and depression persisting since a week was observed in two dogs with thin body condition and slightly dull hair coat. One was vaccinated (DHPPI+L) among two and the other was not vaccinated, one had mild dehydration (6% of b.w.).

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These dogs were graded as moderate cases. Remaining 2 dogs showed anorexia, ascites, anaemia and lethargy since a week. Both were emaciated, not vaccinated, non febrile and had dull hair coat. Out of these two, one had icterus and these were considered as severe cases.

These dogs with hepatic disorders were treated with silymarin and appropriate supportive therapy as per the need. Supportive therapy which includes antibiotics, multivitamins, diuretic (Adams, 2001), fluids (Bunch *et al.*, 2001) and dietary alterations (Rutgers and Haywood, 1988) and were monitored daily. The haematological and biochemical changes were recorded on the day of diagnosis and on 7th day of therapeutic trial.

RESULTS AND DISCUSSION

Clinical signs: The dogs of therapeutic group after initiation of therapy showed improvement in appetite by 2nd day in mild cases, 3rd day in moderate cases and partial improvement by 7th day in severe cases. General activity and alertness was considerably improved by day 3 (mild cases), day 5 (moderate cases) and by day 7 (severe cases). In vomiting cases, emesis was stopped by 2nd day and in one dehydrated case, the hydration status came to normal by 3rd day. In febrile cases, the fever was reduced by 3rd day of the treatment. In ascitic dogs, the considerable reduction in the abdominal size was noticed by 7th day of therapy. Anaemic dogs also showed marked improvement by 7th day, but icterus was not reduced markedly even by 7th day of therapy in a dog with jaundice. This overall improvement in clinical signs is attributed to the effect of therapeutic regimen of silymarin and supportive therapy.

Haematology: In the anaemic dogs, there was a marked improvement in Hb and PCV noticed by 7th day of therapy. These changes could be due to the effect of haematinics and better nutrition and the status of liver, thus resulting in decreased bile acids. Silymarin inhibits the effect of the toxic substances and regulates the functions of hepatocyte (Displaces *et al.*, 1975). The findings are in concurrence with the observations of Vijayakumar *et al.* (2004 a & b).

Table 1 :Haematological findings in dogs with hepatic disorders before and after treatment

Sl. No.	Parameter	Apparently healthy dogs (n = 6)	Dogs with hepatic disorders (n = 6)	
			'0' day	7th day (aftertherapy)
1.	Hb (g/dl)	15.17 ± 0.83	8.03 ± 0.62##	10.83 ± 0.46**
2.	PCV (%)	44 ± 2.08	26.5 ± 2.82##	32.17 ± 2.28**
3.	TLC (Cells / µl)	9596 ± 908	16861 ± 2468#	13.282 ± 1251*
4.	Neutrophils (%)	65.33 ± 2.12	83.33 ± 4.37##	79 ± 4.11**
5.	Lymphocytes (%)	23.5 ± 1.78	13.17 ± 3.06#	16.5 ± 3.04*
6.	Monocytes (%)	8.33 ± 0.42	2.17 ± 1.01##	3.17 ± 1.19NS
7.	Eosinophils (%)	2.83 ± 0.4	1.17 ± 0.4#	1.17 ± 0.3NS
8.	Basophils (%)	---	---	---

: Significant at P < 0.05 with relation to apparently healthy dogs.
: Significant at P < 0.01 with relation to apparently healthy dogs.
* : Significant at P < 0.05 when compared to '0' day.
** : Significant at P < 0.01 when compared to '0' day.
NS : Not Significant (P > 0.01 and P > 0.05)

Marked elevation of TLC (16861±2468/µl) with neutrophilia (86.33±4.37%) was reduced significantly after therapy. The reduction could be due to antibiotics as proved by Vijayakumar *et. al.* (2001) and the anti inflammatory action of Silymarin as quoted by Tanasescu *et.al.* (1998).

Biochemical findings: Though the significantly (P<0.01) elevated mean ALT level reduced by 7th day, it did not reach the normal value. The decrease could be attributed to the membrane stabilizing ability, antioxidant and hepatoprotective properties of silymarin (Mourelle *et. al.*, 1989 and Saraswat *et. al.*, 1997). Saller *et.al.* (2001) reported that silymarin will consistently decrease the transaminase levels. Similar observation was noticed by Paulova.*et. al.* (1990), Vijayakumar *et.al.* (2004 a & b) and Tiwari and Varshney (2005).

Table 2 : Serum biochemical findings in dogs with hepatic disorders before and after treatment

Sl. No.	Parameter	Apparently healthy dogs (n = 6)	Dogs with hepatic disorders (n = 6)	
			'0' day	7th day (after therapy)
1.	ALT (U/L)	21.78 ± 2.09	102.87 ± 8.16##	79.65 ± 5.02**
2.	ALP (KA Units)	5.79 ± 1.76	44.55 ± 12##	34.31 ± 9.85**
3.	Total Serum Protein (g/dl)	6.79 ± 0.26	4.44 ± 0.37##	4.87 ± 0.38**
4.	Serum Albumin (g/dl)	3.52 ± 0.24	1.93 ± 0.3##	2.59 ± 0.28**
5.	Glucose (mg/dl)	95.93 ± 4.97	50.24 ± 5.##	60.57 ± 5.37**
6.	Total Bilirubin (mg/dl)	0.2 ± 0.03	2.97 ± 1.63NS	2.11 ± 1.15*

: Significant at $P < 0.05$ with relation to apparently healthy dogs
: Significant at $P < 0.01$ with relation to apparently healthy dogs.
* : Significant at $P < 0.05$ when compared to '0' day.
** : Significant at $P < 0.01$ when compared to '0'
NS : Not Significant ($P > 0.01$ and $P > 0.05$)

The reduction in mean ALP value and marked decrease of ALP in three dogs which showed high ALP levels was noticed by 7th day of trial. This could be due to the effect of silymarin. Vogel *et al.* (1984), Vijayakumar*et al.* (2004 a & b) and Tiwari and Varshney (2005) also noticed similar findings after therapy with silymarin in hepatic disorders.

Total protein and albumin levels in the dogs of this group showed significant improvement by 7th day of therapy but ascitic dogs (severe cases) showed slight improvement only. The improvement could be due to the correction of hepatic insult by the therapy resulting in improved functional capacity of hepatocyte improved appetite and better protein intake. Displaces *et al.* (1975) concluded that silymarin inhibits the effect of toxic substances and regulates the functions of hepatocyte. Silymarin causes increased hepatocyte protein synthesis (Flora *et al.,* 1998). Vijayakumar *et al.* (2004 a & b) observed significant elevation in total protein and albumin after silymarin therapy in canine hepatic disorders. The above reports are in agreement with the changes noticed in the present study.

Improvement noticed in hypoglycemia by 7th day of therapy could be due to the improved appetite and functional status of hepatocyte exerted by silymarin therapy. The findings are in concurrence with the observations of Vijayakumar *et al.* (2004 a & b).

The bilirubin value was reduced in this group of dogs (including icteric dog) after therapy. The reduction could be attributed to the effect of antibiotics in reducing infection if present as suggested by (Tams, 2001) and Vijayakumar *et al.* (2001) and by the hepatoprotective action of silymarin as reported by Vogel *et al.* (1984) and Vijayakumar (2004 a & b).

To conclude, in the present study, the therapeutic regimen containing silymarin found to be effective in dogs with hepatic disorders.

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Prevalence of Ectoparasites in Poultry

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ABSTRACT

A study was carried out in and around Hassan to know the prevalence of ectoparasite infestation in poultry. A total of 336 birds were screened for ectoparasitic infestation which included 48 desi birds and 288 commercial birds. Out of 48 desi birds screened, 19 were found to be infested with ectoparasites. The desi birds had only lice infestation viz., *Menopon gallinae* on 10 (52.6%) birds, *Lipeurus caponis* on 6 (31.6%) birds, *Goniodes gigas* on 2 (10.5%) birds and *Goniocotes gallinae* (5%) on only one bird. Among 288 commercial birds screened, 82 were found to harbor ectoparasites in which 71 had lice infestation, 8 birds had mite infestation and only 3(3.7%) birds had mixed infestation with lice and mites. The species of lice found in commercial birds were *Menopon gallinae* on 33 (40.2%) birds, *Menacanthus stramineus* on 15 (18.3%) birds, *Lipeurus caponis* on 23 (28%) birds and the mite species found was *Ornithonyssus bursa*.

Key words: Ectoparasites, Prevalence, Poultry, Commercial birds, Desi birds

Poultry production in India has reached industrial proportions on par with the developed countries. The industry involves maintenance of large population of chicken in contrast to the small flocks confined to backyard. Parasitism in poultry is a flock problem. Ectoparasites are important pests of poultry especially where proper management practices are not implemented and are regarded as the basic causes of retardation in growth, lowered vitality and poor conditions of the birds. On one hand, intensification of production is imperative to meet the growing demand for poultry products. On the other hand, however, it is of paramount importance to provide the required health care services against myriads of disease causing agents which affect the productivity of this sector. Where studies have been conducted, parasitic diseases and in particular ectoparasites have been identified as the major impediment to the chicken health world wide owing to the direct and indirect losses they cause (Permin *et al*., 2002; Sonaiya *et al.*, 2004 and Swai *et al.*, 2007). They can affect bird health directly by causing irritation, discomfort, tissue damage, blood loss, toxicosis, allergies and dermatitis which in turn alleviate quality and quantities of meat and egg production. Also they act as mechanical or biological vectors transmitting number of pathogens (Gedion, 1991 and Fabiyi 1996). Despite of their devastating effects the ectoparasites in poultry has not received much attention. Hence, the present study was conducted to determine the prevalence of ectoparasite infestation in poultry in order to devise appropriate control strategies.

MATERIALS AND METHODS

Collection of ectoparasites: The samples taken from chickens were examined by close inspection with naked eyes. A representative of ectoparasites found in the body of the chicken was collected. The plumage of each bird was thoroughly brushed onto a white tray for the collection of ectoparasites. The feathers of the head, neck, wings, body, legs and cloaca were raised and thoroughly examined for ectoparasites. Attached ectoparasites such as lice which could not be removed by brushing, were gently dislodged with a pair of thumb forceps. Birds suspected for mites infestation , deep scrapings were collected by using a scalpel or knife blade dipped in acetic glycerine (1 % glacial acetic acid in glycerine). All types of ectoparasites

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collected were categorized and permanent preparations were made.

Processing of ectoparasites of poultry

Lice: The lice moving over the feathers of the birds were visible to the naked eye. They were collected and preserved in 70 per cent alcohol. Later the specimens were cleared by treating them in 10 per cent NaOH overnight, washing, dehydrating in ascending grades of alcohol, cleared in xylol, then mounted in DPX or Canada balsam and the permanent slides were prepared.

Mites: The feathers with ectoparasites from various parts of the body were plucked and put in plastic containers and brought to the laboratory. The macroscopic examination of feathers revealed mites as specks on the feathers. The infested feathers were kept in a petri dish containing 70 per cent alcohol and examined under stereozoom microscope. The mites were teased by a dissection needle by pressing the feathers down to the petri dish with another needle. The mites were then collected and preserved in 70 per cent alcohol. Then the mites were transferred to a slide containing a drop Berlese's medium, a cover slip was placed on that and allowed to dry at room temperature. The specimens were identified based on their morphological characters.

RESULTS AND DISCUSSION

A total of 336 birds consisting of 48 desi birds and 288 commercial birds were screened for the presence of etoparasites during the study. Among 48 desi birds, 19 were found to harbour ectoparasites and rest of the birds were free of infestation and in case of 288 commercial birds, 82 birds were found to be infested with ectoparasites. The ectoparasites comprised of lice and mites. Among 19 desi birds which were found to harbor ectoparasites, all had lice infestation where as in commercial birds, lice infestation was found on 71 birds, mites on 8 birds, mixed infestation of lice and mites was found on 3 birds.

Table: Prevalence of ectoparasites in poultry

	Desi birds Birds infested		Commercial birds Birds infested	
Species of Ectoparasites	**Number**	**%**	**Number**	**%**
LICE:	10	52.6	33	40.2
a) *Menopon gallinae*	6	31.6	23	28
b) *Lipeurus caponis*	2	10.5	-	-
c) *Goniodes gigas*	1	5	-	-
d) *Goniocotes gallinae*	-	-	15	18.3
e) *Menacanthus stramineus*				
MITES *Ornithonyssus bursa*	-	-	8	9.7
LICE & MITES	-	-	3	3.7

The lice species identified were *Menopon gallinae*, *Menocanthus stramineus*, *Goniodes gigas*, *Goniocotes gallinae* and *Lipeurus caponis*, mite species found was *Ornithonyssus bursa*. The specimens collected during the study were identified as per Soulsby (1982)

Only lice infestation was found on all the desi birds (19) which had ectoparasitic infestation. The lice found were *Menopon gallinae* on 10 (52.6%) birds, *Lipeurus caponis* on 6 (31.6%) birds, *Goniodes gigas* on 2 (10.5%) birds and *Goniocotes gallinae* (5%) on only one bird. The occurrence of *Menopon gallinae* was higher in desi birds when compared to other lice. The findings of the present study were in accordance with Rani *et.al*. (2008) and Sanguaranond (1993) who reported higher prevalence of *Menopon gallinae* followed by *Lipeurus caponis*. Chhabra and Donora (1994) also recorded high prevalence of *Menopon gallinae* while investigating the prevalence of ectoparasites in chicken from households in Zimbabwe. The occurrence of *Lipeurus caponis* (31.6%) and *Goniodes gigas* (10.5%) is in agreement with Rani (1998) and Pandit *et al.* (1993) who carried out a survey on the prevalence of ectoparasites on desifowls in Kashmir valley. *Goniocotes gallinae* (5%) was

found least. The results are similar to the reports of Chhabra and Donora (1994). They recorded Goniocotes gallinae 2% while investigating the prevalence of ectoparasites in chicken from households in Zimbabwe.

In 82 commercial birds which were found to harbor ectoparasites 71 birds had lice infestation. The species of lice found were *Menopon gallinae* on 33 (40.2%) birds, *Menocanthus stramineus* on 15 (18.3%) birds and *Lipeurus caponis* on 23 (28%) birds, Mite species found was *Ornithonyssus bursa* and it was seen on 8 (9.7%) birds, only 3 (3.7%) birds had mixed infestation with lice and mites. The occurrence of *Menopon gallinae* (40.2%) was higher. These observations are comparable with Medjouel Ilyes *et al.* (2013) and Saxena *et al.* (2004). Medjouel Ilyes *et al*. (2013) recorded nine different species of chewing lice in which *Menopon gallinae* (97.2%) was the most prevalent lice identified. The occurrence of *Menacanthus stramineus* (18.3%) was lower when compared to other two lice found on commercial birds. The findings support the reports of Aman *et al.* (2013).

The mite found was Ornithonyssus bursa (9.7%) which is in agreement with Placid (1981) and Deepali Chaddha *et al.* (2005). Sanguaranond (1993) in his prevalence study of ectoparasites in chicken in Thailand has recorded *Ornithonyssus bursa* (9.9%) which is in close agreement with the present study.

Some of the chicken showed mixed infestation of lice and mites. The results are in close agreement with George *et al.* (1992), Pandit *et al.* (1993), Sanguaranond (1993), Chhabra and Donora (1994), Deepali Chaddha *et al.* (2005).

CONCLUSION

In conclusion the results of the study showed lice infestation was more on desi birds as well as commercial birds. In order to reduce morbidity and mortalities due to ectoparasitic problem, it is necessary that strict sanitary control to be followed in rearing facilities. In addition, there is need for extension service to intensify farmer training on health management.

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Comparing Histomorphology of Platinated and Deplastinated Tissues Such as Liver and Kidney of Pig.

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ABSTRACT

The study was conducted to assess the plastination induced changes in the morphology of pig's liver and kidney. The liver and kidneywere processed in a routine procedure for both the tissues under room temperature. Plastination was done with resin solution using plastic tea cup andthermocol. Deplastination was done using organic solvent to remove resin. Both thetissues were stained using Haematoxyllin and Eosin phloxine stain. It was observed that tissues maintained their architecture without histomorphological changes even after the deplastination.

Keywords: Plastination, Deplastination, Liver, Kidney and Histomorphology.

Plastinated specimens can be effectively used for teaching macroscopic and as well microscopic anatomy. While studying histology with plastinated organs, subject of debate is whether plastination induces changes in tissues. *Manjunath et al.,* (2014) has studied the histology of plastinated liver and kidney without deplastination. The present study of histological changes is thus conducted on deplastinated liver and kidney of pig.

MATERIALS AND METHODS

The liver and kidney of pig were collected from the slaughter house and they were divided into two sets. Fixation was doneusing 5 % formal saline for 24 hours. 1st set of liver and kidney were taken for plastination and the other set for deplastination. Tissues were processed up to clearing step, later set one was plastinatedusing plastic tea cup and thermocol solution (15% resin solution) and block was done using paraffin. Set 2 organs were also plastinated and later they weredeplastinated. Deplastination was done using chloroform solution without tea cup and thermocolfor 24 hours. Sections were cut at6 micron and stained using routine haematoxyllin and eosin phloxine method.

RESULTS AND DISCUSSION

On comparison, the histology of plastinatedand deplastinated tissues remained same. The structural integrity and aesthetics of the specimens remained sound. Polymerizing resins, used as embedding media for tissues and organs, produced minimal distortion (Bennet*et al.,* 1976). In the present study, the histology of plastinated liver and kidney tissues showed all the details (Fig. 1, 3) even after the deplastination of the tissues (Fig. 2,4)

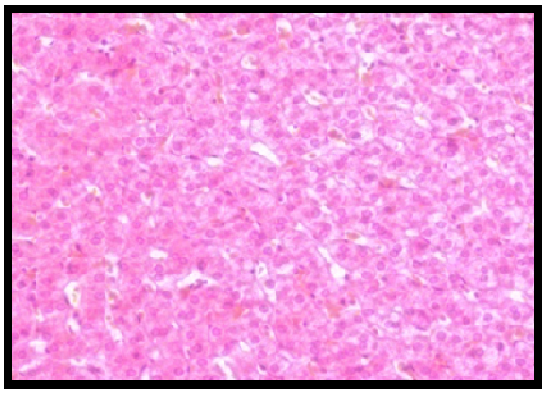

Figure 1: Plastinated liver H & E Phloxine X100

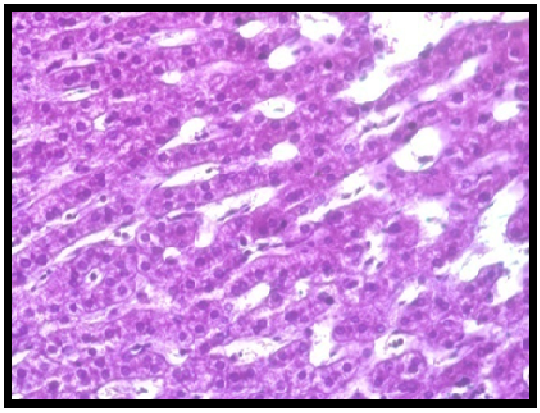

Figure 2: Deplastinated liver H & E Phloxine X100

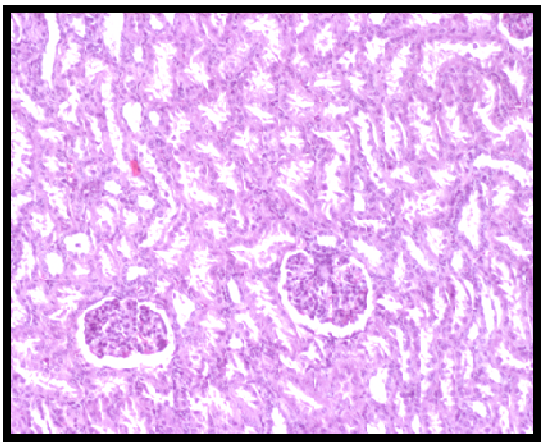

Figure 3: Plastinated kidney H & E Phloxine X100

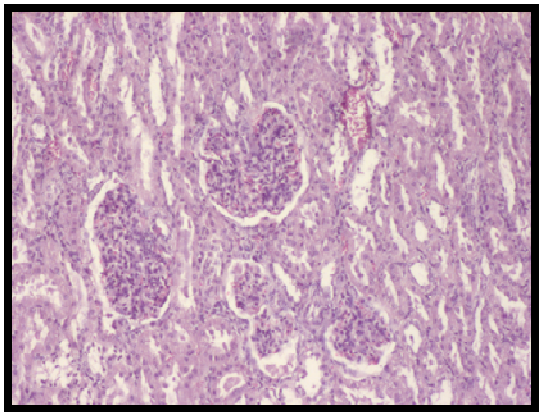

Figure 4: Deplastinated kidney H & E Phloxine X100

Silicone for plastinating the organ such as spleen and pancreas tissue could be used for both light microscopic and ultrastructural studies (Grondin*et al*.,1994). The results in the present study was in accordance with Manjunath*et al.,* (2014) who described the results for plastinated organs. However, they did not do deplastination. In the present study deplastination of the organ was done to know whether there was any significant change in the tissue architecture orin the organ. Eckel*et al.,* (1997) used imprignationof biological materials with curable polymer for plastination. Ripani*et al.* (1996) studied the light microscopy of plastinated tissueusing silicone impregnation. They concluded that silicone embedded sections could be used for both light and electron microscopic studies after deplastination.

It was concluded that plastination and deplastination did not show any difference in the histomorphology of the tissues processed.

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Ripani, M., Boccia, L., Cervone, P. and Macciucca, D. V., 1996. Light microscopy of plastinated tissue can plastinated organs are considered viable for structural observation. *J. Int. Soc. Plastination.,* **11** (1):28-30

Molecular Characterization of *Malassezia sympodialis* from Buffaloes with and without Otitis

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ABSTRACT

Malassezia species are lipophilic unipolar yeasts that can act as opportunistic pathogens in animals. A molecular study of *Malassezia* strains isolated from healthy buffaloes and with otitis from three different geographic regions was carried out by PCR. The PCR assay revealed *M. sympodialis* from both healthy and otitic ears.

Key words: Buffaloe Otitis; Malassezia species; *Malassezia sympodialis*; PCR

Yeasts fitting the description of Malassezia have been recognized on human skin since 1846. Members of the genus Malassezia are lipophilic and/or lipid-dependent, unipolar budding yeasts that can become pathogenic under the influence of predisposing factors like changes in the cutaneous microenvironment and/or alterations in host defenses. It is now widely accepted that Malassezia yeasts are commensal organisms of mammals and may also be associated with various cutaneous diseases.

The taxonomy of Malassezia species has been a matter of discussion since the creation of the genus by Baillon in 1889. An accurate identification of Malassezia species is of relevance as each species plays a specific role in the development of cutaneous and systemic infections (Perez *et. al.,* 2010). Currently, 14 species have been identified within this genus, *M. furfur, M. sympodialis, M. globosa, M. obtusa, M. slooffiae, M. restricta, M. pachydermatis, M. nana, M. dermatis, M. japonica, M. yamatoensis, M. caprae, M. equine and M. cuniculi* which are associated with several common dermatological disorders such as pityriasis versicolor (PV), seborrhoeic dermatitis (SD), atopic dermatitis and folliculitis in human beings (Eidi, 2012). *Malassezia* spp. specific IgE is considered as an important allergen-specific marker to assess the severity of atopic dermatitis in adults. (Glatzl *et. al.*, 2015)

With the exception of *Malassezia pachydermatis*, the remaining species are lipid dependent yeasts that require long chain fatty acids for *in-vitro* growth and are normally found in areas that are rich in sebaceous glands. *Malassezia pachydermatis* is usually associated with external otitis and dermatitis in dog and cat (Guillot and Bond, 1999 and Girao *et. al.*, 2006). *Malassezia sympodialis, Malassezia fufur* and *Malassezia globosa* were the species most frequently encountered in the external ear of cattle with otitis (Duarte *et. al.,* 2001a and Duarte *et. al.,* 2003). However, it is important to look at the distribution of the various *Malassezia* species not only in normal ears but also in otitic ears in buffaloes due to scarcity of reports in India. Further, With the development of molecular techniques, the classification of this genus has improved, allowing a more accurate differentiation between different species (Cafarchia *et. al.,* 2011). The nucleotide

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sequence analysis of the 26S rDNA (D1/D2) region for the differentiation of Malassezia species of cattle was described by Hirai *et. al.* (2004); Cabanes *et. al.* (2005) and Duarte and Hamdan (2008). In the present study an attempt was made to characterize the *Malassezia* species isolated from normal and otitic ears of buffaloes by using PCR.

MATERIALS AND METHODS

The sample for this study consisted of 141 buffaloes aged above 3 years and of either sex from three different places of Andhra Pradesh, India of which 20 were healthy and 121 were diagnosed with otitis. Cerumen, secretions or inspissated pus from the external ear close to the external acoustic meatus from 20 healthy (40 ears) and 121 (166 ears) buffaloes with otitis were collected with the aid of sterile swabs. Isolation of *Malassezia* was performed by inoculating the swab in to Sabouraud's Dextrose broth supplemented with 1% v/v of pure olive oil and incubating at 32^{o}C for 48-72 hours (Lee and Lee, 2010). Broth containing the growth was streaked on to 9 cm petri dishes containing Sabouraud's Dextrose agar added with chloramphenicol @ 150 mg/l. Two drops of olive oil was swabbed on the surface the Sabouraud's Dextrose agar after specimen seeding (Duarte *et. al*., 1999)**.** In the present study SDA with olive oil was used for the isolation of lipid dependent *Malassezia* species as per Duarte *et. al*. (2003) The plates then were incubated at 32°C for 7 days. The smears were made out of the colonies**,** stained with New Methylene Blue and observed under oil immersion for *Malassezia***.** Further confirmation was also done by catalase reaction.

For the identification of species, PCR assay was performed on all positive samples from healthy and otitic ears. The DNA extracted was subjected for amplification by PCR assay with following 3 different sets of primers specific for *M. sympodialis, M. globosa* and *M. furfur.*

The oligonucleotide primers of D1/D2 domains of 26S rDNA sequences specific to *M. sympodialis*, *M. furfur* and *M. globosa* were derived from already published sequences (Fell *et. al .,* 2000 and Hirai *et. al.,* 2004) and the following primers are used:

Species	Primer	Sequence
Malassezia sympodialis	FP	5-'GCA TAT CAA TAA GCG GAG GAA AAG'-3
	RP	5-'GGT CCG TGT TTC AAG ACG'-3
Malassezia furfur	FP	5-'CCG TGC GGC GCT ATG GAC AA'-3
	RP	5-'CGC CAG CAT CCT AAG CGC GA'-3
Malassezia globosa	FP	5-'GTG TGT CTC TGG CGG CTC GT'-3
	RP	5-'GCA AAG TGG CCC AGA GGC GT'-3

The thermal cycler was programmed as follows: denaturation at 95°C for 4 minutes followed by 30 cycles at 94°C for 45 seconds, anneling at 54°C for 20 sec and extension at 72°C for 1 min plus a final extension of 10 min at 72°C and 10°C for 10 minutes

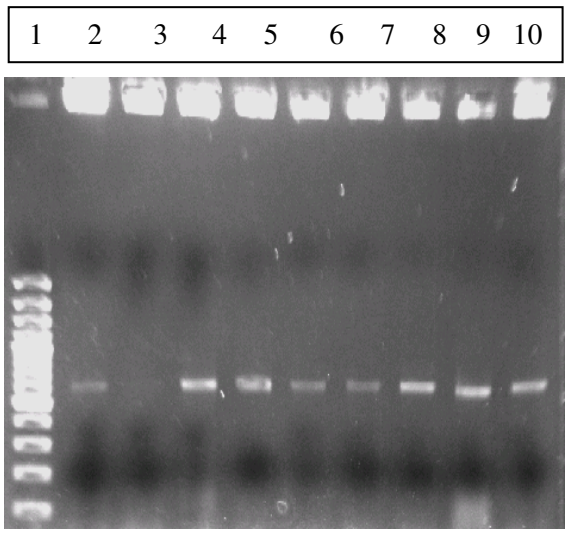
RESULTS AND DISCUSSION

Mycological culture revealed 47.50 % (19/40) of healthy and 59.04% (98/166) of otitic ears were positive for *Malassezia* organisms All the 117 samples (19 from healthy and 98 from otitic ears) subjected for PCR were positive for *M. sympodialis* yielding a product of approximately 580bp size which is specific for 26S rDNA gene sequence.

