Antibiogram Study of *Salmonella* spp. from Field Samples of Small Ruminants

Paramesh, B.N., Basavaraj[1], A., Arun[1], S.J., Bhoyar[2], R., Abhilash, B., Revappaya, M. and Ramesh, S.K.
Department of Veterinary Microbiology, Veterinary College, Bidar
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ABSTRACT

For this study a total number of 140 rectal swab samples of sheep and goats were collected. Out of 140 samples, 8 samples were positive for *Salmonella* spp. All the *Salmonella* isolates revealed the similar morphological, cultural and biochemical characteristics. Eighteen commonly used antibiotics were used for the antibiogram study. All the isolates were resistant to erythromycin, chloramphenocial, trimethoprim and three of them were resistant to cloxacillin, tazobactam and nalidixic acid. All the isolates were sensitive to gentamicine (91.7%), amikacin (90%), ampicilin (80%) and ciprofloxacine (81.8%). The detection of *Salmonella* species in feces from field indicates the need for special attention to prevent incidence in animal and human population in the area.

Keywords: *Salmonella*, Small ruminants, Biochemial and Antibiogram

Salmonella, an important food borne pathogen of zoonotic significance, has often been shown to be endemic in India since decades in humans and animals (Chandra *et al.,* 2005). *Salmonella* has emerged as a pathogen of almost all the vertebrates probably due to its large number of serovars and rapid adaptation to different hosts. Salmonellosis is a hyper endemic disease in India affecting both man and animals (Kumar *et al.,* 1997). There are 2673 serovar of *Salmonella* (Guibourdenche *et al.,* 2010). Extensive usage of antibiotics prior to testing of etiologic agent resulting multi-drug resistant to *Salmonella* strains (Mirza *et al*., 1996) and thus limits the therapeutic success in the treatment of the disease. To select the suitable antibacterial agents for effective therapeutic use against Salmonellosis in sheep and goats, the antibiogram of *Salmonella* isolates should be performed. Therefore, the present research work was undertaken to isolate and identify *Salmonella* spp. from apparently healthy and diseased sheep and goats through cultural, morphological, biochemical characterization and to study the antibiogram of the isolated *Salmonella* spp.

MATERIALS AND METHODS

Collection of samples

One hundred and forty fecal samples were collected from various farms in and around Bidar wherein 76 samples were taken from diarrhoeic cases and 64 samples from healthy sheep and goats. All the samples were subjected to bacteriological isolation.

Isolation of *Salmonella*

The conventional methods for the detection of *Salmonella* was performed following the standard guide lines from ISO 6579:2002. This isolation and identification procedure involved four principle stages: pre-enrichment, selective enrichment, selective plating and confirmation.The collected samples were added to the pre-enrichment media (Selenite F broth and Tetrathionate broth) and were incubated at 37°C, for 24 hours. The suspected cultureswere streaked onto MacConkey's agar, XLD agar, Salmonella Shigella agar and BGA. The inoculated plates were incubated for 24-48 hours at 37°C. Suspected colonies were confirmed primarily by Gram's staining method (Ammar *et al*., 2011).

[1]Department of Veterinary Microbiology, Veterinary College, Shivamogga.
[2]Associate Professor, Department of Veterinary Medicine, Veterinary College, Bidar.

Identification of *Salmonellae*

The pure cultures were the subjected for biochemical test using Sugar fermentation reaction, Citrate utilization test, Hydrolysis of urea, H_2S production, Methyl red test, Voges-Proskauer test and urease test (Karim *et al*., 2008) etc.

Antibiotic susceptibility test

Preparation of standardized inoculum: Four colonies were transferred to a tube containing 5 ml of Muller-Hinton broth and incubated at 37°C for 12hrs.

Inoculation of the test plate: A sterile cotton swab was dipped into bacterial suspension, after removal the excess fluid, the swab was used to streak the dried surface of Muller-Hinton plate by rotating the plate.

Placement of disks: With fine pointed sterile forceps, the selected antibiotic disks were placed on the inoculated plates and the distance between disks was not less than 24 mm.

Reading the results: The degree of sensitivity was determined by measuring the visible and clear zone of inhibition into the surrounding medium.

RESULTS AND DISCUSSION

Colony morphology and biochemical tests for all the isolates were typical for *Salmonella* spp.

Cultural and Morphological characterization

On MacConkey agar plates, all the *Salmonella* isolates appeared lactose non-fermenter, colourless and transparent. On brilliant green phenol red lactose sucrose agar, the colonies were pinkish to red coloured and transparent. On Salmonella-Shigella agar and on Xylose Lysine Deoxycholate agar black color colonies were produced due to production of H_2S. On the basis of cultural characteristics 8 samples were confirmed to be *Salmonella*. On Gram's staining, bacteria were small rod shaped, Gram negative, single or paired in arrangement (Fig.1).

Biochemical characterization

All suspected colonies of *Salmonella* on the basis of cultural and morphological properties were subjected to biochemical tests (Table 2) *viz*., Urea hydrolysis, catalase and triple sugar iron agar. Eight isolates were confirmed to be *Salmonella* species on biochemical test results. Therefore the basis of cultural characteristics and biochemical reaction 8 were confirmed to be *Salmonella* spp.

Antimicrobial sensitivity of isolated *Salmonella* species

All the confirmed isolates were subjected to 18 different antimicrobials. All the isolates were 100 per cent resistant to erythromycin, chloramphenocial and trimethoprim and two of them were resistant to tazobactam. Isolates were 100 per cent sensitive to amikacin, ampicillin, ciprofloxacin and gentamicin.

CONCLUSION

In present study the overall prevalence of *Salmonella* in sheep and goats was 5.7 per cent which is of economic and public health significance for livestock practice. All the isolates of *Salmonella* spp. were resistant to more than one antibiotics.

Table 1: Details of samples collected

Details of farms	Number of fecal samples collected	Diarrhoeic animals	Non diarrhoeic animals	Total number of isolates
Kamatana village	15	8	7	-
Chimakodi village	20	12	8	2
City Hospital	15	6	9	4
ILFC Veterinary College Bidar	10	5	5	-
LRIC Halliked	80	45	35	2
Total	**140**	**76**	**64**	**8**

Table 2: Summary of the biochemical test results of *Salmonella* isolates

Sl. No.	Biochemical test	G1	G2	G3	G4	G5	G6	G7	G8
1	Indole formation	-	-	-	-	-	-	-	-
2	Methyl red test	+	+	+	+	+	+	+	+
3	Voges- Proskauer Reaction	-	-	-	-	-	-	-	-
4	Citrate utilization	+	+	+	+	+	+	+	+
5	Urea hydrolysis	-	-	-	-	-	-	-	-
6	H_2S production on TSI	+	+	+	+	+	+	+	+
7	Catalase	+	+	+	+	+	+	+	+

(+) Positive after 48hrs of incubation

(G) Isolates *Salmonella*

Table 3: Showing antibiotic resistant pattern of *Salmonella* species

Sl. No	Antibacterial agent	G1			G2			G3			G4		
		S	I	R	S	I	R	S	I	R	S	I	R
1	Ampicillin	+	-	-	+	-	-	+	-	-	+	-	-
2	Amoxicillin/clavulanic acid	-	-	+	-	-	+	-	-	+	-	-	+
3	Gentamicin	+	-	-	+	-	-	+	-	-	+	-	-
4	Kanamycin	-	+	-	-	+	-	-	+	-	-	+	-
5	Enrofloxacin	-	-	+	-	+	-	-	+	-	-	+	-
6	Ciprofloxacin	+	-	-	+	-	-	+	-	-	+	-	-
7	Erythromycin	-	-	+	-	-	+	-	-	+	-	-	+
8	Ofloxacin	-	+	-	-	+	-	+	-	-	+	-	-
9	Tetracycline	-	+	-	-	+	-	-	+	-	-	+	-
10	Colistin	-	-	+	-	-	+	-	-	+	-	-	+
11	Cephalexin	-	+	-	-	+	-	+	-	-	+	-	-
12	Nalidixic acid	+	-	-	-	+	-	-	+	-	-	+	-
13	Chloramphenicol	-	-	+	-	-	+	-	-	+	-	-	+
14	Neomycin	-	+	-	-	+	-	-	+	-	-	+	-
15	Sulfatrimethoprim	-	-	+	-	-	+	-	-	+	-	-	+
16	Co-trimaxazole	-	+	-	+	-	-	+	-	-	-	+	-
17	Tazobactam	-	-	+	-	+	-	+	-	-	-	-	+
18	Amikacin	+	-	-	+	-	-	+	-	-	+	-	-

(G) Isolates *Salmonella* (S) Sensitive (I) Intermediate (R) Resistant

Table 4: Showing antibiotic resistant pattern of *Salmonella* species

Sl. No	Antibacterial agent	G5			G6			G7			G8		
		S	I	R	S	I	R	S	I	R	S	I	R
1	Ampicillin	+	-	-	+	-	-	+	-	-	+	-	-
2	Amoxicillin/clavulanic acid	-	+	-	-	-	+	-	-	+	+	-	-
3	Gentamicin	+	-	-	+	-	-	+	-	-	+	-	-
4	Kanamycin	-	+	-	-	+	-	-	+	-	-	+	-
5	Enrofloxacin	+	-	-	-	+	-	-	+	-	-	+	-
6	Ciprofloxacin	+	-	-	+	-	-	+	-	-	+	-	-
7	Erythromycin	-	-	+	-	-	+	-	-	+	-	-	+
8	Ofloxacin	+	-	-	-	-	+	-	+	-	+	-	-
9	Tetracycline	-	+	-	-	+	-	+	-	-	+	-	-
10	Colistin	-	-	+	-	+	-	-	+	-	-	+	-
11	Cephalexin	-	-	+	-	-	+	-	-	+	-	-	+
12	Nalidixic acid	-	+	-	-	+	-	+	-	-	-	+	-
13	Chloramphenicol	-	-	+	-	-	+	-	-	+	-	-	+
14	Neomycin	-	+	-	-	-	+	-	+	-	-	+	-
15	Sulfatrimethoprim	-	-	+	-	-	+	-	-	+	-	-	+
16	Co-trimaxazole	-	-	+	-	+	-	-	-	+	-	-	+
17	Tazobactam	-	+	-	-	+	-	-	+	-	-	+	-
18	Amikacin	+	-	-	+	-	-	+	-	-	+	-	-

(G) Isolates *Salmonella* (S) Sensitive (I) Intermediate (R) Resistant

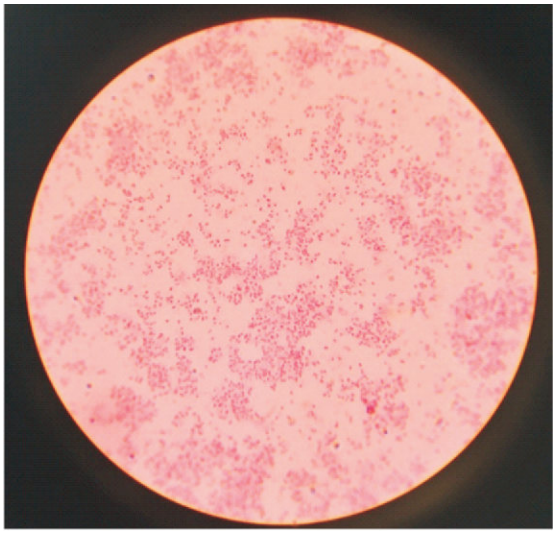

Fig. 1: Gram's staining

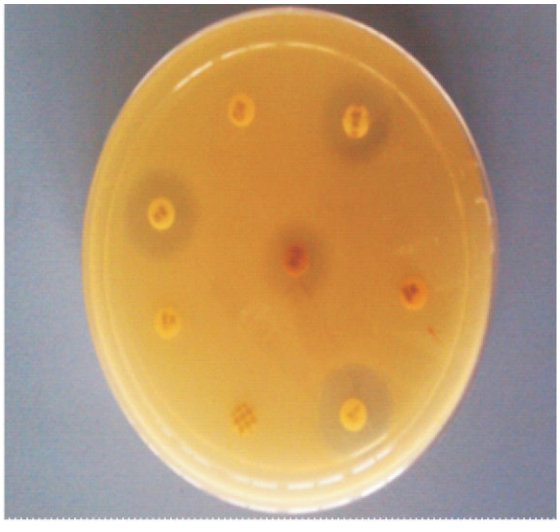

Fig. 2: Plate showing the antibiogram sensitivity pattern of *Salmonella* isolate

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Studies on Prevalence of Salmonellosis in Free Ranging Birds*

Nagaraj, K[1]., Narayana Bhat, M[2]. and Srikrishna Isloor[3]
Wildlife Unit, Veterinary College, Hebbal, Bengaluru-24
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ABSTRACT

The research work was undertaken to study the prevalence of Salmonella infection among free-ranging wild birds in Karnataka during the year 2012-13. A total of 131 fecal samples were collected from free-ranging wild bird sanctuary/congregation locations. The samples were analyzed byculture and biochemical characterization. Two (1.53%) of 131 fecal samples were positive for *Salmonella* organisms. None of the 10 environmental samples such as soil and water from free-ranging wild bird habitats was positive for *Salmonella* organism.

Keywords: *Salmonella*, Free-ranging, Wild birds

Birds are the most populous life forms on the planet. Infectious diseases are one of the possible threats for wildlife conservation, which include Avian Influenza, Psittacosis, Avian Tuberculosis and Salmonellosis especially for the avifauna. Among these, Salmonellosis is "a hundred years old and still going strong" (Wray, 1995). Avian salmonellosis refers to the infection of birds with *Salmonella* bacteria, usually *S. Typhimurium*. Currently, over 2500 distinguishable variants /serotypes are recognized (OIE Terrestrial Manual, 2010), which have been reported from a wide variety of animals including reptiles, birds and mammals.The present study was carried out to know the prevalence of salmonellosis in free ranging wild birds.

MATERIALS AND METHODS

Fecal droppings and environmental (soil/water) samples were collected randomly from bird sanctuary /bird congregation locations such as Bird Sanctuary, Kokkarebellur, Mandya (46 droppings), Kanive and Doddaluvara ponds, Kodagu (47 droppings) and Bird Sanctuary, Mandagadde, Shimoga (38 droppings). A total of 131 fecal droppings and ten environmental samples from above locations were collected. Approximately one gram of feces or soil either fresh or dry from large sized birds and pooled fecal droppings in smaller birds were collected from these areas in to 10 ml buffered peptone water (BPW), about 10 ml water from environment in to 50 ml of BPW and were incubated at 37 °C over night. One ml of the pre-enrichment broth mixture was transferred to tetrathionate brilliant green broth (1:10) and to Rappaport-Vassiliadis soya peptone broth, followed by incubation at 37 °C for 24 hrs. Aliquots of the incubated broth culture were plated on xylose lysine desoxycholate agar (Hi-Media, Mumbai), Hektoen enteric agar and incubated at 37 °C for 24 hrs. Environmental soil samples were processed similar to fecal samples. Colonies showing typical characteristics/features for *Salmonella* were selected for biochemical characterization. Suspicious colonies were subjected for Gram's staining to identify Gram's negative rods and such colonies were inoculated on to triple sugar iron (TSI) agar slants and incubated for 18-24 hrs at 37 °C to identify alkaline slant (red) with H_2S production typical for *Salmonella*. They were further subjected to urease test, oxidase test, nitrate reduction test, indole test, methyl red (MR) test, Voges-Proskauer (VP) test and citrate utilization test for

* Part of M.V.Sc thesis submitted to KVAFSU, Bidar, Karnataka.
[1]Present address: Veterinary Officer, Veterinary Hospital, Somwarpet, Kodagu.
[2]Professor and Head, Department of TVCC, Veterinary College, Hebbal, Bengaluru.
[3]Associate Professor, Department of Microbiology, Veterinary College, Hebbal, Bengaluru.

biochemical characterization of *Salmonella* as per Bergey's manual, 2005.

RESULTS AND DISCUSSION

In the present study, one (2.17%) out of 46 samples from Bird sanctuary, Kokkarebellur, Mandya, one (2.13%) out of 47 samples from Kanive and Doddaluvara ponds of Kodagu yielded positive result for *Salmonella* organism which was confirmed by culturing and biochemical tests. However, no *Salmonella* organism was recovered from 38 samples collected from Bird sanctuary, Mandagadde, Shimoga. Overall two (1.53%) of 131 fecal samplesfrom two free ranging bird sanctuaries/sites revealed *Salmonella* organisms. However, Pennicott *et al*. (2002) reported prevalence of 42-48% at bird feeders and 33% below roost site. Low prevalence in the present study could be attributed to environmental, seasonal and geographical variations. Post mortem results by Refsum *et al*. (2003) and Hall and Saito (2008) recorded 64.8% and 21.5% of cases respectively involving Salmonellosis. Prevalence of *Salmonella* organisms in fecal droppings of free-ranging birds was comparatively low. Nevertheless, it indicates existence of carrier state of Salmonellosis in free-ranging study areas in the state, the increased incidence which can be of zoonotic importance. Arrival of migratory birds to these areas poses threat of introduction of new serotype of *Salmonella*. Constant monitoring of birds in the sanctuary during and after migration is advisable to study the actual status of transmission of *Salmonella* from one geographical area to other. Further, seasonal variations of prevalence of *Salmonella* can be undertaken to understand the impact of environment on spread of salmonella. Some reports of high incidents of *Salmonella* in free ranging birds during post mortem examination indicates that, these organisms can cause severe loss of life under favorable circumstances. However, none of the environmental sample was positive for the *Salmonella* organism.