The nucleotide sequence analysis of the 26S r DNA (D1/D2) region for the differentiation of *Malassezia* species of cattle was used by previous workers (Hirai, 2004; Cabanes *et. al.,* 2005 and Duarte and Hamdan, 2008). Of the 3 sets of primers used in the present study, *Malassezia sympodialis w*as isolated from all the test samples (healthy and diseased), which was in accordance with the findings of Duarte *et. al.* (2003) who reported that *M. sympodialis* was the most common species obtained from both healthy and diseased animals followed by *M. globosa*. Duarte *et. al.* (2001b) reported that the lipid dependent species, *Malassezia globosa, M. sympodialis, M. furfur and M. slooffiae* were associated with bovine parasitic otitis. Though the test samples were obtained from 3 different locations, isolation of *M. sympodialis* at all the locations might be due to existence of similar climatic conditions. However, the genetic

heterogeneity in the DNA profile of *Malassezia* strains isolated from different regions was not studied in the present study.

Fig 1: Agar gel electrophoresis of PCR amplified product of *Malassezia sympodialis*



Lane: 100bp DNA Marker (3000, 2000, 1500, 1200, 1000, 900,800,700,600,500,400,300, 200,100); Lane 2 & 4-10: Test Sample

Although there are 14 proposed species in the genus *Malassezia* based on molecular, morphological and biochemical profiles, *M. sympodialis* was the only species isolated from the ear swabs of 98 otitic and 19 healthy ears subjected to PCR in the present study. However, these findings do not suggest the possibility of the presence of only one species associated with otitis in buffaloes, as in the present investigation, PCR assay was carried out in a limited number of animals. Miranda *et. al.* (2007) reported a variation in Malassezia species between geographical regions and suggested that humidity and temperature may be the main factors explaining the epidemiology of Malassezia species. During the period under report, the maximum temperature recorded varied from 34.2°C during winter to 47.6°C during summer and it is opined that some species of Malassezia do not tolerate temperatures higher than 38°C. Guillot *et. al.* (1994) reported that *M. globosa* has relatively high sensitivity to elevated temperatures and climatic variations. Duarte *et. al.* (2003) reported that otitic samples collected during summer were positive for the thermotolerant *M. sympodialis* while the winter samples were positive for a less thermotolerant *M. globosa*. Probably the above factors might have influenced in the isolation of *M. sympodialis* from the test samples.

In conclusion, the data presented in this study suggests that *M. sympodialis* found as member of normal ear microflora could become active in the presence of inflammation. Further studies on a large population of buffaloes and different geographical locations may be needed to confirm the predominance of the species *Malassezia sympodialis* in buffalo ear microflora.

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Prevalence of Ectoparasites in Small Ruminants

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ABSTRACT

A study was undertaken with the objective, to assess the prevalence of ectoparasites in small ruminants in Bidar district, Karnataka state. The screening was done in 198 sheep and 252 goats from small holder flocks in and around Bidar for the presence of ectoparasites. It was observed that the prevalence of ectoparasites was 43.43 and 58 per cent in sheep and goats respectively. In sheep, 1.51 per cent had mixed infestation of *Sarcoptes* mite and fleas and 36 per cent had ticks only. In goats, 43.47 per cent suffered severely from mixed infestation of *Haemaphysali*, *Hyalomma* and lice *linognathus sp*., 23(9.09%) animals had infestation of *Chorioptes caprae* and *Haemaphysalis sp*., of ticks. One goat was found infested with *Ctenocephalides canis.*

Key words: Prevalence, ectoparasites, small ruminants, ticks, mites.

Livestock rearing plays a significant role in improving socio-economic status of the rural people in India. More than 60 percent of small and marginal landless farmers prefer small ruminant keeping for their sustenance because it requires less investment and can be operated by family members on community pasture land.

Parasitic infestations have significant impact on husbandry, productivity and welfare of livestock. Reduction in weight gain, lowered productivity and damage of hide quality are the major problems of ectoparasites in ruminants. Bidar which is located in the north-eastern part of Karnataka state in India has a sizeable population of goats and sheep maintained as small holder flocks in the district. Since no basic study has been carried, the present study was taken to assess prevalence of ectoparasites in small ruminants.

MATERIAL AND METHODS

The study was carried out in and around Bidar, the north-eastern part of Karnataka state in India. Topographically, it is located at 17.9^0N 77.5^0E, lies at a central position in Deccan, a plateau at an elevation of 2300 ft from the sea level. The average annual precipitation at Bidar is 847 mm with most rainfall during the monsoon season.

From January 2015 to April 2015, 198 sheep and 252 goats of both sexes belonging to various smallholder flocks of three nearby village were included in the study. Ectoparasites encountered either on the skin surface or attached to the hair of infected animals were collected and were preserved in 70 per cent alcohol. Further identification of the species was conducted in the laboratory according to methods described by Soulsby (1982). Skin scraping from suspected cases of mange were collected, examined under stereomicroscope for live mites. The scrapped material processed by standard technique and identified based on their morphological characteristics Soulsby (1982).

RESULTS AND DISCUSSION

In the present study prevalence of ectoparasites were found to be 43.43 and 58 per cent in sheep and goats, respectively. In sheep 1.51 per cent had mixed infestation of mite and ticks and 36 per cent had tick infestation respectively. In goats 43.47 per cent had mixed infestation of ticks and lice, 9.09 per cent has mixed infestation of mite and ticks , 0.39 per cent had infestation of flea and 5.13 per cent had tick infestation alone.

Among the ectoparasite infestation ticks had taken upper hand in sheep and goats. The prevalence of tick, louse and mite was found more among goats than sheep. The different stages of ticks were found in more number in the internal ear

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of the sheep and goats followed by base of horn, eyelids and perianal.

The results in the present study are in consonance with the findings of Sertse and Wossene (2007) who observed that 50 per cent of sheep and 56.4 per cent of goats were infested with one or more ectoparasites. They reported that sheep had mixed infestation with *Damalina ovis*, *Melophagus ovinus*, ticks and *Linognathus* spp whereas, in goats had mixed infection with *Linognathus* spp., ticks, *Sarcoptes* mites and *Ctenocephalides* spp. Rony *et al*. (2010) observed that 69.09 per cent Black Bengal goats found to be infested with several species of ticks, lice and fleas. Similarly Elsaid *et al*. (2013) also reported, 20.1 per cent sheep and 35 per cent goats were found to be infested with one or more ectoparasites. Similar observation was recorded by Seid *et al*. (2014), and also reported that the prevalence of mange mites was significantly higher in goats than in sheep. In another report the tick and flea infestation were predominant in sheep whereas, mite infestations was more common in goats Israel *et al*.(2015). The observation of present study was not in accordance with Tadesse *et al*. (2011) recorded the incidence of tick infestation was higher in sheep than in goats. The difference in the prevalence of ectoparasite infestation could be attributed to the various factors such as climatic conditions of the region, system of management, type of housing, grazing land, presence or absence of acaricidal treatment, host spectrum of the tick species and availability of natural or maintenance host (Khan *et al*., 1993)

This study revealed that *Haemaphysalis* was the predominant tick species in sheep (88%) and goat (86 %). Of 198 sheep examined, 88 per cent of sheep was predominatd by *Haemaphysalis spp*., 22 per cent with *Hyalomma* spp and 1.51 per cent had mixed infestation of *Sarcoptes scabiei var ovis* and *Haemaphysalis.* These observations are in accordance with the findings of Latha *et al.* (2004) who found *Haemaphysalis* spp. was predominant ticks followed by *Hyalomma* spp. Prakasan and Ramani (2007) also mentioned that *Haemaphysalis bispinosa* was the most prevalent tick species of domestic animals in Kerala. The difference in the prevalence of species within the genera could be due to geographical variations as was confined earlier by Latha *et al*. (2004).

Out of 253 goats examined from various small holder flocks, 9.09 per cent were infested by *Chorioptes caprae* whereas, Israel *et al*. (2015) recorded *Demodex caprea* only in goats. It was observed that 43.47 per cent goats suffered severely due to mixed infestation of *Haemaphysalis, Hyalomma* and *linognathus* and one goat being infested by *Ctenocephalides canis*. Israel *et al*. (2015) and Rony *et al*. (2010) also recorded in their studies goat being infested with lice, (*Linognathus*) and fleas (*Ctenocephalides canis*).

The presence of *Hyalomma* sp. In 22 and 14 per cent of sheep and goat respectively was in accordance with Gharbi and Darghouth (2014) who stated that the preferential host for *Hyalomma* spp. was cattle, while sheep and goats were unusual host which could be due to low level of infestation *of Hyalomma* spp tick in sheep and goats. However, present findings are not in akin to the findings of Iqbal *et al*. (2014) where they recorded *Hyalomma sp.* to be more prevalent.

Table: Prevalence of tick infestation in sheep and goats in small holder flocks

Host	Animals screened	Animals infested	Animals infested by ticks only	Animals infested by Mites and ticks	Animals infested by louse and ticks	Animals infested byfleas
Sheep	198	76 (43.43%)	73 (36.06%)	3 (1.51%)	-	-
Goat	253	147 (58%)	13 (5.13%)	23 (9.09%)	110 (43.47%)	1 (0.39%)

None of the animal was found infested with *Boophilus* spp. tick in the present investigation. This finding is not in agreement with report of earlier workers Iqbal *et al*. (2014), Nasibeh *et al.,* (2010) and Rony *et al.* (2010). There is a general belief that *Boophilus* spp ticks is a cattle tick, which

does not feed on other species of animal normally. However, reports of the earlier workers revealed that *Boophilus* tick may also attack other species of animals in the absence of its natural host.

CONCLUSION

It is concluded that, prevalence of ectoparasites was more in goat compared to sheep and tick infestation was more compared to other ectoparasites.

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Physical and Performance Characteristics of Mudhol Hound Dogs – The Pride of Karnataka*

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ABSTRACT

The Mudhol Hound dog, a medium to large sized, sight-hound breed seen in south India, is studied to assess the distribution and breed characteristics. The breeding and home tract is Bagalkot and adjacent areas of Bijapur and Belgaum districts in Northern Karnataka. The trademark features of this breed are aerodynamic body, deep and broad chest, tucked up abdomen, pink/black nostrils, golden brown eyes and white, brown, black and white patchy, white and brown patchy predominant coat colours. The overall means of body length, height at withers and chest girth in adult male and female were 92.90 ± 0.70, 76.62 ± 0.49 and 72.93 ± 0.67 cms and 85.70 ± 0.64, 69.56 ± 0.49 and 66.56 ± 0.56 cms respectively. Sex had highly significant effect ($P< 0.05$) on body length, chest girth and height at withers. The study concludes that Mudhol Hound dog breed is a unique desi canine germ-plasm.

Key words: Morphology, Morphometry, Performance Characteristics, Mudhol Hound, CRIC

The diversity and richness of animal genetic resources has very well made high position for Indian subcontinent across the world. Historical evidences trace the origin of various livestock breeds evolved over a period through natural and man-made selection. In this process they acquired adaptation to hot climatic stress and resistance to diseases, which is an additional feather for desi germplasm. Canines are not an exception to this, but unfortunately no much attention has been paid to understand desi canine genetic resources.

India has the privilege of having excellent dog breeds like Rajapalayam, Chippiparai, Kombai, Kanni, Mudhol Hound, Rampur Hound, Caravan Hound, Banjara Hound, Bhotia sheep dog, Himalayan sheep dog, Alangu, Kaikadi, Indian Spitz,Bakharwal and Jonangi (Ravimurugan, *et al,* 2014). Among these Mudhol hound, the pride of Karnataka is said to be predominant one, the origin of which can be traced from the western region of the Deccan Plateau, which includes parts of Karnataka, Maharashtra and Andhra Pradesh. For centuries, this breed has rich tradition of serving as a companion, hunter and guard dog for both royal families as well as peasants.

The Mudhol hound is a descendent of some of the dogs they brought with them probably the Sloughi (from North Africa), the Saluki (from the Middle East), the Greyhound, These breeds were crossed with the local Indian dogs and evolved into India's native hounds. Over a period of time, these dogs took on the name of the land in which they lived. Historical evidences suggest that Mudhol hounds, known for devotion have been used in Guerrilla warfare to guard and sight interpolers during Maratha regime. Even the Tamil Nadu dog breed, Kanni was considered as a further extension of the Caravan or Mudhol Hound, and was also a descendant of the Saluki (Ravimurugan and Kumaravelu, 2008; Srinivasan, 2011).

In 2010, the Government of Karnataka recognized the importance of conserving this pride canine breed of state and established under Karnataka Veterinary, Animal and Fisheries

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Sciences University, an exclusive first of its kind institute Canine Research and Information Centre – CRIC, (Mudhol Hound) at Timmapur- Mudhol in Bagalkot dist. As a part of standardizing the breed characteristics, the present study was carried out with an objective to record the physical and performance characteristics of Mudhol Hound.

MATERIALS AND METHODS

A total of 150 dogs reared at different villages in Mudhol and Bagalkot talukas of Bagalkot district of Northern Karnataka were selected randomly in this study. Age of the dog was recorded as per the information provided by the owners. Various physical traits like body colour, shape of face, eyes colour, nostrils, patches present on the body along with morphology of ear, chest, abdomen and tail were recorded after careful visual as well as physical examination.

Morphometric measurements were recorded in cm with a precision of 0.5 cm when the animal was in normal standing position. The morphometric parameters such as body length, length of head, length of muzzle, length of tail, height at withers, height at rump, chest girth, belly girth, ear length, ear width and neck length were recorded as suggested by Gonzalez *et al.*,(2011), Leite *et al.,* (2011) and Hisham *et al.,* (2014).

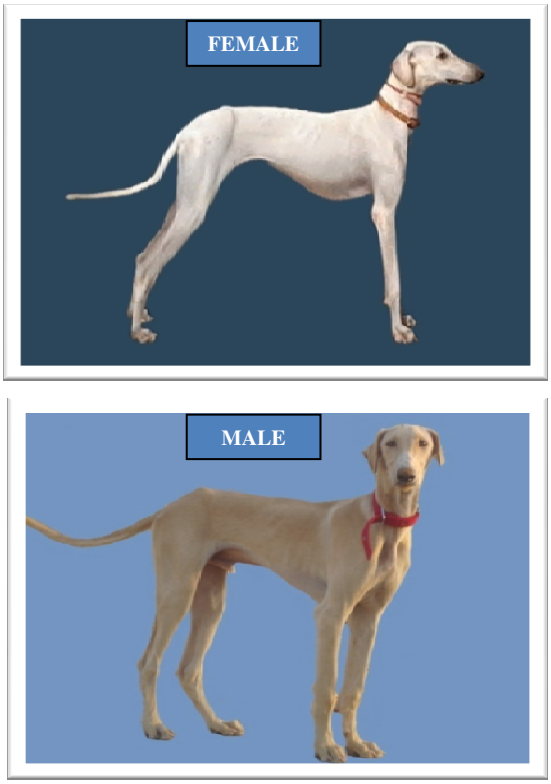
The data collected were subjected to standard statistical analyses as per Snedecor and Cochran (1989). Student's "t"test was carried out to find any significant difference in body length, height at withers and chest girth between sexes.

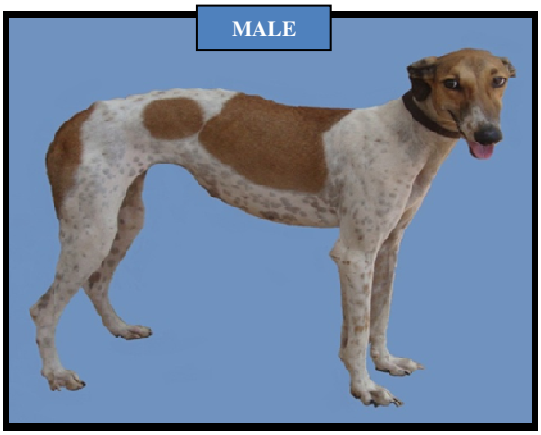
RESULTS AND DISCUSSION

Breeding Tract and population distribution: The main breeding tract of Mudhol hound dogs was observed in Mudhol, Jamkhandi and Bagalkot taluka's of Bagalkot district and parts of its surrounding areas belonging to neighboring districts like Muddebihal and Basavan Bagewadi talukas of Bijapur dist and Raibhag and Athani talukas of Belgaum districts in Northern Karnataka. The breeding tract is having a subtropical climate and generally dry. Annual rainfall of the breeding tract varied from about 562 to 978 mm (the average being 770.00 mm). The minimum and maximum relative humidity ranges between 14 and 77 percent respectively. The annual mean minimum and maximum temperatures are 21.00°C and 41.30°C respectively. They are tolerant of hot, dry weather but not cold weather and can chill in cool weather.

Body Coat Colour and skin: The predominant body colour of Mudhol Hound dog is pristine white (Fig. 1 to 3), rarely brown, black and white and black, white and brown spot markings are found on the back and sometimes on ears. The skin is tight and pink and also black in colour. Two coat varieties are mainly found in this breed *Viz.,* the **smooth coated type** with fine hair that lies close to its body, with no feathering and the **silky coated variety** with hair that feathers on the ears, legs, the back of the thighs, between the hock and the heel, and on the underneath side of the tail. Their fine, taut skin reveals their muscle tone. Any coat color or combination of colors which include fawn, fallow, red, cream, as well as any of those combined with white is acceptable. The most common hues are those that allow the dogs to blend in with their environment, providing natural camouflage (Fig).

Figure: Coat Colors of Mudhol Hound Breed of Dogs





Physical Characteristics: The head is small in proportion to the body and skull is long and narrow. It is flat and moderately wide between the ears. The stop is not pronounced and the dome absent. The head, viewed from above, is wedge shaped with black and pink nostrils. The eyes are dark brown or hazel in colour and oval in shape. They are obliquely placed which gives the dog an intelligent and a piercing expression. The ears are thin, of medium size, mobile, triangular and set fairly high. They are carried flat and close to the head. The neck is elegantly arched; long, supple and well muscled. The body is muscular with small powerful loins. The brisket is long and deep, with a roomy thorax and a well tucked in abdomen. The back is fairly broad. The legs are muscular with forelegs long and straight from elbow to knee. Hip bones are set wide apart, stifle moderately bent, hocks low to the ground. Feet are of moderate length, well arched and have strong pads enabling them for fast running. The tail is long and set on line with the body. It is strong at the base tapering, and slightly curved. It is carried low (Table).

Table: Physical Characters of the Male and Female Mudhol Hound Dogs

Sl No	Morpho-metric variable	Adult male Mudhol Hound Dog (n= 60)	Adult female Mudhol Hound Dog (60)
01	Body Length	92.90 ± 0.70	85.70 ± 0.64
02	Head Length	27.13 ± 0.26	25.88 ± 0.20
03	Muzzle Length	15.90 ± 1.04	12.61 ± 0.13
04	Tail Length	57.85 ± 0.81	50.60 ± 0.60
05	Height at weithers	76.62 ± 0.49	69.56 ± 0.49
06	Height at Rump	76.07 ± 0.37	69.80 ± 0.45
07	Chest Girth	72.93 ± 0.67	66.56 ± 0.56
08	Belly Girth	44.45 ± 0.63	41.70 ± 0.56
09	Ear Length	14.32 ± 0.17	12.87 ± 0.17
10	Ear width	8.88 ± 0.12	8.29 ± 0.12
11	Neck Length	25.47 ± 0.37	23.60 ± 0.32
12	Eye Colour	Brown	Brown
13	Coat Colour	White/ fawn/ brindle/ patchy	White/ fawn/ brindle/ patchy

Figures in parentheses indicate per cent co-efficient of variation.
* - Significant (P < 0.05);
** Highly significant (P < 0.01).

Temperment: The Mudhol is a hardy dog and a keen sight hound. But it has an independent temperament and the male dog has a tendency to attack at times. The Mudhol Hound is a dog that needs plenty of exercise and movement. It cannot be managed in confined areas like any other pet dogs.

Gait and movement: The gait and movement of this hound is an effortless stride, like flying rather than running with the hind legs under the body giving good propulsion. The appearance of the Mudhol Hound will convey a balanced conformation of power, grace and symmetry. Their lean aerodynamic bodies are designed for strength, speed and stamina.

Morphometric measurements: The means for various morphometric measurements are furnished in Table. The overall means for the principal body measurements such as body length, height at withers and chest girth in adult male and female were found to be 92.90 ± 0.70, 76.62 ± 0.49 and 72.93 ± 0.67cms and 85.70 ± 0.64, 69.56 ± 0.49 and 66.56 ± 0.56 cms respectively. The tail lengths of male and female dogs were found to be 57.85 ± 0.81 and 50.60 ± 0.60 cms respectively (Table).

The measurements indicate highly significant ($P < 0.01$) difference between male and female dogs for body length, height at withers and chest girth. Similar observations were made by Porchezhian and Sundaravinayaki (2010) and Hisham *et al.,* (2014).

The mean height at withers suggest that Mudhol hound is a medium-sized dog according to Yilmaz and Ertugrul (2011) who classified the medium sized dogs based on height (51-70 cm). The mean belly girth and chest girth matches with the sight hound anatomy; the hound type dogs should have a deep chest and narrow abdomen. Mean height observed in the present study is larger than that of Rampur hound and Greyhound (60-75cm) and Saluki (58-71cm) dogs (Yilmaz and Ertugrul, 2011). Mudhol Hound dogs are moderate in size and weight. They have shorter coats than the big-size hounds. It is accepted that, generally, they are genuine examples of sight hounds (Pugnetti 2001 and Anonymous 2005).

CONCLUSION

This study shows that Mudhol hound dog breed is a medium to large sized indigenous sight hound dog having its origin it northern part of Karnataka. The dogs of this breed are predominantly pristine white with two coat varities and have small head, long and narrow skull, thin medium sized triangular ears, elegantly arched neck, muscular body and long, straight, muscular legs. A significance difference ($P<0.05$) has been observed between male and female animals with respect to body length, chest girth and height at withers. Further there is vast scope for conservation of this desi germ plasm seeing the pet lovers attraction and the craze of people to rear in this region.

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Comparison of Growth Performance of Kenguri Strain-1 Lambs Fed Cow Milk or Milk Replacer

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ABSTRACT

Fifteen Kenguri strain-1 lambs were divided into three groups of five each and allocated one of the following feeding regimen: Group-1 was allowed with mother with no additional milk (T1), group-2 was fed 100ml of cow's milk daily in addition to dam's milk (T2) and group-3 was given milk replacer alone (T3). All groups were fed in five phases; 0-3, 4-14, 15-30, 31-60 and 61-90 days of age during a three month growth trial. The feeding of cow's milk and milk replacer was started from 15th day of their birth. The milk replacer was increased gradually from 25, 50 and 75g/day by reconstituting in water in the ratio of 3:1 and all the groups were offered lamb starter and good quality hay of 20, 100 and 200g/day each during 15-30, 31-6- and 61-90 days, respectively. . The CP and ME in lamb starter and milk replacer were 22.0 and 22.15%; 10.5 and 25.54MJ/kg feed respectively. Average body weight gain in T1, T2 and T3 groups were 100.2, 123.9 and 124.5 g/day and no significant difference among the groups was observed. There was an improvement (24%) in weight gain of lambs due to additional CP and ME supply through cow milk and milk replacer in T2 and T3 groups. It was concluded that milk replacer fed up to 75g/day /lamb (300ml) improved weight gain without any adverse effect on lambs.

Key words: Milk replacer, Lambs, Gain.

In most of the developing countries of the world, resource-poor landless labourers and marginal farmers depend on small ruminants for their livelihood and the feed resource available for these animals is common grass land which is also depleting every year. Irrespective of the systems of feeding, high mortality, poor growth rate of young ones and delay in onset of puberty are common in small ruminants because feeding of young one is not given much attention. Since the young ones are the future meat and milk producing stock, these should be nourished with food that can substitute for dam's milk which are commonly known as milk replacer and starter feeds (FAO, 2011). This practice is much useful for orphan, weaned lambs and in case of twins or triplets where dam's milk is not sufficient to nourish the young ones (ICAR, 2013). Even feeding of milk from other animals is also in practice in lamb rearing when dam's milk is not sufficient. Therefore promoting the feeding of milk replacer and starter feeds is not only advantageous in improving the survivability and growth of young ones but also reduces the milk feeding and labor cost (Heaney *et al.* 1982). Hence, the present experiment was taken up on Kenguri strain-1 lambs to compare the growth performance in response to feeding cow milk and milk replacer during their early life.

MATERIALS AND METHODS

Fifteen Kenguri strain-1 lambs (Birth wt.2.83 to 3.0kg) obtained by crossing indigenous Kenguri sheep breed and garole plus Nari swarna were chosen immediately after lambing and divided into three groups of five lambs each and housed in individual pen with respective dam/ewe. The lambs of group-1 were allowed with mother with no additional milk (T1), lambs of group-2 were fed 100ml of cow's milk daily in addition to ewe's milk (T2) and the lambs of group-3 were given milk replacer (T3). All the lambs of three groups were fed in five phases; 0-3, 4-14, 15-30, 31-60 and 61-90 days of age during three months of trial as per the standard feeding schedule of lambs (ICAR, 2013). The cow's milk and milk replacer feeding was started from 15th day of their birth and the feeding of dam's milk was stopped only in milk replacer fed group from 15th day onwards. The quantity of milk replacer was increased gradually from 25, 50 and 75g/day by reconstituting in water in the ratio of 3:1 between 15-30, 31-60 and 61-90 days of age respectively for the lambs of T3 group. Lambs of all the groups were offered lamb starter and good quality hay of 20, 100 and 200g/day each

from 15th day of age onwards during last three phases i.e. 15-30, 31-60 and 61-90 days of age. All the ewes were allowed for 3-4 hrs of grazing daily and offered 300-400g of concentrate feed supplement (CFM) to support milk production. The daily feed intake, weekly body weight and nutritional status of lambs were recorded. The milk yield of the ewes was assessed by hand milking once in a week and milk samples were drawn for milk composition analysis (Milkotest). The feed samples collected were subjected for proximate composition (AOAC, 2000) and fiber fractions (Van Soest, 1991).

RESULTS AND DISCUSSION

The chemical composition of lamb starter, milk replacer and concentrate feed supplement is given in Table 1. The CP and ME in lamb starter and CFM were 22.0 and 16.69%; 10.5 and 11.9MJ/kg feed respectively. The milk replacer contained 22.15% CP, 20.15MJ/kg ME, 25.54% fat, 1.0% Ca and 0.8% P. The composition was similar to the high quality lamb milk replacers which usually have 22-24% CP, 25-35% fat (Umberger, 1997) while the Whereas CP level in lamb starter was higher than the value (20.84%) reported by Emsen *et al*. (2004) for Awasi lambs.

Table 1. Ingredient composition (%) and chemical composition (% on DMB) of lamb starter and concentrate feed mixture (fed to ewes)

Ingredients	Lamb starter	Milk replacer#	CFM
Casein	-	2	-
Skim milk powder	-	58	-
Vegetable oil	-	25	-
Starch	-	14	-
Maize	36	-	60
SBM	27	-	21
WB	33	-	15
Salt	01	-	01
Mineral mix	01	-	01
DCP	02	01	02
Total	**100**	**100**	**100**
Cost, Rs/kg	30.4	55.2	27.4
CP	22	22.2	16.7
ME (MJ/kg)$	10.5	20.2	11.9
CF	5.0	-	3.6
EE	1.3	25.5	2.3
Ca	-	1.0	-
P	-	0.8	-
NDF	26.7	-	23.5
ADF	8.1	-	9.4
ADL	1.8	-	1.8

Citric acid-2g, Sporlac powder-one sachet ($1.5x10^8$ spores/kg MR) was added $ Calculated

The average weekly milk yield of ewes and composition during different phases of feeding is given in Table 2. No significant differences were observed among between the treatment groups with respect to milk yield since the ewes were maintained on an uniform feeding regimen whereas composition of the milk differed significantly ($P<0.01$) except in total solids among the groups.

Table 2. Milk yield (ml/d) and composition of dam's milk

Particular	T1	T2	T3	SEM	P Value
Dams's milk yield, ml/day					
0-3	313	295	296	12.83	NS
4-14	308	283	277	13.63	NS
15-30	296	283	272	13.38	NS
31-60	260	256	249	10.72	NS
61-90	209	212	214	12.98	NS
Composition of dam's milk					
Fat,%	5.7	5.9	4.7	0.156	0.01
LR	34.3	35.8	33.0	0.560	0.01
Lactose, %	4.3	4.8	3.8	0.075	0.01
TS, %	10.4	10.6	11.8	1.253	NS
CP, %	3.70	3.90	3.90	0.048	0.01

Average body weight gain in T1, T2 and T3 groups were 100.2, 123.9 and 124.5 g/day and finishing weight after 90 days of feeding in corresponding groups was 11.44, 13.23 and 13.46 kg respectively which were not significant among the groups (Table 3). The similar trend of improvement in weight gain was observed in T2 and T3 groups when compared to T1group in all five phases of feeding schedule. Average daily gain in group T2 and T3 were higher than the value (115g/d) obtained by Emsen et al. (2004) for Awassi lambs and lower than the value (180g/d) reported by Sezen Ocak and Soner Cankaya (2013). No mortality in lambs was observed in this study due to feeding of milk replacer whereas in other studies mortality was 9% (Sezen Ocak and Soner Cankaya, 2013) and 15% (Emsen et al. 2004) in artificially reared lambs. Better survivability rate of lambs fed milk replacer than reared with ewes (55-75%) was reported due to higher plane of nutrition in milk replacer fed lambs (Kumar *et al*. 2014).

Because of additional CP and ME supply through cow's milk and milk replacer in T2 and T3 groups respectively there was an improvement (24%) in weight gain of lambs. However, no difference was seen between T2 and T3 groups. Bimczok, Röhl and Ganter (2005) reported significantly higher ($P<0.05$) average daily gain of 262 g/d when lambs were fed ad libitum milk replacer when compared to the lambs fed with milk starter. The cost of producing one kg body weight was lower (Rs.63.7) in milk replacer fed group (T3) followed by T2 (Rs.116.6) and T1 (Rs.121.9) which indicated that rearing of lambs on milk replacer was profitable in terms of higher body weight gain (Table 3). Though, milk from other animals can equally support as that of milk replacer but it is not advisable in order to save milk and earn maximum profit instead, milk replacer was found to be better alternative as far as economical rearing of lambs are concerned.

Table 3. Average overall body weight gain, the daily gain of experimental lambs in different periods and economics of feeding cow milk and milk replacer.

Particular	T1	T2	T3	SEM	P Value
Initial B.wt,kg	3.02	2.83	3.00	0.34	NS
Final B.wt., kg	11.44	13.23	13.46	1.28	NS
Overall gain, kg	8.24	10.41	10.46	1.08	NS
Gain/d, g	100.2	123.9	124.5	12.9	NS
Weight gain, g/d in different periods					
0-3	86.3	163.0	165.8	55.39	NS
4-14	69.1	114.8	142.6	39.79	NS
15-30	91.2	113.4	115.6	30.61	NS
31-60	98.6	113.0	112.8	20.38	NS
61-90	97.4	116.4	116.7	14.17	NS
Economics of feeding cow milk and milk replacer to the lambs					
Cost of ewe's milk fed for 90 days (Rs.)	675.9	659.4	109.8		
Cost of cow milk/milk replacer fed for 90 days (Rs.)	-	225.0	227.6		
Cost of lamb starter fed for 90 days (Rs.)	282.6	282.6	282.6		
Cost of hay fed for 90 days (Rs.)	46.5	46.5	46.5		
Total cost (Rs.)	**1005.0**	**1213.5**	**666.6**		
Cost/kg B.wt. (Rs.)	121.97	116.57	63.73		

Note: Cost of ewe's milk and cow milk –Rs.30.0, hay-Rs.5.0, lamb starter-Rs.30.39 and milk replacer –Rs.55.18

Table 4. Average intake of dam's milk and cow's milk (ml/d) or milk replacer (g/d), CFM and hay (g/d) and their CP (g/d) and ME (MJ/d) contribution in experimental lambs and their body weight gain (g/d) in different periods of growth

Treatment	Age, days	Intake				CP intake				CP Total	ME intake				ME Total	B.wt. Gain
		Dams milk	Cow's milk, /MR	CFM	Hay	Dams milk	Cow's milk/ MR	CFM	Hay		Dams milk	Cow's milk/ MR	CFM	Hay		
T1	0 -3	313	-	-	-	11.4	-	-	-	11.4	1.44	-	-	-	1.44	86.3
	4 -14	308	-	-	-	12.7	-	-	-	12.7	1.42	-	-	-	1.42	69.1
	15 -30	296	-	20	20	11.6	-	4.4	0.8	16.8	1.36	-	0.21	0.13	1.70	91.2
	31 -60	260	-	100	100	8.9	-	22.0	4.0	34.9	1.20	-	1.05	0.65	2.90	98.6
	61 - 90	209	-	200	200	8.1	-	44.0	8.0	60.1	0.96	-	2.10	1.30	4.36	97.4
T2	0 -3	295	-			13.8	-	-	-	13.8	1.36	-	-	-	1.36	163.0
	4 -14	283	-			12.4	-	-	-	12.4	1.30	-	-	-	1.30	114.8
	15 -30	282	100	20	20	12.1	3.0	4.4	0.8	20.3	1.30	0.52	0.21	0.13	2.16	113.4
	31 - 60	256	100	100	100	8.4	3.0	22.0	4.0	37.4	1.18	0.52	1.05	0.65	3.40	113.0
	61 - 90	212	100	200	200	8.5	3.0	44.0	8.0	63.5	0.97	0.52	2.10	1.30	4.89	116.4
T3	0 -3	296	-	-	-	11.2	-	-	-	11.2	1.36	-	-	-	1.36	165.8
	4 -14	277	-	-	-	8.9	-	-	-	8.9	1.28	-	-	-	1.28	142.6
	15 -30	-	25	20	20	-	5.54	4.4	0.8	10.7	-	0.50	0.21	0.13	0.84	115.6
	31 - 60	-	50	100	100	-	11.08	22.0	4.0	37.1	-	1.01	1.05	0.65	2.71	112.8
	61 - 90	-	75	200	200	-	16.61	44.0	8.0	68.6	-	1.51	2.10	1.30	4.91	116.7

It was concluded that milk replacer up to 75g/day /lamb (300ml) can be used to rear lambs when dam's milk is insufficient to meet the lambs' requirement in case of twins or triplets or for orphan lambs.

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Effect of Urea Treated Sugarcane Bagasse Supplemented with Fibrolytic Enzymes on Performance of Kenguri Sheep

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ABSTRACT

The experiment was conducted on nine Kenguri sheep by dividing into three groups of three animals each in 3x3 LSD design to know the effect of urea treated sugarcane bagasse (SCB) supplemented with fibrolytic enzymes on the performance. 2% urea treated SCB was the basal diet for all the treatment groups.T-1 group (control) fed with only 2% urea treated SCB, T-2 was supplemented with 1% xylanase enzyme and T-3 with 1% mixed enzymes. All the groups were given 250g Concentrate feed mixture (CFM) per day per animal. The DMI improved significantly ($P \leq 0.05$) in T2 and T3 when compared to T1. The nutrient digestibility of DM, OM, CF, NDF, ADF and hemicelluloses was found to be non significant among the three groups. The intake of DCP was found to be significantly ($P \leq 0.01$) higher in T-3 group, whereas no significant difference in TDN intake. The rumen pH among treatment groups were within the normal range but significantly ($P \leq 0.01$) higher TVFA in T-3 group was noticed. The results of the study indicated that the palatability and total DMI were improved when 2% urea treated SCB was supplemented with 1% xylanase. However, further studies are required by increasing the level of both urea and xylanase addition to know the effect on nutrient utilization in SCB.

Key Words: Fibrolytic Enzymes, Sheep, Sugarcane Bagasse, Urea

In India Livestock production in ruminants is mainly extensive system. Most of the feed requirements are met either from grazing on community lands or on crop residues based rations, without supplementing concentrates, due to the poor economic condition of the farmers. Hence, crop residues, containing high level of ligno-cellulosic materials with low levels of fermentable carbohydrates and other byproducts are used as main source of dry matter for ruminants. The sugar cane bagasse (SCB) is highly lignified material when compared to other crop residues and less palatable, with low digestibility of nutrients (Kaur and Kaushal, 2001). The major constraints in utilization of crop residues for growth and other productive performance are low voluntary feed intake, low digestibility and imbalance of absorbed nutrients which are partly due to physico-chemical structure (Minson, 1990) and partly due to low microbial protein production in the rumen. Urea treatment is a better option to improve the nitrogen content thereby improving the protein value of low quality roughages. Inclusion of fibrolytic enzymes in the straw based rations of ruminant livestock improved the digestibility and body weight gains (Singh and Das, 2009). Hence, an attempt was made to evaluate effect of fibrolytic enzymes on intake, digestibility and nutrient utilization in Kenguri Sheep fed with 2% urea treated SCB based rations.

MATERIALS AND METHODS

A pilot study was conducted on four sheep prior to actual experiment to study the voluntary intake of SCB for 10 days. As the intake was too low, later SCB was treated with 2% urea and 20% moisture and stored for seven days. The intake of urea treated SCB was improved in pilot study. Based on this pilot study 2% urea treated SCB taken as basal diet. The experiment was conducted on nine Kenguri sheep (Age; 6 to 12 months, BW; 20-23kg) by dividing in to three groups of three animals each in 3 X 3 Latin Square design with preliminary period of 10 days, followed by 4 weeks collection period. The SCB treated with urea 2% (w/w) with 20 per cent moisture level and stored for seven days was fed to all groups of sheep as basal diet. The control (T-1) group was fed 2% urea ammoniated SCB plus 250g Concentrate feed mixture (CFM), the treatment

group (T-2) was fed with urea treated SCB +250g CFM + 1% xylanase enzyme (added to SCB 24 hours before offering SCB) and T-3 fed with urea treated SCB+ 250g CFM+ 1% mixed enzymes (Cellulase - 346.93IU/g; Xylanase - 1974.70 IU/g; Pectinase - 317.94 IU/g and Phytase - 288.73IU/g) added to SCB 24 hours before offering SCB. Daily feed intake and weekly body weight were recorded during the experiment. Rumen fluid was collected at two hours post feeding before start of the experiment and at the end of each trial period. The pH was recorded immediately using electronic digital pH meter and total volatile fatty acids (TVFA) was analyzed (Barnet and Reid, 1956). Blood samples were collected at the beginning and at the ending of each trial period using heparinized tube and blood serum was separated for analysis of total protein (Gornall, 1981) and serum urea nitrogen (Fawcett and Scott, 1960). Digestion trial for five days was conducted at last week of each period, during which daily intake of SCB and CFM as well as output of dung was recorded. The samples of feed, fodder and dung were analysed for proximate constituents (AOAC, 1995) and forage fibre fractions (Vansoest, 1991). The data was analysed statistically using SAS (2012).

RESULTS AND DISCUSSION

The chemical composition of SCB, CFM and experimental diets are given in Table 1. The SCB contained (%) 1.95 CP, 2.67 TA, 89.44 NDF, 62.65 ADF, 1.93 ADL, 49.61 Cellulose and 26.79 Hemicellulose. The CFM contained 14.85% CP and 2% urea treated SCB supplemented with enzyme also contained similar protein. The CP content of SCB was increased to 4.59 to 5.00% after ammoniation with 2% urea. Addition of 1% xylanase to 2% urea treated SCB increased the NFE content by 3.4% (43.97% *v/s* 45.46%) than the 2% urea treated SCB alone, which might be due to the release of soluble carbohydrate due to the pre-consumptive effect of xylanase that was added 24 hours before feeding after ammoniation. While the effect was not noticed after mixed enzyme addition to 2% urea treated SCB. The findings were similar to the values reported by Hristov *et al,* (2000). The average daily gain of experimental sheep (Table 2) was not statistically significant among the groups as they were fed for maintenance, but all groups have gained on an average of 32-37 g/d. The mean DMI (g/d) from SCB and CFM, the total DMI (g) , total DMI as per cent body weight and as a proportion of metabolic body weight are given in Table 2. The DMI of SCB was higher ($P<0.05$) in T2 compared to T3, whereas DMI as % body weight $W^{0.75}$ kg was non-significant among the groups. The total DMI (g/day) was significantly higher ($p\leq 0.05$) in T-2 group compared to other groups. This indicated that the addition of 1% xylanase improved the palatability and intake of 2% urea treated SCB when compared to the total DMI among the T-2 and T-3 groups. Mixed enzyme added group T3 also showed the similar effect on total DMI. The findings are corroborated with the results of experiments on steers with mixed enzyme at 0.4% of the diet (Elkady *et al.*, 2006), and in Murrah buffalo calves (Thakur *et al.,* 2010).

Table 1. Chemical composition (% DMB) of CFM, SCB and experimental feeds.

Parameter	CFM	SCB	T1	T2	T3
OM	85.91	97.33	96.92	97.19	96.52
CP	14.85	1.95	4.59	5.40	5.04
EE	1.88	0.58	0.53	0.53	0.58
CF	9.01	45.50	47.83	45.79	46.63
NFE	60.17	49.30	43.97	45.46	44.28
TA	14.09	2.67	3.08	2.81	3.42
AIA	1.58	1.88	1.90	2.02	2.26
NDF	29.13	89.44	89.51	88.84	88.69
ADF	12.28	62.65	61.00	62.17	62.68
ADL	1.03	1.93	2.33	2.07	2.34
Cellulose	9.70	49.61	47.31	48.90	49.07
Hemicellulose	16.85	26.79	28.51	26.72	26.01

Table 2. Average daily gain (g/d), Mean dry matter intake (g/d) of experimental sheep.

Parameter	T1	T2	T3	P
Initial weight (kg)	22.76	22.55	22.56	NS
Final weight (kg)	23.52	23.54	23.60	NS
ADG (g)	30.48	35.28	37.10	NS
DMI, g/d				
CFM (g/d)	231.16	231.16	231.16	
SCB (g/d)	229.74^{b}	256.64^{a}	250.01^{ab}	*
SCB (% B.W.)	0.98	1.13	1.10	NS
SCB ($BW^{0.75}$)	21.53	24.62	23.97	NS
Total DMI (g/d)	460.91^{b}	487.80^{a}	481.17^{ab}	*
TDMI, % B.W.	2.06	2.21	2.17	NS
TDMI, kg $B.W^{0.75}$	44.48	47.59	46.78	NS

* P≤0.05, Means bearing different superscript in a row differ significantly.

The mean digestibility of nutrients of experimental sheep is given in Table 3. The digestibility of DM, OM, CF, NDF, ADF and hemicelluloses was non significant among the treatment groups. While improved digestibility of CP reported by El-Badwai et al., (1990b) in treated SCB. The same trend was not observed in group T-3 where the basal diet was supplemented with mixed enzyme. The mean intake of digestible nutrients (g/d) of experimental sheep was non significant except intake of digestible CP. The difference in the digestible CP intake was due to marginal variations in the CP content of the total ration and the influence of urea significantly improved the DMI from SCB. The nutritive value in terms of DCP (%) of the T-3 diet was significantly (P≤0.01) higher followed by T-2 and T-1. However, The DCP and TDN content of all the rations were within maintenance requirement of experimental sheep according to (Ranjhan, 1998). There was a significant (P≤0.01) difference in the rumen pH among the treatment groups, and which was well within the normal range (William, 2005). The significantly (P≤0.01) higher TVFA (mmol/dl) in T-3 followed by T-2 and T-1 groups (Table 4) might be due to the higher hemicelluloses digestibility in T-3 and T-2 groups when compared to the T-1 group. Significantly (P≤0.01) higher serum urea nitrogen was noticed in T-3 group when compared to the T-2 and T-1 groups. The values were within the normal range (William, 2005). The higher serum urea nitrogen in T-3 is attributed to higher DCP intake and efficient metabolism in the animals. The similar results were reported by Elkady *et al.,* (2006) in calves fed with exogenous enzyme supplemented diets.

Table 3. Mean digestibility (%) and Nutritive Value of diets fed to experimental sheep.

Parameter	T1	T2	T3	P
Digestibility(%)				
DM	53.58	56.19	53.68	NS
OM	56.78	59.23	57.01	NS
CP	72.20	73.59	72.25	NS
EE	69.89	70.73	69.62	NS
CF	50.97	53.98	51.48	NS
NFE	59.41	59.72	59.95	NS
NDF	45.51	48.24	46.08	NS
ADF	39.80	41.35	39.74	NS
Cellulose	49.92	50.61	48.68	NS
Hemicellulose	53.71	57.95	60.61	NS
DOMD	53.22	53.63	53.69	NS
Nutritive Value (%)				
DCP (%)	7.47^{b}	8.04^{ab}	8.44^{a}	*
TDN (%)	59.36	60.34	62.86	NS
DCP intake (g)	32.09^{c}	34.68^{b}	36.01^{a}	*
TDN intake (g)	254.25	259.68	268.95	NS
ME(MJ/d) intake	3.85	3.93	4.07	NS

* P≤0.05, Means bearing different superscript in a row differ significantly.

Table 4. Mean Rumen pH, TVFA (mmol/dl), Total serum protein (g %) and Serum urea nitrogen (mg %) of experimental sheep.

Parameter	T1	T2	T3	P
pH	6.55^{b}	6.94^{a}	6.87^{a}	**
TVFA	8.18^{c}	10.65^{b}	12.96^{a}	**
Total serum protein	6.37	6.78	6.01	NS
Serum urea nitrogen	12.44^{b}	11.79^{b}	16.14^{a}	**

***** P≤0.01, Means bearing different superscript in a row differ significantly.

It was concluded that, addition of 1% xylanase to 2% urea treated SCB supplemented

with CFM has significantly improved the DMI in sheep when compared to 1% mixed enzyme supplemented or control group. Improvement in the nutritive value interms of DCP suggestive of increasing the level of urea for ammoniation and xylanase addition, may further improve the nutrient utilization in SCB.

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Sequencing Error in Polyadenine Repeat Chromatogram Readings of Ovine RXFP2 Gene Fragment

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ABSTRACT

The sequencing of RXFP2 fragment by single pass method using forward primer used for PCR amplification resulted in good sequencing chromatogram but the reverse primer even on repeated attempts failed to give desirable sequencing chromatogram and showed noisy mixed peaks. The RXFP2 sequence data just based on forward sequencing revealed a deletion of a nucleotide A (CAAG instead of CAAAG) at 28th nucleotide of the amplicon, which would be major deletion mutation leading to non functional RXFP2. The same result was obtained for all the samples analyzed. Cloning of the RXFP2-II fragment into pGEMT vector and sequencing with SP6 primer revealed no deletion (CTTTG), indicating the sequencing error in single pass method of sequencing. The error in poly single nucleotide repeat region could be either due to error during PCR amplification or chromatogram reading. Similar results from several samples analyzed rules out the error due to PCR amplification and findings support the fact that, the sequence chromatograph reading error is more in A/T run homopolymers rather than G/C runs. The chromatograph in polyA regions tended to become wavy, especially when present within 25-35 nucleotides of the sequencing PCR product. Sequencing through a poly-A in a plasmid usually works better than in PCR. Thus vector cloned sequencing with reconfirmation of the sequence by reverse primer is to be adopted before declaring SNP in the amplified products with poly A tracts.

Key words: RXFP2, Sequencing error, Polyadenine, Chromatogram.

DNA sequencing is the ultimate method in identification of variations at DNA level. Proper sequence chromatogram reading is crucial in identifying single nucleotide polymorphisms (SNPs). Even though advanced technique as next generation sequencing is available, older methods of sequencing are still used in identification of SNPs. Homopolymer error is one of the several errors in sequence chromatogram reading. Hence, before declaring any SNP one needs to through check for repeatability and possible errors in sequence reading. One such Poly 'A' reading error in Ovine RXFP2 fragment sequence reading which would have functional relevance is reported in the present study.

MATERIALS AND METHOD

Genomic DNA was extracted from venous blood samples collected from 30 cryptrochid and 30 normal male Mandya sheep by using the High salt method (Miller *et al*., 1988). Primers were designed based on the sequence obtained by BLAST of Bovine RXFP2 Ensemble sequence (ENSBTAT 00000020135) on to Ovine whole genome database (Oar_v3.1) using CLCBIO software. The Polymerase Chain Reaction was carried out in 25 ul volume with 1.5mM $MgCl_2$, 200 µM of dNTPs each, 100 ng of genomic DNA, 20 pM of each primer and 1 unit of Taq DNA polymerase with cyclic conditions of initial denaturation for 94°C / 7 min followed by 30 cycles of denaturation (94°C / 45 sec) annealing (58°C / 30 sec) and extension (72°C / 30sec) and final extension for 72°C / 7 min. The amplified product custom sequenced by single pass sequencing using primers used for amplification of the products. The sequencing was done at Chromus Bio, Bangaluru and Amnion, Bangaluru. Resultant chromatogram sequences were analyzed using Chroms lite (Version 2.1.1) and CLC BIO programme (CLC BIO, 2011).

The amplified products were eletrophorized on 0.8% low EEO agarose gel, the band was excised and DNA isolated from gel by Gel Extraction kit (Promega PCR Purification kit). The product was cloned in pGEMT-Easy cloning

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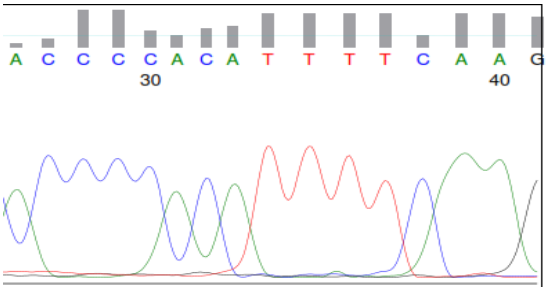
vector system (Promega) as per the manufacturer's conditions. Transformation was carried out using DH5α strain of *E.coli.* following the protocol of Sambrook *et al.* (1989). White colonies grown in the media containing Ampicillin, X-Gal and Isopropyl thiogalactoside (IPTG) were selected and confirmed by Colony PCR to check the insert of desired fragment. Plasmid DNA was isolated from the freshly grown culture of white colonies and subjected to custom sequencing using T7 and SP6 primers. The sequence chromatograms were analyzed for polymorphism.

RESULTS AND DISCUSSION

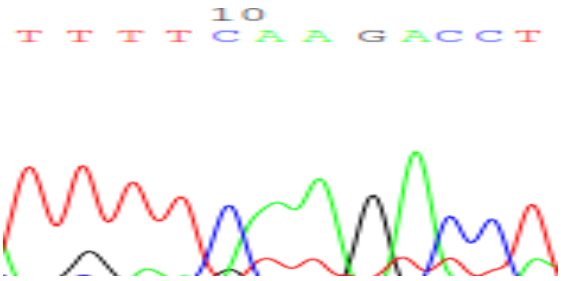
RXFP2 is the only receptor for INSL3. Decreased or defective expression of the receptor is involved in intra abdominal cryptorchidism in mice and Human (OMIA, 2014; Bathgate *et al*., 2006). RXFP2 gene is relatively conserved across broad spectrum of species and has 18 exons (Overbeek *et al.,* 2001). A SNP in exon 8, corresponding to T222P mutation of RXFP2 in humans (Nuti *et al.,* 2007) is reported to be involved in cryptorchidism. Hence, in the present study exon 8 of Ovine RXFP2 gene was studied for polymorphism and its probable association with cryptorchidism.

The PCR amplification resulted in a fragment of 258 bp from all the sixty samples analyzed. The amplicon encompassed part of exon 8 and intron 8 region of Ovine RXFP2 gene and was submitted to Genbank (KC906244.1). The primer sequence, amplicon details are presented in table 1.

Fig.1. The forward sequence chromatogram of Ovine RXFP2 fragment



Cryptorchid sample



Normal sample

Fig.2. The reverse sequence chromatogram of Ovine RXFP2 showed noisy mixed peaks.

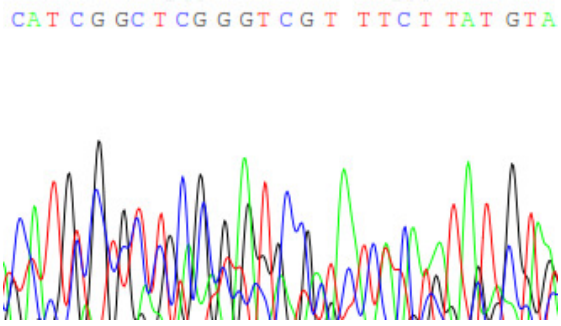


Table.1. Primer sequence, PCR amplicon details of the Ovine RXFP2 gene

Gene I.D	Primer Sequence		Genome position	Size	Gen Bank. No
Ovine RXFP2	F	GTCAGAATTCTAGATGACAATCCC	Exon 8 & part of Intron 8	258	KC906244.1
	R	AAATATGCCATGAGCCATGG			

The custom sequencing of Ovine RXFP2 fragment by single pass method with forward primer used in PCR amplification yielded satisfactory chromatogram for all the samples sequenced (Fig. 1). The reverse primer used in PCR amplification failed to give desirable sequencing chromatogram even on repeated attempts and showed noisy mixed peaks (Fig.2).

The analysis of RXFP2 sequence data just based on forward sequencing revealed a deletion of a nucleotide A (CAAG/CAAAG) at 43 nucleotide of exon 8 for samples from both cryptorchid and normal group. The deletion would result in frame shift in sequence reading and thus abrupt stoppage in RXFP2 protein synthesis, resulting in cryptorchid phenotype (Fig.3). Thus to conform the

deletion mutation the samples were subjected to cloning and sequencing. The RXFP2 sequence based on cloning and sequencing with SP6 primer revealed no deletion (CTTTG) (Fig.4).

Fig.3. Effect of deletion mutation in exon 8 of Ovine RXFP2

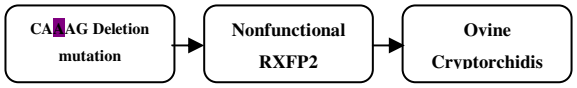
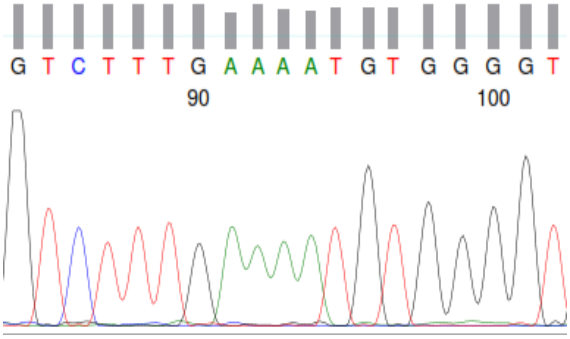


Fig.4. The reverse sequence chromatogram of Ovine RXFP2 by cloning and sequencing method revealed no deletion (CTTTG)



The error in poly single nucleotide repeat region could be either due to error during PCR amplification or during sequencing. Clarke *et al.* (2001) have reported that PCR amplification introduces errors into mononucleotide and dinucleotide repeat sequences, and the usual error is introduction of extra nucleotide. Such PCR introduced errors were accounted for by sequencing several samples. Moore *et al.* (2006) reported that while reading sequence chromatograph, the common error observed usually is in the regions of homopolymer, especially with A/T runs rather than G/C runs. In the present study it is observed that the chromatograph in polyA regions tends to become wavy, especially when present within 25-30 nucleotides of the sequencing PCR product and thus caused difficulty in identifying exact number of A in the region. Sequencing through a poly-A tail in a plasmid usually works better than in PCR. Thus Vector cloned sequencing with reconfirmation of the sequence by reverse primer is to be adopted for PCR products with poly A tracts. No associative SNP for cryptorchidsim was identified in the sheep population studied.

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Economics of Utilization of Fibrin from Buffalo Blood in Buffalo Meat patties

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ABSTRACT

Buffalo blood was collected from a local slaughterhouse and fibrin is separated by whipping action over a wooden stick. The physico-chemical and microbiological properties of fibrin were determined. Buffalo meat patties were prepared by replacing lean meat at 0, 3, 6 and 9% with fibrin. The physico-chemical properties of patties i.e emulsion stability, pH, cooking yield increased significantly ($P<0.05$). Replacement of lean meat had significant ($P<0.05$) effects on proximate composition of patties. The sensory attributes were also significantly affected but remained good to very good upto a level of 9%. Results from this study indicated that meat could be replaced up to 9% with fibrin in patties formulations without adversely affecting quality and reduction in cost of production upto Rs 20/kg in treated meat patties.

Key words: Buffalo blood, Fibrin,Utilization, Buffalo meat, Patties, Quality,Economics

After addressing the calorie hunger, there is immense need to supply animal proteins at reduced cost in the diet of common mass to fulfil the dietary requirement of proteins in developing countries like India. Blood is one of the under - utilized by-product from slaughter animals, accounting for 3-5% of live weight and contains 18-19% proteins. Meat industry is under constant pressure to improve the utilization of whole blood from its slaughter operations. Some of this meat by-product is used in feed and fertilizer formulations, but profits from these outlets are marginal (Caldironi and Ockerman1982). High quality proteins prepared from edible blood represent a potential source of large quantities of dietary protein. Though much of the protein from blood has been utilised in the form of blood meal for feed industry and to a certain extent the serum proteins and plasma proteins for food purpose, the clotted blood lumps remain attached to the bleeding trough in abattoir is being wasted in many local abattoirs of developing nations like India (Divakaran ,1982). Fibrin recovered from clotted mass can serve as potential protein source. Being globular protein, the fibrin solubility in salt solutions is fairly good, hence can be utilized in processed meat products (Kondaiah and Lakshmanan, 1985).

Modern consumers are no longer satisfied with the traditional meat products. Rapid urbanization and change in life style have increased demand for more nutritious and ready to eat products. Novel processing technique for meat resulted in the production of meat products which are tasty and convenient with superior sensory qualities. However, high cost of these products makes it difficult for an average consumer to use these products regularly in their diet (Malav *et. al.,* 2013). Therefore development of technology which aims at designing low cost meat products along with utilization of abattoir waste like blood may solve the problem of environmental pollution also.