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Cutaneous Squamous Cell Carcinoma in Dogs: Ki 67 and Survivin Expression*

Kavya, N., Suguna Rao, Sathyanarayana, M.L., Narayanaswamy, H.D., Ranganath, L[1]., Kamran, A[2]. and Byregowda, T.R.

Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru

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ABSTRACT

The present study was carried out to demonstrate the expression of Ki 67 proliferation antigen and survivin genes in 8 cases of canine spontaneous cutaneous squamous cell carcinoma and to know the correlation between the expression profile of both the markers and the outcome of tumour condition through follow up study using immunohistochemistry (IHC) and Quantitative Taqman Real Time PCR assay (qRT-PCR). Ki 67 and Survivin expression was detected in all cases of squamous cell carcinoma encountered, survivin expression positively coincided with the cell proliferation in squamous cell carcinoma and over expression of Ki 67 and survivin was associated with poor post surgical outcome. The expression of Ki67 and survivin between various post surgical outcome groups showed significant difference ($P<0.05$). Ki67 index and survivin expression correlated well and were useful in predicting prognosis of canine spontaneous cutaneous squamous cell carcinoma.

Keywords: Cutaneous squamous cell carcinoma, Ki67, Survivin, IHC, qRT-PCR, Post surgical follow up

In cancer biology assessment of cell proliferation is an important factor which indicates prognosis in many types of tumours. Recent advances have identified a number of tumour markers that may form a basis for tumour stratification (Donnay *et al*., 1995). Inhibitors of apoptosis proteins (IAPs) are a family of such markers that interfere with the activation of caspases by various mechanisms (Salvesen and Duckett, 2002). Survivin is one of the members of IAP that regulates cell division and suppresses apoptosis. Currently survivin protein expression is being used as a prognostic factor in several human neoplasms. Knowledge of the distribution of survivin in canine tissues, and its role in neoplastic processes is important as high survivin expression by neoplasms correlates with more aggressive behaviour, decreased response to chemotherapeutic agents and shortened survival time as compared to cancers that are survivin negative (Li, 2003). Survivin expression is also associated with increased expression of those proteins that influence proliferation such as Ki 67 and PCNA. Ki 67 protein (pKi 67) is a large non-histone nuclear protein present during all active phases of the cell cycle (G1, S, G2 and M) but absent in quiescent or resting cells (Madewell, 2001 and Mukaratirwa, 2005). Ki 67 expression has been found to be an excellent independent prognostic factor for cell proliferation, metastasis, disease-free survival rate, and overall survival rate.With this back ground, the current study was taken up to demonstrate the expression of survivin and Ki 67 in canine spontaneous squamous cell carcinoma in order to evaluate and predict the outcome of cutaneous squamous cell carcinoma in dogs.

MATERIALS AND METHODS

The study was carried out in the Department of Veterinary Pathology, Veterinary College, Hebbal Bengaluru, during 2015-2016, on 8 cases of squamous

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[1] Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru.

[2] Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru.

cell carcinoma out of 83 cutaneous and subcutaneous neoplasms in dogs presented to Department of Veterinary Surgery, Veterinary College, Hebbal, Bengaluru. A follow up study for a minimum period of 8 months was carried out post surgically in all the dogs.

Histopathology: Representative tissue samples obtained after surgical excision were fixed in 10 per cent neutral buffered formalin and processed by routine paraffin embedding technique. Sections of 4-5 ìm thickness were taken and cut sections were stained with hematoxyline and eosin.

Quantitative real-time qRT-PCR for survivin mRNA expression: Total RNA was isolated using TRIzol® ready to use solution procured from M/s Invitrogen (USA) and used as per manufacturer's recommendations. Complementary DNA (cDNA) was prepared for RNA sequences encoding survivin gene of dog using gene specific primers. Quantitative Taqman Real Time PCR assay was carried out for survivin (anti apoptotic protein) and GAPDH (housekeeping gene) mRNA using the Thermal cycler (EPPENDORF realplex 2.2) instrument according to the manufacturer's instructions. Published sequences available in the gene bank were used for the designing of required primers for thestudy. Primers were designed using primerblast sequence analyzing softwares. The published reference sequence of survivin for dog was from NCBINo: NM-001003348.

Real-time PCR amplification reaction was carried out in a 20 µl reaction mixture containing 10 µl each of mastermix (3B quantimix) and samples were used in duplicate. Relative gene quantification was done by comparative Ct method, and the values were expressed as relative to the reference sample used, as calibrator.

Immunohistochemistry of Ki 67 proliferation antigen: For IHC staining, 4-5 µm sections were deparaffinised and hydrated. Slides were then subjected to heat induced epitope retrieval by incubation in Tris –EDTA Buffer of pH 9 in a microwave oven, followed by 20 min cool-down and treatment with 3% hydrogen peroxide for 10 mins before antibody application. Sections are covered with anti-Ki 67 antibody (monoclonal mouse anti-human antibody for Ki-67 antigen clone GM001; ready to use, PathnSitu, Bengaluru) for one hour at room temperature. After washing with PBS the section were incubated with PolyExel Target binder (Polyclonal goat anti-mouse immunoglobulins; PathnSitu, Bengaluru) for 25 mins at room temperature. Then sections were washed in PBS and incubated with PolyExel HRP (PathnSitu, Bengaluru) for 20 mins followed by washing in two changes of PBS and

Primers and probes used for qRT-PCR

Primer code		Primer sequence	Product size (bp)
Canine survivin F	5'-3'	TCATCTGGTTGTGCTTTCCT	88
Canine survivin R	5'-3'	TGGCTCTTTCTTTGTCCAGT	
Survivin probe	3'-5'	TCTGTCAAGAAGCAGTTTGAAGA	

Thermal cycling conditions for amplification of dog survivin gene

Stage	Temperature (°C)	Duration	No. of cycles
Initial denaturation	95	3 minutes	1
Denaturation of cDNA	95	5 seconds	40
Annealing of primers	59.6	20 seconds	
Extension	65	20 seconds	

incubation with StunnDAB (PathnSitu, Bengaluru) for five mins at room temperature. Then the sections were counterstained with Harris hematoxylin. To determine Ki 67 index per tumour section, approximately 1000 neoplastic cells were counted in 10 representative fields of vision at high magnification. The number of positive cells per 1000 cells was expressed as percentage.

Statistical analysis: Statistical analysis was performed using the statistical software R version 3.2.4. Mean values and standard error of mean were calculated and all values were expressed as (Mean±SE). The data were analyzed by *t* test-unpaired, ANOVA Tukey test was used for finding the source of the differences in multiple groups. For all statistical analysis, p value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Among 83 cases of spontaneous cutaneous and subcutaneous tissue tumours 64 cases of malignant and 19 benign tumours were recorded. Out of 64 cases of malignant tumour, epithelial malignant neoplasms were the most common type of tumours observed (40 cases) among which 8 cases were diagnosed as squamous cell carcinoma based on the predominant cell type and histological characteristics, which formed a source of materials for the present study.

In this study, nondescript dog accounted for 3 cases followed by Labrador retriever (2 cases), Boxer (1 case), Dalmatian (1 case) and Irish setter (1 case). The age of susceptibility of dogs with squamous cell carcinoma varied from 5 to 10 years with an average age of 7.625 years. Grossly, the size of tumour growths was 3 to 10 cm at their highest diameter. Growths were round to oval in shape and some tumours showed ulceration with moderately firm consistency and pale brown or cream coloured cut surface as shown in Fig.1. In one case cut surface of the tumour showed multiple small cauliflower like growths with cystic fluid filled cavities. Occurrence of squamous cell carcinoma based on breed, age, sex and site are shown in Table 1. Histopathologically, squamous cell carcinoma revealed neoplastic squamous epithelial cells arranged in varying sizes of cell nests consisting of proliferating immature cells at periphery and mature cells in the centre with central keratinisation as shown in Fig. 1. The degree of cellular pleomorphism and the mitotic activity varied from moderate to high. The occurrence of squamous cell carcinoma with respect to breed probably depends upon popularity of these breeds and their relative number in different geographical areas. Gross and histopathological features of squamous cell carcinoma in the present study are adequately supported by the findings of Dayananda *et al. (*2009), Kashyap *et al.* (2013) and Al-akraa and Mostafa (2015). Immunohistochemistry was done to measure the growth fraction of tumours using anti-human Ki 67 antibody clone (GM001) raised against Ki 67 nuclear proliferation antigen. The expression of Ki 67 in squamous cell carcinoma is shown in the Table

Table 1: Occurrence of squamous cell carcinoma based on breed, age, sex and site

Cases	Breed	Age(years)*	Sex	Site
1	Non descript	10	Female	Flank
2	Labrador retriever	7	Female	Hip
3	Boxer	8	Male	Digit
4	Non descript	7	Female	Perianal
5	Non descript	10	Male	Paw
6	Dalmatian	6	Male	Shoulder
7	Irish setter	5	Male	Shoulder
8	Labrador retriever	8	Male	Perianal

* Mean age of occurrence 7.625 years

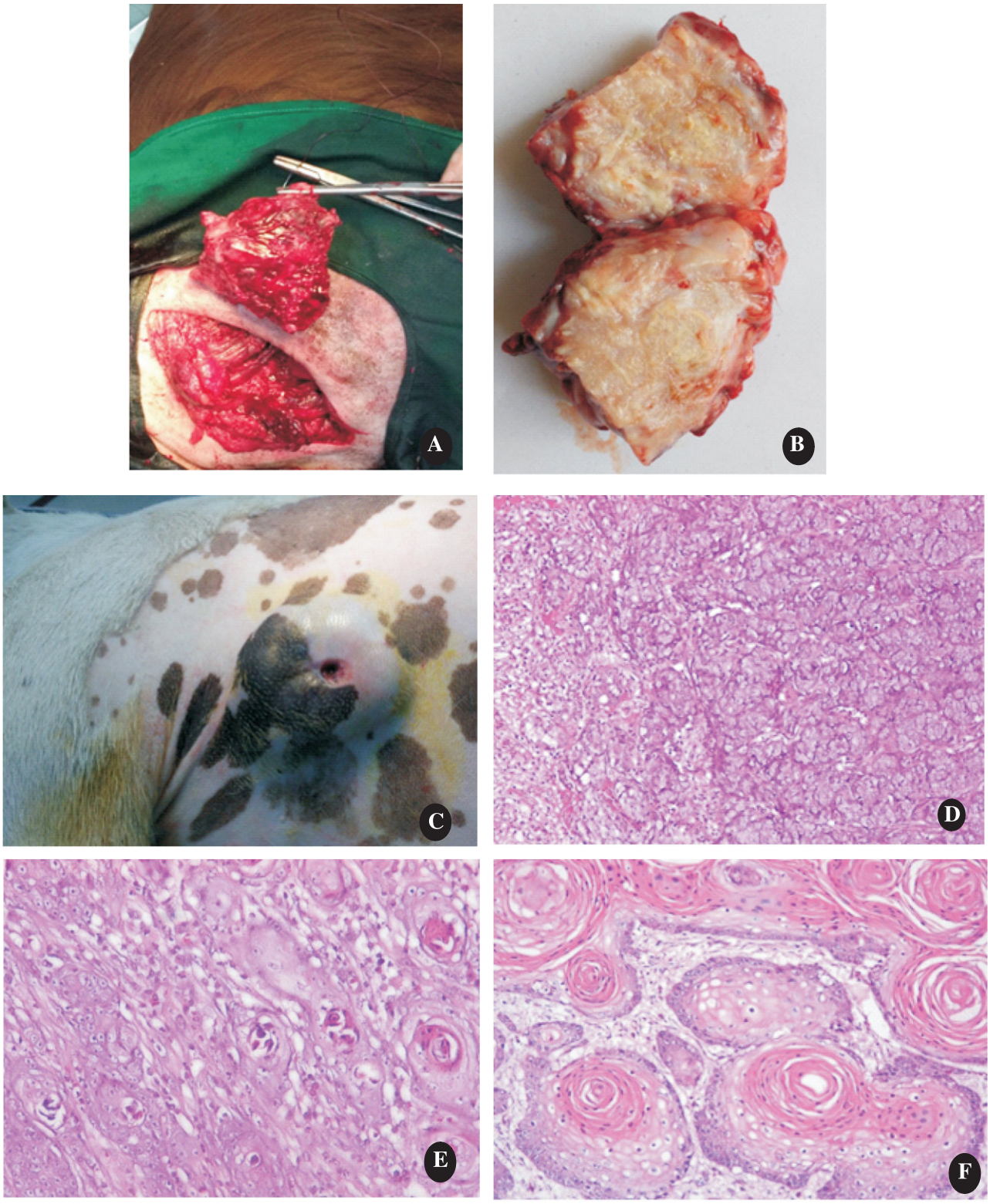

Fig. 1: Gross and histopathological sections of squamous cell carcinoma. An oval shaped growth with firm consistency removed from skin at shoulder region (A), Cut surface of tumour mass (B), Round shaped moderately firm tumour mass at shoulder region (C), Poorly differentiated Squamous cell carcinoma (D), Moderately differentiated Squamous cell carcinoma (E), Well differentiated Squamous cell carcinoma (F)

2. The positive reactivity for Ki 67 proliferation antigen was observed as dark brown coloured granular material restricted to nucleus and cells at the periphery of cell nest revealed more number of Ki 67 positive cells. The cells under mitoses were intensely stained and cell layers immediately next to keratin pearls were negative for Ki 67. In moderately and poorly differentiated squamous cell carcinoma where keratin pearls were not formed, Ki 67 positive cells were found distributed throughout the nests as shown in Fig. 2. The mean±SE of Ki 67 index of squamous cell carcinoma in the present investigation was 43.1±3.0.

The post surgical case follow up revealed that out of 8 cases two dogs were dead, three cases showed recurrence and other three were alive with disease free status till the end of follow up study. The mean Ki 67 index among dogs which were dead, alive and had recurrence of tumours during postsurgical follow was found to be highest in the dogs which died during follow up (54.45±6.05), followed by dogs which showed recurrence of tumour (41.86±1.67) and lower in the dogs which were alive till the end of follow up (36.76±2.33).The Ki 67 expression values were compared between the various post-surgical outcome groups which showed a statistically significant ($P \leq 0.05$) difference in expression between dead and disease free alive groups. This indicated that higher the expression of Ki 67 poorer will be the post surgical outcome. The results obtained in the present study are in accordance with those of Nieto *et al.* (2000), Zuccari *et al.* (2004), Dayananda *et al.* (2009), Kadthur *et al.* (2011) and Brodzki *et al*. (2014) who reported that Ki 67 is valuable marker in tumour grading as it's expression indicates the degree of malignancy, metastatic potential and low survival rate in various types of canine malignancies. In the present study however, no significant difference was observed between alive and recurrence groups.

The relative quantification of survivin gene was carried out to determine the level of expression of survivin gene in squamous cell carcinoma using real time PCR assay which revealed expression of survivin in all the cases tested. The mean±SE of survivin gene expression in squamous cell carcinoma was 22.16±7.83 (Table 2). The survivin gene expression in postsurgical follow up groups revealed that highest survivin expression was in the dogs which died during follow up (61.23±22.64), followed by dogs which showed recurrence of tumour (12.99±3.88) and lower survivin expression in the dogs which were alive at the end of follow up (5.29±1.24). Survivin expression values were compared between the various post surgical outcome groups which showed a statistically significant ($P \leq 0.05$) difference in expression of survivin between dead and disease free alive groups. This indicated that higher expression of survivin gene is associated with poor post surgical outcome which is in accordance with that of Altieri (2008), Wimmershoff *et al. (*2010), Lechler *et al. (*2011), Shoeneman *et al. (*2011) and Renn *et al. (*2014) who reported the diagnostic and prognostic value of survivin in various types human and animal malignancies. In the present study however, no significant difference was observed between alive and recurrence groups. The present study also revealed a positive correlation between the expression of Ki 67 and Survivin which indicated that inhibition of apoptosis favoured the proliferating cells.

CONCLUSION

In the present study there was an increase in the expression of survivin in those neoplasms that also showed higher expression of Ki 67. The results indicated that survivin expression positively coincided with the cell proliferation in squamous cell carcinoma of dogs and over expression of Ki 67 and survivin is associated with poor post surgical outcome.

On the basis of expression of both the markers it was concluded that Ki 67 and Survivin expression in canine squamous cell carcinoma could be considered as a prognostic aid in the Veterinary Medicine, however the results from the current study need to be further validated in large number of squamous cell carcinoma cases.