Reports are available on various uses of blood proteins in different food products including meat products (Seidman *et. al.,*1979; Caldironi and Ockerman 1982; Kondaiah and Lakshmanan 1985; Hazarika and Biro 1993). But literature on utilization of buffalo fibrin in meat food products is scanty. Hence an attempt was made to recover fibrin from buffalo blood and to incorporate in buffalo meat patties.

MATERIALS AND METHODS

Collection of blood: Blood was collected in clean stainless steel cans under hygienic conditions from buffaloes slaughtered in a local slaughterhouse.

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Blood was agitated with a mechanical agitator and the threads of fibrin were formed over it.

Preparation of fibrin: The clotted lump was separated by whipping action over a wooden stick. Such lumps of clots were collected and washed with cold distilled water. The water required was almost twenty times the volume of the clot. The final wash was done with normal saline solution to prevent proteins of fibrin being denatured; the fibrin so obtained was in the form of large mass of meaty, stringy, fibrous material. The wet fibrin was further oven dried in hot air oven at 35^0C for a period of 8 hours. Such ten batches of fibrin separation task were undertaken till separated fibrin became four kg. The dried fibrin was stored in clean glass jars and stored at room temperature.

Recipe of patties: Deboned buffalo meat was packed in clean polyethylene bags and frozen at -20^0C until use. Analar and food grade chemicals were procured from Qualigens, Mercks and BDH. Refined salt (Tata Chemicals Ltd., Mumbai), Sun flower oil, Low density polyethylene films (200 gauges) bags, onion and garlic were procured from local market, Bidar (Karnataka state). To prepare condiment, onion and garlic were peeled off, cut into small pieces and homogenised in a mixer to obtain a fine paste. Spices were prepared in laboratory as per pre- standardised formulation as shown below.

Spice ingredients	Percent
Aniseed	10
Black pepper	10
Capsicum	8
Caraway seed	10
Cardamom	5
Cinnamon	4
Clove	1
Coriander powder	20
Cumin seed	22
Turmeric	10

Recipes for buffalo meat patties were standardised through eight preliminary trials. The standardized recipe contained 85 parts buffalo meat with 15 parts of sun flower oil and green condiments 5%, table salt 2%, dry spices mix1%, sugar 1%, phosphate 0.5%, sodium nitrite 0.02% and ice water 12%. Buffalo meat patties for the present study were prepared by incorporating fibrin at 0, 3, 6, and 9 percent levels by replacing lean meat. The dried fibrin used in these trials was rehydrated by soaking it in potable water overnight to a moisture level equivalent to lean meat and this was assessed by checking moisture level as per AOAC (1995).

Preparation of patties: Meat emulsion was prepared using Bowl chopper (Mado Germany). Partially thawed minced meat was chopped with chilled water. Salt, phosphate, sugar, nitrite and fibrin were mixed and chopped. Refined mustard oil was added gradually and blended with spices and green condiments. Finally sixty grams of emulsion was moulded in aluminium circular mould and placed on perforated trays and cooked for 18 minutes in a preheated oven at 180 ^{0}C to obtain an internal temperature of about 75^0C . Five such trials were conducted for each level of incorporation.

Analysis of sample: Proximate composition i.e., moisture, protein, lipid, total ash contents of fibrin and patties were determined according to AOAC (1995) methods. Solubility of fibrin was determined by the method of Beuchat (1977). Microbiological analysis of standard plate counts (SPC), coliforms, salmonella, staphylococcus and yeasts and molds was done as per APHA (2001).

The pH of raw emulsion as well as cooked patties was determined by the method of AOAC (1995) using pH meter. Emulsion stability was determined by the method of Baliga and Madiah (1970) with slight modifications. Percent cooking yields for control and treated samples were calculated by taking the weights of raw and cooked patties. The sensory attributes of the product were evaluated by eight semi trained panelists, using an 8 point Hedonic scale as per Keeton (1983). Data obtained were analysed statistically using Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The yield of fibrin from raw blood is less than one percent whereas it is 40-45% from the clotted mass of blood (Kondaiah and Lakshmanan, 1985). The dried fibrin contained about 89-90% protein, 0.75% of lipids and about 1.10% of ash. The proximate composition, functional and microbiological qualities are evaluated so as to study their suitability for incorporation in patties and the results are presented in Table1. Earlier workers have reported similar values for proximate composition of fibrin (Kondaiah and Lakshmanan, 1985). Microbiological qualities of fibrin were within acceptable limits as reported for other blood plasma proteins (Tybor *et. al.,* 1975; Hazarika and Biro 1993).

Table1. Characteristics of fibrin used for patties preparation

Parameters	Fibrin
Proximate composition	
Moisture %	7.78 ± 0.08
Proteins %	89.22 ± 0.57
Lipids %	0.92 ± 0.09
Total ash %	1.16 ± 0.09
Functional properties	
Solubility %	67.88 ± 0.85
Microbiological qualities	
SPC log/g	2.67 ± 0.25
Coliforms	Nil
Salmonella	Nil
Staphylococcus	Nil
Yeast and molds	2.34± 0.28

Values are Mean ± SE

Physical properties of patties: Results of physical properties viz., emulsion stability, pH, cooking yield are presented in Table 2.The results revealed the incorporation of fibrin improved the emulsion stability (92.73, 93.26, 93.78 and 94.26 %) significantly ($p<0.05$).

The pH values of emulsion increased (6.01, 6.09, 6.13, and 6.18) significantly ($p<0.05$) between different levels of incorporation of fibrin. The rehydration of fibrin before patties preparation might be responsible for increase in the pH as water binds with myofibrillar proteins and contributes to increase in their net ionic charge. Rusig (1979) also reported that the pH of the sausages incorporated with plasma protein isolate was higher than the control due to high pH of plasma protein isolate.

There was a significant ($p<0.05$) increase in the cooking yield (95.35, 96.20, 97.28 and 98.45%) of patties with increase in the level of fibrin. Similar increases in cooking yields were reported by Seideman *et. al*., (1979), Rusig (1979), Hazarika and Biro(1993) in beef and pork sausages respectively, on incorporation of different blood proteins in sausage formulation.

Proximate composition: The moisture contents (65.70, 63.41, 61.37 and 60.08%) of patties decreased significantly ($p< 0.05$) with increased level of fibrin incorporation. The decreases in moisture contents might be due to dried protein isolate, which brought down the moisture levels (Terell *et.al.,* 1982 ; Hazarika and Biro 1993).

The protein contents (16.62, 17.83, 19.15 and 20.46%) and ash contents (2.65, 3.01, 3.70 and

Table 2 : Physico- Chemical attributes of patties prepared by incorporation of fibrin

Parameters	% of Lean replaced with fibrin			
Physical	**0**	**3**	**6**	**9**
Emulsion stability, %	92.73 ± 0.08^{A}	93.26 ±0.04^{B}	93.78 ± 0.04^{C}	94.26 ± 0.05^{D}
pH of the emulsion	6.01 ± 0.01^{A}	6.09 ± 0.01^{B}	6.13 ± 0.02^{BC}	6.18 ± 0.01^{CD}
Cooking yield, %	95.35 ± 0.10^{A}	96.20 ± 0.05^{B}	97.28 ± 0.09^{C}	98.45 ± 0.10^{D}
Chemical				
Moisture, %	65.70 ± 0.13^{D}	63.41 ± 0.12^{C}	61.37 ± 0.07^{B}	60.08 ± 0.12^{A}
Proteins, %	16.62 ± 0.09^{A}	17.83 ± 0.07^{B}	19.15 ± 0.06^{C}	20.46 ± 0.09^{D}
Total lipids %	12.80 ± 0.05^{C}	12.45 ± 0.16^{B}	12.29 ± 0.11^{AB}	12.10 ± 0.05^{A}
Ash %	2.65 ± 0.07^{A}	3.01 ± 0.13^{B}	3.70 ± 0.09^{C}	4.47 ± 0.08^{D}

Values are Mean ± SE

Means with different superscripts (row – wise) differ significantly ($P< 0.05$)

4.47%) increased significantly ($p<0.05$) with the increased fibrin levels in patties formulation. Similar results were reported by Terrel *et. al.,* (1979) and Hazarika and Biro (1993) while working with beef and pork sausages using plasma protein isolate. This might be due to the higher protein and ash contents of the fibrin.

The lipid contents (12.80, 12.45, 12.29 and 12.10%) of the patties decreased significantly ($p<0.05$) with increased levels of fibrin incorporation. Similar results are reported by Terell *et. al.,* (1982) while working with increased levels of plasma protein isolate in frankfurters.

Sensory evaluation: Sensory evaluation results are presented in Table 3.The incorporation of fibrin up to 9% did not affect ($p<0.05$) the appearance, juiciness, texture, mouth coating and overall acceptability of patties. On the other hand, the flavour scores decreased ($p<0.05$) significantly by the addition of fibrin. The reduction in flavour scores might be due to bland taste of fibrin Patties up to 9% of lean replacement with fibrin were graded as very good by taste panel members. Similar observations were also reported by Rusig (1979) and Caldironi and Ockerman (1982) in beef sausages incorporated with plasma protein isolate. It may be concluded that fibrin prepared from buffalo blood may very well be used as a substitute of lean meat up to 9% in buffalo patties with better yield, higher protein and lower lipid contents.

Economics of incorporation of fibrin in patties: The comparative cost for formulation of 50kg control and patties prepared by replacing lean meat at 9% is presented in Table 4. It includes the cost of raw materials required for preparation of patties like deboned buffalo meat, table salt, spice mixture, condiments, phosphate, sodium nitrite. In addition transportation cost for the purchase of raw materials was included. The retail prices for these ingredients are no relatively stable in our marketing system. However, the cost of these ingredients can be lowered if purchased in bulk quantities from distributors/ wholesale agents that may further reduce the cost of production. The formulation cost of 50 kg product was Rs. (Indian rupee) 9,654/- for control and treatment with 9% replacement of lean with fibrin was Rs.8,888/-. The reduction in cost for treated product was mainly due to fibrin incorporation. Overhead cost involved in product preparation is presented in Table5. It includes labour charges (skilled and unskilled), electricity charges, rent for buildings, packaging material cost, water charges and maintenance cost and equipment depreciation (@ 10% per annum). It is similar for both control and patties prepared by 9% lean replacement with fibrin and amounts to be Rs. 1,048/- for the production of 50 kg product. Similar factors were considered while calculating cost of formulation of restructured meat products by Malav (2013).

Table 3 : Sensory evaluation of patties prepared by incorporation of fibrin

Sensory evaluation Parameters	% of Lean replaced with fibrin			
	0	3	6	9
Appearance	6.73 ± 0.20	6.77 ±0.17	6.96 ± 0.16	6.76 ± 0.18
Flavour	6.97 ± 0.17^{A}	6.47 ± 0.15^{B}	6.37 ± 0.17^{BC}	6.33 ± 0.14 ^{BC}
Juiciness	6.61 ± 0.19	6.09 ± 0.20	6.46 ± 0.18	6.24 ± 0.19
Texture	7.05 ± 0.18	6.89 ± 0.19	6.73 ± 0.29	6.84 ± 0.18
Mouth coating	7.17 ± 0.16	7.16 ± 0.16	7.12 ± 0.19	6.97 ± 0.21
Overall palatability	6.98 ± 0.15	6.71 ± 0.11	6.59 ± 0.18	6.49 ± 0.13

Values are Mean ± SE

Means with different superscripts (row – wise) differ significantly (P< 0.05)

Table 4 : Comparative cost for formulation of 50 kg control and Patties prepared by 9% lean replacement with fibrin

Ingredients	Rate Rs/kg	Control			Patties prepared by 9% lean replacement with fibrin	
		Percentage used	Qt(Kg)	Rs	Qt(Kg)	Rs
Buffalo meat(Deboned)	200	85	42.50	8500	38.67	7734
Sunflower oil	90	15	7.50	675	7.50	675
Fibrin	-------	--------	-------	--------	3.83	-----
Green Condiments	25	5	2.5	62.5	2.5	62.50
Spice mix	250	2	1	250	1	250
Sugar	30	1	0.5	15	0.5	15
Salt	20	2	1	20	1	20
Phosphate	100	0.5	0.25	25	0.25	25
Sodium nitrite	600	0.02	0.01	6	0.01	6
Transportation cost				100		100
Total Cost (Rs.)				9654		8888

Table 5 : Overhead production cost of approximately 50 kg Patties prepared by 9% lean replacement with fibrin

Overhead production sections	Expenditure Details in Rs.
Labour charges	Skilled staff= 200x1= 200/day Unskilled staff= 140x2= 280/day Total= 480/day
Electric charges	30KWhx Rs.3.5/KWh= 105/day
Equipment depreciation	@ 10% per annum=11000/annum= 30.14/day
Cost of packaging material	(8"x6" LDPE pouches)= 200x0.60= 120
Water charges (200 litre)	200x0.06= 12
Maintenance cost	100 / day
Rent of building	200 / day
TOTAL OVERHEAD COST	1047.14= 1048

Production cost of 50 kg Patties prepared by 9% lean replacement with fibrin are presented in Table 6. The product yield was around 95% for control and 98% for treatment products. However, a safety margin of 1 to 2 % should be considered to compensate the losses that might occur during various steps of processing, packaging and marketing hence considering a final yield at 93% for control and 96% for treatment products. Therefore the cost of 1kg product comes to Rs. 208/- for control and Rs 188.20/- for treatment products. This study indicated that patties prepared by 9% lean replacement with fibrin resulted reduction in cost of production by Rs. 20 / kg of the product prepared when compared to control without compromising sensory qualities except flavour.

Table 6 : Production cost of 50 kg Patties prepared by 9% lean replacement with fibrin

Parameter	Control	Patties prepared by 9% lean replacement with fibrin
Cost of formulation in Rs.	9654	8888
Overhead production cost in Rs.	1048	1048
Total expenditure in Rs.	10702	9936
Product yield (kg)	51.15	52.80
Actual cost per kg product in Rs.	208	188.20

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Evolutionary Relationship of Porcine Beta-Defensin-1 Gene with Beta-Defensins from Different Species*

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ABSTRACT

Porcine beta defensin-1 (PBD-1) gene encodes an antimicrobial peptide that is active against several bacteria and fungi. They arecationshaving six highly conserved cysteine residues.Present study was undertaken todetermine the evolutionary relationship between coding sequence of PBD-1andbeta-defensins from different species. Total RNA was extracted from the tongue of Ankamali pigs and cDNA were synthesized by Reverse Transcription –Polymerase Chain Reaction (RT-PCR). The PBD-1 gene was amplified from the cDNA. Nucleotide sequencing revealed that the product was exactly 243 bp long encompassing the 192 bp long complete coding sequence (CDS). The nucleotide sequences were subjected to multiple sequence alignment using "MegAlign". The PBD-1 sequence displayed relatively higher per cent identity with cattle tracheal antimicrobial peptide (TAP) and goat β-defensin-2 (74.7). The sequence identities with cattle bovine neutrophil β-defensin-4 (BNBD-4), enteric β-defensin (EBD), buffalo BNBD4, EBD, TAP and sheep β-defensin-2 (SBD-2)were also higher at 70.2, 71.6, 72.3, 73.7, 73.2 and 71.1 per cent, respectively in comparison toβ-defensin-2 of human (61.5), chimpanzee (60.5), monkey(62.1), rat BD-2 (61.3) and gallopavin-2 (GAL-2) of chicken(47.8). Phylogenetic tree constructed from the derived PBD-1 nucleotide sequences illustrated two primary branches from the root for birds and mammals. Among mammals, β-defensin-2 genes of primates formed a separate cluster. Genes of cattle and buffalo were clustered together. Sequences from sheep and goat shared a common node. PBD-1 gene formed a separate branch away from other artiodactylesequences indicating its evolutionary difference from beta-defensins of other species in terms of structure, organisation and composition.

Keywords: Evolution; Beta-defensins; Sequence Analysis; Phylogenetic Analysis.

Recent advancements in molecular genetics have led to a revolution making it possible to study evolutionary relationship at molecular level using DNAor protein sequences. Originally, the purpose of mostmolecularphylogenetic trees was to estimate the relationshipsamong the species represented by those sequences, buttoday the purposes have expanded to include understandingthe relationships among the sequences themselves withoutregard to the host species(Hall,2013).

Antimicrobial peptides are polypeptides made up of 100 or fewer amino acid residues and act against a wide range of microbes (Ganz, 2003). Based on the net charge present, AMPs are broadly classified into anionic and cationic peptides (Hancock, 1997).Among cationic AMPs, defensins are a subclass with broad spectrum antimicrobial activity against various bacteria, fungi and viruses. Based on intramolecular disulphide bonds between highly conserved cysteine residues, three families of defensins are defined *viz*. α-, β- and θ-defensins (Lai & Gallo, 2009). The porcine β-defensin-1 (PBD-1) peptide found at higher concentrations in the oral cavity of pigs with direct action against several bacteria and fungiplaysa vital role in the innate immunity of pigs (Zhang *et al*., 1999; Shi *et al*., 1999; Jiang *et al.,* 2006; Li *et al*., 2013).

Coding sequences of beta-defensin genes from important livestock species have been sequenced and characterized (Bagnicka *et al.*, 2010). However,studies on evolutionary relationship amongbeta-defensin sequences are scanty. Therefore, the present study was undertaken to determine the evolutionary relationship between coding sequence of PBD-1, obtained in our study andbeta-defensinsequences from different species available in the National Centre for Biotechnology Information (NCBI)database.

MATERIALS AND METHODS

Collection of tissue samples andextraction of RNA: Tongue tissue samples were collected from adult Ankamali pigs reared at Centre for Pig Production and Research, Mannuthy, Thrissur,

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Kerala, Indiaimmediately after the slaughter of the animal. About 100 mg of tissue was used for isolation of total RNA using 1 ml TRI reagent (Sigma Aldrich) as per manufacturer's instructions. The RNA was quantified in NanoDrop™ 2000c spectrophotometer (ThermoScientific) and its integrity was assessed by 1.2% denaturing agarose gel electrophoresis.

DNase treatment and cDNA synthesis: Genomic DNA contamination, if any, was removed by Deoxyribonuclease (DNase) treatment of RNA samples with a commercially available DNase (DNase I, Amplification Grade, Sigma Aldrich). Complementary DNA (cDNA) was synthesised from 1 µg of DNase treated RNA using Revert Aid first strand cDNA synthesis kit (Thermo Scientific, K1622). Random hexamer primers were used for amplification. Resultant cDNA was stored at -20 °C until further use.

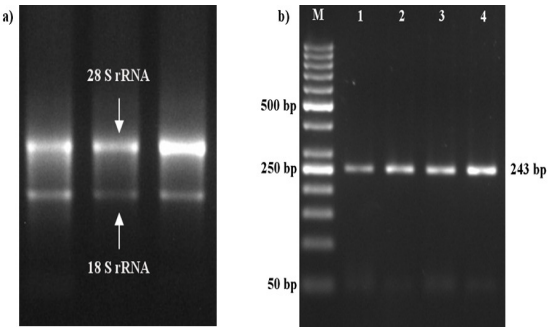
Amplification of PBD-1 gene: The complete coding sequence (CDS) of PBD-1 gene was amplified using cDNA as template. Published primers (Qi *et al*., 2009)used in the study were: Forward 5'-ACCAGCATGAGACTCCACC-3' and reverse 5'-GCTTCTGAGCCATATCTGTG-3'. PCR was carried out in a thermal cycler (Bio-Rad, USA). Each 50 µl volume reaction contained 1X Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTP mixture, 10 pM/µl each of forward and reverse primers, 1.5 units of Taq polymerase and nuclease free water to make up the volume. The PCR conditions followed were initial denaturation for 3 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 20 sec at 58.8°C, 30 sec at 72°C and final extension at 72°C for 8 min. The amplified PCR products were resolved in 2% (w/v) agarose gel in 1X TBE.

Nucleotide sequencing and analysis: PCR products obtained were sequenced using respective forward and reverse primers in an automated sequencer (ABI prism) using Sanger's dideoxy chain termination method (Sanger *et al*., 1977) at SciGenom Labs Pvt. Ltd., Cochin. The sequences obtained were analysed using the 'MegAlign' tool of Lasergene Software (DNASTAR, USA) to generate sequence alignment reports, sequence distances and phylogenetic trees.

RESULTS AND DISCUSSION

Concentration, Purity and Quality of RNA: The concentration of total RNA extracted from tongue epithelium of Ankamali pigs was 506.38 ng/µl. The Optical Density (OD) ratio (260:280) of extracted RNA was 2.06. The integrity of extracted RNA was verified by agarose gel electrophoresis(Figure Ia). The 28S and 18S rRNA bands were clear and intensity of the 28S rRNA band was almost twice that of the 18S rRNA indicating good quality RNA. The mRNA was observed as smear spanning between 28S and 18S rRNA. The absence of band near the well indicated the purity of RNA sample from genomic DNA contamination. The PCR product at optimum annealing temperature (58.8°C) yielded a specific product of 243 bp upon 2 % agarose gel electrophoresis (Figure Ib).

Figure I. **a)** Total RNA isolated from different tissues of Ankamali and LWY pigs.
b) PCR amplification of 243 bp fragment of PBD-1 gene from cDNA. Lane 1-4: 243 bp PCR product of PBD-1 gene. Lane M: 50 bp DNA marker.



Sequence analysis: Nucleotide and predicted amino acid sequences obtained in the present study were aligned with that of other beta-defensingenes available inthe NCBI GenBank. The genes included in the analysis werebovine neutrophil β-defensin-4 (BNBD-4), enteric β-defensin (EBD) and tracheal antimicrobial peptide (TAP) genes of cattle, BNBD-4, EBD and TAP genes of buffalo, goatβ-defensin-2 (GBD-2), sheepβ-defensin-2 (SBD-2), gallopavin-2 (GAL-2) of chicken, β-defensin-2 (DEFB-2) genes from human, chimpanzee, rhesus monkey and rat. Multiple sequence alignment of nucleotide (Figure II) and peptide (Figure III) sequencewas performed using "MegAlign" programme ofLasergene software (DNASTAR Inc., USA).

Figure II. Nucleotide sequence alignment report of PBD-1 sequence with that of other beta-defensin genes. Shaded portion indicates residues that differ from the Consensus.

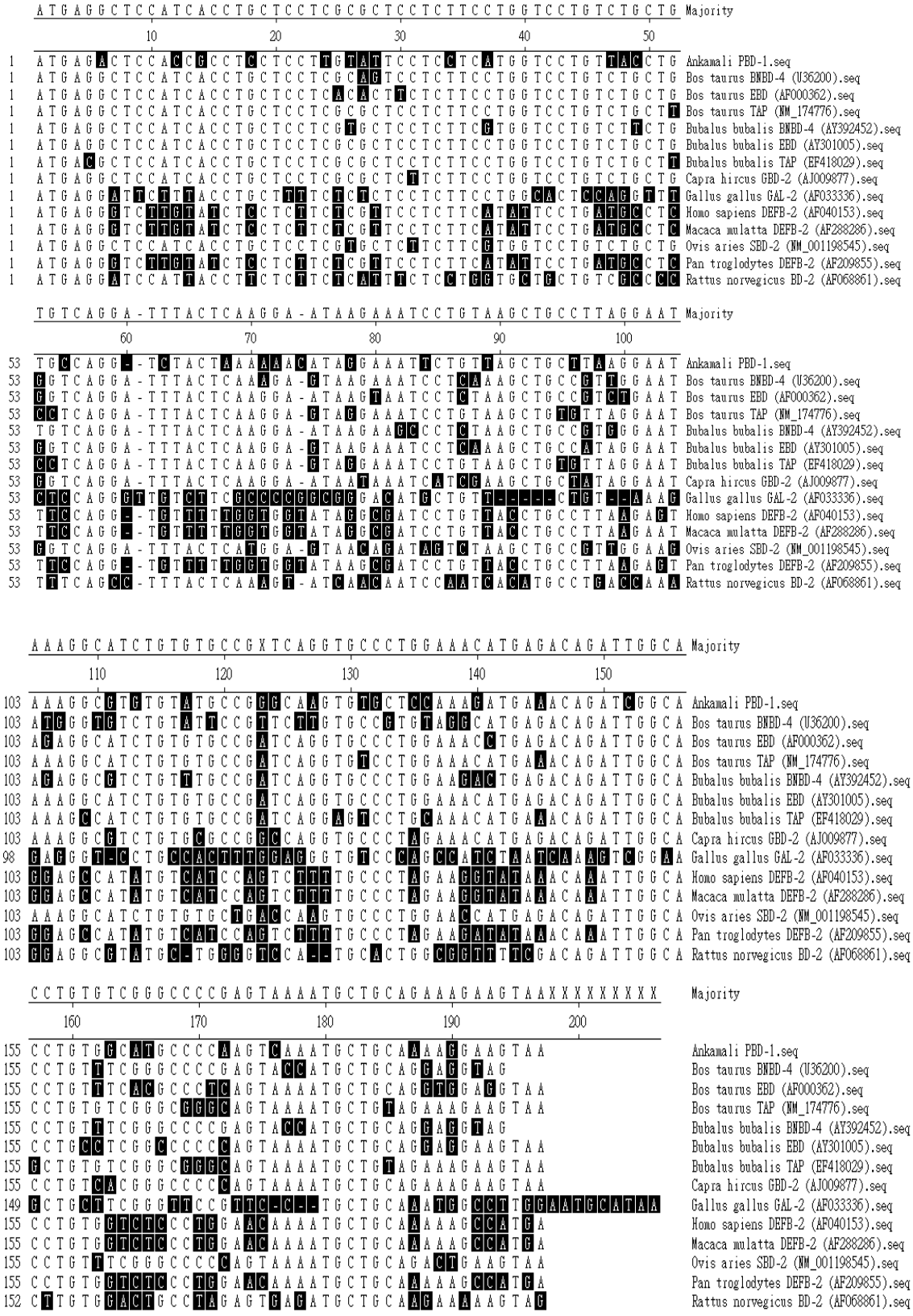
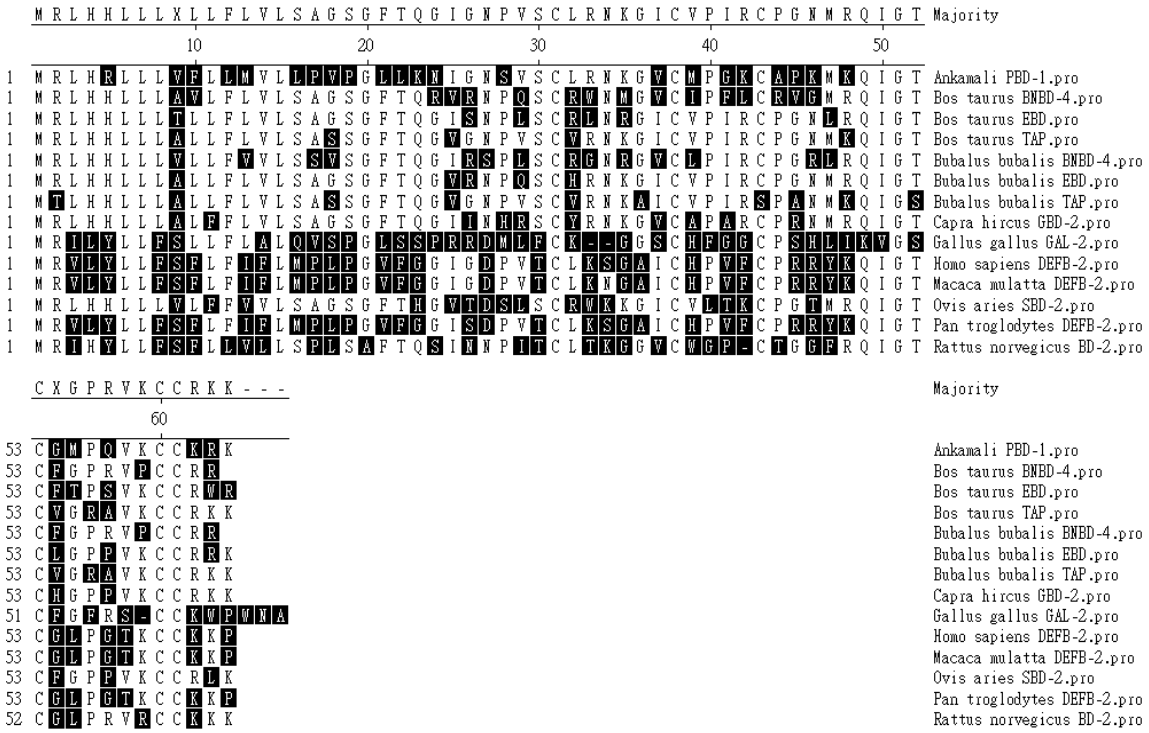


Figure III. Peptide sequence alignment report of PBD-1 sequence with that of other beta-defensin genes.Shaded portion indicates residues that differ from the Consensus.



Nucleotide sequence homology: The PBD-1 sequence from Ankamali displayed relatively higher per cent identity with cattle TAP and goat GBD-2, both at74.7 per cent. The sequence identities with cattle BNBD-4, EBD, buffalo BNBD4, EBD, TAP and SBD-2were also higher at 70.2, 71.6, 72.3, 73.7, 73.2 and 71.1 per cent, respectively in comparison to human DEFB-2 (61.5), chimpanzee DEFB-2 (60.5), monkeyDEFB-2 (62.1), rat BD-2 (61.3) and chicken GAL-2(47.8). Consequently,divergence analysis showed highest divergence of PBD-1sequence from chicken GAL-2 and the least divergence from cattle TAP and goat GBD-2.

Peptide sequence homology: In case of predicted amino acid sequences, cattle TAP showed 54.7 per cent identity with the predicted amino acid sequence of PBD-1. The goat GBD-2 and buffalo EBD had 53.1 per cent similarity. Buffalo BNBD-4 and cattle BNBD-4 had 50.8 and 49.2 per cent identity, respectively. Buffalo TAP, monkey BD-2, sheep SBD-2 showed 48.4 per cent whereas rat BD-2 had 47.6 per cent similarity. Cattle EBD and human DEFB-2 had 46.9 per cent and chimpanzeeDEFB-2 showed 45.3 per cent similarity.Chicken GAL-2 had the least similarity at 31.1 per cent.

Phylogenetic analysis: The phylogenetic trees were constructed fromnucleotide(Figures IV) and predicted amino acid(Figure V) sequences of related genes to estimate the evolutionary relationship using "MegAlign" programme of Lasergene software. Bootstrap method was applied to assess the reliability of the trees. Hall (2013) noticed that the bootstrap test estimates the reliability of each node instead of reliability of the whole tree. It was also suggested that the nodes with < 70 per cent reliability may not be informative.

Similarity was present between the trees constructed from nucleotide and predicted amino acid sequences. Sequence of chicken GAL-2 gene was used as an outgroupin order to obtain a rooted tree. Outgroup is a sequence that is more distantly related to the remaining sequences. Primary branching from the root was between GAL-2 (chicken) and all other mammalian sequences. Among mammals, DEFB-2 genes of the three primates (human, chimpanzee, monkey) formed a

Figure IV. Phylogenetic tree based on the deduced peptide sequence of PBD-1and other beta-defensin genes.NCBI accession numbers are shown in parentheses.

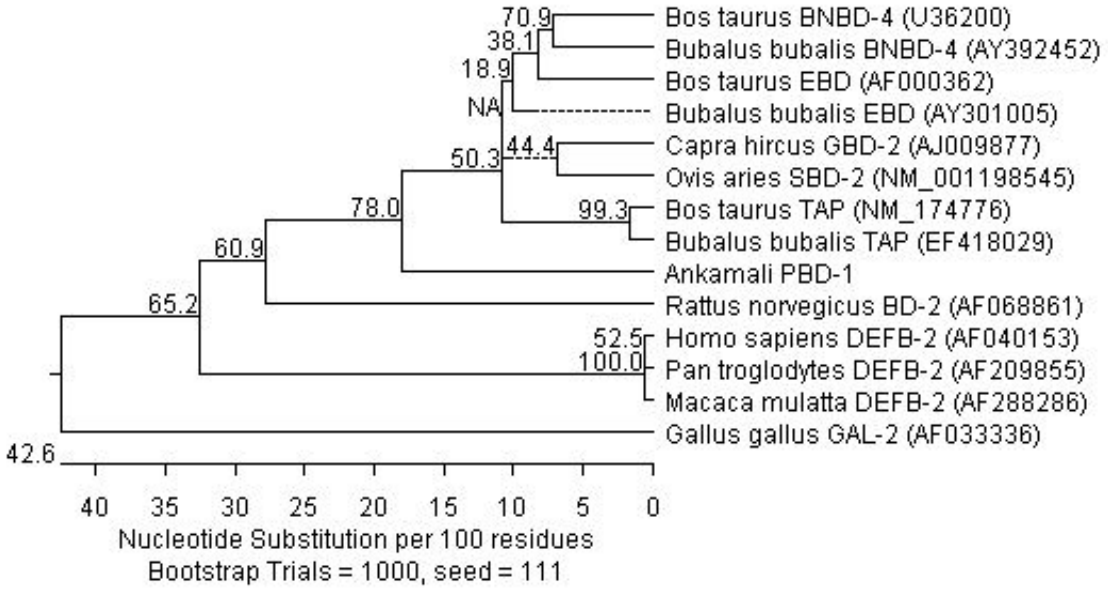
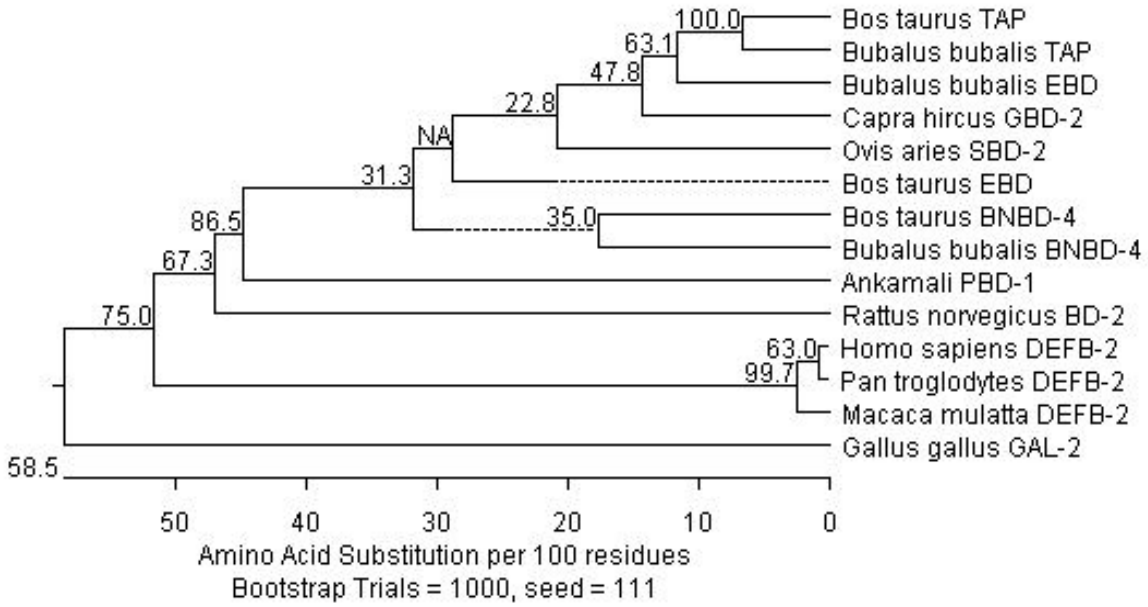


Figure V. Phylogenetic tree based on the nucleotide sequence of PBD-1 and other beta-defensingenes. NCBI accession numbers are shown in parentheses.



separate cluster and rat BD-2 formed a unique branch. Related genes of ruminants like TAP and BNBD-4 of cattle and buffalo were clustered together. Sheep and goat BD-2 genes shared a common node. PBD-1 gene formed a separate branch away from other artiodactyle genes.

It may be observed from above results that the evolutionary classification from molecular data does not show much deviation from the classical systems. It may also be inferred that among the different species compared, the PBD-1 gene was quite different from beta-defensins of other species in terms of structure, organisation and composition.

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Effect of Supplementation of Exogenous Fibrolytic Enzymes to Crop Residues on *In Vitro* Gas Production Kinetics, Rumen substrate degradability and Microbial Biomass Synthesis*

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ABSTRACT

Five roughages viz. maize stover, sorghum stover, paddy straw, finger millet straw and maize husk were subjected for *in vitro* study to evaluate different levels of a fibrolytic enzyme (0, 8, 10, 12, 15, 20 and 25g/12kg DM) on fermentation kinetics, substrate degradation, microbial biomass synthesis and volatile fatty acid production. Among the roughages, paddy straw had the highest (P<0.01) $t_{1/2}$ (29.73 h) value, lowest (P<0.01) rate (k) of gas production (0.0234 h^{-1}) and highest gas production at t1/2 (148.0 ml/g DM) whereas maize husk had significantly higher (P<0.01) truly digestible organic matter (TDOM) at t1/2 (440.8 mg/g DM). Significantly higher (P<0.01) partitioning factor (PF), microbial biomass production (MBP) and efficiency of microbial biomass synthesis (EMBS) but significantly lower ammonia nitrogen (NH3-N) were observed in maize husk. The k values were significantly improved (P<0.01) with addition of enzyme but not the gas production at t1/2. The TDOM and neutral detergent fiber digestibility (NDFD) at t1/2 were significantly (P<0.01) higher in 10, 12 and 15g enzyme supplemented group. In the same group there were significant (P<0.01) improvements in PF, MBP and EMBS to that of control group. The results indicated that maize husk was more efficient in MBP at 10 and 12g enzyme levels which cab improve rate of fermentation and microbial biomass production.

Key words: Fermentation kinetics, Fibrolytic enzyme, Microbial biomass synthesis. Roughage.

Crop residues constitute the major ingredient of ruminants' diets in developing countries. These are low in energy due to high fibre and lignin content which makes them less palatable and less digestible. The forage degradability can be improved by various physical, chemical and biological treatments. The use of exogenous fibrolytic enzymes (EFE) to enhance the production system. The effectiveness of EFE is influenced by many factors such as dose, activity and composition (Eun and Beauchemin, 2007), the prevailing pH and temperature (Arriola et al., 2011), the animal performance level (Schingoethe et al., 1999), the experimental design (Adesogan *et al.,* 2014), and the fraction and proportion of the diet to which the enzyme is applied (Krueger et al., 2008; Dean et al., 2013), type and dose of enzyme and type of diet fed to animals. Therefore enzyme should be tested on different forages at different doses to identify the optimum enzyme dose for particular forage. *In vitro* gas production technique can be used to evaluate the efficacy of EFE on fiber digestibility as it is less expensive and less laborious, can be used for evaluation of more number of feed samples when compared to *in vivo* studies. Most of studies on EFEs have been conducted with good-quality forages, but studies involving fibrous crop residues are limited. Hence, the present study was carried out to determine the effects of different levels of a commercial enzyme mixture on *in vitro* rumen fermentation characteristics of five crop residues viz. maize stover, sorghum stover, paddy straw, finger millet straw and maize husk.

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MATERIAL AND METHODS

Forage samples and enzyme mixture: Samples of maize stover, sorghum stover, paddy straw, finger millet straw (FMS) and maize husk were selected as substrates to be tested. The forages were dried at 65°C, ground in a mill to pass through a one mm sieve and stored in plastic bags for subsequent determination of chemical components and *in vitro* studies. The fibrolytic enzyme (Fibromase™) used in the study was procured from Alltech Inc., Nicholasville, KY, USA and contained fermentation extracts and solubles from *Aspergillus niger* and *Trichoderma longibrachiatum* and xylanolytic activity of 100 IU of xylanase/g of product, as indicated by the manufacturer.

Level of inclusion of enzyme for *in vitro* studies:The amounts of the fibrolytic enzyme for the *in vitro* study were based on daily amounts that would be provided to a dairy cow consuming 12 kg/d of DM and emulated 8, 10, 12, 15, 20 and 25 g enzyme/day/cow (Elwakeel *et al*., 2007). The enzyme preparation (20mg Fibromase) was suspended in 50 ml, 1mM sodium phosphate buffer (pH 6.5). The amounts used for the *in vitro* fermentations were scaled based on the substrate provided to each *in vitro* syringe (0.2 g) relative to daily feed intake by a dairy cow (12 kg) and the amount of enzyme added to emulate 8 g/d/cow was 332µl (0.133 mg/syringe).

***In vitro* gas production kinetics and ruminal fermentation:** A crossbred (Holstein Friesian x Bos indicus) lactating dairy cow (B.wt.: 400 kg, milk yield:9 kg/day) fitted with a flexible rumen cannula of large diameter (Bar Diamond, Inc. USA), served as the donor of the rumen inoculum. Cow was fed with basal diet consisting of finger millet straw at the rate of 6.5 to 7 kg per day and CFM (Maize- 50%, Wheat bran - 45%, mineral mixture - 2%, salt -1%, urea – 2%) of 4.0 kg/day in two equal portions at the time of milking at 6.00 A.M and 1.30PM. The rumen fluid was collected between 9.00 to 9.30 AM before offering finger millet straw. Air equilibrated feed samples (200 ± 10 mg) of maize stover, sorghum stover, paddy straw, finger millet straw and maize husk were incubated in 100 ml calibrated glass syringes in triplicate according to Menke and Steingass, (1988) with 30 ml mixed rumen suspension with three blank incubations and standards. Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60, 72, 84 and 96 h of incubation. The rate and extent of gas production were calculated by non-linear regression using the model $Y = D\ (1 - e^{-k*t})$ where, Y is gas volume (ml) at time t, D is potential gas production (ml) and k is rate (per hour) at which gas is produced (Krishnamoorthy *et al*., 1991). The time at half asymptotic gas production (t1/2) was calculated as ln2/k. Gas production at 24 h, corrected for the blank and standards was used for determination of ME using the following equations, Roughages $ME = 2.2 + 0.1357\ GP + 0.0057\ CP + 0.0002859\ EE^2$ Where, ME= Metabolizable energy, MJ/kg DM; GP= gas, ml/200 mg DM; CP, EE and TA are crude protein, ether extract and total ash, respectively, in g/kg DM.

The microbial biomass synthesis of maize stover, sorghum stover, paddy straw, finger millet straw and maize husk was obtained by determining the ratio of truly digested organic matter (TDOM) and gas production at 24 h as well as at the time at which half maximum gas production was achieved (t1/2) as described by BlÜmmel et al., (1997a). Two sets of incubations were run separately for all the feed stuffs to determine PF values at $t_{1/2}$ and 24 h of incubation. In each incubation, three replicates of 500 ± 10 mg of air equilibrated feed samples were weighed into 100 ml calibrated syringes and incubated with 40 ml of mixed rumen suspension at 39°C with parallel incubations of blanks (Makkar *et al*., 1995). Incubations were terminated by recording gas production at $t_{1/2}$ and 24 h for the respective feed samples by immersing in ice water bath to prevent further microbial activity. The contents of the syringes were quantitatively transferred through the nozzle of the syringe into 600 ml spout less Berzelius beakers.

The syringes were rinsed with 100 ml neutral detergent solution by dispensing 25 ml NDS into the syringe each time. The incubation residue was refluxed for 1 h followed by filtration on preweighed Gooch crucibles to recover true

undigested matter (Goering and Van Soest, 1970). Crucibles with undigested residue were dried at 100°C overnight and weighed to determine true undigested residue. Residue was ashed at 500°C for 3 hours to determine the true undigested organic matter. The TDOM was calculated as the difference between OM incubated and the undigested OM of feed origin recovered in the residue. The PF was calculated as the ratio of mg of TDOM per ml gas produced at $t_{1/2}$ and 24 h. *In vitro* apparent DM degradability, ammonia-N (NH3-N) and total volatile fatty acids (TVFA) were determined as per the procedures described by Blummel *et al*., (2003). After terminating the incubation at 24 h, the entire syringe content was transferred into centrifuge tubes and centrifuged at 20000 x g for 30 min at 4°C. The supernatant fraction was carefully removed with a Pasteur pipette and stored for NH3-N analysis by Kjeldhal method using direct steam distillation. The syringes were washed three times with 5-6 ml of distilled water to transfer the contents quantitatively into the centrifuge tubes. After complete rinsing of the syringes, centrifugation was once again repeated at 20,000 x g for 30 min at 4°C and the supernatant was siphoned off and discarded. The residues were dried for 48 h in the forced air oven at 60°C and weighed. *In vitro* ADDM was calculated as 24h ADDM= mg DM of substrate incubated – (mg DM residue – mg DM blank). Microbial biomass production was calculated as [$t_{1/2}$TDOM-(Gas volume at t1/2 x stoichiometric factor)]. The stoichiometric factor (SF) of 2.20 for roughages was used (Blümmel *et al.,* 1997b).

The data was subjected to two way analysis of variance. Individual differences between means were tested using Bonferroni't' test when treatment effect was significant. All the statistical procedures were carried out as per the methods described by Snedecor and Cochran (1994) by programming and processing in computer. The computer program Graph Pad Prism (2004, graph pad Inc, USA) was used to calculate the rate and extent of gas production in the non-linear equation.

RESULTS AND DISCUSSION

Chemical composition: The CP content of the roughages ranged from 1.94% in maize husk to 3.45% in sorghum stover and ME values ranged from 5.62 MJ/kg DM in sorghum stover to 9.08 MJ/kg DM in maize husk (Table 1). The chemical composition all the roughages were similar to the values reported in earlier studies (Kiran and Krishnamoorthy, 2007 and Tang *et al*., 2008).

Kinetics parameter: There was significant difference ($P<0.01$) between the roughage sources in $t_{1/2}$ values where highest $t_{1/2}$ value was noticed for paddy straw and lowest for FMS. The higher

Table 1. Chemical composition (% on DMB), *in vitro* gas production at 24 h (GP-24, ml/g DM) and predicted metabolizable energy (ME, MJ/kg DM) of some roughages used in the study.

Parameter	Maize stover	Sorghum stover	Paddy straw	FMS	Maize husk
DM	90.05	89.89	91.22	90.0	90.05
OM	91.60	91.24	91.80	91.8	97.48
CP	3.08	3.45	2.94	3.44	1.94
EE	0.95	1.11	0.90	1.12	0.68
TA	8.45	8.19	8.21	8.20	2.51
NDF	72.74	68.04	74.34	65.67	76.33
ADF	48.27	40.42	51.85	36.69	43.45
ADL	6.60	6.78	7.18	5.20	2.45
GP-24	181	189	119	182.0	249
ME	7.32	7.57	5.62	7.37	9.08

Table 2. Effects of fibrolytic enzymes on *in vitro* fermentation kinetics and gas production at t1/2 of five roughages

	Enzyme level[1]									P	
Particulars	**Control**	**8**	**10**	**12**	**15**	**20**	**25**	**Mean**	**SEM**	**Treatment**	**Straw**
t1/2 (h)											
Maize stover	18.25	16.36	17.13	16.38	15.91	15.24	15.72	**16.43^{a}**	0.28	0.01	0.001
Sorghum stover	17.91	18.02	17.72	18.49	18.37	17.79	17.42	**17.96^{b}**			
Paddy straw	31.86	31.81	28.46	29.45	29.67	28.10	28.78	**29.73^{c}**			
FMS	13.90	13.59	12.97	13.62	13.61	13.55	14.39	**13.66^{d}**			
Maize husk	15.86	14.72	13.98	14.28	14.54	14.94	14.60	**14.70^{d}**			
Mean	**19.56^{C}**	**18.90^{BC}**	**18.05^{AB}**	**18.44^{AB}**	**18.42^{AB}**	**17.92^{A}**	**18.18^{AB}**				
k (/h)											
Maize stover	0.0380	0.0424	0.0405	0.0423	0.0436	0.0455	0.0441	**0.0423^{a}**	0.00055	0.01	0.001
Sorghum stover	0.0387	0.0385	0.0391	0.0375	0.0377	0.0390	0.0398	**0.0386^{b}**			
Paddy straw	0.0218	0.0218	0.0244	0.0235	0.0234	0.0247	0.0241	**0.0234^{c}**			
FMS	0.0498	0.0510	0.0530	0.0509	0.0509	0.0511	0.0480	**0.0507^{d}**			
Maize husk	0.0437	0.0471	0.0496	0.0485	0.0477	0.0464	0.0475	**0.0472^{e}**			
Mean	**0.0384^{A}**	**0.0401^{B}**	**0.0413^{B}**	**0.0406^{B}**	**0.0406^{B}**	**0.0413^{B}**	**0.0407^{B}**				
Gas at t1/2 (ml)											
Maize stover	127.1	125.0	124.7	123.3	120.9	117.3	120.0	**122.6^{a}**	0.72	0.06	0.001
Sorghum stover	142.5	142.6	144.1	143.3	143.5	143.6	143.6	**143.3^{b}**			
Paddy straw	149.1	148.8	147.0	147.6	148.1	147.9	147.5	**148.0^{c}**			
FMS	126.6	130.8	131.2	131.5	132.3	132.3	129.2	**130.6^{d}**			
Maize husk	141.9	142.5	143.6	141.5	142.9	144.3	146.2	**143.3^{b}**			
Mean	**137.4**	**137.9**	**138.1**	**137.4**	**137.5**	**137.1**	**137.3**				
	Enzyme level[1]									**P**	
Particulars	**Control**	**8**	**10**	**12**	**15**	**20**	**25**	**Mean**	**SEM**	**Treatment**	**Straw**
t1/2 (h)											
Maize stover	18.25	16.36	17.13	16.38	15.91	15.24	15.72	**16.43^{a}**	0.28	0.01	0.001
Sorghum stover	17.91	18.02	17.72	18.49	18.37	17.79	17.42	**17.96^{b}**			
Paddy straw	31.86	31.81	28.46	29.45	29.67	28.10	28.78	**29.73^{c}**			
FMS	13.90	13.59	12.97	13.62	13.61	13.55	14.39	**13.66^{d}**			
Maize husk	15.86	14.72	13.98	14.28	14.54	14.94	14.60	**14.70^{d}**			
Mean	**19.56^{C}**	**18.90^{BC}**	**18.05^{AB}**	**18.44^{AB}**	**18.42^{AB}**	**17.92^{A}**	**18.18^{AB}**				
k (/h)											
Maize stover	0.0380	0.0424	0.0405	0.0423	0.0436	0.0455	0.0441	**0.0423^{a}**	0.00055	0.01	0.001
Sorghum stover	0.0387	0.0385	0.0391	0.0375	0.0377	0.0390	0.0398	**0.0386^{b}**			
Paddy straw	0.0218	0.0218	0.0244	0.0235	0.0234	0.0247	0.0241	**0.0234^{c}**			
FMS	0.0498	0.0510	0.0530	0.0509	0.0509	0.0511	0.0480	**0.0507^{d}**			
Maize husk	0.0437	0.0471	0.0496	0.0485	0.0477	0.0464	0.0475	**0.0472^{e}**			
Mean	**0.0384^{A}**	**0.0401^{B}**	**0.0413^{B}**	**0.0406^{B}**	**0.0406^{B}**	**0.0413^{B}**	**0.0407^{B}**				
Gas at t1/2 (ml)											
Maize stover	127.1	125.0	124.7	123.3	120.9	117.3	120.0	**122.6^{a}**	0.72	0.06	0.001
Sorghum stover	142.5	142.6	144.1	143.3	143.5	143.6	143.6	**143.3^{b}**			
Paddy straw	149.1	148.8	147.0	147.6	148.1	147.9	147.5	**148.0^{c}**			
FMS	126.6	130.8	131.2	131.5	132.3	132.3	129.2	**130.6^{d}**			
Maize husk	141.9	142.5	143.6	141.5	142.9	144.3	146.2	**143.3^{b}**			
Mean	**137.4**	**137.9**	**138.1**	**137.4**	**137.5**	**137.1**	**137.3**				

^{abc} Means between rows not bearing a common superscript letters differ significantly (P<0.01).
^{ABC} Means between columns not bearing a common superscript letters differ significantly (P<0.05).
[1]Levels mimicking the addition of 8, 10, 12, 15, 20 and 25 g/d of enzyme to the diets of lactating cows consuming 12 kg/d of DM

$t_{1/2}$ in paddy straw was due to higher lignification when compared to other roughage sources (Table 2.). The $t_{1/2}$ values were significantly (P<0.01) reduced due to addition of enzyme to the roughages when compared to control group (Table 2.). Similarly, $t_{1/2}$ values have greater influence on the 'k' values wherein paddy straw had significantly (P≤0.001) lowest 'k' value ($0.0234h^{-1}$). However, addition of enzyme had improved 'k' values compared to control but there was no significant

Table 3. Effect of firbolytic enzyme on *in vitro* substrate degradability of five roughages

	Enzyme level[1]									P	
	Control	**8**	**10**	**12**	**15**	**20**	**25**	**Mean**	**SEM**	**Treatment**	**Straw**
TDOM at t1/2(mg/g DM)											
Maize stover	417.0	408.7	411.7	405.4	396.5	388.4	394.9	**403.2^{a}**	2.23	0.05	0.001
Sorghum stover	463.2	470.4	472.6	475.6	469.2	465.2	468.2	**469.2^{b}**			
Paddy straw	453.2	455.4	458.7	456.2	460.7	458.6	452.7	**456.5^{c}**			
FMS	402.7	414.7	418.7	415.6	424.7	420.7	409.6	**415.2^{a}**			
Maize husk	475.2	480.3	482.7	478.3	480.2	483.7	485.2	**480.8^{b}**			
Mean	**442.2^{A}**	**445.8^{AB}**	**448.9^{B}**	**446.2^{AB}**	**446.2^{AB}**	**443.3^{A}**	**442.1^{A}**				
NDFD t1/2 (%)											
Maize stover	36.5	37.4	36.9	37.5	38.4	36.1	36.0	**37.0^{bc}**	0.64	0.01	0.001
Sorghum stover	33.3	34.3	36.8	37.0	35.6	36.7	34.7	**35.5^{ab}**			
Paddy straw	41.4	43.5	42.1	43.1	40.8	40.5	40.2	**41.7^{d}**			
FMS	30.7	32.1	33.8	33.6	30.6	30.6	30.6	**31.7^{e}**			
Maize husk	38.4	39.9	39.5	39.8	38.6	39.8	39.1	**39.3^{cd}**			
Mean	**36.1^{AB}**	**37.4^{ABC}**	**37.8^{BC}**	**38.2^{C}**	**36.8^{BC}**	**36.8^{A}**	**36.1^{A}**				

	Enzyme level[1]									P	
	Control	**8**	**10**	**12**	**15**	**20**	**25**	**Mean**	**SEM**	**Treatment**	**Straw**
TDOM at t1/2 (mg/g DM)											
Maize stover	417.0	408.7	411.7	405.4	396.5	388.4	394.9	**403.2^{a}**	2.23	0.05	0.001
Sorghum stover	463.2	470.4	472.6	475.6	469.2	465.2	468.2	**469.2^{b}**			
Paddy straw	453.2	455.4	458.7	456.2	460.7	458.6	452.7	**456.5^{c}**			
FMS	402.7	414.7	418.7	415.6	424.7	420.7	409.6	**415.2^{a}**			
Maize husk	475.2	480.3	482.7	478.3	480.2	483.7	485.2	**480.8^{b}**			
Mean	**442.2^{A}**	**445.8^{AB}**	**448.9^{B}**	**446.2^{AB}**	**446.2^{AB}**	**443.3^{A}**	**442.1^{A}**				
NDFD t1/2 (%)											
Maize stover	36.5	37.4	36.9	37.5	38.4	36.1	36.0	**37.0^{bc}**	0.64	0.01	0.001
Sorghum stover	33.3	34.3	36.8	37.0	35.6	36.7	34.7	**35.5^{ab}**			
Paddy straw	41.4	43.5	42.1	43.1	40.8	40.5	40.2	**41.7^{d}**			
FMS	30.7	32.1	33.8	33.6	30.6	30.6	30.6	**31.7^{e}**			
Maize husk	38.4	39.9	39.5	39.8	38.6	39.8	39.1	**39.3^{cd}**			
Mean	**36.1^{AB}**	**37.4^{ABC}**	**37.8^{BC}**	**38.2^{C}**	**36.8^{BC}**	**36.8^{A}**	**36.1^{A}**				

[abc] Means between rows not bearing a common superscript letters differ significantly (P<0.01).
[ABC] Means between columns not bearing a common superscript letters differ significantly (P<0.05).
[1]Levels mimicking the addition of 8, 10, 12, 15, 20 and 25 g/d of enzyme to the diets of lactating cows consuming 12 kg/d of DM

Table 4. Effects of fibrolytic enzymes on *in vitro* microbial biomass synthesis and NH3-N level of five roughages

	Enzyme level[1]									P	
Particulars	**Control**	**8**	**10**	**12**	**15**	**20**	**25**	**Mean**	**SEM**	**Treatment**	**Straw**
PF at $t_{1/2}$ (mg/ml)											
Maize stover	3.28	3.27	3.30	3.29	3.28	3.31	3.29	**3.29^{a}**	0.008	0.01	0.01
Sorghum stover	3.25	3.30	3.28	3.32	3.27	3.24	3.26	**3.27^{a}**			
Paddy straw	3.04	3.06	3.12	3.09	3.11	3.10	3.07	**3.08^{b}**			
FMS	3.18	3.17	3.19	3.16	3.21	3.18	3.17	**3.18^{c}**			
Maize husk	3.35	3.37	3.36	3.38	3.36	3.35	3.32	**3.36^{d}**			
Mean	**3.22^{A}**	**3.23^{AB}**	**3.25^{B}**	**3.24^{B}**	**3.24^{B}**	**3.23^{AB}**	**3.22^{A}**				
MBP at $t_{1/2}$ (mg)											
Maize stover	137.3	133.7	137.4	134.1	130.5	130.3	130.8	**133.4^{a}**	1.11	0.01	0.01
Sorghum stover	149.7	156.8	155.6	160.5	153.6	149.3	152.3	**154.0^{c}**			
Paddy straw	125.2	128.0	135.2	131.4	134.8	133.3	128.3	**130.9^{ab}**			
FMS	124.1	126.9	129.9	126.3	133.6	129.6	125.3	**128.0^{b}**			
Maize husk	163.2	166.8	166.6	167.0	165.8	166.3	163.7	**165.6^{d}**			
Mean	**139.9^{A}**	**142.4^{ABC}**	**145.0^{C}**	**143.8^{BC}**	**143.7^{BC}**	**141.8^{AB}**	**140.1^{B}**				
EMBS at $t_{1/2}$ (g/kg)											
Maize stover	329.3	327.2	333.6	330.7	329.2	335.4	331.3	**330.9^{a}**	1.60	0.01	0.001
Sorghum stover	323.1	333.4	329.3	337.4	327.2	321.0	325.4	**328.1^{a}**			
Paddy straw	276.3	281.0	294.9	288.0	292.6	290.6	283.4	**286.7^{b}**			
FMS	308.2	306.0	310.4	303.8	314.7	308.2	306.0	**308.2^{c}**			
Maize husk	343.3	347.2	345.3	349.1	345.3	343.9	337.4	**344.5^{d}**			
Mean	**316.0^{A}**	**319.0^{AB}**	**322.7^{B}**	**321.8^{B}**	**321.8^{B}**	**319.8^{AB}**	**316.7^{A}**				
NH_3-N/dl											
Maize stover	13.2	14.2	14.7	14.2	13.2	14.2	14.7	**14.0^{b}**	0.22	0.06	0.001
Sorghum stover	15.6	16.6	17.1	17.6	15.6	15.6	16.1	**16.3^{a}**			
Paddy straw	15.6	16.1	17.1	16.6	16.1	16.1	17.1	**16.4^{a}**			
FMS	17.1	17.6	17.6	16.6	17.6	16.6	16.6	**17.1^{a}**			
Maize husk	12.2	12.2	12.7	12.7	12.2	14.2	12.7	**12.7^{b}**			
Mean	**14.8^{A}**	**15.3^{ABC}**	**15.8^{C}**	**15.5^{BC}**	**14.9^{AB}**	**15.3^{ABC}**	**15.4^{ABC}**				

^{abc} Means between rows not bearing a common superscript letters differ significantly (P<0.01).
^{ABC} Means betewn columns not bearing a common superscript letters differ significantly (P<0.05).
[1]Levels mimicking the addition of 8, 10, 12, 15, 20 and 25 g/d of enzyme to the diets of lactating cows consuming 12 kg/d of DM

difference among the level of enzymes supplemented. These results were in agreement with Diaz *et al*., (2013), who reported an increase in 'k' and decrease in $t_{1/2}$ when maize stover was supplemented with four level of enzyme. The gas production at $t_{1/2}$ was significantly (P≤0.001) highest in paddy straw when compared to other roughage sources and which was due to higher $t_{1/2}$ value. Tang *et al*., (2008) reported increased 'k' values and 48h gas production when maize stover was supplemented with fibrolytic enzyme at 5.0 or 7.5 g/kg DM when compared to non supplemented group. The increase in total gas production was associated with higher OM and NDF degradability in substrate due to

increase in bacterial number and rate of fermentation. As the dose of the enzyme decreased the $t_{1/2}$ and 'k' values increased and vice-versa which clearly indicated that lower level of enzyme was advantageous than higher level.