Table 2: Ki 67 and Survivin mRNA expression values in squamous cell carcinoma of dogs (n=8)

Type of tumor	Ki 67 expression (%) (Immunohistochemistry)	Survivin gene expression folds (q-RT PCR)	Follow up study
Well differentiated Squamous cell carcinoma	32.1	3.17	Alive
Well differentiated Squamous cell carcinoma	39.2	20.68	Recurrence
Poorly differentiated Squamous cell carcinoma	60.5	83.87	Death
Well differentiated Squamous cell carcinoma	40.4	10.06	Recurrence
Moderately differentiated Squamous cell carcinoma	45.2	38.59	Death
Well differentiated Squamous cell carcinoma	48.4	8.23	Recurrence
Well differentiated Squamous cell carcinoma	39	5.25	Alive
Well differentiated Squamous cell carcinoma	40	7.47	Alive
	Mean±SE 43.1±3.00	**Mean±SE 22.16±7.83**	

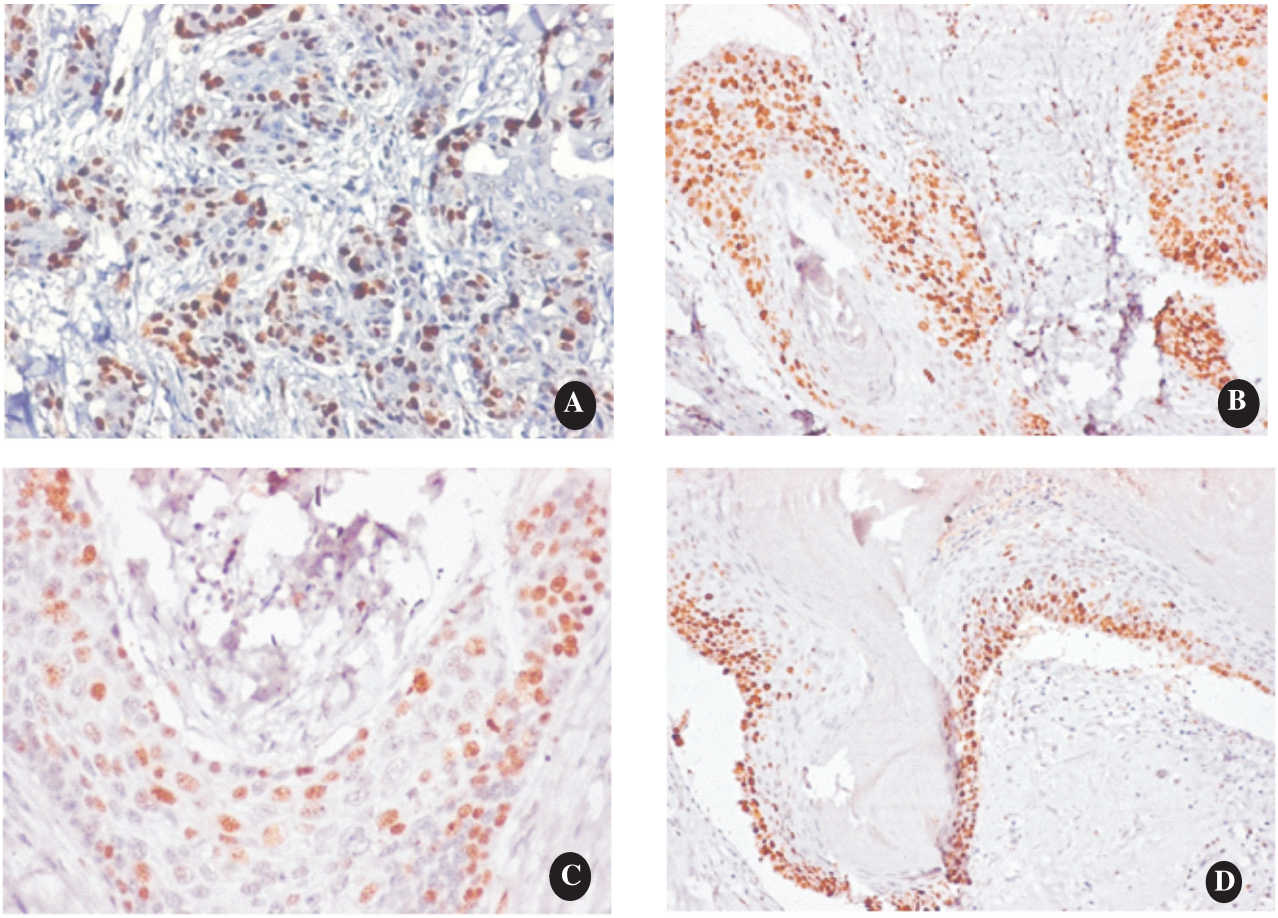

Fig. 2: Sections of squamous cell carcinoma showing Ki 67 positive nuclei IHC. Poorly differentiated Squamous cell carcinoma (A), Moderately differentiated Squamous cell carcinoma (B), Well differentiated Squamous cell carcinoma (C), Well differentiated Squamous cell carcinoma (D)

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Studies on Haemato-biochemical Changes in Dogs with Chronic Kidney Disease

Lathamani, V.S. and Ramesh, P.T.
Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru
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ABSTRACT

Chronic kidney disease (CKD) in dogs is azotaemia of renal origin, is a major cause of morbidity and mortality, especially in older dogs and cats. Dogs with progressive chronic kidney disease shows hematological alteration of different intensities which is reflected on the blood parameters. Fifteen dogs with progressive kidney disease were assessed by studying the hematological parameters in conjunction with serum parameters characteristic for kidney functional alteration (blood urea nitrogen and creatinine). Clinical signs observed were inappetance to anorexia, vomiting, oral ulcers, pain in the lumbar regin, polyurea, polydypsia etc. Increased levels in serum creatinine (4.2 to 10.2 mg/dl) and blood urea nitrogen (48 to 122 mg/dl) were noticed in all dogs which were included under study. In 9 out of the 15 (60 %) dogs taken into study, an increase in hematocrit levels was noticed with values ranging from 16.8 % to 31.5% and a decrease in hemoglobin levels was noticed in 12 out of 15 dogs (80 %) with values ranging from 5.5 to 10.3 g/dl. Thrombocytopaenia observed in 5 dogs (78,000 to 1,19,000/µl). Total leukocyte count revealed lymphopenia in 7 dogs (46.66 %) and lymphocytosis in 3 dogs (20%).

Keywords: Hemato-biochemical, Chronic kidney disease, Dog

Chronic kidney disease (CKD) in dogs can be defined as azotaemia of renal origin for more than two weeks duration. CKD is a major cause of morbidity and mortality, especially in older dogs and cats. Nephron damage associated with CKD is usually irreversible and can be progressive. Prevalence of CKD was estimated to be 0.5% to 1.5% of all dogs. Dogs with progressive chronic kidney disease shows hematological alteration of different intensities which is reflected on the blood parameters. In case of chronic nephropathies, anemia is usually normochromic and normocytic but with a very small reticulocytes number and reduced hematocrit (Falca and Vulpe, 2011).

The present study was undertaken to correlate hematologic and biochemical parameters with intensity of renal failure in fifteen dogs which were diagnosed for chronic kidney disease.

MATERIALS AND METHODS

Fifteen dogs presented to Veterinary College Hospital with clinical signs suggestive of CKD were included in this study. The dogs were diagnosed as CKD based on routine serum biochemistry analysis with continuous increase in blood urea nitrogen and creatinine levels in conjunction with patient history, clinical findings, urinalysis and ultrasonographic examination.

The study group consists of 9 females and 6 males from different breeds, with an average age of 11 years (from 9 to 14 years). Patient blood samples were collected from the cephalic vein by venipuncture in EDTA tubes for hematologic examination and standard tubes without anticoagulant for biochemical examination.

The EDTA blood was analysed for hemoglobin, hematocrit, reticulocytes, total leukocyte, neutrophils, eosinophils, lymphocytes and platelet counts. Biochemical evaluation of renal function alteration was carried out with biochemical analyzer for blood urea nitrogen and creatinine.

RESULTS AND DISCUSSION

Fifteen dogs with progressive kidney disease were assessed by studying hematological parameters in conjunction with serum parameters characteristic for kidney functional alteration (blood urea nitrogen and creatinine). Clinical signs observed were inappetance to anorexia, vomiting, oral ulcers, pain in the lumbar regin, polyurea, polydypsia etc. Increased levels in serum creatinine (4.2 to 10.2 mg/dl) and blood urea nitrogen (48 to 122 mg/dl) were noticed in all of the dogs which were included under study. In 9 out of the 15 (60 %) dogs taken into study, an increase in hematocrit levels was noticed with values ranging from 16.8% to 31.5% and a decrease in hemoglobin levels was noticed in 12 out of 15 dogs (80 %) with values ranging from 5.5 to 10.3 g/dl (Table 1). Thrombocytopaenia, lymphopenia, lymphocytosis and neutrophilia were observed in 33.33% (5/15), 46.66% (7/15), 20% (3/15) and 26.66% (4/15) of dogs respectively.

There was a direct correlation between the degree of anemia and serum creatinine levels which aided in establishing the extent of the chronic renal failure. King *et al.* (1992) reported the extent of chronic kidney disease correlated with the degree of anemia. Anemia in chronic kidney disease is induced by reduced erythropoietin secretion at kidney level. Erythropoietin is produced primarily in the peritubular interstitial cells of the inner renal cortex and outer medulla in the kidney (Erslev and Besarab, 1997). In CKD as there is progression of kidney damage, there are fewer erythropoietin producing cells within the kidneys (Lulich *et al.,* 1992). Uremia is known to decrease red blood cell survival (Weiss and Goodnough, 2005). Chronic renal failure can be associated with leukopenia, which reflects the effects of endogenous glucocorticoids or stress of chronic disease (Jane and Seguin, 2006). Changes in white blood series were observed in the dogs taken into study which didn't correlate to intensity reported in the study, where almost all of the patients showed lymphopenia (Simona *et al.,* 2010). Thrombocytopaenia seen in the present study may be correlated to immune mediated thrombocytopaenia due to several infectious diseases affecting the kidney. This finding was in agreement with Gafter *et al.* (1987), who noticed mild thrombocytopenia in patients with CKD.

CONCLUSION

Haematological analysis in CKD provides useful information about the progress of the disease as well as anemia, providing additional information for therapeutic protocol adjustment for amending induced hematological consequences.

Table 1: Haematobiochemical values in 15 dogs with chronic kidney disease

Parameters	Average value	Range	Normal value
Creatinine (mg/dl)	5.25±2.3	4.2 – 10.2	0.3-1.3
BUN (mg/dl)	78±21.6	48 – 122	6 -25
Haematocrit (%)	23.10±6.4	16.8 – 31.5	36- 55
Haemoglobin (g/dl)	7.2±2,2	5.5 – 10.3	12.1- 18.2
RBC (millions/µl)	3.5±1.2	2.1 – 4.0	4.8- 9.3
Total leukocyte count (cells$X10^3$/µl)	3.8±1.3	2.9- 24.3	4- 12
Platelets (cells/µl)	98,800±2,300	78,000-1,19,000	1.5 – 4.5 lakhs
Neutrophills (%)	78%	54- 92 %	60-77 %
Lymphocytes (%)	17%	8- 40 %	12-30 %
Eosinophils (%)	2%	2-3 %	2-10 %
Monocytes (%)	3%	3-5 %	3-10%

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Effect of AREG, NRG-1 and TNF-α at Lower Concentration on Development and Survivability of Sheep Preantral Follicles In Vitro*

Ramesh, H.S., Girish Kumar, V., Nandi, S[1]. and Ramesh, P.T[2].
Department of Veterinary Biochemistry, Veterinary College, Hebbal, KVAFSU, Bengaluru
Received: 05/11/2017 ***Accepted:*** 28/12/2017

ABSTRACT

The present study was aimed to assess effects of amphiregulin (AREG), neuregulin-1 (NRG1) and Tumor necrosis factor (TNF-α) on growth and growth rates of preantral follicles (PFs). PFs were isolated from slaughterhouse ovaries by a mechanical cum enzymatic method. PFs were cultured in media containing AREG, NRG1 and TNF-α at concentration of 0.5ng/ml *in vitro* for 7 days and were examined for their sizes and growth. Size of the follicles in all the groups was significantly more ($P \leq 0.05$) on day 3 of the culture compared to that of day 0. On day 3, 5 and 7 the growth observed in the AREG and NRG-1 group was significantly more ($P \leq 0.05$) than that of TNF-α group. Our study may contribute for optimization of improvement of IVF technology and its use as an option for treatment of infertility.

Keywords: Preantral follicles, Oocytes, Amphiregulin, Neuregulin-1, Tumor necrosis factor-α

Current methods for in vitro production of embryos in sheep are dependent upon a supply of developmentally competent oocytes from large antral or preovulatory follicles, which are present in the ovary in relatively small numbers. The production of embryos through this technology had a limitation because of availability of very less number of cultivable and fertilizable oocytes in buffalo, which is an important dairy animal in India. Also, using oocytes only from antral follicles limits the number of offspring's per animal and thus under exploitation of the superior female genetic material (Van den Hurk *et al.,* 1997). Since the ovaries of sheep contain a large number of preantral follicles which could be a potential source of cultivable oocytes for production of embryos. AREG enhance oocyte developmental competence, possibly by increasing the metabolite supply from the cumulus cells to the oocyte through extended gap-junction coupling (Sugimura *et al*., 2014). Ovarian neuregulin-1 (NRG-1) types I and III are potential ligands for the ERBB3 receptor, analyzed the promoter regions of the Nrg1 gene that are expressed in granulosa cells and determined the interactions of NRG and AREG in regulating granulosa cell and cumulus cell functions in culture. Ovarian immune effector cells, such as macrophages and lymphocytes, also secrete cytokines including TNF-α, which have been implicated in oocyte development, ovulation, and progesterone production (Wu *et al*., 2001). The objective of this study was to determine the effect AREG, NRG-1 and TNF-α on growth of preantral follicles during different days of culture period.

MATERIALS AND METHODS

Isolation of large preantral follicles

Ovaries were collected from mature non-pregnant sheep from a civil slaughter house, Bangalore and were brought to the laboratory in warm (32 to 33°C) normal saline supplemented with gentamicin (50 µg/ml) for the isolation of preantral follicles.Thin and small sections (approximately 1 mm) were made

*Part of Ph.D. thesis work submitted to Karnataka Veterinary, Animal and Fisheries University, Bidar, India.
[1]National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru.
[2]Department of Veterinary Medicine, Veterinary College, Hebbal, KVAFSU, Bengaluru.

from the ovarian cortex and these ovarian sections were digested with trypsin (0.25%) by incubating at 37°C for 5 minutes. Combined mechanical cum enzymatic method was followed for the isolation of large preantral follicles. Preantral follicles of 100 to 250 µm size were isolated from collected specimens by micro-dissection method using 26 G disposable needle and scalpel blade.

Culture of preantral follicles

Preantral follicles were cultured in 100ml droplets of culture medium. Composition of culture medium varied according to the experiment as mentioned below. They were cultured under mineral oil in 35 mm petridish placed in CO_2 incubator (38^0C, 5% CO_2 in air, 90-95% relative humidity) for different periods as indicated below in different experiments. The culture medium was replaced every alternate day. Every time the culture medium was prepared fresh and kept for incubation in CO_2 incubator for 30 minutes before using for culture (Gupta *et al.,* 2002).

Experimental design: Two to three PFs each were allocated into the following 3 groups: (1) Culture with standard culture media containing AREG (0.5 ng) for 7 days (2) Culture with standard culture media containing NRG-1 (0.5ng) for 7 days (3) Culture with standard culture media containing TNF-α (0.5 ng) for 7 days.

Statistical analysis

The differences between the size and vitality of follicles on different days of in-vitro culture were analysed by ANOVA and the respective means were compared using Bonneferoni Multiple comparison test (Graph Pad Prism, Graph Pad Software Inc., San Deigo, USA). Differences between the mean values were considered singnificant when the P values were less than 0.05.

RESULTS AND DISCUSSION

There was no significant difference ($P \geq 0.05$) in the size of preantral follicles used in the all groups on day 0 of the experiment. But the size of the follicles in all the groups was significantly more ($P \leq 0.05$) on day 3 of the culture compared to that of day 0. Thereafter there was no significant ($P \geq 0.05$) change in the size of the follicles in all the 3 groups. There was no significant difference ($P \geq 0.05$) in the size of follicles between the all groups on day 3, 5 and 7. The growth achieved by preantral follicles by either 5th day or 7th day of culture was not significantly different ($P \geq 0.05$) from the growth achieved by 3rd day of culture in all the experimental groups. On day 3, 5 and 7, the growth observed in the AREG and NRG-1 group was significantly more ($P \leq 0.05$) than that of TNF-α group (Table 1).

In vitro technology represents not only an important tool to understand regulative processes underlying follicle development, but also a future option for the preservation of fertility (Cecconi, 2002). The requirements for the continued growth of large preantral follicles, which can be isolated for *in vitro* studies, have been extensively explored in rodents and to a lesser extent in domestic species. Taken together, the results highlighted the need for a better understanding of the earliest stages of follicular development in domestic ruminants, particularly follicle activation and the primary to secondary follicle transition (Fortune, 2003). In the above context, studies were made on the *in vitro* culturing and growth of sheep preantral follicles isolated from the ovaries collected from slaughter house.