Substrate degradation: Maize husk had significantly (P≤0.01) higher TDOM at $t_{1/2}$ and was due to lower lignification when compared to other roughage sources (Table 3). Among enzyme supplemented levels, 10g, 12g and 15g levels significantly (P≤0.001) improved TDOM when compared to other groups. Because of higher incubation time (t1/2) paddy straw had significantly(P≤0.001) higher NDFD at t1/2 among roughage sources and 12g of enzyme supplementation improved (P≤0.001) NDFD at t1/2. Similarly, Tang *et al*., (2008), reported significant improvement in 24h and 48g IVDMD and IVDOM when maize stover was supplemented with two (8 and 12g) levels of enzyme. Chopra *et al*., (2007) reported increased DM, NDF, ADF and cellulose degradability of wheat straw, maize stovers, bajra stalks and berseem hay on account of addition of fibrolytic enzymes. Similarly Ganai *et al*., (2011) reported increase in IVDMD and NDFD in jowar stover supplemented with enzyme mixture containing cellulase and xylanase activities. Addition of enzymes in the substrate might have caused fibre hydrolysis which may have increased digestion rate and/or extent of digestion (Feng *et al*., 1996; Krueger *et al*., 2008). Enzyme application to the substrate enhances the attachment of rumen microorganisms to the feed particles thereby increasing hydrolytic capacity of the rumen (Wang *et al*., 2001). Increased NDF degradability enhances the energy density of diets and stimulates the microbial production (Oba and Allen, 1999). Another possibility is that exogenous enzymes can access greater surface area compared with cell bound microbial enzymes (Thakur *et al*., 2008). The decline in DM and NDF degradability at higher levels might be due to the fact that beneficial disruption of the feed area may get diminished because the excess exogenous enzyme attached to feed may have restricted microbial attachment and limited digestion of feed.

Microbial biomass indices: PF at $t_{1/2}$ was significantly (P≤0.001) higher in maize husk than in other roughage sources (Table 3). Because of lesser lignifications, maize husk had significantly (P≤0.001) higher TDOM at $t_{1/2}$ and lesser gas production. The same was reflected in MBP at $t_{1/2}$ in maize husk. However, lower MBP at $t_{1/2}$ was observed in FMS among roughage sources. Similar trend was observed in EMBS between roughages. The results were corroborated with the findings of Soltan *et al*., (2013) who reported higher PF (Partition factor) value for different grass species supplemented with cellulase. The results indicated that the supplementation of fibrolytic enzymes had improved microbial biomass production at 10g, 12g and 15g level when compared to control.

NH3-N level was significantly (P≤0.01) higher in FMS and lowest in maize husk whereas in enzyme supplemented groups, 10and 12g level significantly improved NH3-N level when compared to the control and other enzyme supplemented groups. However, the NH3-N levels were within the permissible level of 5mg/dl of rumen liquor (Satter and Slyter, 1974).

CONCLUSION

Even though some variations were observed between roughage sources with respect to fermentation characteristics and substrate degradation due to variation in chemical composition of roughages, maize husk was found to be more efficient in microbial biomass synthesis followed by sorghum stover. Enzyme levels 10g and 12g were found to be optimum to improve the rate of fermentation and microbial protein synthesis and higher level of enzyme supplementation did not show any additional benefit.

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Utilization of Azolla as Feed Supplement in Large White Yorkshire Weaned Piglets

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ABSTRACT

An experiment to study the effect of feeding fresh and dried Azolla (*Azolla pinnata*) on the growth and feed consumption of growing piglets was conducted on 24 weaned Large White Yorkshire piglets (12 males +12 females) for 12 weeks duration. Each treatment group consisted of 8 piglets which were housed in 4 individual pens with two pigs (1male +1female) in each. The three diet groups were *viz.,* Control group **(C),** 10% green fresh azolla supplemented group **(F)** and 10% dry azolla supplemented group **(D)**. The nutrient analysis of dry Azolla revealed dry matter (94.50 %), crude protein (23.84%), ether extract (3.83%) and total ash (15.21%). The results shows non-significant body weight gains in all the treatment groups. The weekly cumulative feed intake for 12 weeks (3 m) was 37.25±2.25, 36.90 ±0.95 and 37.94±1.02 kg in C,F and D groups respectively and the values were non significant. The piglets of control group (C) had Non significantly higher feed efficiency (2.97±0.20) compared to F (3.06±0.09) and D (3.21±0.17) groups. It can be concluded that wherever available, azolla can be safely incorporated into feeds of growing piglets either in fresh or dried form @10% without affecting its growth rate of piglets. However, further studies on digestibility are necessary to study the proper utilization of azolla in pigs.

Key words: Growth rate, body weight, Azolla, Piglets.

Azolla (*Azolla pinnata*) is an aquatic fern that has been useful as a bio fertilizer and green manure for rice crop due to its Nitrogen fixing abilities(Van Hove and Lejeune, 1996). In recent times it has been used as feed supplement in different species of farm animals and poultry with varied results (Khatun, *et al.*1999, Prasanna *et al*., 2012). Research and promotion of azolla as a livestock feed has been growing. Because azolla has a higher protein content (19-30%) than most green forage crops, it is a valuable protein feed supplement for many species including ruminants, poultry, pigs and fish (Hasan *et al*.,2009).Azolla can be used as an ideal source of feed for cattle, sheep, goats, pigs, rabbits and fish as an alternate source for concentrate / feed / fodder to improve the production status of the animals (Mahadevappa *et al.* 2012).

MATERIALS AND METHODS

The present experiment of 12 weeks duration was conducted to study the influence of Azolla as a supplemental feed. Twenty four (12Male +12Female) weaned piglets of Large White Yorkshire, weaned at 8 weeks with uniform body weights were selected and distributed into 3 Treatment groups with each group comprising of 8 piglets in 4 replicated pens, with each pen housing 1 male +1female. The study was conducted for 12 weeks.

The experimental diet was prepared by grinding and mixing the individual feed ingredients *viz.*, SBM-15%, WB-35%, and Maize-47%, Mineral Mixture-2% and salt-1%, so as to provide 16% Crude Proteins. The experimental piglets were housed under uniform standard managemental conditions. All piglets were previously given Iron dextran and vitamin B Complex, had needle tooth cut as per the schedule. The piglets were weaned from sows on 56th day of age and ear tagged for identification and were randomly allotted to three treatment groups *viz.,*Control group **(C),** 10% green fresh azolla supplemented group **(F)** and 10% dry azolla supplemented group **(D)**. All the piglets were offered the same Compounded feed mixture diet *ad libitum*. The feed offered and left over was recorded daily to measure the actual feed intake. Data on weekly body weight changes, Average Daily Gain and feed

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consumption were recorded. Feed efficiency was calculated in terms of FCR based on body weight gained and feed consumed for 12 weeks.

Azolla was cultivated in 12 X 18 ft. silpauline sheet as well as cement rings with 4ft diameter and 1ft depth following NARDEP method (Kamalasanana *et al*., 2002). The nutritional value of feed and Azolla were analyzed for proximate constituents as per AOAC (1995). Azolla was produced in sufficient quantities and the sundried dried azolla was stored in plastic drums of 75litre capacity with air tight lid before the experiment. Azolla was offered both in fresh and dry forms at the rate of at the rate of 10% of feed offered based on previous weeks feed intake.

Statistical analysis: The data generated for various parameters of body weight gain, feed intake and Feed Conversion Ration were analyzed as per the statistical methods provided by Snedecor and Cochran, (1989) to record the mean, standard error and ANOVA test was performed to test the significance of mean values.

RESULTS AND DISCUSSION

Composition of experimental diets: The Proximate composition of Compound feed mixture (CFM) ration used for all the experimental groups was found to be dry matter 92%, crude protein 18%, ether extract 4.5%, crude fiber 8.2% and total ash 11.5%. The chemical composition (%) of Azolla cultivated and used in the experiment was found to have Dry Matter 94.5%, Crude Protein 23.44%, Crude Fiber 12.37%, Ether Extract 2.93% and Total Ash 16.21%. The fresh azolla had 4.6% dry matter. The composition of azolla was almost similar with earlier observations (Pannaerker, 1988, Tamang and Samanta, 1993, Basak, *et al.*,2002 and Cherryl *et al*.2014) who reported that the *Azolla pinnata* contained in the range, crude protein 24-30%, ether extract 3.0-3.36%, crude fibre 9.1-13.01%, Ca-0.40-1.64% and P-0.50-0.90%. Due to the better amino acid profile of Azolla it has been successfully used as a partial replacement for soya bean in pig fattening diets.(Wang and Fuller, 1989 and Alvaro, 1994)

Effect on feed consumption: The Mean ± SE of cumulative feed intake (g) has been presented in Table 1 and the mean body weight changes along with mean FCR have been presented in Table-2. Piglets in both treatment groups F and D readily consumed the offered Azolla. The average daily feed consumed during the study was obtained by adding both azolla and concentrate consumed on Dry matter basis. All the values of feed consumed were non significant for entire period from 0 week to 12th week of experiment. Inclusion of Azolla has shown ready acceptance in both fresh and dry form. There is increased palatability as revealed by increased feed consumption by weaned piglets. Cherryl *et al*.,(2013) found that Mean daily feed intake recorded was 1.75, 1.69 and 1.64 kg, respectively during growing phase and 2.03, 1.96 and 1.91 kg respectively during finishing phase in pigs fed with rations containing Azolla as protein replacement at 0, 10 and 20%. In similar earlier experiments Becerra *et al.* (1990) and Alvaro 1994) also found that there were no differences in Total Dry Matter intake among the experimental groups. However Becerra *et al.* (1990) reported that feeding of azolla at higher levels of 30% affected the palatability of the feed and reduced the feed consumption.

Table 1: Cumulative feed intake (g) of 12 Wks.

S.No.	Group	Particulars	Feed intake (g)
1	F	Fresh Azolla offered (4.6%DM)	10,400 ± 53.94
2	F	Fresh Azolla offered on DMB	488.80 ± 2.54
3	F	Concentrate	4,784.02 ± 24.79
4	F	Total DMI	5,272.82 ± 8.06
5	D	DryAzolla on DMB	675.71 ± 5.09
6	D	Concentrate	4,744.62 ±24.63
7	D	Total DMI	5,420.27 ±29.67
8	C	Concentrate	5,321 ±29.29

Table 2: Mean ± SE of body weight changes and FCR

Treat-ment Group	Cumulative Body Weight	ADG (g)	Cumulative Feed Intake (Kg)	FCR
C	12.84 ±1.01	152.82 ±12.06	37.25 ±2.25	2.90 ±0.20
F	12.16 ±0.58	144.79 ±6.87	36.90 ±0.95	3.06 ±0.09
D	12.06 ±0.75	143.60 ±8.95	37.94 ±1.02	3.15 ±0.17

Effect on body weight: Though the weight gains were lower in the fresh and dry azolla fed groups , there were no significant differences in body weight gains noticed at all weeks among all the groups. The total weights gained in the three groups were 12.84±1.01, 12.16±0.58 and 12.06±0.75 kg in C, F and D groups respectively. The average daily weight gains were similar and non significant at 152.82±12.06,144.79±6.87 and 143.60±8.95 gram/day in C, F and D groups respectively.

Becerra *et al.,* (1990) conducted feeding trial on growing-fattening pigs with basal diet of fresh sugarcane juice in control and substituted with azolla at 15 and 30 per cent of diet. They found that in growing phase, pig performance decreased as the amount of Azolla in the diet is increased and these effects were reversed in the finishing phase. However final result was that there were no differences in growth rate among treatment groups. This is in agreement with the present study. In another study by Parthasarathy *et al*.,(2006), Twenty desi pigs were randomly distributed into four groups of five pigs each and fed with iso-nitrogenous concentrate mixture replaced with sun-dried Azolla at 0, 10, 20 and 30% levels. Body weight gain and feed intake were recorded fortnightly during the 90-day growth trial period to determine the growth rate and feed efficiency. The average body weight gain ranged from 11.20 to 13.10 kg. The weight gain of pigs fed with 10% Azolla was more but the differences among the groups were non-significant. The highest level of dietary Azolla decreased feed efficiency significantly. It was concluded that Azolla can be incorporated up to 30% in the ration of desi pigs without any considerable adverse effect on growth. In similar experiment on grower pigs Cherryl et al., (2013) concluded that inclusion of Azolla at 20% is not harmful rather it is beneficial, in the way of reducing the feed cost wherever the azolla cultivation is cheaper.

Effect on Feed Conversion efficiency (FCR): The mean FCR in the three groups were 2.90±0.20, 3.03±0.09 and 3.15±0.17 in C, F and D groups respectively. The results indicate that the FCR was comparably similar in all the groups and non significant. Becerra *et al.*(1990) have reported FCR of 4.3,5.2 and 5.7 in Control, 15% and 30% azolla replaced feeds in weaned grower pigs. In another experiment by Alvaro (1994), Fresh azolla was offered at levels of 0, 1.7, 3.48 and 5.21 kg/day with replacement rate of the soya bean protein of 0, 10, 20 and 30%. The FCR was 2.1, 1.98, 2.0 and 2.2.

CONCLUSION

It may be concluded that Azolla can be supplemented at 10% both in fresh and dry form without any deleterious effect on body weight gains, feed consumption and FCR of grower pigs. However, a further study on digestibility is necessary to study the proper utilization of azolla in pigs.

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Efficacy of Individual and Combined Effects of Graded Levels of Abana^{tm} and Garlic Paste Supplementation on Meat Cholesterol and Serum Lipid Profile in Broilers*.

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ABSTRACT

A study was conducted to assess the efficacy of cholesterol lowering herbal preparation (Abana^{TM}) and garlic paste on muscle cholesterol and serum lipid profile in broilers. A total of one hundred eighty day old broiler chicks were randomly distributed into six experimental groups, comprising three replicates each with 5 male and 5 female chicks. Six experimental diets viz., diet without supplement as Control and with Abana^{TM}, an herbal preparation at 80 or 120 mg/kg body weight and garlic paste at 0.5% level individually and in combinations as test diets were allotted to each group. The diets were prepared for pre-starter (1-14 days), starter (15 - 21 days) and finisher phase (22 to 42 days). The cholesterol content of muscle and total cholesterol, LDL, HDL and triglycerides in serum samples from each group (n=6) were analyzed at 4th, 5th and 6th week. The inclusion of Abana^{TM} at 80 or 120 mg/kg body weight or garlic paste at 0.5 per cent levels or it combination in the broilers diets showed significant reduction in serum cholesterol, triglycerides, HDL, LDL and VLDL levels when compared to control diet at different intervals. The mean meat cholesterol values were also significantly lower in test groups when compared to control at 4, 5th and 6th week. The supplementation of broilers diet with Abana^{TM} (80 or 120 mg/kg body weight) or garlic paste (0.5 per cent) or together can reduce cholesterol and various lipid fractions in serum including muscle cholesterol level which are desirable features from human health point view.

Keywords: Cholesterol, Serum lipid, Abana, Garlic Paste, Broilers.

Although, India occupies 5th place in poultry meat production in the world, but in consumption aspect India has to go a long way. According to the National Nutrition Council, each individual requires 11 kg of meat per annum. Currently, India's per capita consumption of poultry meat is around 2.7kg (BAHS., 2014).

Irrespective of religious taboos, poultry meat has curved a niche for itself and has replaced other red meat cuisines for itself meant for table purposes. Now a day's people are more and more health conscious. Hence the cholesterol content of food products of animal origin had become a primary area of consumer concern due to the increased awareness of the link between higher dietary cholesterol intake and the incidence of coronary diseases. Therefore, the demand for the low cholesterol food products has been greatly increasing in the market.

Reduction of cholesterol content in poultry meat is one of the better approaches to control dietary cholesterol intake. There are several cholesterol reducing agents such as copper, garlic, linseed oil, chitosan, amaranthus plant, lovastatin, ketoisocapric acid, etc. (Vivek *et al*., 2004).

The cholesterol content of the edible muscle tissue of broiler chickens can be reduced approximately by 25 per cent after feeding a supra normal level of copper for 42 days without altering growth of the chickens or substantially increasing the copper content of edible meat (Raju *et al*., 1995).

Premkumar *et al*. (2001) reported that the dietary supplementation of copper and garlic individually and in combination significantly reduced serum total cholesterol, breast meat cholesterol by 23.5 per cent in broilers at market age of seven weeks.

Konjufca*et al*. (1997) found that feeding dietary garlic or copper for 21 days reduced cholesterol levels of broiler meat without altering growth of the chickens or feed efficiency.

Garlic (*Allium sativium*) is widely distributed all over the world. In the past two decades particular attention has been focused on its cholesterol lowering activity (Reddy *et al*., 1991). Garlic contains active principles like allium,

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allylicsulphide, which lowers the low density lipoprotein (LDL) cholesterol and also act as anti-carcinogenic (Carrijo *et al*., 2005). Abana™ a herbal preparation from Himalaya Drug Company containing 40 herbal ingredients like Arjuna (*Teminalia arjuna*), Ashvagandha (*Withania somnifera*), etc., is reported to regulate serum lipids by lowering the cholesterol, triglycerides, LDL, very low density lipoprotein (VLDL) levels and increasing high density lipoprotein (HDL) levels which in turn restore cardio protective mechanism in humans.

Hence, the present study has been designed to study the individual and combined effects of graded levels of Abana™ and Garlic paste supplementation on meat cholesterol and serum lipid profile in broiler.

MATERIALS AND METHODS

The sample of Abana™ which was in powder form obtained from Himalaya Drug Company and garlic in the form of paste obtained from Dabur Food Ltd. These were incorporated in the experimental diets at appropriate levels.

One hundred eighty day old broiler chicks (Vencobb) procured from a local hatchery were weighed, wing branded and randomly distributed into six experimental groups, comprising three replicates each with 10 birds having equal number of male and female birds. Six experimental diets were formulated and allotted to each group.

The experiment was conducted from day one to 42 days of age on litter floor. Chicks were vaccinated for Newcastle disease on 7th, day using 'F' strains and for Infectious bursal disease on 14th.day strict management practices were followed with *ad libitum* feed and water.Pre-starter mash was fed to chicks from day one to 14 days, while the starter from 15 to 21 days, finisher from 22 to 42 days. In the test diets the Abana™, an cholesterol lowering herbal preparation was included at two different levels (80 and 120 mg/kg body weight) and garlic paste at 0.5% levels individually and in combinations as described in Table I.

Broilers were slaughtered randomly among treatments at the end of 4th, 5th and 6th weeks of age. The muscle samples were collected, chopped, minced and frozen at –20^{0}C for the further analysis of total cholesterol. For extraction of total lipid and cholesterol the tissues were boiled in ethanol for few hours and then kept overnight after centrifugation at 5,000 rpm for 10 minutes the supernatant was obtained and used for the estimation of total lipids by Vanillin reagent (Hinda and Mohan, 1995) and cholesterol content according to Liebermann and Burchardt reaction (Henry and Henry, 1974).

For the determination of serum cholesterol, LDL, HDL and triglycerides, the blood samples of two birds were collected from each replicate group at every 4th, 5th and 6th week. Serum was separated individually and all the three replicates in each treatment were pooled and representative samples were subjected to serum cholesterol estimation using auto analyzer. The design of the experiment is CRD with one way analysis. The analysis of data is carried as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The findings on meat cholesterol (mg/g), Serum cholesterol (mg/dl), Serum LDL (mg/dl), Serum HDL (mg/dl), Serum VLDL (mg/dl) and Serum triglycerides (mg/dl) of experimental birds under different treatments at different periods of the experiment are given in Table.

The mean meat cholesterol mg/g of meat from 22.50 mg/g of meat in diet with Abana™ 120 mg and Garlic paste 0.5 per cent (T6) significantly differed from (T1) without Abana™ or Garlic paste at 42nd day. These values are in agreement with the work conducted by Kunjufua*et al*. (1997), who stated that the supplementation of three per cent garlic powder to Ross x Ross male broilers for 21 days significantly lowered the muscle cholesterol by 23 per cent.

The supplementation of Abana™ at 120 mg/kg and garlic paste at 0.5 per cent (T_6), significantly reduced the serum cholesterol level. The results on serum cholesterol concentration as influenced by Abana™ and garlic paste at different levels individually and in combination in broiler diets revealed reduction in serum cholesterol level in the treatment diets as compared to the control diet, variations among different dietary treatments and different weeks was significant (P>0.05) (Pesti and Bakalli, 1998).

Table. Mean±SE Meat cholesterol (mg/g), Serum cholesterol (mg/dl), Serum LDL (mg/dl), Serum HDL (mg/dl), Serum VLDL (mg/dl) and Serum triglycerides (mg/dl) of experimental birds under different treatments at different periods of the experiment.

Parameters/weeks	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Meat cholesterol						
IV Week	8.07^{a}±0.29	8.00^{ab}±2.55	4.87^{ab}±0.73	12.93^{ab}±2.13	7.10^{ab}±0.91	8.80^{b}±1.56
V Week	16.27^{a}±0.87	7.30^{b}±1.02	18.70^{b}±2.57	15.77^{ab}±2.13	9.57^{b}±0.44	14.57^{b}±2.12
VI Week	24.23^{a}±0.52	25.90^{b}±3.49	10.53^{c}±0.30	10.17^{b}±1.75	19.13^{bc}±4.52	22.50^{c}±0.47
Serum cholesterol						
IV Week	149.50^{a}±7.84	130.10^{ab}±2.37	113.70^{b}±9.32	127.40^{ab}±1.48	124.20^{b}±1.79	109.90^{b}±1.77
V Week	151.40^{a}±8.70	129.60^{ab}±1.52	108.90^{b}±12.55	126.60^{ab}±1.29	116.80^{ab}±12.71	97.83^{b}±8.82
VI Week	152.90^{a}±1.20	123.50^{ab}±2.98	107.50^{b}±3.39	125.20^{ab}±7.02	116.00^{b}±11.04	97.49^{b}±9.42
Serum LDL						
IV Week	48.44^{a}±2.62	40.38^{ab}±3.03	26.24^{c}±2.67	42.51^{a}±0.73	27.74^{bc}±3.58	20.95^{c}±2.45
V Week	48.89^{a}±4.81	40.18^{abc}±4.52	24.12^{bc}±2.58	41.38^{ab}±2.24	26.93^{bc}±6.03	20.06^{c}±4.77
VI Week	52.05^{a}±3.58	29.62^{bc}±0.78	24.18^{c}±2.78	40.69^{ab}±0.32	26.45^{c}±3.89	18.98^{c}±1.03
Serum HDL						
IV Week	84.53±0.93	79.05±4.49	78.24±6.53	82.18±2.51	77.38±1.26	76.90±5.43
V Week	84.03±2.27	78.50±4.97	76.57±8.20	81.50±3.41	76.90±5.43	69.30±4.34
VI Week	85.00^{a}±7.77	74.60^{ab}±2.65	73.71^{ab}±1.78	81.63^{ab}±3.53	75.37^{ab}±1.04	65.18^{b}±3.41
Serum VLDL						
IV Week	16.21±0.75	14.87±0.80	11.86±0.89	14.25±1.48	14.12±1.65	11.68±0.34
V Week	16.60^{a}±0.37	14.15^{ab}±0.16	11.86^{ab}±0.97	14.19^{ab}±1.42	13.66^{ab}±1.93	11.37^{b}±0.64
VI Week	16.80^{a}±0.56	13.59^{ab}±1.21	11.27^{b}±0.67	14.17^{ab}±1.53	13.06^{ab}±0.36	10.68^{b}±0.28
Serum triglycerides						
IV Week	1946 ± 380	1988 ± 356	1923 ± 9	1959 ± 250	1907 ± 300	1955 ± 169
V Week	2002 ± 273	1445 ± 82	1486 ± 279	1575 ± 198	1876 ± 162	1419 ± 87
VI Week	1969 ± 158	1394 ± 23	1429 ± 238	1509 ± 132	1840 ± 97	1352 ± 58

* Mean bearing at least one common superscript within a column are statically similar (P>0.05)
NS - Non-significant (P≥0.05) with in a column

The serum LDL concentration as influenced by Abana™ at 80 mg/kg body weight and 120 mg/kg body weight and garlic paste at 0.5 per cent level fed to broilers individually and in combination, revealed significant (P<0.05) reduction in serum LDL levels with in column at 4th 5th and 6th week.

The serum HDL concentration as influenced by Abana™ and garlic paste at 0.5 per cent at different levels individually and in combination in broiler diets, revealed significant reduction in serum HDL levels within the column at 6th week. The values of serum HDL showed non-significant (P>_0.05) difference at fourth and fifth weeks.

The serum VLDL concentration as influenced by Abana™ at 80 mg/kg body weight and 120 mg/kg body weight and garlic paste at 0.5 per cent levels fed to broilers individually and in combination in broilers diet revealed significant reduction in serum VLDL with in column at 5th and 6th weeks whereas at 4th week the values of serum VLDL remained non-significant.

The serum triglycerides level as influenced by Abana™. 80 mg/dl and Abana™ 120 mg/kg body weight and garlic paste at 0.5 per cent level fed to broilers individually and in combination in broilers diet revealed non-significant (P≥0.05) difference with in column at 4th, 5th and 6th week.

CONCLUSION

It is concluded that the feeding Abana^{TM} at 80 mg/kg body weight and 120 mg/kg body weight and garlic paste at 0.5 per cent levels in broilers could be beneficial in reducing serum and meat cholesterol decreased significantly at 4th, 5th and 6th week.

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Effect of Herbal Liquid Feed Supplements on the Performance of Broilers

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ABSTRACT

An experiment was conducted to evaluate the effect of herbal liquid feed supplements on the growth, FCR, immune response and economic benefit in broiler chickens reared on deep litter system. A total of ninety-six 30-day old commercial broiler chicks with similar body weight (1.476 ± 0.007 kg) were used for study. The herbal preparation was administered orally through drinking water at the rate of 20 ml per 100 birds per day from 31st to 42nd day. Significant (P<0.05) improvement in final body weight of the treatment group (2.27 ± 0.01 kg) when compared with control group (2.23 ± 0.01 kg) was observed. The overall FCR was 1.767 and 1.747 in control and treatment group, respectively. Considerable increase in antibody titres against Newcastle disease was observed in treatment group as compared to control group, indicating better immunomodulatory effect. The cost per unit weight gain during experimental period was found to be Rs. 62.91 in treatment group as compared to Rs. 66.22 in control group, indicating greater profitability.

Key words: Broiler chickens, herbal feed supplement, growth, immune response

Many feed supplements like antibiotics, probiotics (Naik *et al.,* 2000), prebiotics (Panda, 2003) are added to the diet of broilers for optimum utilization of feed nutrients. Synthetic drugs and chemicals have certain disadvantages like toxicity, contraindications, drug resistance, health hazards, high cost etc. Generally, herbal products do not have any side effects like resistance and residual effects. As per a WHO report, up to 80% of people still rely on traditional remedies as their medicine (Arunkumar and Muthuselvam, 2009). It has been found that turmeric, tulsi, amla and aloe vera preparations increase the body weight gain, feed efficiency and decrease the feed intake. These preparations were found to decrease the mortality rates and the cost of feed with significant reduction of serum cholesterol, serum triglycerides and increased humoral response against Ranikhet disease (Eevuri and Putturu 2013).

In view of the above, the study was planned to test the impact of herbal liquid feed supplement (coded as '*Lastende' which is a mixture of 21 herbs*) on the growth and performance of broilers. The product was developed by M/s. VetPet Formulations, Mangalore, Karnataka.

MATERIALS AND METHODS

A total of ninety-six 30-day old commercial broiler chicks reared on deep litter system were employed for the study. The birds were divided into two groups, each having three replicates of 16 birds. Each group had almost similar average initial body weight (1.476 ± 0.007 kg). Plain commercial finisher mash was given to both control and treatment groups. For treatment group, liquid herbal feed supplement through drinking water at the rate of 20 ml per 100 birds per day for a period of 12 days from 31st to 42nd day was given as recommended by the manufacturer. Initial weight, Final weight, Feed intake and mortality were recorded every 3 days from 31st day until 42nd day. For Humoral Immune response study, Haemagglutination Inhibition (H.I) titres were estimated as per the method of Allan and Gouch (1974). Data thus collected was subjected to statistical analysis, as per Snedecor and Cochran (1998). The net returns from herbal liquid feed supplement were assessed at the end of the trial.

RESULTS AND DISCUSSION

Observations on weight gain, feed intake and feed conversion ratio (FCR) during the experimental period (31st to 42nd day) showed no cognizable difference among the replicates. Hence, data was pooled together for statistical analysis and the same is presented in Table 1. The results revealed that there was significant

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(P<0.05) improvement in final body weight of the treatment group (2.27 ± 0.01 kg) when compared with non-supplemented group (2.23 ± 0.01 kg). The weight gain during the experimental period was significantly (P<0.01) higher in treatment group (0.79 ± 0.01) as compared to control group (0.74 ± 0.01), the feed consumption was significantly (P<0.05) higher in treatment group (1.776 ± 0.004) as compared to control group (1.750 ± 0.006) and the FCR was significantly (P<0.05) lower in treatment group (2.365 ± 0.022) as compared to control group (2.247 ± 0.021). Similar to the present study, significant increase in body weights of broilers were also observed with supplementation of *Ocimum sanctum* leaves by Lanjewar *et al.* (2008) and Gupta and Charan (2007). However, Mehala and Moorthy (2008) reported that a combination of aloe vera and *Curcuma longa* resulted in non-significant difference in body weights of broilers.

Table 1. Weight gain, Feed intake and FCR in experimental broilers (31st to 42nd day)

Parameter	Control	Treatment	*P* value
Initial body weight (kg)	1.47 ± 0.01	1.48 ± 0.01	NS
Final body weight (kg)	2.23 ± 0.01	2.27 ± 0.01	0.020
Weight gain (kg)	0.74 ± 0.01	0.79 ± 0.01	0.007
Feed intake (kg)	1.750 ± 0.006	1.776 ± 0.004	0.026
FCR	2.365 ± 0.022	2.247 ± 0.021	0.017

The antibody titres recordings at 30, 33, 36, 39, 40 and 42 days are presented in Table 2. There was a considerable increase in antibody titres of treatment groups in comparison with birds of control group, thus indicating better immunomodulatory effect. Eevuri and Putturu (2013) reviewed several herbs used in poultry industry and reported that feeding of broilers with diet supplemented with amla, tulsi and turmeric powder, either alone or in combination, resulted in high HI titre values for ND vaccination.

Table 2. Haemagglutination Inhibition results (Using 4 HA units of Lasota virus)

Period	Antibody titres	
	Control	Treatment
30th day	1:64	1:64
33rd day	1:128	1:256
36th day	1:128	1:512
39th day	1:128	1:256
42nd day	1:64	1:128

Hundred per cent livability was observed in treatment group while 4.17 % mortality was observed in control group. Vidhyarthi *et al*. (2008) had reported less mortality in broilers fed with herbal growth promoter containing amla as one of the ingredients while Mehala and Moorthy (2008), similar to present findings had reported hundred per cent livability with inclusion of aloe-vera and *Curcuma longa* and their combinations in broiler diets.

The analysis of production cost and economic benefit are given in Table 3. The total feed consumed during entire rearing period (42 days) was higher in treatment group (3.965 kg) as compared to control group (3.940 kg). However, broilers in treatment group had a lower FCR (1.747) as compared to control group (1.767). The gross profit per bird was higher in treatment group (Rs. 17.34) as compared to control group (Rs. 15.32), thus giving an added benefit of Rs. 2.02 per bird, or Rs. 0.77 per kg live weight in treatment group. Dinodiya *et al.* (2015) obtained highest net profit in birds supplemented with herbal feed supplements compared to synthetic antimicrobial feed supplements. Jadhav *et al.* (2008) studied the economic impact analysis of feeding synthetic and herbal choline to broiler chickens and reported that birds fed polyherbal formulations recorded higher net returns when compared with control as well as synthetic choline inclusion. Similar findings have been reported by Shivakumar *et al*. (2008) who observed higher feed cost in birds supplemented with herbal growth promoter but with an extra gain of Rs. 0.74 per bird as compared with unsupplemented group. They also reported an extra profit of Rs. 0.43/kg live weight, and attributed the extra gain to better performance.

Table 3: Economic benefit analysis of incorporation of herbal liquid feed supplement in broilers

Sl. No	Particulars	Control	Treat-ment
Performance of broilers (1st to 42nd day)			
1.	Average live weight (kg)	2.23	2.27
2.	Feed consumed (kg)	3.940	3.965
3.	FCR	1.767	1.747
Economic benefit analysis			
4.	Cost of day old chick (Rs)	26	26
5.	Feed cost @ Rs 28/kg	110.32	111.02
6.	**Total cost (4 + 5)**	**136.32**	**137.02**
7.	Income from sale of birds @ Rs 68/kg	151.64	154.36
8.	**Gross Profit per bird Rs. (7 – 6)**	**15.32**	**17.34**
9.	**Gross Profit per unit weight Rs. (8 ÷ 1)**	**6.87**	**7.64**

CONCLUSIONS

The present study indicated that incorporation of herbal liquid feed supplement (*Lastende*) from 31st to 42nd day of broiler rearing significantly improved growth and overall performance of broilers and also resulted in higher antibody titres against Newcastle disease, indicating better protection of birds.

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Microbial Quality of Salted and Sun Dried Anchovies (*Stolephorus spp.*) and White Sardines (*Escualosa thoracata*) of Dry Fish Market, Mangalore

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ABSTRACT

The present investigation was carried out to elucidate the microbial and biochemical quality of salted and sun dried fishes such as Anchovies and White Sardines sold at the local fish market of Mangalore. The samples were collected from the fish market packed in polyethylene bags and stored at ambient temperature. Sampling was done on fortnight basis. The total bacterial load of salted and sun dried Anchovies and White Sardines (WS) showed variations with different sampling intervals. The total plate count of WS were 3.78 log cfu/g. 5.53 log cfu/g, 4.59 log cfu/g and 5.80 log cfu/g at 1st, 2nd, 3rd and 4th sampling, respectively and total plate count of Anchovies at 1st, 2nd, 3rd and 4th sampling were 3.81 log cfu/g, 4.57 log cfu/g, 3.99 log cfu/g and 4.61 log cfu/g, respectively. The halophilic counts of both the fish samples indicated the presence of halophilic bacteria. The bacteria such as *E. coli* indicated their presence in both the samples which implicated in faecal contamination. TVB-N values were above the acceptable limits. The samples obtained from the fish market had poor microbial and biochemical quality which could be due to unhygienic handling of the samples.

Key words: *Anchovies, White Sardines, TVB-N, TMA-N, Halophilic bacteria*

Fish is highly nutritious food supplying all the essential amino acids and fatty acids in the recommended proportion to the consumers. On the other hand fish is also highly perishable commodity due to low post mortem pH, less glycogen reservoir, low connective tissue and high moisture content which support the rapid proliferation of spoilage microflora after the death of the fish (Wang *et al*., 2011; Akinneye *et al*., 2010). Fish is one of the most important sources of animal protein available and has been widely accepted as a good source of protein for the maintenance of good health (Andrew, 2001). Consumption of fish provides an important nutrient to a large number of people worldwide and thus makes a very significant contribution to nutrition. Preservation of fish is necessary due to the rapid change in enzymatic and bacterial activity which results in spoilage (Djendoubi *et al*., 2009). Spoilage affects the odour, flavour, texture, colour and chemical compositions of fish (Agbabiaka *et al*., 2012) and these in turn affect the nutritional quality, consumer acceptability and commercial value of fish (Daramola *et al*., 2007). There are various methods of fish preservation such as icing, freezing, salting, drying, canning, freeze-drying, non thermal methods such as irradiation and other processes. However, freezing, chilling, canning and irradiation needs sophisticated equipments and the cost of process is very expensive. Salting is one of the oldest fish preservation techniques and widely applied around the world (OECD, 2008).

Traditionally salting and drying of fish is being practised since thousands of years. Salting and drying of fish is simple, economical and effective method of preservation. Preservation of fish by salting, drying, smoking and fermentation together termed as curing preservation. Among the cured preservation methods, salting and drying predominates and nearly 8.9% of the total marine catch in the world (FAO, 2013). In India about 5% of the total catch is diverted for salting and drying preservation of fish and shell fish (Handbook of Fisheries Statistics, Govt. of India, 2013). Salting is a preliminary step in fish drying process to obtain a commercial product with adequate shelf life by decreasing water activity (Gallart-Jornet *et al*., 2007) and provide specific organoleptic and sensory characteristics (Boudhrioua *et al*., 2009) of the final products (Andres *et al*., 2005).

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The preservative effect of salting is mainly attributed to decrease the water activity (a_w) for inhibiting the growth of many spoilage microorganisms. Drying is the process of removing water from a solid by evaporation. It is a simultaneous process of heat and mass transfer (Belessiotis and Delyannis, 2011). Drying of aquatic products decreases water activity by removal of water and thus inhibits microbial growth as well as undesirable chemical reactions related with enzymes which enables storability of the product under ambient temperatures (Al-Harahsheh *et al*., 2009). Drying helps to extend shelf-life, improves quality, reduces post-harvest losses and packaging costs, lowers shipping weights, enhances appearance, retains original flavour and maintains nutritional value (Bala and Mondol, 2001).

The quality of salted and sun dried fishes are adversely affected by the contamination of different microorganisms. The determination of microbiological and biochemical quality of salted and dried fishes commercially available from the market is very important for guarding health, hygiene and sanitation of the consumer (Lilabati *et al*., 1999). In India, cured fish and fishery products are popular in local markets and some commercially important fish species are exported to other countries. The export of cured fish and fishery products has been declined due to their poor quality (Sugumar *et al*., 1995). Fish must be dried quickly using good sanitation and hygienic practice in plenty of sunlight and air, which protects fish from insect infestation and dirt. Fish and shellfish harbours a large number of microorganisms, one of the major factors contributing to poor quality of fish and fishery products in retail trade is unhygienic handling, unsanitised storage leading to off odour, physical damage and cross contamination with dust and objectionable microorganisms (Sugumar et al., 2000). The majority of these microorganisms are non pathogenic causing only spoilage of fish but some are pathogenic and causes food poisoning. Fungal contamination is a common problem and it adversely affects the quality of salted and dried fish. The quality deterioration of foods during processing, storage and distribution is mainly caused by microorganisms. Preservation of fish by salt curing has been practised in fish drying yards of Mangalore as a traditional technique. Different varieties of fish are washed in sea water and immersed in saturated brine for 24 hours and dried for two to three days in open sunlight on a sandy sea shore area or on palm leaves. The same practice is being followed till date with little modification. Therefore, the microbiological and biochemical quality attributes of salted and dried fish assessments are necessary to ensure good quality of product before consumption. Against this background the present study was carried out on the microbial quality of salted and sun dried Anchovies (*Stolephorus* spp.) and White Sardines (*Escualosa thoracata*) of dry fish market, Mangalore.

MATERIALS AND METHODS

Sample collection: The fish samples were collected from the dry fish market of Mangalore. The samples were brought to the laboratory in a clean polyethylene bags which were stored at ambient temperature. The biochemical (AOAC, 2005), and microbiological characteristics (APHA, 2005) of sun dried fish samples were carried out. Total bacterial load and halophilic bacterial count were estimated according to the procedure described in APHA, (2005). Most probable number (MPN) technique was used to enumerate total coliforms of a sample.

Microbial analysis

Isolation of Bacterial Pathogens:

Escherichia coli: From the positive tubes of EC broth, a loopful of inoculum was taken and streaked onto the Eosin Methylene Blue (EMB) agar. Plates were incubated at 37° C for 24-48 hours. Typical colonies having greenish metallic sheen were picked from the plates and sub-cultured in Tripticase Soya Agar (TSA) slants and preserved for further studies.

Vibrios: 25 g of sample was homogenized and enriched in 225 ml of alkaline peptone water (APW) at 37° C for 6-8 hours. A loopful of enriched sample was inoculated into Thiosulphate citrate bile salt sucrose agar (TCBS) and incubated at 37° C for 24 hours. Presence of Vibrio shows yellow and green colonies (Sucrose fermenter and non-fermenter) and the green colonies were picked and sub cultured onto TSA slants for further confirmation.

Salmonella:10 g of sample was inoculated in to 90 ml of pre-enrichment Lactose Broth and incubated at 37°C for 24 h. After incubation 1 ml of the pre-enrichment broth was inoculated further into a selective enrichment broth, (Selenite Cystine Broth)) aseptically and incubated at 37° C for 24-48 hours. An inoculum from SCB was streaked on to Bismuth Sulphite Agar (BSA) and incubated for 24-48 hours at 37° C and were examined for the presence of colonies suggestive for *Salmonella*. Morphologically, typical *Salmonella* colonies appeared brown, grey or black with or without black centres often with metallic sheen. Characteristic colonies were picked from the plates and aseptically transferred to TSA slants for further confirmation.

Staphylococcus aureus: 10 g of sample was enriched in 90 ml of the Lactose broth and incubated at 37° C for 24 h. After the incubation a loopful of the inoculum was streaked on to Baird Parker Agar (BPA) and incubated at 37°C for 24-48 hours. After incubation plates were examined for the presence of colonies suggestive for *Staphylococcus aureus*. Characteristic colonies were picked from the plates and aseptically transferred to TSA slants for further confirmation.

Biochemical Analysis:

The biochemical spoilage indicators such as Total volatile base nitrogen (TVB-N) and Tri-methylamine nitrogen (TMA-N) of salted and dried fish sample were determined by Conway micro diffusion method (Beatty and Gibbons,1937).

RESULTS AND DISCUSSION

Microbial analysis: The Total bacterial load of salted and sun dried anchovies and white sardines were analysed and the results are shown in Table 1. The TPC of white sardine and anchovies showed variance with different sampling intervals. The counts of white sardine were 3.78, 5.53, 4.59 and 5.80 $\log_{10}$ cfu/g at the 1st, 2nd, 3rd and 4th sampling respectively. Anchovies at 1st, 2nd, 3rd and 4th sampling showed total bacterial counts of 3.81, 4.57, 3.99 and 4.61 $\log_{10}$ cfu/g respectively. The total bacterial load of salted, sun dried anchovies and white sardines showed an increasing trend throughout the study (Table 1). The acceptable limit of total bacterial load for dried fish is $1.0x10^5$ cfu/g (Surendran *et al*., 2006). In the present investigation the total bacterial load of anchovies and white sardines were within the acceptable limit.

Table 1: Total bacterial load (TPC) of salted and sun dried Anchovies and White Sardine

White sardine	TPC ($\log_{10}$cfu/g)	Anchovies	TPC ($\log_{10}$ cfu/g)
1st sampling	3.78±0.045	1st sampling	3.81±0.190
2nd sampling	5.53±0.586	2nd sampling	4.57±0.183
3rd sampling	4.59±0.227	3rd sampling	3.99±0.627
4th sampling	5.80±0.085	4th sampling	4.61±0.257

The halophilic counts of white sardine were 5.08, 5.90, 4.20 and 3.25 $\log_{10}$ cfu/g for 1st , 2nd, 3rd, and 4th samplings respectively, whereas for anchovies the counts were 3.69, 4.77, 4.36 and 3.31 $\log_{10}$ cfu/g respectively for 1st , 2nd, 3rd, and 4th sampling (Table 2). The halophilic bacterial counts associated with salted and dried fish were quantitatively carried out and shown in the Table 2. The halophilic counts of both the fish samples indicated the presence of halophilic bacteria but load did not exceed the permissible limit.

Table 2: Total halophilic counts of salted and dried fish at different sampling intervals

White Sardine	Halophilic Count ($\log_{10}$cfu/g)	Anchovies	Halophilic Count ($\log_{10}$cfu/g)
1st sampling	5.08±0.851	1st sampling	3.69±0.198
2nd sampling	5.90±1.766	2nd sampling	4.77±1.420
3rd sampling	4.20±0.713	3rd sampling	4.36±0.494
4th sampling	3.25±0.663	4th sampling	3.31±0.277

Fish samples were screened for bacterial pathogens such as *E. coli*, *Salmonella*, *Staphylococcus*, Vibrios and faecal coliforms, are shown in the Table 3. *Salmonella* was present in anchovies (1st sampling), *E. coli* and Vibrios showed positivity in 2nd and 3rd sampling in Anchovies. In samples of white sardine *Salmonella* and *E. coli* were present in 1st and 2nd sampling respectively. The results of bacterial pathogens (*Salmonella*, *E.coli*, Vibrios, *Staphylococcus*) associated with salted and sun dried fishes are shown in the Table 4. The bacteria such as *E.coli* and *Salmonella* indicated their presence in both the samples which implicated in faecal contamination. Vibrio is a halophilic bacterium usually present in the marine environment but in the case of *Salmonella* it does not occur naturally in marine waters and its presence is due to unhygienic handling or use of polluted coastal water for washing (Clucas and Ward, 1996). Incidence of pathogens in the samples of fish market may be attributed to external contamination and poor handling practice (Jedah *et al*., 1998). The total coliforms and faecal coliforms were also enumerated by using MPN technique. The counts were $2.3x10^1$, $4.3x10^1$, $4.0x10^1$, $1.1x10^1$ MPN/100g for different samplings respectively (Table 3). The faecal coliforms are derived from faeces of human and other warm blooded animals. Washing the fishes after harvesting in polluted coastal water definitely adds to the number of faecal indicator organisms. Drying done in unhygienic manner may also add faecal coliforms to the fishes (Anand *et al*., 2002).

Biochemical analysis: The results of total volatile base nitrogen of salted, sun dried white sardine and anchovies were shown in the Table 4. The TVB-N values were showing an increasing trend during different sampling intervals throughout the experiment for white sardine. TVB-N was found to be 158.66, 82.56, 70.93 and 64.51 mg N/100 g during 1st, 2nd, 3rd and 4th sampling respectively. In case of anchovies the TVB-N values were 37.06, 94.32, 71.08 and 74.32 mg N/100 g during 1st, 2nd, 3rd and 4th sampling respectively. Tri-methylamine-nitrogen of salted, sun dried white sardine and anchovies analysed and results were shown in the table 4. The TMA-N values were showing increasing trend during different sampling intervals throughout the experiment for white sardine. TMA-N was found to be 43.78, 12.73, 5.54 and 7.33 mg N/100g during 1st, 2nd, 3rd and 4th sampling respectively. In case of anchovies the TMA-N values were 1.39, 15.01, 11.0 and 4.98 mg N/100g during 1st, 2nd, 3rd and 4th sampling respectively. The TVB-N and TMA-N indicates freshness of fish (Beatty and Gibbons, 1936). The acceptability level of TVB-N in dried fish is 30-40 mgN/100g. Above this limit the products are considered as unfit for human consumption. In the present study both the samples have TVB-N value above the acceptable limits. Higher levels of TVB-N level of fish in retail markets are obtained by several investigators. The TVB-N level of fish recorded in retail market was higher 98 mg N/100g (Iyer *et al*., 1986). The production of TMA is dependent on the bacterial

Table 3: Pathogens associated with the salted and sun-dried fish

Sample type	Sampling intervals	Bacterial Pathogens					
		Total coliforms (MPN/100g)	Faecal coliforms	*E. coli*	*Salmonella*	*Staphyloc occus aurues*	Vibrios
Anchovies	1st Sampling	23	4	-	+	-	+
	2nd Sampling	39	7	+	-	-	+
	3rd sampling	75	11	+	-	-	+
	4th sampling	43	7	-	-	-	+
White Sardine	1st Sampling	43	4	-	+	-	+
	2nd Sampling	43	4	+	-	-	+
	3rd sampling	39	7	-	-	-	+
	4th sampling	23	4	-	-	-	+

activity as well as from endogenous enzymes. The recommended level of TMA value is 10-15 mg N/ 100g (Connel, 1980). In the present study TMA-N values of the samples were within the limit except samples of white sardine during Ist sampling rose to the higher level (43.78 mg N/100g) than the acceptable limit. This can be elucidated bacterial putrefaction of spoilage bacteria is the reason for sudden increase of TMA in fish muscle (Huss, 1988).

Table 4: Biochemical characteristics of salted, sun dried white sardine and anchovies at different sampling intervals.

Characteristics/ Sampling	White Sardine		Anchovies	
	TVB-N	TMA-N	TVB-N	TMA-N
I	158.66± 21.385	43.78± 1.046	37.06± 1.850	1.39± 0.280
II	82.46± 6.175	12.73± 0.225	94.32± 3.042	15.01± 0.843
III	70.93± 9.636	5.54± 0.403	71.08± 0.271	11.0± 0.173
IV	64.51± 4.734	7.33± 0.288	74.32± 1.082	4.98± 0.699

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Quality Assessment of Canned Indian Mackerel (*Rastrelliger kanagurta*) using different Filling Media

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ABSTRACT

The present study was undertaken to investigate the microorganisms associated with the raw material, Indian Mackerel (*Rastrelliger kanagurta*) used for development of canned product in different filling media (brine, vegetable oil,curry) and its storage stability. Fresh Mackerel was analysed for microbial and biochemical quality. The present study showed the absence of bacterial pathogens such as *Salmonella*, *Staphylococcus* aureus and *Vibrio parahaemolyticus* in the raw material, whereas presence of *E. coli* and Faecal coliforms were observed in the fresh fish sample. The tin free steel (TFS) cans were used for preparing canned products in different filling media , heat processed and were stored at ambient temperature (30 °C ± 2°C) for a period of 8 months. The incubated cans were opened under aseptic condition and analysed for physical and sensory attributes.Sensory attributes such as flavour, colour and texture were good and acceptable. Hence the canned Mackerel in different filling media were suitable for consumption after a period of eight months with evidence of no microbial growth and also not much of variation in the sensory attributes of stored cans.

Key words: *Mackerel, brine, curry, oil, microbial quality, sensory attributes*

Seafood play an indispensable role in the human diet worldwide and have been the cheapest source of protein for the population in the under developed nation, which cannot afford a balanced diet for their livelihood. In addition, the seafood trade is a key revenue earner to many countries and also provides employment to a significant population. Indian scenario is a classical example of this and during the financial year 2013-14, the seafood exports recorded a growth of 5.98% in quantity, 60.23% in rupees and 42.6% growth in US $ earnings respectively. During the year, the foreign exchange revenue earned by marine product export is record US$ 3511.67 million (MPEDA, 2013). The seafood industry in India is growing at a very rapid pace and a vast proportion of fish and shell fish resources in deep water area of Indian ocean remain under exploited. The production of fish and shrimp by aquaculture (86.66 lakh tonnes as on 2013) in increasing steadily and is becoming a major source of raw material for processing and export. The continuous increase in the production of fishery products by wild capture and aquaculture and rapid growth of industry in India calls for a parallel improvement in marketing, quality standards and food safety management from production to consumption. Fish is an extremely important component of the human diet and provide nutrients such as protein, omega-3 (n-3) fatty acids (Burger et al., 2005; Daviglus *et al*., 2002). Fish can be preserved in many ways including freezing, salting, pickling, smoking and drying. But the most useful way of preserving seafood is through canning (Okyere *et al.,* 2015). Fish canning' is a classic example of this type of processing consciously evolved to meet specific needs. It is a sophisticated method of processing of raw fish and other edible aquatic products which keeps the final product suitable for consumption at ambient temperature over a longer period of time than any other processing method. Qualities like durability, availability in large varieties, easy storage and transportation makes canned fish popular all over the world utilizing about 20% of the global catch.

Fish spoils easily, the cause of which has been attributed to series of post mortem changes caused by bacteria, indigenous enzymes and chemical reactions. Bacteria occur naturally on the skin and in the slime of living fish, where they do no harm, but immediately after the death, the bacteria begins to proliferate in its tissues. In terms of microbial spoilage *Clostridium botulinum* has been identified especially in canned fish, food poisoning has also been attributed to *Escherichia*

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coli, *Salmonella*, *Listeria monocytogens, Bacillus cereus, Staphylococcus aureus* etc. Bio- chemical and physiological changes that occur in harvested fish include formation of volatile bases particularly Trimethylamine (TMA-N), Dimethylamine (DMA-N), Total volatile base nitrogen (TVB-N) and ammonia. Against this backgroundThe present study was carried out to assess the quality of canned Indian mackerel (*Rastrelliger kanagurta)* using different filling media (oil, brine and curry). Indian mackerel is the commonly used and affordable commercial fish species for canned product in India, and thus chosen as the raw material for this work.

MATERIALS AND METHODS

Materials for the study were procured (on fortnightly basis) from the major fish landing centre, Mangalore. The samples were brought to the laboratory in iced condition until further study. The biochemical (AOAC, 2005), and microbiological quality (APHA, 2005) of fresh Mackerel were carried out was used as raw material for canning. Most probable number (MPN) technique was used to enumerate total coliforms of the sample.

Isolation and Identification of Bacterial Pathogens

Isolation of *Escherichia coli*: A loopful of inoculum from EC broth was taken and streaked onto Eosin Methylene Blue (EMB) agar. Plates were incubated at 37° C for 24-48 hours. A typical colonies having greenish metallic sheen were picked from the plates and sub cultured on to Tripticase Soya Agar (TSA) slants and preserved for further studies.

Isolation of Vibrios: 25 g of the sample was homogenized and enriched in 225 ml of alkaline peptone water (APW) and incubated at 37° C for 6-8 hours. A loopful of enriched sample was inoculated into thiosulphate citrate bile salt sucrose agar (TCBS) and incubated at 37° C for 24 hours. Presence of Vibrio shows yellow and green colonies (Sucrose fermenter and non-fermenter) and the green colonies were picked and subcultured onto TSA slants for further studies.

Isolation of *Salmonella*: 10 g of sample was inoculated into 90 ml of the pre-enrichment Lactose Broth and incubated at 37° C for 24 h. After incubation 1 ml of the pre-enrichment broth was inoculated further into a selective enrichment broth, Selenite Cystine Broth (SCB) aseptically and incubated at 37° C for 24-48 hours. An inoculum from SCB was streaked on to Bismuth Sulphite Agar (BSA), and incubated for 24-48 hours at 37° C and were examined for presence of colonies suggestive for *Salmonella*. Morphologically, typical *Salmonella* colonies appeared brown, grey or black with or without black centres often with metallic sheen. Characteristic colonies were picked from the plates and aseptically transferred to TSA slants for further confirmation.