Results presented here show that AREG and NRG-1 group supported the growth of preantral follicles *in vitro*. The observation made in this study was similar to the findings of Richani *et al.* (2013) wherein COCs cultured with AREG have increased oocyte developmental competence. AREG is needed to propagate the LH stimulus from the mural granulosa cells to the cumulus cells which are insensitive to direct LH stimulation (Conti *et al*., 2012). AREG induces nuclear maturation more rapidly than FSH (Park *et al*., 2004). The observation made in this study coincides with the findings of Noma *et al.* (2011) wherein NRG1 participates both in autocrine and paracrine manner during ovulation and may impact luteinization and oocyte maturation. The NRG1 enhances AREG-induced progesterone production in granulosa cells, and also regulates oocyte maturation

Table 1: Effect of AREG, NRG-1 and TNF-α on size (mean ± SEM) profile by preantral follicles during *in vitro* culture

Group	No	Size (diameter in mm) of preantral follicles			
		Day 0	Day 3	Day 5	Day 7
AREG	46	$180.70^{1,a}$± 8.07	$225.20^{2,a}$± 9.40	$257.80^{2,a}$±9.63	$260.30^{2,a}$± 9.50
NRG-1	50	$183.70^{1,a}$± 8.16	$224.20^{2,a}$± 9.50	$252.80^{2,a}$±9.46	$256.30^{2,a}$± 9.50
TNF- α	47	$103.80^{1,a}$± 10.35	$137.50^{2,a}$±10.9	$150.00^{2,a}$±11.09	151.00^{2a}± 11.16

N: Number of preantral follicles.
Values with different superscript letters and numbers differ significantly ($P \leq 0.05$) within column and rows, respectively.

via cumulus cell-dependent mechanism. Paradoxically, TNF-α knockout mice also exhibit increased granulosa cell proliferation as well as reduced oocyte apoptosis (Lin *et al*., 2004). From the experiments conducted in the present study, it can be concluded that culture with AREG and NRG-1 was beneficial for the gowth of sheep preantral follicles in-vitro.

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Blood Biochemical Profile of Broiler Rabbits Fed with Detoxified Karanj (Honge) (*Pongamia glabra* vent*)* Seed Meal in TMR Based Diets*

Giridhar, K.S., Glori Doss, R.G., Reddy, B.S.V., Prabhu, T.M., Thirumalesh, T. and Manju, G.U.
Departmentof Animal Nutrition, Veterinary College, Bengaluru, Karnataka, India
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ABSTRACT

The feeding trial was conducted for 70 days to study the effect of incorporation of Solvent treated karanj seed meal (SKSM) and Alkali (2% NaOH) treated karanj seed meal (AKSM) based total mixed ration (TMR) diets on blood biochemical profile of broiler rabbits. TMR made of napier hay and concentrate at the ratio of 81:19 was fed to thirty weaned (6-7 weeks old) broiler rabbits (T_1control), In T_2& T_3, Groundnutcake (GNC) was replaced by SKSM at the rate of 25 per cent and 50 per cent respectively and in T_4& T_5 AKSM replaced GNC at the rate of 25 per cent and 50 per centrespectively. Blood samples collected at 30th, 50th and 70th day of the experimental period revealed Hb (g/dl), SGPT (IU/L), SGOT (IU/L), creatinine (mg/dl), total protein (g/dl), serum albumin(g/dl) and serum globulin(g/dl)values were within the normal physiological range and found no significant difference between treatments indicating no adverse effect of SKSM or AKSM on blood biochemical profile of rabbits.

Keywords: Karanj seed meal, Broiler rabbit, TMR diet

Among various unconventional feeds, Karanj (honge) (*Pongamia glabra* vent) seed meal (KSM), a by-product of honge seed, rich in protein (24-32% CP), one of the most important agro industrial by-product harbored in India. The annual production of honge seeds in India is estimated to be 1.37 lakh tones and 9 to 90kg seed/tree/annum (Gaurav Dwivedi *et al.,* 2011). Utilization of raw honge seed meal as such has limitation due to presence of various toxic factors such as Karanjin, a furanoflavonoid (Roy *et al.,* 1977; Prabhu *et al.,* 2002; Vinay and Sindhu Kanya, 2008), furanodiketone, chromenoflavones (Naikstam and Bringi, 1973), tannins and trypsin inhibitors (Vinay and Sindhu Kanya, 2008).

Various methods were developed to reduce the karanjin content of cake such as water washing, water soaking, dry heat treatment, pressure cooking, urea ammoniation, alkali (calcium hydroxide, potassium hydroxide, sodium hydroxide and sodium bicarbonate) treatments, biological treatments (*Saccharomyces cerevisiae, Aspergillus oryzae*) and toxin binder (HSCAS). Among them pressure cooking was found to be the most effective method, followed by sodium hydroxide treatment (Soren *et al.,* 2009). Detoxified karanj cake might replace conventional oil cakes (soybean meal or groundnut cake) (FAO, 2012). Rabbit rearing has become popular in various parts of the country. Owing to the wider adaptability, rabbits unlike other monogastric species such as swine and poultry do not compete directly with human beings (Cheeke, 1987). Reducing the cost of production is the major concern by means of utilizing other alternative feed source.

Efforts are required to utilize this unconventional agro industrial by-product in rabbit ration as an alternative to the expensive conventional protein supplement by evolving economically and easily adaptable processing technology to make wholesome protein supplement. Hence, the present investigation was undertaken with the objective of nutritional evaluation of detoxified KSM on certain hematological parameters of broiler rabbits.

*Part of M.V.Sc thesis of first author submitted to KVAFSU, Bidar.

MATERIALS AND METHODS

The study was conducted to evaluate the nutritive value of SKSM and AKSM (2% NaOH) in TMR based diet of broiler rabbits. Thirty weaned broiler rabbits of similar age (6-7 weeks old), weight and breed were randomly allotted to 5 groups comprising of 6 (3 male and 3 female) rabbits in each treatment group. Five iso-nitrogenous and iso-caloric complete diets based on their chemical composition were formulated as per NRC (1977). TMR Diets containing concentrate and Napier hay (18% CP and 66% TDN) in the ratio of 81:19 containing GNC, SKSM, AKSM and sunflower cake as protein source (Table 1). Napier fodder was dried, chopped and ground to 1-2 mm size before it was incorporated into concentrate mixture to make complete diet in mash form.

TMR Diets containing concentrate and Napier hay (81:19) containing GNC and sunflower cake as protein source in control (T_1). In T_2 and T_3GNC was replaced by SKSM at the rate of 25 and 50% and in T_4 and T_5 GNC was replaced by AKSM at the rate of 25 and 50% in the concentrate mixture.

Proximate analysis of both SKSM and AKSM samples was carried as per AOAC (1995) and fibre fractions as per Van Soest *et al.* (1991).

The blood samples were collected from marginal ear vein of each rabbit at 30th, 50th and 70th day of the experimental period and analysed for Hb, SGPT, SGOT, Creatinine and Serum proteins, Albumin and Globulins. Sahli's haemoglobinometer used to estimate Hb and routine biochemistry analyser under continuous flow analysis (CFA) technique was used to analyse the rest of blood biochemicals. The data was subjected to statistical analysis using analysis of variance techniques as described by Snedecor and Cochran (1980) and accordingly results were interpreted.

Table 1: Ingredient composition of experimental diets

	Treatments				
	T_1	T_2	T_3	T_4	T_5
Maize	23.00	28.00	27.00	28.05	27.00
Deoiled rice bran	22.20	11.00	7.60	11.00	7.60
Groundnut cake	25.00	18.75	12.50	18.75	12.50
Sunflower cake	6.00	10.40	12.60	10.40	12.60
Napier hay	19.00	19.00	19.00	19.00	19.00
Molasses	2.50	2.50	2.50	2.50	2.50
Di calcium phosphate	0.80	0.80	0.80	0.80	0.80
Salt	0.50	0.50	0.50	0.50	0.50
Mineral mixture[1]	1.00	1.00	1.00	1.00	1.00
SKSM	-	8.00	16.50	-	-
AKSM	-	-	-	8.00	16.50
Total	100.00	100.00	100.00	100.00	100.00
Additives[2]	0.05	0.05	0.05	0.05	0.05

SKSM-solvent treated karanj seed meal; AKSM- alkali treated karanj seed meal

[1] Mineral mixture composition (%):

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

[2]Additives composition (g/kg):

Ecare-Se -10 (a-tocopherol-10%, and Se-200 ppm); Doxycycline -10, Vitamin AB_2D_3K- 30.

Table 2: Chemical composition[1] of the experimental diets, SKSM and AKSM on DMB

Particulars	Experimental diets					SKSM	AKSM
	T_1 (Control)	T_2 (25% SKSM)	T_3 (50% SKSM)	T_4 (25% AKSM)	T_5 (50% AKSM)		
Proximate composition (%)							
Dry matter	91.22	90.29	91.47	90.92	90.67	91.49	95.58
Organic matter	89.87	89.63	90.15	90.09	89.59	93.76	93.02
Crude protein	18.12	18.39	18.32	18.52	17.98	32.00	32.00
Crude fibre	13.12	13.34	12.97	11.98	12.22	7.97	9.67
Ether extract	3.51	3.47	3.12	3.47	3.02	0.98	0.78
Nitrogen free extractives	55.12	54.43	55.74	56.12	56.37	52.81	50.57
Total ash	10.13	10.37	9.85	9.91	10.41	6.24	6.98
Fibre fractions (%)							
Neutral detergent fibre	33.48	32.47	32.57	31.27	31.33	36.97	38.47
Acid detergent fibre	17.76	16.99	18.72	17.28	16.82	13.90	14.60
Hemicellulose	15.72	15.48	13.85	13.99	14.51	23.07	23.87
Cellulose	15.09	14.87	16.51	14.83	14.85	11.48	12.05
Acid detergent lignin	2.67	2.12	2.21	2.45	1.97	2.42	2.55

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

RESULTS AND DISCUSSION

1. Proximate composition

The average chemical composition of SKSM and AKSM, which was used in the present study, is shown in Table 2. The reason for low moisture in AKSM is due to sun drying after alkali treatment. CP content of both types of KSM is same, suggesting that alkali treatment had no effect on the CP content of KSM. However with respect to other proximate principles slight variation exists, which is attributed to alkali treatment.

The SKSM and AKSM's CP values analysed in present study were similar to Prabhu *et al.* (2002) and Soren *et al*. (2009). The average NDF, ADF, hemicellulose, cellulose and ADL values of SKSM and AKSM were in accordance with those reported by Chandrasekaran *et al*. (1989), Prabhu *et al*. (2002) and Soren *et al*. (2009). Based on overall chemical composition, the potential of including SKSM and AKSM as an unconventional protein supplement for broiler rabbits can be justified.

2. Chemical composition of diets

The average chemical composition of all treatment diets of rabbits during feeding trial is presented in Table 2. The levels of CP, CF, EE, NFE, TA and fibre fractions were similar among different treatments.

3. Hemoglobin (Hb)

The haemoglobin of either SKSM or AKSM fed rabbits was similar to those of control with respect to period of collection and there was no significant difference between the treatments with respect to sex, indicated that there was no variable effect of KSM on Hb percentage. Present Hb values were in agreement with the results of Gowda (1994) and Vasanth Kumar *et al.* (1999), who reported that there was no effect of feeding neem seed cake on Hb concentrations in rabbits.

4. Serum Glutamate Pyruvate Transaminase (SGPT)

SGPT values were found similar between treatments at different time intervals and also with respect to sex. Similar trend was observed in broiler rabbits when fed with Neem seed cake (Gowda, 1994 and Vasanth Kumar *et al*., 1999) and Cashew apple waste (Fanimo *et al*., 2003). Ravi *et al*. (2001) found that the activities of SGPT did not vary significantly among the treatments and values were within the normal range when lambs were fed with either SKSM or AKSM.

5. Serum Glutamate Oxaloacetate Transaminase (SGOT)

Usually rise in SGOT activity occurs in acute liver and muscle damages (Pen Sent, 1983). From the earlier studies of Samantha and Sasmal (1986) it was identified that enhanced SGOT activity and mortality in chicks was due to hepatic or cardiac damage, when fed with extract of karanj cake (neutral part in petroleum ether). After detoxification of karanj cake SGOT activity found to be normal upon experimentation on lambs (Ravi *et al*., 2001).

The present findings are consistent with Gowda (1994), Vasanth Kumar *et al.* (1999) and Fanimo *et al*. (2003) who also reported that neem seed cake and cashew apple waste did not influence serum SGOT activities.

6. Serum Creatinine

Irrespective of diet, serum creatinine values were similar among treatments and influence of sex in between treatment was also found to be non-significant.Creatinine is commonly used to indicate level of renal function and possible damage to kidney architecture (Slunnil, 1974). Similar serum creatinine values among treatments in present trial indicate that activity of kidney was in normal condition.

The observations in present study are supported by Fanimo *et al*. (2003) found no difference among dietary treatments and the serum creatinine values when rabbits were fed with cashew apple waste.

7. Serum Protein

The serum protein, albumin and globulin concentration levels did not differ significantly between the control and KSM fed groups. This clearly shows that feeding of SKSM or AKSM did not alter

Table 3: Average hematological parameters of different treatments in growth trial (Cumulative of 30, 50 and 70th day)

	T1 (Control)	T2 (25% SKSM)	T3 (50% SKSM)	T4 (25% AKSM)	T5 (50% AKSM)
H b (g%)					
Combined^{NS}	14.02±0.15	13.73±0.15	13.72±0.12	13.61±0.17	13.58±0.21
Male^{NS}	14.17±0.17	13.94±0.25	13.99± 0.06	13.89±0.16	13.84±0.31
Female^{NS}	13.87±0.25	13.51±0.18	13.46±0.19	13.33±0.27	13.31±0.28
SGPT (IU/litre)					
Combined ^{NS}	31.27 ± 0.57	31.93 ± 0.56	32.89 ± 0.85	33.14 ± 0.65	32.37 ± 0.83
Male^{NS}	30.64 ± 0.72	31.97 ± 0.77	33.14 ± 1.06	33.14 ± 1.10	31.47 ± 0.97
Female^{NS}	31.89 ± 0.87	31.88 ± 0.86	32.63 ± 1.38	33.13 ± 0.78	33.27 ± 1.33
SGOT (IU/litre)					
Combined^{NS}	38.38 ± 0.73	40.08 ± 0.52	37.11 ± 1.01	38.02 ± 0.91	38.85 ± 0.70
Male^{NS}	38.57 ± 0.99	39.94 ± 0.92	37.28 ± 1.46	37.57 ± 1.11	37.29 ± 0.99
Female^{NS}	38.19 ± 1.13	40.22 ± 0.55	36.93 ± 1.49	38.47 ± 1.49	40.41 ± 0.70
Creatinine (mg/dl)					
Combined^{NS}	1.30 ± 0.06	1.27 ± 0.04	1.22 ± 0.06	1.26 ± 0.03	1.27 ± 0.05
Male^{NS}	1.29 ± 0.11	1.28 ± 0.07	1.17 ± 0.09	1.28 ± 0.05	1.27 ± 0.08
Female^{NS}	1.30 ± 0.06	1.27 ± 0.04	1.27 ± 0.08	1.25 ± 0.04	1.26 ± 0.07
Protein (g/dl)					
Combined^{NS}	6.27 ±0.09	6.11 ± 0.09	6.25 ± 0.07	6.18 ± 0.08	6.19 ± 0.10
Male^{NS}	6.27 ± 0.09	6.11 ± 0.09	6.25 ± 0.07	6.18 ± 0.08	6.19 ± 0.10
Female^{NS}	6.29 ± 0.15	6.05 ± 0.09	6.26 ± 0.09	6.16 ± 0.13	6.12 ± 0.12
Albumin (g/dl)					
Combined ^{NS}	3.45 ± 0.06	3.38 ± 0.07	3.38 ± 0.05	3.53 ± 0.06	3.37 ± 0.06
Male^{NS}	3.32 ± 0.09	3.37 ± 0.07	3.43 ± 0.07	3.52 ± 0.11	3.46 ± 0.09
Female^{NS}	3.58 ± 0.06	3.40 ± 0.12	3.33 ± 0.07	3.55 ± 0.07	3.29 ± 0.09
Globulin (g/dl)					
Combined^{NS}	2.82 ± 0.13	2.73 ± 0.12	2.87 ± 0.07	2.64 ± 0.10	2.81 ± 0.12
Male^{NS}	2.93 ± 0.18	2.81 ± 0.19	2.81 ± 0.09	2.67 ± 0.14	2.79 ± 0.17
Female^{NS}	2.71 ± 0.19	2.65 ± 0.15	2.93 ± 0.12	2.61 ± 0.14	2.83 ± 0.17

the various serum protein concentrations indicating maintenance of normal functioning of vital organs.

Present values were in agreement with the findings of Gowda (1994) and Fanimo *et al.* (2003). In general, the serum total protein concentration between 6 and 8 g/dl, indicates general health of an animal. (Kaneko, 1989).

The average concentration or activities of all biochemical constituents of blood of present study were in normal range (Kaneko, 1989) and are represented in (Table 3).

CONCLUSION

It is concluded that SKSM or AKSM can be incorporated in the diet of broiler rabbits replacing costly GNC at 25 and 50 per cent without any adverse effect on health status.

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Sugarcane Press Residue as a Profitable Mineral Supplement for Layer Production*

Suma, N., Venkatarami Reddy, B.S., Suresh, B.N[1]., Jaishankar, N[2]., Manju, G.U[3]. and Veena, N.
Department of Animal Nutrition, Veterinary College, KVAFSU, Bengaluru
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ABSTRACT

A study was carried out to know the importance of Sugarcane Press Residue (SPR) as a valuable mineral source for economic layer production. The chemical composition of SPR revealed: CP–12.67, EE–7.50, CF–17.50, TA–24.62, NFE–37.71, AIA–9.51%. Whereas it's mineral composition was: Ca–4.52%, P–1.25%, Mg–1.28%, K–1.81%, S-2.62%, Fe-2042 ppm, Mn–228 ppm, Zn–36.5 ppm, Cu–22.6 ppm and Co–236.7 ppm. Eight isonitrogenous layer diets were prepared by incorporating SPR at 0, 5, 10 and 15 per cent in soya based and fish based diets to form T_1 (soya-control), T_2, T_3, T_4(soya-test) and T_5 (fish-control), T_6, T_7, T_8(fish-test) groups, respectively to cater the relevant minerals as well as organic nutrients. Each of such formulated diets was offered to 5 replicates of four layers (32 weeks) each for 84 days.The mean net returns ranged non-significantly (P>0.05) from Rs. 7.64 (T_8) to 10.33 (T_6); from 6.19 (T_8) to 9.18 (T_7); from 3.77 (T_3) to 7.45 (T_7) and from 6.12 (T_3) to 8.98 (T_7), respectively, during Period I, Period II, Period III and Cumulatively. Thus study inferred that otherwise wasteful minerals and organic nutrients in the SPR can form a valuable substitute/partial replacement for the conventional sources of layer diets.

Keywords: Sugarcane press residue, Isonitrogenous, Minerals, Layers and Net returns

Poultry production has gained tremendous momentum during the last five decades in the socio-economic development of India. In the advancement of science of nutrition, the chick infact has played a vital role in unraveling the nutritional importance of many minerals. Marginal deficiencies of both major and minor mineral elements invariably cause significant reduction in the birds' performance (Scott *et al*., 1982). There is, therefore, both a need and scope for improvement in the mineral nutrition of poultry through its sourcing as well as enhancing its availability.

Energy and protein are by far the most important nutrients in formulating the poultry rations and that the importance of the role of minerals in the overall performance of birds cannot however, be ignored (McDonald, *et al.,* 2002). Thus, certain proportion of the layer diet (9-12 per cent) indeed needs to be made up with mineral supplements to accomplish proper growth and production. For this purpose, invariably addition of mineral supplements such as bone meal, dicalcium phosphate, calcite powder, shell grit etc., are mandatory, but however, with variable levels of inclusion as the case (fish-based and soya-based diets) may be. In view of their periodical scarcity as well as cost, alternate sourcing of minerals assumes significance.

Sugarcane Press Residue also known as pressmud/filter residue is obtained after the sugarcane juice being boiled and filtered to remove the accompanying mud and other organic particles before the juice is subjected for sugar extraction in sugar industry (Singh and Solomon, 1995).

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[1] Dept. of Animal Nutrition, Veterinary College, KVAFSU, Hassan.
[2] ICAR-Krishi Vigyan Kendra, Kalaburgi-II, Raddewadagi, Jewargi, Kalaburgi.
[3] Dept. of Animal Nutrition, Veterinary College, KVAFSU, Shimoga.

Layer farming is a well-organized sector requiring about 6 million Metric Tons (mMT) of feed annually. As a result, such huge quantity of feed requires the conventional mineral mixture to an extent of 0.18mMT (*i.e*. about 3 per cent of feed). Even if 10 per cent of such required mineral mixture is spared by SPR, there would be an annual utilization of 20,000 tons of dried SPR.

A preliminary trials on growing sheep (Suresh *et al.,* 2006) and broiler birds (Budeppa *et al*., 2008) revealed that SPR as a valuable ingredient for feeding. More studies of SPR utilization in livestock and poultry have showed as a source of organic as well as inorganic nutrients (Suresh *et al.*, 2009a; Suresh *et al.*, 2009b; Suresh *et al.*, 2010a; Suresh *et al.*, 2010b; Suresh and Reddy, 2011; Suresh *et al.,* 2012; Suma *et al*., 2015).

Hence, a study in layers with the possibility of inclusion of higher levels of SPR is being taken up with an objective to reduce the cost of feed preparation for layers.

MATERIALS AND METHODS

Collection/Screening of SPR: The sufficient amount of SPR was procured from Mysore paper mills, Shimoga for experimental purpose and was dried under sun till it became air dried and was stored for further evaluation.The sugar cane press residue samples were first screened for proximate composition (AOAC, 1995) and then for microbiological examination to identify the presence of any microbes such as *E. coli, Bacillus* and *Salmonella* species.

Experimental Design: Two BIS (1992) specified practical control diets for both soya (T_1) and fish based (T_5) test diets were formulated, while the SPR was included at three levels (5%, 10% and 15%) in test diets of both soya based (T_2-T_4) and fish based (T_6-T_8) types to form a total of 8 treatment diets. The detailed ingredient composition of each diet is being given in Table 1. Inclusion of SPR in any given diet was at the expense of deoiled rice bran, sunflower extractions, jowar and soybean meal. Consequently, in view of low energy and protein levels in SPR, there had been a steady decline in the calculated energy and protein concentrations of such SPR based diets. Further, enough care was exercised to optimize the levels of most of essential minerals and Ca:P ratio among various diets, but however, there occurred undue incremental levels of certain minerals as the SPR inclusion level was increased in such diets.

Using 160 BV-300 commercial layers, an experiment was conducted to study the effect of inclusion of sun dried SPR as a source of minerals for layers. At the age of 32 weeks, all birds were divided randomly into 40 groups of 4 birds each. Each of the 8 diets described earlier were offered to five such replications of 4 birds each in colony cage units. A completely randomized design was employed to carry out the experiment. Further, care was taken to maintain uniform managemental and health conditions.

Performance parameters: Every day in late hours, the number of eggs produced in a particular replicate group was recorded and the hen day egg production was arranged on the basis of three 28-day periods as per various treatments for further statistical analysis. The rate of egg production was calculated both for the individual periods and on the basis of cumulative period as well.

Cost effectiveness: The cost of each diet including that of mineral salts and SPR in such diets was arrived at by considering the prevailing prices of the constituent compounded feed ingredients, mineral salts and SPR. The sale value of eggs produced was also considered at the prevailing market prices. The relative cost effectiveness of each diet was thus assessed.

Statistical analysis: Data pertaining to various parameters obtained during the experimental trial were analyzed in Completely Randomized Design according to the methods described by Snedecor and Cochran (1980).

Table1: Ingredient composition of experimental layer diets (in kg.)

Ingredient	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Maize	435.75	435.00	435.00	435.00	420.25	425.75	430.00	436.25
Jower	0	15.00	30.00	45.00	0	10.00	20.00	30.00
Deoiled rice bran	170.00	115.00	57.00	0	200.00	141.00	82.00	20.00
Soya bean meal	129.00	131.25	134.00	135.75	80.00	82.00	83.50	85.00
Groundnut extractions	50.00	50.00	50.00	50.00	40.00	40.00	40.00	40.00
Sun flower extractions	100.00	92.00	86.00	80.00	99.00	94.00	91.00	89.00
Fish, dry	0	0	0	0	60.00	60.00	60.00	60.00
Dicalcium phosphate	12.50	11.75	11.00	10.25	6.00	5.25	4.50	3.75
Calcite powder	29.75	27.00	24.00	21.00	24.25	21.50	18.50	15.50
Shell grit	70.00	70.00	70.00	70.00	70.00	70.00	70.00	70.00
Salt	3.00	3.00	3.00	3.00	0.50	0.50	0.50	0.50
SPR	**0**	**50.00**	**100.00**	**150.00**	**0**	**50.00**	**100.00**	**150.00**
Zinc oxide	0.1000	0.0960	0.0910	0.0860	0.0920	0.0870	0.0830	0.0780
Copper sulphate	0.0800	0.0599	0.0399	0.0196	0.0819	0.0620	0.0422	0.0224
Potassium iodide	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034
Sodium selenite	0.0044	0.0044	0.0044	0.0044	0.0041	0.0041	0.0041	0.0041
Manganese sulphate	0.2500	0.2110	0.1710	0.1320	0.2480	0.2090	0.1700	0.1310
Total	1000.4	1000.4	1000.3	1000.3	1000.4	1000.4	1000.3	1000.2
Additives*	+	+	+	+	+	+	+	+
Mineral profile (calculated)								
Calcium, %	3.92	3.92	3.92	3.92	3.92	3.93	3.92	3.92
Total phosphorus%	0.80	0.76	0.72	0.68	0.81	0.77	0.73	0.69
Available phosphorus,%	0.35	0.35	0.35	0.35	0.35	0.35	0.34	0.34
Sodium, %	0.15	0.15	0.14	0.14	0.15	0.15	0.14	0.131.41
Chloride,%	0.23	0.22	0.21	0.14	0.22	0.21	0.21	0.20
Potassium, %	0.75	0.70	0.65	0.60	0.71	0.66	0.61	0.56
Magnesium, %	0.28	0.24	0.20	0.15	0.30	0.25	0.21	0.17
Sulphur, %	0.17	0.29	0.41	0.53	0.17	0.30	0.42	0.54
Iron, ppm	206.03	279.28	352.30	425.39	225.17	299.84	374.02	448.36
Iodine, ppm	2.01	2.01	2.01	2.01	2.01	2.01	2.01	2.01
Copper, ppm	25.47	24.94	24.44	23.85	25.44	24.96	24.49	24.01
Cobalt, ppm	0.00	11.84	23.67	35.51	0.00	11.84	23.67	35.51
Manganese, ppm	89.68	89.56	89.10	88.94	89.64	89.41	89.17	88.89

Table1: Ingredient composition of experimental layer diets (in kg.) (*Contd...*)

Ingredient	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Zinc, ppm	93.94	93.72	92.63	91.55	93.96	92.88	92.57	91.39
Sodium, %	0.15	0.15	0.14	0.14	0.15	0.15	0.14	0.131.41
Chloride,%	0.23	0.22	0.21	0.14	0.22	0.21	0.21	0.20
Potassium, %	0.75	0.70	0.65	0.60	0.71	0.66	0.61	0.56
Magnesium, %	0.28	0.24	0.20	0.15	0.30	0.25	0.21	0.17
Sulphur, %	0.17	0.29	0.41	0.53	0.17	0.30	0.42	0.54
Iron, ppm	206.03	279.28	352.30	425.39	225.17	299.84	374.02	448.36
Iodine, ppm	2.01	2.01	2.01	2.01	2.01	2.01	2.01	2.01
Copper, ppm	25.47	24.94	24.44	23.85	25.44	24.96	24.49	24.01
Cobalt, ppm	0.00	11.84	23.67	35.51	0.00	11.84	23.67	35.51
Manganese, ppm	89.68	89.56	89.10	88.94	89.64	89.41	89.17	88.89
Zinc, ppm	93.94	93.72	92.63	91.55	93.96	92.88	92.57	91.39
Selenium, ppm	2.02	2.02	2.02	2.02	2.00	2.00	2.00	2.00
Metabolisable energy, Kcal/kg	2434.1	2408.0	2383.0	2357.8	2438.8	2414.0	2387.8	2363.6
Crude protein, %	17.58	17.38	17.21	17.02	17.59	17.40	17.24	17.07
Methionine, %	0.34	0.33	0.33	0.33	0.34	0.33	0.33	0.33
Lysine, %	0.75	0.74	0.73	0.73	0.83	0.82	0.82	0.81
C/P ratio	138.49	138.58	138.43	138.51	138.61	138.70	138.54	138.50
Ca: Pav ratio	11.19	11.23	11.24	11.25	11.34	11.38	11.39	11.41

* Consisted Breevit – 500g, Doxycycline – 40g, Toxin binder – 750g, Choline chloride – 500g, DL-Methionine – 700g, L-Lysine – 200g, Enzymes – 500g per ton of feed in T_1 through T_4 diets while it was same in T_5 through T_8 diets excepting no DL-Methionine and L-Lysine.

RESULTS AND DISCUSSION

Composition of Sugarcane Press Residue (SPR) and experimental diets: Composition of SPR on DM basis revealed that it resembles several cereal grains on the basis of crude protein of 12.67 per cent, although the ether extract, crude fibre and total ash values of 7.50, 17.50 and 24.62 per cent, respectively were quite high. The mineral profile of SPR revealed that it is a good source of both major as well as minor minerals especially of Calcium (4.52 %), Phosphorus (1.25 %), Potassium (1.81 %), Sulphur (2.62 %), Iron (2042 ppm) and Manganese (228 ppm).

The proximate composition including that of calcium and phosphorus of experimental layer diets compounded on different occasions of the 84-day experimental period is given in Table 2. The results revealed that the proximate analysis of layer diets was similar among all the 8 diets and such values are by and large in conformation with BIS (1992) recommendations. As expected, the contents of crude protein and NFE in layer diets tended to decline with incremental levels of SPR in such diets. Such a trend was quite opposite for rest of the nutrients especially for ether extract and crude fiber.

Egg production: The mean hen day egg production under different treatment groups recorded during different periods as well as cumulatively in terms of both egg number and per cent production are presented in Table 3.

The 28-day mean egg number values ranged from 24.14 (T_8) to 26.82 (T_6) during Period I; from 23.34 (T_8) to 26.72 (T_1) during Period II; from 22.70

Table 2: Proximate composition of experimental layer diets (% on DM basis)*

Dietary Description			Treat-ments	DM	CP	EE	CF	TA	NFE	Ca	P
Protein source		SPR (%)									
Soya based	Control	0	T1	89.90	17.57	1.91	7.15	13.09	60.28	3.92	0.80
	Test	5	T2	89.85	17.36	2.36	7.20	13.25	59.83	3.93	0.76
		10	T3	89.85	17.20	2.91	7.36	14.46	58.07	3.93	0.72
		15	T4	89.37	17.01	3.39	7.42	14.36	57.82	3.93	0.68
Fish based	Control	0	T5	89.56	17.48	2.26	7.19	15.17	57.91	3.92	0.81
	Test	5	T6	89.37	17.39	2.74	7.36	15.37	57.15	3.93	0.77
		10	T7	89.35	17.23	3.30	7.50	15.26	56.71	3.93	0.73
		15	T8	89.26	17.09	3.83	7.60	15.81	55.67	3.93	0.69

Table 3: Mean hen day egg production (No. and %) of experimental birds fed different diets during different periods of the experiment

Dietary Description		Treat-ments	Egg production (Number) NS				Egg production (%) NS			
Protein source	SPR (%)		Period I	Period I	Period I	Cumulative	Period I	Period I	Period I	Cumulative
Soya based	**0**	**T1**	26.68±0.33	**26.72±0.23**	25.08±0.55	**26.12±0.35**	95.16±1.14	**95.36±0.87**	89.46±1.98	**93.33±1.24**
	5	**T2**	26.76±0.71	24.74±1.24	25.24±1.17	25.56±0.72	95.52±2.53	88.21±4.43	90.00±4.15	91.25±2.59
	10	**T3**	25.58±0.82	25.02±1.83	**22.70±2.44**	24.40±1.55	91.26±2.97	89.29±6.54	**80.89±8.70**	87.14±5.51
	15	**T4**	25.78±0.36	25.14±0.72	**25.36±0.24**	25.40±0.39	91.98±1.29	89.64±2.52	**90.54±0.88**	90.71±1.37
Fish based	**0**	**T5**	26.72±0.52	25.36±0.77	24.52±1.33	25.52±0.87	95.34±1.90	90.54±2.76	87.50±4.73	91.13±3.08
	5	**T6**	**26.82±0.32**	25.76±0.32	24.82±0.52	25.78±0.34	**95.72±1.17**	91.96±1.13	88.57±1.90	92.08±1.26
	10	**T7**	26.62±0.28	26.00±0.20	25.08±0.43	25.90±0.25	95.02±0.99	92.68±0.71	89.46±1.53	92.38±0.91
	15	**T8**	**24.14±1.42**	**23.34±1.43**	23.14±1.59	**23.52±1.45**	**86.06±5.05**	**83.21±5.12**	82.50±5.67	**83.93±5.19**

NS - Non significant (P>0.05)

(T_3) to 25.36 (T_4) during Period III and from 23.52 (T_8) to 26.12 (T_1) on cumulative basis. Comparison of treatment groups is more appropriate when egg production is on per cent basis and thus the egg production expressed as per cent ranged from 86.06 (T_8) to 95.72 (T_6) per cent during Period I; from 83.21 (T_8) to 95.36 (T_1) per cent during Period II and from 80.89 (T_3) to 90.54 (T_4) per cent during Period III. The cumulative egg production ranged from 87.14 (T_3) to 93.33 (T_1) per cent in soya based diets and 83.93 (T_8) to 92.38 (T_7) per cent in fishmeal based diets which however, were non-significantly different ($P \geq 0.05$) from each other. During all the periods in general, the birds fed diets with the incremental levels of SPR showed non significantly ($P \geq 0.05$) reduced egg production. However, there was inconsistent trend among the test diets during different periods. The results obtained in the present study are in accordance with those of the experiments conducted by Combs *et al.* (1979), Karanakaran and Purushothaman (1998), Paton and Cantor (2000), Ekweozor *et al.* (2002) and Surai (2002), where in, the variation in nutrient profile of diets of present study was more or less similar to their reports.