Isolation of Staphylococcus: 10 g of sample was enriched in 90 ml of the Lactose broth and incubated at 37° C for 24 h. After the incubation a loopful of the inoculum was streaked on to Baird Parker Agar (BPA) and incubated at 37°C for 24-48 hours. After incubation plates were examined for presence of colonies suggestive for *Staphylococcus aureus*. Characteristic colonies were picked from the plates and aseptically transferred to TSA slants for further confirmation.

Biochemical Analysis: Total volatile base nitrogen (TVB-N) and Tri-methylamine nitrogen (TMA-N) by Conway micro diffusion method (Beatty and Gibbons, 1937), TBARS (Raghavan and Hultin, 2005) and pH (Vyncke, 1981) were analysed.

Unit operations in Canning of fish (Saralaya, 1978): Fresh Fish was washed with potable water and dressed to remove the inedible portions such as head, fins, scale and off entrails and again washed with good quality of water. Dressed fish were subjected to size cutting (approximately 3cm size) to accommodate in TFS cans. Saturated brining was followed for dressed fish for 15 minutes. Fish were packed in TFS cans and subjected to pre cooking at 100^{o}C for 10-15 minutes. After draining the liquid from pre cooking, different filling media (vegetable oil, Curry and brine) were filled into the cans leaving 10mm head space (as per FDA). Filling hot medium (at 80^{o}C) into the cans helps in proper exhausting to yield good vacuum at the end. Exhausted cans were subjected to double seaming using manually operated double seaming machine The closed cans were then washed with potable

water and were subjected to heat processing (thermal processing) at 121°C for 70 -90 minutes to get final Fo values of 4-6 minutes. The processed cans were cooled in water, wiped, dried and labelled and stored at ambient temperature for further studies. The representative cans were incubated at 37°C and 55°C for a week to look for any spoilage.

Cut out test analysis was done to appraise the general quality of a canned food. In this test, the condition of the food contents, the external and internal conditions of the can and other characteristics of the product are examined by certain organoleptic and physical tests such as gross weight, net weight, drained weight of content, liquid weight, vacuum, head space, condition of can interior, meat adhesion etc. The test results are noted and tabulated for each type of product in standard profarma.

RESULTS AND DISCUSSION

The total bacterial load of fresh mackerel was analysed and results are depicted in Table 1. Total bacterial loads of mackerel were 3.74, 3.53, 3.59 and 4.80 $\log_{10}$cfu/g respectively for first, second, third and fourth sampling intervals, which were found to be within the limits (ICMSF, 1986). The total and faecal coliforms were 460 and 420 MPN/g respectively in the first sampling but subsequently the counts were reduced considerably and were within the limits. Sample showed the presence of *E. coli.* and Vibrios. However, pathogens such as *Salmonella, Staphylococcus aureus* were absent in the sample.

Biochemical Characteristics of Mackerel: The biochemical spoilage indicators such as TVBN, TMAN and pH were analysed and results are shown in the Table 2. All the parameters were within the acceptable limits.

Table 2: Biochemical characteristics of fresh Mackerel:

Sample	TVB-N (mgN/ 100g)	TMA (mgN/ 100g)	pH	TBARS (mgMDA/ Kg)
1st sampling	11.20	1.4	6.51	0.10
2nd sampling	10.30	3.20	6.41	0.31
3rd sampling	12.70	4.86	6.42	0.32
4th sampling	11.64	10.40	6.50	0.48

Cut out test analaysis of Canned Mackerel: Prepared cans were analysed for their quality by performing Cut Out test and the results are depicted in Table 3. The present investigation was carried out to elucidate microbial and biochemical quality of mackerel as a raw material for the development of canned product in different filling media such as brine, oil and curry, and to assess its quality by "Cut out" test. The acceptable limit of total bacterial load for fresh fish is 10^6 $_{\log10}$cfu/g at 37°C (Surendran *et al.,* 2006). In the present investigation the total bacterial load of fresh mackerel were within the acceptable limit. Bacterial pathogens such as *E. coli* and Vibrios were present in the sample which are implicated in faecal contamination. Incidence of pathogens in the samples of fish from landing centre may be attributed to cross contamination and poor handling practice (Jedah *et al.,* 1998). The total coliforms and faecal coliforms were also enumerated by MPN technique. The counts were 460 and 240 MPN/ 100g respectively. The feacal

Table 1: Microbial quality of fresh fish (Indian Mackerel)

Sample	TPC ($\log_{10}$cfu/g)	Total coliforms (MPN/g)	Faecal coliforms (MPN/g)	Pathogens			
				E. coli	*Salmo-nella*	*Staphy lococcus aureus*	Vibrios
1st sampling	3.74±0.045	460	240	+	-	-	+
2nd sampling	3.53±0.586	43	4	-	-	-	+
3rd sampling	3.59±0.227	43	4	-		-	+
4th sampling	4.80±0.085	23	4	-	-	-	+

coliforms are derived from faeces of human beings and other warm blooded animals. Washing the fishes after harvesting in polluted coastal water definitely add the faecal indicator organisms. Tanikawa (1971) reported that TVB-N content of 20 mg N/100g as the maximum limit of freshness of raw material suitable for canning. Above this limit the products are considered not suitable for canning.However all the biochemical spoilage indicators such as TVBN, TMAN, pH and TBARS were below the acceptable limits. Madhavan *et al.*, (1970) worked on the suitability of ice stored mackerel for canning, and found that mackerel with 2.36 mg N/100g TMA-N were suitable and those with 2.81 mg N/100g were not suitable.

The recommended level of TMA value is 10-15 mg N/ 100g (*Connel*, 1980). In the present study TMA-N values of fresh mackerel sample was within the acceptable limits. 'Cut- out' tests are generally conducted to evaluate the qualities of the product which can be judged by physical measurements and visual observations. As can be seen from the tabulated data, net weight of the steam precooked products of different packs were around 200g and slight variations in the values may be due to packing and filling differences. The solid weight as the percentage of net weight were around 70% for the steam precooked products. The I.S.I specifications (1963, 1966) state that the minimum drained weight of solids for mackerel should be 65 to 70%. But, Japanese Export Standards (1976) for canned and bottled food require drained weight percentage ranging from 77 to 82% of the net weight in case of sardine, mackerel and tuna. However, in the present study the net and solid weight were satisfactory for the can size of 301×203. Head space specified by I.S.I (1968) for canned fishes is a maximum of 10 mm. The head space of all the products varied from 6.5 to 9.5 mm which is well within the above limit. pH values of

Table 3: Results of cut out test analysis of canned mackerel in different filling media

Sl. No.	Particulars/attributes	Description		
1	Product	Mackerel in brine	Mackerel in Oil	Mackerel in Curry
2	Date of production	10-6-14	10-6-14	10-6-14
3	Date of testing	6-2-15	6-2-15	6-2-15
4	Can type	TFS	TFS	TFS
5	Std. Net weight	140g	138g	140g
6	Gross weight	160g	160g	166g
7	Empty can weight	42g	42g	42g
8	Solid weight	82g	96g	96g
9	Liquid volume	74ml	46ml	48ml
10	Colour	Good	Good	Good
11	Flavour	Good	Good	Good
12	Texture	Good	Good	Good
13	No of pieces	3	3	3
14	Appearance	Good	Good	good
15	Turbidity	Absent	Absent	absent
16	pH	6.5	6.5	6.45
17	Broken pieces	Absent	Absent	Absent
18	Adhesion	Absent	Absent	Absent
19	Curd formation	Absent	Absent	Absent
20	Head space	8- 10 mm	10mm	10mm
21	TPC/(cfu/g)	Nil	Nil	Nil

different products were in the range of 6.0 to 6.5 and these values were in the normal pH range for canned fish. There were no broken pieces in the steam precooked products. This indicates steam precooked products are resistant to breakage and high temperature processing as they are packed before precooking. Adhesion of meat to the can surface also affects the quality of the product. There was absolutely no adhesion in steam precooked products. Hence this aspect of steam precooking is found to be best for canned fishery products. Turbidity was not observed in any of the steam precooked products. Hence the solutions in different filling media were clear and were more attractive. Sensory attributes of different steam precooked products such as colour, flavour and acceptability were good. Hence in the experiment it was proved that the overall acceptability of canned products were good with different filling media.

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Black Quarter in Crossbred Dairy Cow- A Case Report

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ABSTRACT

A four year old cross bred Jersey cow was presented to Teaching Veterinary Clinical Complex, Veterinary College, Hassan with history of anorexia and lateral recumbency. On clinical examination an increase in body temperature, respiratory and heart rates were observed. The animal was found recumbent with bloody discharges from rectum and vagina, swelling of right hip and lumbar region which were hot to touch and showed crepitations. Black quarter in the cow was confirmed on observation of *Clostridium chauvoei* on Gram's staining of aspirate samples collected from hip region. Haematological studies showed haemoconcentration and leucocytosis with neutrophilia and lymphopenia. On treatment with Inj. Streptopenicillin Inj. Meloxicam and parentral fluids the cow failed to respond and died within 3 days of onset of the signs.

Keywords - Black quarter, *Clostridium chauvoei*, treatment

Black quarter is an economically important disease in cattle and other domestic animals. It is caused by *Clostridium chauvoei* a Gram-positive, rod shaped, spore forming and toxin-producing anaerobic organism. The disease typically occurs during the late summer or early fall, cattle of all ages but mostly occurs in young ones aged 6 to 24 months.

The disease is mainly transmitted through ingestion of resistant spores which reach the muscular tissue through the blood circulation and remain in latency for years. On development of tissue hypoxia, the spores germinate and multiply, producing several potent toxins (Radostits *et al.,* 2006). Among them the alpha toxin was found to be the one of the major involved in the pathogenicity of the disease (Useh *et al.,* 2003; Hang'ombe *et al.,* 2006) due to its hemolytic, necrotic and histotoxic effects (Quinn *et al.,* 2005).

A four year old crossbred Jersey crossbred cow was presented to Teaching Veterinary Clinical Complex, Veterinary College, Hassan with history of anorexia and lateral recumbency. On detailed clinical examination the body temperature (103.8^{o}F), respiratory rate (54breaths/min) and heart rate (88beats/min) were turns to be increased. The cow was found recumbent with blood discharges from rectum and vagina, swellings on right hip and lumbar region which were hot to touch and showed crepitations. Blood sample and needle aspirates from the affected muscles were collected and subjected to haematological examination and Grams staining respectively as per the standard lab procedures (Jain., 1986).

Based on the history, clinical signs and observation of Gram positive rod shaped, single or small irregular clumps of *Clostridium chauvoei* organisms in the affected tissue the disease black quarter was confirmed. Treatment was undertaken in the cow with Inj.Streptopenicillin @ 40000 IU/Kg body weight (inj. Dicristicine®), Inj.Meloxicam @ 0.3 mg/Kg body weight (Inj.Melonex®) and supported with parentral fluid therapy for three days. However the cow did not respond to the above treatment and died within 3 days of onset of the disease.

The present clinical findings of high body temperature, anorexia, recumbency, hot and crepitative swellings in hip and lumbar muscles are in agreement with Radostitis *et al*., 2006. Van Vleet and Valentine (2007) and Langroudi *et al*., (2012) reported predilection of the clostridium chaouvei organisms in the hind limbs, fore limbs and tongue muscles. In the early stages of the disease, hot swellings in the affected muscles are observed. Which later becomes cold and gas filled leading to crepitations. The affected animal shows marked depression, lameness and recumbency. If untreated death occurs in 24 to 48 hours.

The haematological evaluation revealed increase in packed cell volume, haemoglobin concentration and total erythrocyte count indicating haemoconcentration due to anorexia, dehydration

and oedematous accumulations. Similar observations were made by Ritchie (1990), Singh *et al.* (1993) and Useh, *et al.*, (2003). However El-sawi *et al.,* (1989) and Idrees *et al.,* (2013) reported decrease in total erythrocyte counts and attributed it to hemorrhagic and hemolytic effects of toxins produced by the bacteria. The platelets count was normal however El-sawi *et al.,* (1989) and Singh *et al.,* (1993) reported thrombocytopenia in their studies. The above variations may be due to the course and nature of occurrence of disease in the natural and experimental conditions. Further leucocytosis with neutrophilia and lymphopenia were recorded. Similar observations were reported by Ritchie (1990), and Idrees *et al.,* (2013), whereas Singh *et al.,* (1993), reported leucopenia with neutrophilia. The death of cow could have resulted due to the hemolytic, necrolytic and histotoxic effects of the *Clostridium chauvoei* (Quinn *et al.,* 2005).

Black quarter has an acute onset and high case fatality rates in dairy cattle especially Jersey cows. Early detection of the disease and use of high dose of penicillin's could save the affected animals. It can be prevented and controlled in endemic areas by vaccination of young calves at an early age with booster vaccination at sixth month, which gives good immunity and protection for several years.

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Macerated Fetus in a Bitch – A Case Report

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ABSTRACT

A Great Dane dog aged three and half years, delivered two months back was presented with history of recurrent blood tinged foul smelling vaginal discharge. Abdominal palpation revealed presence of a hard mass in the abdomen. On transabdominal ultrasonography bright hyperechoic areas were seen in the region of the uterus. Laprohysterotomy was performed. Fetal bones were found embedded in the uterus. The fetal bones were removed and the patient was treated with broad spectrum antibiotics for one week and made an uneventful recovery.

Key words: Fetal maceration, bitch, hysterotomy

Pregnancy loss may occur at any stage of gestation and has been observed in all species of domestic animals (Roberts, 1971). If fetal death occurs in the first half of pregnancy, fetal resorption takes place, whereas fetal death occuring during the second half of pregnancy result is an abortion or the birth of a stillborn. In cases where there is a failure of expulsion of fetus at the end of gestation, perhaps due to uterine inertia and intrauterine infections, fetal emphysema and maceration occurs (Johnston et al., 2001).

Although maceration of the foetus can occur in any species, it is described less frequently in dogs (Roberts, 1971). Bacteria enter the uterus through the dilated cervix, and by a combination of putrefaction and autolysis, the soft tissues are digested, leaving a mass of fetal bones within the uterus (Jones *et al*., 1997). Sometimes these become embedded in the uterine wall and are difficult to remove other than by hysterotomy. Under these circumstances a chronic endometritis ensues and there is severe damage to the endometrium (Noakes et al., 2001). The condition of retained fetal bones *in utero* is recognized in canine practice, but rare. Bitches that retain macerated fetuses are usually systemically ill (England, 1998). The presented study describes a case of fetal maceration and its management in a female dog.

HISTORY AND CLINICAL SIGNS

A Great dane dog aged three and half years, delivered two months back was presented to the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bengaluru with the complaint of persistant, blood tinged, serosanginous, mucoid foul smelling vaginal discharge for the past two months. The animal delivered 14 puppies of which 6 were still born by normal vaginal delivery. The owner reported reduced appetite and loss of body condition of the animal after whelping.

On clinical examination the animal's temperature, heart hate and pulse rate was found to be normal. General body condition of the animal was poor. Complete blood count and serum biochemistry values were found to be within the normal range (RBC: 8.26 X 10^6 cells/µl; WBC: 17,200 cells/µl; PCV: 43.6%; Hb: 14.9g%; platelet count: 2,72,000 cells/µl; creatinine: 1mg/dl; SGPT: 16 IU; BUN: 8.7).

DIAGNOSIS AND TREATMENT

Abdominal palpation revealed presence of a hard mass in the abdomen. Transabdominal ultrasonography was performed which revealed presence of hyperechoic areas in the uterus (Fig 1.), which was confirmed by radiograph of the lateral abdomen.

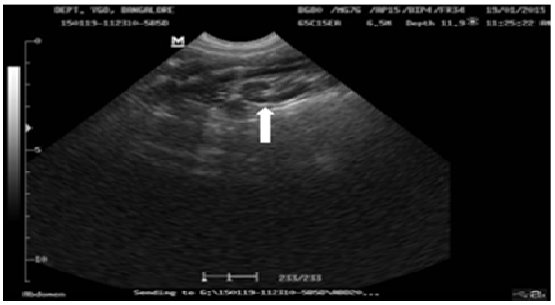

Fig 1. Real time 2D ultrasonography of the abdomen with hyperechoic areas (indicated by arrow) in the region of the uterus.

Radiograph revealed presence of a radio dense mass around 2.5 cm in diameter in the abdomen, suggestive of fetal maceration (Fig 2.). Based on these diagnostic approaches, it was diagnosed as a case of fetal maceration. The dog was subjected to laparohysterotomy.

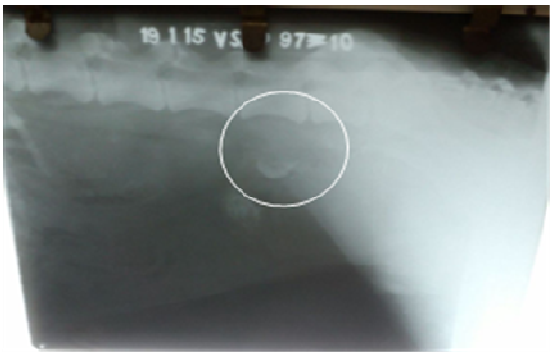

Fig 2. Radiograph of the caudal abdomen with radio dense mass (encircled).

Laparohysterotomy was performed under general anesthesia with 12.5 mg/kg body weight of thiopentone sodium 2.5% solution (Inj. thisol®) intravenously after premedication with atropine sulphate at the dose rate of 0.04mg/kg *(Inj Atropine®, 2.5ml,s/c)* and diazepam at the dose rate of 0.5mg/kg body weight*(Inj. Calmpose 4ml i/v)*. The ventral abdomen was aseptically prepared and a mid-ventral celiotomy was performed. The uterus was exteriorized and hard, firm swelling was detected in the left uterine horn closer to the ovarian end. A longitudinal incision was made on the uterine horn and the retained fetal bones (Fig 3.) which were found to be embedded in the uterus was removed.

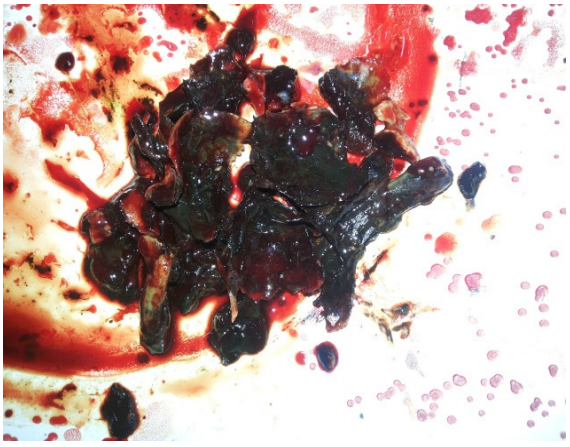

Fig 3: Retained fetal bones which were blood tinged and embedded in the endometrium

The hysterotomy incision was closed as per standard procedure. Since the animal was still young and the owner wished to breed the animal in the future, ovario-hysterectomy was not performed. Post-operative care included a course of broad spectrum antibiotic- cephalexin 500mg bid for 7 days (Tab Lixen palatab®). The surgical wound was dressed on alternate days and the sutures were removed on the 10th day after surgery and the dog made an uneventful recovery.

DISCUSSION

Fetal maceration may occur at any stage of gestation and has been observed in all species. It is more commonly observed in cattle (Roberts, 1971). In multiparous animals, maceration of early embryos and fetuses usually ends in their being resorbed. The other fetuses develop normally or occasionally some become macerated in turn by extension of infection. However, it can also occur in full term fetuses that fail to expel from uterus (Jackson, 2004). In long standing cases of fetal maceration such as this case report, the acute emphysematous stage has passed, straining is seldom observed, and the cervix is usually quite contracted. Generalized symptoms of elevated temperature and pulse are usually absent In the present case report, the dog had delivered live pups and the unexpelled fetus would have probably become macerated. Johnston et al. (2001) reported presence of a foul and fetid uterine discharge in dogs that retain macerated fetuses. On the contrary, presence of recurrent odorless, serosanguinous vaginal discharge since three months seen in this case finds agreement with the observations of Serin and Parin (2009) who reported serosanguinous vaginal discharge for two months in a case of fetal maceration in a non-descript female dog. Diagnosis of fetal maceration can be made by abdominal palpation, ultrasonography and radiography (Jackson, 2004; Kealy *et al*., 2011). Confirmatory diagnosis of retained fetal bones here was done by visualization of echogenic fetal bones on ultrasound scanning. Management of fetal maceration cases involves ovariohysterectomy or hysterotomy and removal of fetuses. Prostaglandin therapy may be successful only in cases of fetal maceration where the skeletal material is not embedded into the

uterine tissues (Feldman and Nelson, 1996). However in cases where it is embedded into the uterus, surgical intervention is the only recommended procedure. Laparohysterotomy performed in the present case justifies the approach because the fetal bones were embedded. On laprohysterotomy, the uterus was shrunken and thick walled. Severe degenerative and sclerotic changes had occurred in the endometrium. Surgical management for this case for successful and the dog made an uneventful recovery.

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Pityriasis in a piglet - A case report

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ABSTRACT

A three month old cross breed male piglet was presented to the veterinary hospital, Chikkanayakanahalli, Tumkur, Karnataka, with a complaint of skin lesions on the ventral aspect of the body. Clinical examination revealed large coalescing ringworm like lesions on ventral abdomen and inner aspect of thighs. The lesions were ring like small erythematous papules with raised and reddened borders. As the laboratory examination revealed negative for fungal infection and mite infestation, the condition was diagnosed as Pityriasis Rosea by recognizing the characteristic lesions. Topical antibiotic cream application on lesions was advised twice daily, the lesions were improved slowly by a week and complete recovery was observed by the end of 6th week.

Key words: Piglet, pityriasis, Erythematous lesions

Pityriasis rosea is a sporadic disease of unknown etiology of pigs, usually 8-14 week old, but occasionally as young as 2 weeks and very rarely in pigs up to 10 months. One or more pigs from the litter may be affected. The disease is mild, but transient anorexia and diarrhea have been reported. But as soon as the skin rash appears, these digestive disturbances disappear. The initial skin lesions were small erythematous papules, which rapidly expand to form a ring (collarette) with distinct raised borders. The lesions enlarge at their periohery, and adjescent lesions may coalesce. The centre of the lesion is flat and covered with bran- like scale overlying normal skin. The lesions are found predominantly on the ventral abdomen and the inner thighs but occasionally may be seen over the back, neck and legs. Characteristically, there is no pruritus and recovery may be spontaneous in 6-8 weeks.

History: A three month old cross breed male piglet was presented to the Veterinary Hospital, Chikkanayakanahalli, Tumkur, with a complaint of skin lesions on the ventral aspect of the body since last two weeks. The piglet was weaned and ready to sale. There was no history of diarrhea but the piglet was slightly anorectic. Other piglets of the same age group in the same herd were normal. Owner seeked veterinary assistance because of the appearance of an unsightly skin disease was adversely affecting the saling price of the piglet. The piglet was already treated with topical antifungal cream for the last one week.

Clinical examination: Affected piglet had large coalescing ringworm like lesions on ventral abdomen spread up to groin region and inner aspect of thighs (fig). The lesions were small erythematous papules, forming a ring with raised and reddened borders. In the centre of the ring a light scab developed and the area was covered with normal bristles.

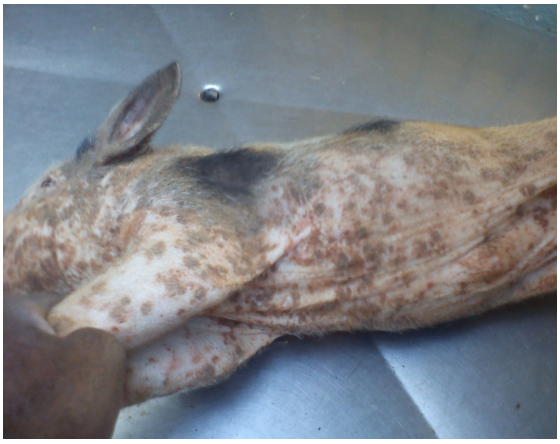

Fig: Piglet with Pityriasis skin lesions on ventral side of abdomen, even over neck and legs.

Laboratory findings and Diagnosis: Brisles and deep skin scrapings were examined microscopically to find out mite infestation. The examination revealed negative for the presence of mites. Even though the lesions were identical to ring worm lesions, it has been ruled out because the lesions not responded to antifungal cream.

As the other piglets of same herd were normal, it is confirmed as non contagious nature of

infection. Laboratory examination revealed no infectious cause, hence the condition was diagnosed as Pityriasis rosea by recognizing the characteristic lesions on the body.

Treatment: Though the spontaneous recovery is anticipated application of antibiotic cream was advised on lesions twice daily to prevent secondary bacterial infections. The lesions improved slowly by a week and 50% of the lesions disappeared in 3 weeks. Almost complete recovery was observed by the end of 6th week except few lesions in groin region, which were worsened by maggot infestation. Maggot wounds were cleaned and treated with meggoticidal sprays regularly and the piglet was completely recovered in 6½ weeks.

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Foetal Mummification in a Crossbred Jersey Heifer: A Case Report

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ABSTRACT

A primipara crossbred Jersey heifer was presented with a history of prolonged gestation and straining from last 2 days. On the basis of history and clinical examination, heifer was diagnosed as mummified foetus entangled in the mucosal folds of the uterus and was successfully removed by traction after induction of the epidural anaesthesia and lubricating the birth canal. Heifer was treated with lenova-AP intra uterine and with other supportive theraphy for three days. Animal was not served in the subsequent oestrus and inseminated thereafter.

Key words*:* Mummification; traction; cervix

Foetal mummification is a gestational disorder and is responsible for economic loss to the farmer. The incidence of mummified foetus might be higher in some herds but the incidence was usually lower and sporadic in cattle (Roberts, 1971). The incidence of foetal mummification in cattle was 0.13-1.8% (Barth, 1986). Talbot & Hafs (1974) treated the mummified foetus in cow with Prostaglandin F2α. Mummified foetus might also be treated with intramuscular administration of Stilbestrol, Estradiol, Respositol diethylstilbestrol or manual removal of the persistent corpus luteum (Roberts, 2002). Dabas and Chaudhari (2011) successfully managed the foetal mummification using Prostaglandin F2α in a crossbred cow. Most of the mummified foetuses would remain in the uterus until they were treated to expel or until were removed by caesarean section (Wenkoff & Manns, 1977). Arthur et al. (1996) showed that treatment of mummified foetus with Prostaglandin F2α created some complexicity in cattle like maceration of mummified foetus and packed in the birth canal instead of expelled out. It occurs as a consequence of uterine infection, pyometra, chronic endometritis and finally the animal should therefore be sent for slaughter. Azizunnesa, *et al., (*2010) preferred caesarean section for removal of the mummified foetus rather than Prostaglandin F2α injection. Sumit Singhal *et al.,* (2012) had successfully managed a case of foetal mummification with open cervix by applying simple traction. The present case was treated by obstretic manoeuvres and medications.

CASE HISTORY AND OBSERVATION

A three years old Jersey cross bred heifer with a history of prolonged gestation and straining from the last 2 days was presented to the Teaching Veterinary Clinical Complex, Veterinary College, Shimoga. Animal was inseminated 10 months back. Animal was found normal. On per-rectal examination revealed that absence of foetal movements, fremitus and cotyledons and there was a hard mass in the uterus. Per-vaginal examination revealed that cervix was relaxed and was dilated more than four fingers. Orbit of the foetus was palpated with no eye ball and body surface of the foetus was found leathery, tough with sticky mucus. There was no bad odour coming out during examination, which indicated absence of infection in the uterus. Finally, the case was diagnosed as foetal mummification.

TREATMENT AND DISCUSSION

The mummified foetus was removed by obstetrical manoeuvres (traction) procedure (Fig:1) after induction of epidural anaesthesia(2% lignocaine hydrochloride) and sufficient lubrication of birth canal. On examination of the foetus revealed skeleton of about 3 months of age with skin adhered tightly over skeletal mass and eye orbits without eyeball. Post operative care involved Lenovo-AP (Levofloxacin, Ornidazole and Alpha tocopheral) 30 ml IU, Inj. Anistamin (Chlorpheniramine maleate)10 ml i/m, Inj. Belamyl 10 ml i/m for 3 days and Inj. Pragma (Cloprostenol) 2 ml i/m to enhance uterine contractions and further involution of the uterus. The animal was recovered

an showed regular oestrus cycle. The present case of examination and treatment were in agreement with the Roberts (2002) and Sumit Singhal *et. al.,* (2012). On examination, the ovaries were found smooth and cervix was dilated. It was possibly due to repeated rough manipulation of ovaries leading to removal of pregnancy corpus luteum resulting in the uterine contractions or initiation of parturition.

Fig 1: Mummified foetus removed by traction.

CONCLUSION

Foetal mummification can be easily diagnosed based on the history of prolonged gestation and per-rectal examination.

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