Net returns: The net returns under various dietary groups obtained as the difference between the cost of feed as input (Table 4) and the sale price of eggs as the output factor during different periods as well as cumulatively, have been worked out and are presented in Table 5 while the main factor wise returns are presented in Table 6.

The cost of control soya diet (T_1) was Rs. 25.15/ kg which got reduced to Rs. 22.78/kg as the SPR level got increased to 15 per cent, which was partly due to the progressive decreasing cost of mineral salts at the expense of SPR as mineral source. Likewise, the cost of fish based diets ranged from Rs. 27.01 (T_5-control) to Rs. 24.69/kg (T_8-15 % SPR).

The mean net returns ranged non significantly ($P>0.05$) from Rs. 10.10 (T_8) to 17.86 (T_4); from 6.17 (T_8) to 14.87 (T1); from 5.11 (T_6) to 11.90 (T2) and from 8.71 (T_6) to 13.76 (T_2), respectively, during Period I, Period II, Period III and Cumulatively.

The effect of main factors (Table 6) revealed that throughout the experimental period, the net returns were not significantly ($P>0.05$) affected by any of the main factors. With regard to the protein source as main factor, the returns from fish based diets were quite lower than those from soya based diets because of the prevailing higher price of fish meal.

The cost of layer diets ranged from Rs. 27.01 (T_5) to Rs. 22.78 (T_1) per kg. As the SPR level increased, there was a gradual decrease in the cost of the diets both in soya and fish based diets while mean net returns ranged from Rs. 6.12 (T_3) to Rs. 8.98 (T_7) per bird.

Sun dried Sugarcane Press Residue (SPR) when included at 5-15 per cent either in the soya-based or fish-based layer diets, the results has shown that the SPR can become prospective nonconventional feedstuff in contributing different nutrients (Table 7). It was observed that as the level of SPR increased from 5 to15 per cent either in the soya-based or fish-based diets, the contribution (%) of energy and protein ranged from 2.5 to 7.5 and 3.7 to 11.0, respectively. Similarly, the respective ether extract and crude fibre contribution (%) in the diet by SPR ranged from 21 to 44 and 15 to 50.

The range of contribution (%) of other nutrients in the diets with the incremental levels of SPR was 3 to 9 for calcium, 8 to 28 for total phosphorus, 6 to 17 for available phosphorus, 2 to 8 for potassium, 0.5 to 2.6 for magnesium, 44 to 74 for sulphur, 28 to 55 for iron, 18 to 55 for copper, 14 to 43 for manganese and 5 to 15 for zinc.

Assuming that the level and quality (amino acid composition) of SPR being equal to that of rice bran (Suma *et al.,* 2015b), it was calculated that the magnitude of contribution of various amino acids ranged from 2 to 7 per cent at 5 per cent SPR inclusion level and from about 7 to 22 per cent at 15 per cent SPR included diets. Thus SPR can be an effective contributor of various nutrients with particular emphasis of minerals.

It may be inferred that the otherwise wasteful minerals and organic nutrients in the SPR can form a

Table 4: Ingredient composition (kg/ton) and cost (Rs.) of layer diets

Ingredient	Rate (Rs./Kg)	T1	T2	T3	T4	T5	T6	T7	T8
Maize	19.89	435.8	435	435	435	420.3	425.8	430	436.3
Jowar	15.20	0	15	30	45	0	10	20	30
Deoiled rice bran	20.68	170	115	57	0	200	141	82	20
Soya bean meal	41.98	129	131.3	134	135.8	80	82	83.5	85
Ground nut extraction	31.00	50	50	50	50	40	40	40	40
Sun flower extraction	23.00	100	92	86	80	99	94	91	89
Fish	76.00	0	0	0	0	60	60	60	60
Dicalcium phosphate	40.00	12.5	11.75	11	10.25	6	5.25	4.5	3.75
Calcite	02.50	29.75	27	24	21	24.25	21.5	18.5	15.5
Shell grits	16.83	70	70	70	70	70	70	70	70
Salt	15.00	3	3	3	3	0.5	0.5	0.5	0.5
SPR	03.00	0	50	100	150	0	50	100	150
Zinc oxide	180.0	0.1	0.096	0.091	0.086	0.092	0.087	0.083	0.078
Copper sulphate	108.0	0.08	0.06	0.04	0.02	0.08	0.06	0.04	0.02
Potassium iodide	1550	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Sodium selinite	4,000	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Manganese sulphate	32.00	0.25	0.211	0.171	0.132	0.248	0.209	0.17	0.131
Total		1000.4	1000.4	1000.3	1000.3	1000.4	1000.4	1000.3	1000.2
Additives		+	+	+	+	+	+	+	+
Cost of diet (Rs./kg)		**25.15**	**24.36**	**23.58**	**22.78**	**27.01**	**26.24**	**25.47**	**24.69**
Cost of mineral salts (Rs./kg)		**1.853**	**1.962**	**2.070**	**2.178**	**1.540**	**1.649**	**1.757**	**1.865**

valuable substitute/partial replacement for the conventional sources. Such observation is further based on the fact that the SPR based diets also supported net returns as well.

CONCLUSION

From the different parameters studied, it may be inferred that the otherwise wasteful minerals and organic nutrients in the Sugar cane Press Residue (SPR) can form a valuable substitute/partial replacement for the conventional sources of economical layer production.

Table 5: Period-wise and cumulative mean net returns (Rs./bird)[1] under different treatments

Dietary description			Treatments	Mean net returns[1]			
Protein source		SPR (%)		Period I [NS]	Period II [NS]	Period III [NS]	Cumulative [NS]
Soya based	**Control**	**0**	**T1**	15.99	14.87	7.06	12.51
	Test	**5**	**T2**	17.16	11.15	11.90	13.76
		10	**T3**	16.34	14.61	6.05	12.52
		15	**T4**	17.86	12.67	13.44	16.99
Fish based	**Control**	**0**	**T5**	10.90	8.45	5.39	7.96
	Test	**5**	**T6**	12.09	9.08	5.11	8.71
		10	**T7**	13.72	11.43	9.04	12.78
		15	**T8**	10.10	6.17	6.18	8.90

[1] Values are obtained as the difference of the input cost of feed (Table IV) and the output value of eggs produced (Table III) at the prevailing egg prices of Rs. 3.69 per egg.
[NS] - Non significant

Table 6: Period wise and cumulative mean net returns (Rs./bird)[1] as affected by main factors at different intervals

i) SPR as main factor

SPR level (%)	Mean net return[1]			
	Period I [NS]	Period II [NS]	Period III [NS]	Cumulative [NS]
0	13.45	11.66	6.23	10.24
5	14.63	10.12	8.51	11.24
10	15.03	13.02	7.55	12.65
15	13.98	9.42	9.81	12.95

ii) Protein source as main factor

Protein source	Period I [NS]	Period II [NS]	Period III [NS]	Cumulative [NS]
Soya	16.84 ± 0.42	13.33 ± 0.88	9.61 ± 0.87	**13.95 ± 0.91**
Fish	11.70 ± 0.79	8.78 ± 0.92	6.43 ± 0.89	**9.59 ± 0.90**

[1] Values are obtained as the difference of the input cost of feed (Table IV) and the output value ofeggs produced (Table III) at the prevailing egg prices of Rs. 3.69 per egg.
[NS] - Non significant

Table 7: Magnitude of contribution of Sugarcane Press Residue and its selected nutrients in various experimental diets

Treatment No.	T1	T2	T3	T4	T5	T6	T7	T8
% By SPR	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Cost Rs/kg	0.00	0.73	1.43	2.10	0.00	0.75	1.47	2.16
ME kcal/kg	0.00	2.47	4.95	7.46	0.00	2.47	4.95	7.46
CP %	0.00	3.65	7.31	10.97	0.00	3.66	7.31	10.96
EE%	0.00	21.13	34.71	44.11	0.00	18.62	31.30	40.47
CF %	0.00	14.93	31.31	49.69	0.00	14.60	30.57	48.04
Ca %	0.00	3.06	6.12	9.19	0.00	3.06	6.12	9.19
TP %	0.00	8.12	17.29	27.58	0.00	7.94	16.97	27.30
Pav %	0.00	5.70	11.34	16.91	0.00	5.75	11.45	17.11
K %	0.00	2.12	4.46	7.00	0.00	2.24	4.75	7.57
Mg %	0.00	0.45	1.18	2.55	0.00	0.42	1.08	2.28
S %	0.00	45.29	63.83	73.90	0.00	44.41	62.91	73.08
Fe ppm	0.00	30.43	46.11	55.22	0.00	27.91	43.50	53.34
Cu ppm	0.00	18.33	36.67	54.98	0.00	18.33	36.67	55.01
Mn ppm	0.00	14.18	28.42	42.55	0.00	14.20	28.37	42.50
Zn ppm	0.00	4.88	9.78	14.66	0.00	4.91	9.78	14.73
Lys %	0.00	3.86	7.54	10.99	0.00	3.41	6.68	9.82
Arg %	0.00	3.22	6.49	9.80	0.00	3.09	6.22	9.40
Met %	0.00	4.25	8.62	13.10	0.00	3.33	6.73	10.20
Cys %	0.00	2.22	4.52	6.96	0.00	2.21	4.48	6.82
M+C %	0.00	3.30	6.72	10.27	0.00	2.88	5.82	8.83
Try %	0.00	3.02	6.08	9.18	0.00	2.99	6.00	9.04
Thr %	0.00	3.18	6.32	9.39	0.00	3.00	5.95	8.85
Ileu %	0.00	2.38	4.98	7.92	0.00	2.32	4.81	7.51
Leu %	0.00	2.57	5.28	8.19	0.00	2.49	5.07	7.77
Val %	0.00	3.81	8.06	12.94	0.00	3.57	7.47	11.76
Phe %	0.00	2.42	5.06	8.00	0.00	2.40	4.98	7.76
His %	0.00	2.76	5.80	9.24	0.00	2.66	5.54	8.67
Gly %	0.00	3.14	6.53	10.27	0.00	3.02	6.23	9.67
Ser %	0.00	6.59	13.78	21.70	0.00	6.39	13.35	20.96

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Effect of Feeding Tannin Containing Jowar (*Sorghum bicolor*) Varieties on Digestibility and Hematology Parameters in Deccani Sheep*

Bhalerao, S.M[1]., Mallikarjunappa, S., Ramachandra, B. and Nagabhushana, V.
Department of Animal Nutrition, Veterinary College, KVAFSU, Bidar
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ABSTRACT

An experiment was conducted on twenty-eight Deccani lambs which were randomly divided into four equal groups and fed for 60 days experimental trial period. The experimental lambs were fed with four different concentrate mixtures using four different sorghum variety grains *viz*., MAULEE, CSV-216-R, CSH-15-R and LOCAL varieties and other common concentrate ingrenedits. As a sole source of roughage straws of respective grain varieties were used. Total tannin content of grain of each variety of sorghum grain was found to be 0.68 ±0.30, 0.61 ±0.03, 0.46 ±0.03 and 0.31 ±0.03 in MAULEE, CSV-216-R, CSH-15-R and local varieties respectively. The *In-vitro* dry matter digestibility (IVDMD %) of concentrate mixture was recorded as 84.16 ±0.39, 84.24 ±1.13, 87.65±03.79 and 88.75 ±0.72 in MAULEE, CSV-216-R, CSH-15-R and local varieties respectively. The IVDMD (%) of sorghum straw was recorded as 60.33 ±0.65, 60.78±1.69, 63.34 ±01.07 and 64.24 ±0.75 in MAULEE, CSV-216-R, CSH-15-R and local varieties respectively. The IVDMD (%) of total mixed ration (TMR) was observed as 67.80 ±0.88, 68.81 ±0.86, 71.05 ±0.77 and 69.98 ±0.72 in MAULEE, CSV-216-R, CSH-15-R and local varieties respectively. There was significant ($P \leq 0.05$) difference observed in IVDMD of concentrate mixture, straw and in mixed ration. The blood hematology parameters like TLC, TEC, PCV and Hemoglobin were similar to across the treatment groups. Thus, the present study concludes that, tannin content of jowar varieties may have adverse effect on IVDMD (%), with no adverse effect on the hematology parameters of sheep fed on jowar varieties.

Keywords: Sheep, Jowar, Tannin, *In-vitro* digestibility, Hematology

Sheep rearing is becoming an important source of livelihood through wool, meat, skin and manure especially among small and marginal farmers as well as landless labours. India posses over 65 million sheep and 135 million goats contributing over 42 per cent of the total ruminant population (Livestock Census, 2012).

Jowar (*Sorghum bicolor*) is one of the predominant millet crop cultivated in dry lands, both in Kharif and Rabi seasons. Kharif sorghum grains are available for poultry and animal feeding (Rajashekher Reddy *et al*., 2005); while Rabi sorghum grain is used for human consumption (Subramanium and Metta, 2000). Further, sorghum straw is available in majority of Indian states for feeding of animals but main limitation of sorghum grain is presence of anti-nutritional factors such as phytates and tannins. The phytates get destroyed in rumen of ruminant animals. The tannins bind to proteins including enzymes of digestive tract which affect its availability and utilization (Nunez-Hernandez *et al*., 1991). The scientific information on feeding different varieties of jowar grains along with straw is limited especially under the intensive system of sheep rearing. Therefore, the present experiment was designed to study the effect of feeding diets based on different varieties of jowar

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[1]Department of Animal Nutrition, Krantisingh Nana Patil College of Veterinary Science, MAFSU, Shirwal-412801, Dist-Satara (Maharashtra)

on *in-vitro* dry matter digestibility and blood parameters of the sheep in intensive sheep rearing.

MATERIALS AND METHODS

The seeds of different sorghum varieties namely MAULEE, CSV-216-R, CSH-15-R were procured from NRCS (National Research Centre for Sorghum) Hyderabad, Telangana and one local variety available in market. They were grown, cultivated, harvested and straw and grains of these varieties were used for feeding of animals. An experiment was conducted on twenty-eight Deccani lambs (12 males and 16 females) of four months old with comparable body weights were randomly divided into four equal groups. The jowar straw, concentrate mixture and jowar grains were analyzed for their proximate principles (AOAC, 1995). The lambs were fed with four types of concentrate mixture which were formulated using 40 parts of sorghum grains *viz.,* MAULEE, CSV-216-R, CSH-15-R and local varieties and other common concentrate ingredients like ground nut (*Arachis hypogaea*) cake 20 parts, sunflower (*Helianthus annus*) cake 05 parts, safflower (*Carthamus tinctorius*) cake 05 parts, field bean (*Phaseolus vulgaris*) 10 parts, rice (*Oryza sativa*) polish 10 parts, wheat (*Triticum aestivum*) bran 07 parts, mineral mixture 02 parts and salt 01 part. The concentrate mixture was offered according to DM and DCP requirement of lambs (Ranjhan, 1991). Similarly the straws of these different varieties were used as sole source of roughage in respective groups.

The animals were fed with experimental diets for 60 days. Total tannin content of grain of each variety was estimated in the laboratory of NRCS, Hyderabad as per the method described by NRCS (1981). The *In-vitro* digestibility studies were done according to Tilley and Terry (1963) method to estimate IVDMD for all concentrate mixtures, sorghum straw varieties and concentrate-straw mixed rations. The blood samples were collected once in a month for the study of hematological parameters like total leukocyte count (TLC), total erythrocyte count (TRC), hemoglobin, and packed cell volume (PCV) etc. The experimental observations obtained were subjected to statistical analysis as per the method described by Snedecor and Cochran (1989).

Table 1: Composition of concentrate mixture

Sr.No.	Ingredients	Level of inclusion (kg)
1	Sorghum grain	40
2	Ground nut cake	20
3	Sunflower cake	05
4	Safflower cake	05
5	Avre nuchu (field bean)	10
6	Rice polish	10
7	Wheat bran	07
8	Mineral mixture	02
9	Salt	01

Table 2: Chemical composition of experimental concentrate mixture (% DM basis)

Parameters	Treatment Groups			
	A	B	C	D
Crude protein (%)	21.49	21.75	23.09	23.68
Organic matter (%)	91.12	91.99	92.05	91.91
Ether extract (%)	04.91	04.93	04.93	05.01
Crude fiber (%)	06.80	06.94	06.60	06.62
Total ash (%)	08.86	08.06	07.95	08.09
Nitrogen free extract (%)	56.33	56.42	58.78	58.79
Acid insoluble ash (%)	01.55	01.33	01.27	01.43
Calcium (%)	01.97	01.98	01.96	01.96
Phosphorus (%)	0.62	0.77	0.65	0.78
Neutral detergent fibre (%)	28.19	28.38	27.91	27.96
Acid detergent fibre (%)	08.59	08.62	08.35	07.99

Table 3: Chemical composition of experimental sorghum straw (% DM basis)

Parameters	Treatment Groups			
	A	B	C	D
Crude protein (%)	03.12	03.23	03.71	03.78
Organic matter (%)	92.10	92.17	92.48	92.34
Ether extract (%)	01.34	01.29	01.24	01.31
Crude fiber (%)	29.57	31.56	31.65	29.71
Total ash (%)	07.90	07.83	07.52	07.66
Nitrogen free extract (%)	57.38	55.89	55.78	58.02
Acid insoluble ash (%)	02.58	04.39	03.79	02.71
Calcium (%)	0.30	0.35	0.35	0.37
Phosphorus (%)	0.06	0.04	0.06	0.05
Neutral detergent fibre (%)	74.32	74.86	77.69	77.74
Acid detergent fibre (%)	62.41	63.34	64.12	64.17

RESULTS AND DISCUSSION

The results of tannin content of jowar, *in-vitro* digestibility of dry matter and the hematology parameters are presented in Table 4 and discussed as here under.

Tannin content of sorghum grain

The estimated Tannin content were 0.68%, 0.61%, 0.46%, and 0.31% in treatment groups A, B, C and D respectively. Tannin content of jowar in treatment Group A (0.68%) and treatment Group B (0.61%) were recorded significantly ($P \leq 0.05$) higher compared to Group C (0.46%) and D (0.31%). Brandt *et al.* (1992) recorded tannin contents in white sorghum as 0.09 per cent and in reddish white sorghum as 0.27 per cent. The tannin content of sorghum grain in present study was higher than the normal value. Tannins bind to both exogenous and endogenous proteins including enzymes of digestive tract, affecting the utilization of proteins and exert negative effects on dry matter and protein digestibility (Lohan *et al*., 1980).

In-vitro digestibility studies

There was a significant ($P \leq 0.05$) difference observed in IVDMD of concentrate mixture, sorghum straw and a mixed ration (Table 4). The IVDMD of straw in treatment Group A (60.33%) was significantly ($P \leq 0.05$) lower and was significantly ($P \leq 0.05$) higher in treatment Group C (66.34%) the significant difference might be due to higher tannin content in sorghum grains, which may inhibit ruminal bacteria. In addition *in-vitro* IVDMD are reported to be more in less tannin content varieties (Dhore *et al.,* 2005; Reddy *et al.,* 1988).

Hematology Parameters

All the hematology values were well within the normal physiological range. The TLC values ranged from 10.42 (D) to 11.75 (C) which was in accordance with the normal values of TLC (4 to 12 $x10^3$/cumm) in sheep (William, 2005.). Galip *et al*. (1997) observed that $6.91x10^3$/cumm leukocyte count in Merino lambs fed with sorghum grains and straw. The TRC values ranged from 9.61 (A) to 10.41 (B) which were also well within normal physiological range of 8 to $12x10^6$ cells/µl and showed no significant differences in treatment groups. The PCV values were well within normal physiological range and recorded as 29.57, 29.80, 31.14 and 28.42 per cent in treatment groups A, B, C and D respectively. There was a no significant difference in the PCV value between treatment groups. Sukhvir Kaur *et al.* (2001) observed non-significant difference in PCV by feeding complete ration containing sorghum. The hemoglobin values were within normal physiological range and were 9.27, 9.53, 8.92 and 8.40 g% in treatment groups A, B, C and D respectively. There was a no significant difference in the Hb value between treatment groups. Sukhvir kaur *et al.* (2001) observed a non-significant difference in Hb by feeding complete diets. There was no significant difference in the TLC, TRC, PCV and hemoglobin values between treatment groups A, B, C and D.

CONCLUSION

The present study concludes that, the tannin content of jowar varieties has shown adverse effect on *in-vitro* dry matter digestibility (IVDMD) of the diet without any effect on hematology parameters of sheep. Therefore, the findings made in a present study can be more emphasized if further research is performed under different feeding regimes with more detailed study.

Table 4: Mean Values of Tannin in Sorghum Grain, *In-Vitro* Digestibility and Hematology Parameters of Experimental Sheep

Parameters	Treatment Groups			
	A (MAULEE)	B (CSV-216-R)	C (CSH-15-R)	D (LOCAL)
Tannin in jowar grains (%)	$0.68^{a} \pm 0.30$	$0.61^{a} \pm 0.03$	$0.46^{b} \pm 0.03$	$0.31^{b} \pm 0.03$
In-Vitro digestibility studies (%)				
Concentrate mixture	$84.16^{a} \pm 0.39$	$84.24^{a} \pm 1.13$	$87.65^{b} \pm 03.79$	$88.75^{b} \pm 0.72$
Sorghum straw	$60.33^{a} \pm 0.65$	$60.78^{a} \pm 1.69$	$63.34^{b} \pm 01.07$	$64.24^{b} \pm 0.75$
Concentrate-straw mixed ration	$67.80^{a} \pm 0.88$	$68.81^{a} \pm 0.86$	$71.05^{b} \pm 0.77$	$69.98^{b} \pm 0.72$
Hematology parameters				
Total leukocytes count (10^3)	11.19 ± 0.35	11.96 ± 1.56	11.75 ± 0.32	10.42 ± 0.07
Total erythrocytes count (10^6)	9.61 ± 0.10	10.41 ± 0.06	9.90 ± 0.14	9.85 ± 0.04
Packed cell volume (%)	29.57 ± 2.98	29.80 ± 2.24	31.14 ± 2.06	28.42 ± 2.01
Hemoglobin (g %)	9.27 ± 0.26	9.53 ± 0.20	8.92 ± 0.34	8.40 ± 0.31
Nutritional and other important parameters				
Average Total DMI (g)	667.88±67.84	663.68±68.80	666.15±61.19	663.12±60.53
Dry matter digestibility (%)	$53.11^{a} \pm 1.23$	$55.60^{a} \pm 1.30$	$62.21^{b} \pm 1.11$	$62.89^{b} \pm 0.56$
Organic matter intake (g)	$555.94^{a} \pm 3.19$	$558.85^{a} \pm 2.20$	$563.09^{b} \pm 1.79$	$556.99^{a} \pm 3.92$
Organic matter Digestibility (%)	$50.56^{a} \pm 1.01$	$52.99^{a} \pm 1.10$	$60.06^{b} \pm 0.71$	$60.68^{b} \pm 0.65$
Crude Protein Digestibility (%)	$43.83^{a} \pm 1.20$	$43.55^{a} \pm 1.02$	$54.77^{b} \pm 1.25$	$55.56^{b} \pm 1.02$
TDN % of diet	61.72 ± 1.12	62.84 ± 1.15	63.38 ± 1.22	64.94 ± 0.33
DCP of diet (%)	$7.09^{a} \pm 0.66$	$8.02^{b} \pm 0.74$	$8.14^{b} \pm 0.41$	$8.34^{b} \pm 0.57$
Initial body weights (kg)	13.99 ± 0.95	13.98 ± 0.53	13.94 ± 0.36	13.99 ± 0.52
Final body weights (kg)	$16.88^{a} \pm 0.39$	$17.43^{a} \pm 0.60$	$17.66^{b} \pm 0.97$	$18.01^{b} \pm 0.59$

Note: ^{a,b,c} Means with different superscript in a row differ significantly ($P<0.01$)

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Effect of Supplementation of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) Powder on the Performance of Broiler Birds*

Jadhav, S.A., Bhalerao, S.M., Khanvilkar, A.V[1]., Shende, T.C[2]. and Changan, S.D.
Department of Animal Nutrition, Krantisingh Nana Patil College of Veterinary Science, MAFSU, Shirwal-412801, Dist-Satara (Maharashtra)
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ABSTRACT

An experiment was carried out on a day-old, 240 broiler chicks to study the performance of broiler birds fed diets based on turmeric and ginger powder. The experimental chicks were divided randomly into four treatment groups having three replicates of 20 birds each. The four treatments were *viz.* control Group (T_0), T_0+0.50% turmeric powder (T_1), T_0+0.50% ginger powder (T_2) and the T_0+combination of turmeric and ginger powder at the level 0.50% each (T_3). The experimental feed was fed for six weeks in three phases *viz.,* pre-starter (0-7 day), starter (8-21 day) and finisher (22-42 day). The observations pertaining to feed consumption and feed conversion ration (FCR) were recorded. At end of 6th week, a metabolic trial of three days was conducted for nutrient retention studies. The data obtained for growth parameters, feed consumption and feed conversion ratio were subjected to statistical analysis. The treatment Group T_1 (2003.86gm) showed significantly higher ($P<0.05$) final body weights than T_2 (1919.59gm), T_3 (1919.43gm) and T_0 (1906.81gm) treatment groups. The data revealed that, the treatment groups receiving 0.50% turmeric powder in Group T_1 (1960.5gm) had higher body weight gains than control and other treatment groups. The average cumulative feed consumption, FCR and nutrient retention (%) differed non-significantly in all treatment groups.

Keywords: Broiler birds, Turmeric, Ginger

Poultry plays an important role in the economy development of the country since it has tremendous employability and income generating potential. Today, India is the third largest egg producer and sixth largest broiler producer in world (GOI, published in 2015). Indiscriminate use of antibiotics in poultry industry is deleterious. Therefore, there is a great demand to produce quality organic poultry meat and eggs.

In view of this, herbal and plant derivatives could be a valuable alternative in promoting growth and health of poultry especially broilers. Many plants have beneficial multifunctional aspects derived from their specific bio-active components. The rhizome part of turmeric (*Curcuma longa*), contains curcuminoids, zingiberene, turmerone etc. The curcumin (diferuloylmethane) is the main bioactive component that has a wide spectrum of biological actions and protective effects of turmeric as food additives. The rhizome part of ginger (*Zingiber officinale*) contains gingerol, shogoals, zingiberene. The zingiberen and zingerol stimulates the digestive system by controlling the digestive pH and digestive enzymes. Hence, it may be of immense benefit and value in poultry nutrition especially for broilers due to their antibacterial, anti-inflammatory, and immune-modulatory properties (Onu, 2010). Considering the above facts, the present study was planned to investigate the effect of turmeric and ginger powder supplementation on growth, nutrient digestibility and nutrient retention in broiler birds.

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[1]Associate Professor & HOD, Department of Livestock Production & Management.
[2]Assistant Professor & HOD, Department of Animal Genetics & Breeding.

MATERIALS AND METHODS

An experiment was carried out on day-old, 240 broiler chicks for a period of six weeks at poultry farm of KNP College of Veterinary Science, MAFSU, Shirwal (Dit-Satara). The experimental chicks were divided randomly into four treatment groups having three replicates of 20 birds each. All standard managemental practices were followed strictly during rearing of birds on deep litter system. The four treatments were *viz.* control group (T_0), T_0+0.50 % turmeric powder (T_1), T_0+0.50 % ginger powder (T_2) and the T_0+combination of turmeric and ginger powder at the level of 0.50% each (T_3). The experimental diets were formulated as per BIS (2007) specifications. The treatment feed was fed in three phases *viz.*, pre-starter (0-7 day), starter (8-21 day) and finisher (22-42 day). For the first three weeks, the feed was offered in the chick feeders. Thereafter, it was offered in grower feeders throughout the experiment. The *ad-lib* feed and clean drinking water was made available during the experiment. Every day, weighed amount of feed was offered to all the treatment groups and the residue was collected and weighed separately on the next day. The average daily and weekly feed consumption per bird for all the groups was calculated. The feed samples were analyzed for its proximate principles as per AOAC (2005). The body weight of individual chick from all the groups was recorded at beginning (day old) of the experiment and thereafter at weekly intervals. The body weight gain per week, average daily and weekly feed consumption, FCR were recorded and tabulated. At the end of 6th week of age, a metabolic trial was conducted for nutrient retention study. The data obtained was subjected to statistical analysis by using completely randomized block (CRD) design as per Snedecor and Cochran (1994).

Table 1: Per cent ingredient composition of broiler pre-starter mash

Ingredients (Kg)	Pre-starter			
	T_0	T_1	T_2	T_3
Maize	53.50	54	54	54
Soybean meal (deoiled)	32.55	29.29	29.9	29.9
Maize gluten meal	6.10	8	8	8
Soybean oil	3.05	2.8	2.8	2.8
Common salt	0.50	0.50	0.50	0.50
Limestone powder	1.10	1.10	1.10	1.10
Dicalcium phosphate	2.00	2.00	2.00	2.00
Trace-mineral mix	0.50	0.50	0.50	0.50
L-lysine	0.01	0.01	0.01	0.01
DL-methionine	0.30	0.30	0.30	0.30
Coccidiostat	0.062	0.062	0.062	0.062
Selvit E	0.02	0.02	0.02	0.02
Toxin binder	0.055	0.055	0.06	0.06
Rovimix	0.20	0.20	0.20	0.20
Choline chloride	0.05	0.05	0.05	0.05
Turmeric powder	-	0.50	-	0.50
Ginger powder	-	-	0.50	0.50
Total	**100**	**100**	**100**	**100**

Table 2: Per cent ingredient composition of broiler starter mash

Ingredients (Kg)	Starter			
	T_0	T_1	T_2	T_3
Maize	54.86	54	54	54.5
Soybean meal (deoiled)	29.8	28.4	28.4	27.5
Maize gluten meal	6.2	8	8	8
Soybean oil	4.3	4.3	4.3	4.3
Common salt	0.5	0.5	0.5	0.5
Limestone powder	1.1	1.1	1.1	1.1
Dicalcium phosphate	2.0	2.0	2.0	2.0
Trace-mineral mix	0.5	0.5	0.5	0.5
L-lysine	0.02	0.02	0.02	0.02
DL-methionine	0.32	0.32	0.32	0.32
Coccidiostat	0.062	0.062	0.062	0.062
Selvit E	0.02	0.02	0.02	0.02
Toxin binder	0.055	0.055	0.055	0.055
Rovimix	0.2	0.2	0.2	0.2
Choline chloride	0.05	0.05	0.05	0.05
Turmeric powder	-	0.50	-	0.50
Ginger powder	-	-	0.50	0.50
Total	**100**	**100**	**100**	**100**

RESULTS AND DISCUSSION

Body weight and cumulative body weight gain

The average live body weights at the end of 6th week were 1906.81, 2003.86, 1919.59 and 1919.43 gm in treatment groups T_0, T_1, T_2 and T_3 respectively. The average body weight of broiler chicks in T_1 group was significantly ($P<0.05$) higher than all treatment groups and did not differ between groups T_0, T_1 and T_3. The result thus, indicated that supplementation of 0.50% turmeric powder in standard broiler diet is beneficial. The findings of Rajput *et al.* (2013), Abou-Elkhair *et al.* (2014) and Mondal *et al.* (2015) are comparable with that of present study who reported higher body weight in turmeric supplemented group as compared to control. The significant increase in body weight in turmeric supplemented group could be due to antioxidant activity of turmeric (*Curcuma longa*) at level of 0.50% which might have promoted hepatic function.

The average body weight gain at sixth week was 348.56, 357.17, 371.93 and 374.11g in T_0, T_1, T_2 and T_3 group respectively. The group T_2 and T_3 showed higher average body weight gain than T_0 and T_1 groups. T_3 group recorded highest average body weight gain than other groups. In the present study, the average weekly cumulative body weight gain at the end of six week was observed to be 1863.41, 1960.5, 1876.26, 1876.03 g in T_0, T_1, T_2 and T_3 groups, respectively. The treatment group T_1 revealed significantly ($P<0.05$) higher cumulative body weight gain than other groups during experimental trial. The findings by Moorthy *et al*. (2009), Wadhwa *et al.* (2011) Arkan *et al.* (2012), Suriya *et al.* (2012), Barazesh *et al.* (2013), Hussein (2013), Abou-Elkhair *et al.* (2014) and Mondal *et al.* (2015) regarding incorporation of turmeric and ginger powder in standard broiler diet can be compared with present findings.

Table 3: Per cent ingredient composition of broiler finisher mash

Ingredients (Kg)	Finisher			
	T_0	T_1	T_2	T_3
Maize	59.8	60.1	60.0	60.7
Soybean meal (deoiled)	21.8	20.6	20.6	19.6
Maize gluten meal	9	9.4	9.5	9.5
Soyabean oil	4.5	4.5	4.5	4.5
Common salt	0.5	0.5	0.5	0.5
Limestone powder	1.1	1.1	1.1	1.1
Dicalcium phosphate	2.0	2.0	2.0	2.0
Trace-mineral mix	0.5	0.5	0.5	0.5
L-lysine	0.1	0.1	0.1	0.1
DL-methionine	0.27	0.27	0.27	0.27
Coccidiostat	0.062	0.062	0.062	0.062
Selvit E	0.02	0.02	0.02	0.02
Toxin binder	0.06	0.06	0.06	0.06
Rovimix	0.2	0.2	0.2	0.2
Choline chloride	0.05	0.05	0.05	0.05
Turmeric powder	-	0.50	-	0.50
Ginger powder	-	-	0.50	0.50
Total	**100**	**100**	**100**	**100**

Feed Consumption and FCR

At the end of 6th week, average weekly feed consumption (g) per bird was recorded as 916.90, 879.66, 898.40, 924.21gm in T_0, T_1, T_2 and T_3 groups, respectively. The Average weekly cumulative feed consumption was observed as 3184, 3110, 3134; 3127 g in T_0, T_1, T_2 and T_3 groups, at 6th week respectively. The experimental broilers fed on control diet and other dietary treatments did not exhibit significant difference in feed intake at 1st and 6th week. The present study thus revealed that, turmeric and ginger supplementation in diet of broiler chickens did not exert any negative effect on weekly feed consumption. The present observations are in corroboration with those recorded by Doley *et al.* (2009), Kehinde *et al.* (2011), Nouzarian *et al.* (2011), Akbarian *et al.* (2012), Ahmed *et al.* (2014), Abou-Elkhair *et al.* (2014) and Mondal *et al.* (2015) who reported non-significant effect of turmeric and ginger powder on feed intake.

The average weekly feed conversion ratio of third week differed significantly and showed better FCR in treatment groups as compared to control, but during first, second, fourth, fifth and sixth the difference in weekly FCR was found non-significant. The cumulative feed conversion ratio during 3rd and 4th week differed significantly and showed better feed conversion ratio in T_1 group as compared to other groups including control. The average cumulative feed conversion ratio at the end of 1st, 2nd, 5th and 6th week however differed non-significantly. The corresponding average cumulative feed conversion ratio at the end of sixth week was calculated as 1.70, 1.58, 1.67 and 1.66 in T_0, T_1, T_2 and T_3 groups, respectively. The T_1 group showed numerically better average weekly cumulative feed conversion ratio than other groups but was statistically non-significant. The findings of present study are in agreement with the results reported by Mohammed and Yusuf (2011), Zomrawi *et al.*

(2011) and Akbarian *et al.* (2012) who reported non-significant difference in FCR when turmeric and ginger were added to basal broiler diet.

Retention of Nutrients

The highest dry matter retention was observed in T_1 (80.20%) and in T_0, T_2 and T_3 the values were 78.38%, 79.58% and 78.55% respectively. However the values did not differ significantly. The DM retention in turmeric supplemented group (T_1) was highest indicating better utilization of nutrients. The findings of present study are in line with findings of Minh *et al*. (2010) and EL-Matty *et al*. (2014) who reported similar values for DM digestibility ($P \geq 0.05$) in turmeric (78.01) and ginger (78.29%) supplemented groups. The highest OM retention was observed in T_1 (82.11%), followed by treatment T_3 (81.66%), T_2 (80.01%) and T_0 (79.87%). There was no significant difference between groups. EL-Matty *et al.* (2014) also reported no significant effect of turmeric (77.66%) and ginger (77.76%) powder supplementation on OM digestibility as compared to control (76.88%) in broilers fed turmeric and ginger. The highest CP retention was observed in T_1 (82.68%) followed by T_2 (82.30%), T_3 (80.28%) and the least in T_0 (77.55%) which differed significantly. The findings of the present study are in line with the findings of EL-Matty *et al.* (2014) who reported significantly higher digestibility of CP in broilers that received the diet containing turmeric (79.88%) and ginger (79.95%) powder as compared to control (76.09 %). The highest CF retention was observed in control group T_0 (42.41%) followed by treatment T_2 (41.52%), T_3 (41.02%) and T_1 (38.19%). With a nonsignificant differences. Similarly, EL-Matty *et al.* (2014) reported no significant effect on CF digestibility among the treatment groups in broilers fed on turmeric (24.14%) and ginger (24.75%) containing powders at the level of 0.50 g/kg in comparison to control (24.56%).

The highest EE retention was observed in T_1 (84.59%) followed by T_2 (84.57%), T_3 (84.30%) and the least in T_0 (80.86%). With a nonsignificant difference. The findings of present study are in line with findings of EL-Matty *et al.* (2014) who reported significant reduction in digestibility of EE in broilers fed on control diet (75.78) as compared to diet supplemented with turmeric (79.30%) & ginger (79.07%) powder. The NFE retention values were 81.88%, 82.24%, 81.93% and 82.07% in T_0, T_1, T_2 and T_3 respectively with a nonsignificant difference. The findings of the present study are non- significant. Our results are in agreement with those of EL-Matty *et al.* (2014) who reported that digestibility of NFE did not differ significantly ($P \geq 0.05$) between the broilers that received turmeric (77.33%) and ginger (77.53%) supplemented diets over broilers fed control (76.86%) diet.

Nitrogen, Calcium and Phosphorous Balance studies

The per cent N retained in metabolic trial was 78.33, 82.25, 80.50 and 78.97 (g/day) per bird. It was highest in T_1 followed by T_2, T_3 and T_0. With a nonsignificant difference. The higher retention of N by broilers fed 0.50 % turmeric powder supplemented diet could be due to higher body weight gain in comparison to broilers of other treatment groups.

The per cent retention of calcium was 41.4, 41.22, 40.90 and 39.41 (g/day) per bird for T_0, T_1, T_2 and T_3, respectively with no significant difference. However, the highest Ca retention percentage was observed in T_0 among different treatment groups. The per cent retention of phosphorus was 41.78 ± 1.73, 36.05 ± 3.45, 35.63 ± 3.94 and 38.58 ± 2.11 (g/day) per bird for T_0, T_1, T_2, and T_3 respectively with no significant difference. However the retention percentage of P was higher for T_0 group. The present study results are in agreement with Dono (2012) who reported the effect of turmeric meal on ash and nitrogen retention in broilers as non-significant. The data on N, Ca and P retention are not much available in literature for the broilers fed turmeric and ginger supplemented diets. Hence these retention values were compared with different feed additives like probiotics supplemented in the broilers diet. Munj *et al.* (2010) reported 76.92 and 81.28% N retention in broilers fed diets without and with probiotics. Kokje (1999) reported higher values for Ca and P retention in control group (45.51% and 53.46%) over probiotic supplemented group (34.04% and 45.86%), respectively. The improvements in the nutrient and energy utilization in current study might be attributed to the antimicrobial properties of phytochemicals in ginger and turmeric.

Table 4: Average proximate composition of the broiler pre-starter mash (% DM basis)

Particulars	Pre-starter			
	T_0	T_1	T_2	T_3
Moisture%	8.40	8.64	8.24	7.94
Crude protein%	23.07	23.07	23.12	23.12
Crude fibre%	5.53	5.64	5.32	5.28
Ether extract%	4.56	4.56	4.44	4.44
Total ash%	10.10	8.22	8.98	9.98
Nitrogen free extract%	56.74	58.51	58.14	57.18
Acid insoluble ash%	2.88	2.76	2.12	2.90
Calcium%	1.42	1.42	1.35	1.35
Total Phosphorus%	0.90	0.90	0.88	0.88
Calculated ME (kcal/kg)	3096	3094	3016	3016

Table 5: Average proximate composition of the different broiler starter mash (%DM basis)

Particulars	Starter			
	T_0	T_1	T_2	T_3
Moisture %	8.55	8.84	8.86	9.09
rude protein %	22.09	22.09	22.04	2.04
Crude fibre %	5.56	5.75	5.87	5.66
Ether extract %	3.59	3.73	3.66	3.78
Total ash %	10.53	11.62	10.40	9.04
Nitrogen free extract %	58.23	56.81	58.06	59.48
Acid insoluble ash %	2.74	2.81	2.75	2.82
Calcium %	1.42	1.40	1.41	1.42
Total Phosphorus %	0.64	0.72	0.70	0.68
Calculated ME (kcal/kg)	3110	3110	3116	3116

Table 6: Average proximate composition of different broiler finisher mash (%DM basis)

Particulars	Finisher			
	T_0	T_1	T_2	T_3
Moisture%	6.44	6.92	6.02	5.40
Crude protein%	20.86	20.99	20.26	20.89
Crude fibre%	5.51	5.56	5.76	5.60
Ether extract%	4.67	4.76	4.65	4.71
Total ash%	9.59	7.86	8.43	10.18
Nitrogen free extract%	59.37	60.83	60.90	58.62
Acid insoluble ash%	2.31	1.99	1.89	2.12
Calcium%	1.24	1.25	1.26	1.26
Total Phosphorus%	0.52	0.56	0.58	0.55
Calculated ME (kcal/kg)	3196	3196	3202	3202

Table 7: Details of Body weights, Body weight gains, Feed intake and FCR of Broilers Birds

Parameters	Treatment groups			
	T_0 (Control feed)	T_1 (To+0.5% turmeric)	T_2 (To+0.5% ginger)	T_3 (To+turmeric & ginger 0.5% each)
Average live body weight (g) of broilers				
Day-old body weights	43.40±0.75	43.36±0.54	43.33±0.14	43.40±0.46
Body wt 6th week of age	1906.81±10.92^b	2003.86±3.88^a	1919.59±1.84^b	1919.43±2.14^b
Average body weight gain (g) of broilers				
Body wt gain at 1st Week	109.3±1.65^c	121.9±0.83^a	111.03±0.56^c	115.43±0.54^b
Body wt gain at 6th Week	348.56±12.51	357.17±3.54	371.93±10.39	374.1±3.91
Average cumulative body weight CBW() gain (g) of broilers				
CBW gain at 1st Week	109.3±1.65^c	121.9±0.83^a	111.03±0.56^c	115.43±0.54^b
CBW gain at 6th week	1863.41±11.58^b	1960.5±3.44^a	1876.26±1.73^b	1876.03±2.24^b
Average feed consumption (g) per bird				
1st week	118.86±7.97	116.23±10.12	130.41±3.19	114.1±2.84
6th week	916.9±14.35	879.66±10.17	898±4.16	924.21±15.40
Average weekly cumulative feed consumption				
1st week	118.86±7.97	116.23±10.12	130.41±3.19	114.1±2.84
6th week	3184±65.01	3110.58±59.47	3134.43±40.04	3127.21±42.82
Average weekly mean feed conversion ratio and cumulative FCR per bird				
FCR at 1st week	1.08±0.06	0.95±0.08	1.17±0.02	0.98±0.02
Mean FCR at 6th week	1.60±0.22	1.48±0.23	1.56±0.20	1.54±0.22
CFCR at 6th week	1.70±0.03	1.58±0.02	1.67±0.02	1.66±0.02

Note: ^{a,b,c} Means with different superscript in a row differ significantly ($P<0.01$)

Table 8: Average nutrient retention of experimental broilers from different treatment groups

Nutrient Retention (%)	Treatment groups			
	T_0	T_1	T_2	T_3
Dry Matter	78.38±0.84	80.20±1.03	79.58±2.65	78.55±1.49
Organic Matter	79.87±1.31	82.11±0.70	80.01±0.71	81.66±0.99
Crude Protein	77.55±0.83^{b}	82.68±1.44^{a}	82.30±1.81^{a}	80.28±0.83^{ab}
Crude Fibre	42.41±2.04	38.19±4.76	41.52±2.44	41.02±1.14
Ether Extract	80.86±0.66	84.59±0.99	84.57±1.15	84.30±1.00
Nitrogen Free Extract	81.88±1.01	82.24±1.76	81.93±0.95	82.07±0.88

Note: ^{a,b,c} Means with different superscript in a row differ significantly (P<0.01)

Table 9: Average daily Nitrogen, Calcium, Phosphorous retention (g/bird) in broilers

Parameters	T_0	T_1	T_2	T_3
Nitrogen Retention (g/bird) Studies				
Nitrogen intake (g)	2.63±0.10	2.77±0.13	2.78±0.09	2.80±0.08
Nitrogen excretion (g)	0.57±0.04	0.48±0.04	0.54±0.03	0.59±0.02
Nitrogen retention (g)	2.06±0.08	2.29±0.15	2.24±0.11	2.21±0.08
Nitrogen retention%	78.33±1.41	82.25±1.89	80.50±1.41	78.97±1.08
Calcium Retention (g/bird) Studies				
Calcium intake (g)	0.98±0.03	1.04±0.02	1.07±0.03	1.03±0.01
Calcium excretion (g)	0.58±0.04	0.61±0.02	0.64±0.03	0.62±0.02
Calcium retention (g)	0.40±0.03	0.43±0.01	0.43±0.03	0.41±0.01
Calcium retention%	**41.4±3.36**	**41.22±1.11**	**40.19±2.32**	**39.41±1.03**
Phosphorous retention (g/bird) in broilers				
Phosphorous intake (g)	0.44±0.01	0.47±0.01	0.47±0.01	0.46±0.01
Phosphorous excretion (g)	0.26±0.01	0.30±0.01	0.30±0.02	0.28±0.01
Phosphorous retention (g)	0.19±0.01	0.18±0.01	0.20±0.03	0.18±0.01
Phosphorous retention%	41.78±1.73	36.05±3.45	35.63±3.94	38.58±2.46

Note: ^{a,b,c} Means with different superscript in a row differ significantly (P<0.01)

CONCLUSION

The average body weight of broiler chicks in T_1 group was significantly ($P \leq 0.05$) higher than all treatment groups which indicated that supplementation of 0.50% turmeric powder in standard broiler diet is beneficial. The present study also recorded that, turmeric and ginger supplementation in diet of broiler chickens did not exert any negative effect in weekly feed consumption and retention studies. Thus, in general the results of present study indicated better live body weight, FCR and nutrient retention in turmeric supplemented group compared to all other treatment groups.

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Effect of Partial Replacement of Concentrate Mixture by Wet Distillers Grain with Solubles (WDGS) on Feed Intake, Nutrient Digestibility and Feed Efficiency of Crossbred Lactating Cows (*Bos taurus*)*

Changan, S.D., Bhalerao, S.M., Khanvilkar, A.V[1]., Dhande, V.U. and Jadhav, S.A.
Department of Animal Nutrition, Krantisingh Nana Patil College of Veterinary Science, MAFSU, Shirwal-412801, Dist-Satara (Maharashtra)
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ABSTRACT

An experiment was undertaken on twelve Holstein Friesian (HF) crossbred cows for a period of thirteen weeks to study the feed intake, nutrient digestibility and feed efficiency. The experimental cows were allotted randomly into three groups of four and offered pelleted concentrate feed, maize silage as routine practice of farm. The control group (T_0) was maintained without replacement of concentrate mixture by Wet Distillers Grain with Solubles (WDGS), while in treatment groups the concentrate feed was replaced with WDGS on dry matter basis @ 10 and 20 per cent, for T_1 and T_2 groups, respectively. The average Dry Matter intake (DMI) during experimental period was recorded as 14.014, 14.076 and 14.089 kg in T_0, T_1 and T_2 groups respectively and the treatment groups showed DM intake on par with the control group. The average Digestible Crude Protein (DCP) intake was recorded as 1.370, 1.434 and 1.476 kg while, Total Digestible Nutrients (TDN) intake was ranged from 9.974 kg (T_0) to 10.404 kg (T_2). The DCP and TDN intake was significantly ($P \leq 0.01$) higher in treatment groups. The efficiency of feed utilization in terms of DMI, DCP and TDN required per kg of FCM proved beneficial when diet of crossbred lactating cows were supplemented with WDGS. The digestibility coefficients for Dry Matter, Organic Matter, Crude Protein, Ether Extract, Crude Fiber and Nitrogen Free Extract was recorded as 71.34, 70.90 and 71.62; 69.26, 70.20 and 70.42; 70.26, 71.55 and 72.60; 70.95, 71.58 and 73.86; 68.53, 70.22 and 72.64; 73.97, 75.81 and 76.63 in T_0, T_1 and T_2 groups, respectively. The results indicated improvement in overall digestibility of nutrients but differed non-significantly among treatment groups. Thus, the inclusion of WDGS in diet of lactating cows could not affect the overall intake of dry matter, digestibility coefficients of nutrients and the feed efficiency.

Keywords: WDGS, Crossbred lactating cows, Feed intake and nutrient digestibility

In India, present livestock composition indicates that, farmers are interested in dairy business as there is increase in the population of milking crossbred cattle from 14.40 to 19.42 million, and milking buffalo population increased from 48.64 to 51.05 million over previous census (*19th livestock census, published by GOI in 2014*) but there is shortage of feeds and fodders due to increased urbanization, rapid shrinkage of grazing lands. At present, the country faces a net deficit of 35.6% green fodder, 26% dry crop residues and 41% concentrate feeds *(Ministry of Agriculture, GOI, published in 2014).* Furthermore, cost of conventional feeds and fodders is also high and increasing day by day, which leads to under-feeding of dairy animals. Therefore, due to high cross breed cattle population and shortage of feeds and fodder to meet the daily nutrient requirement of high yielding dairy animals, there is low production of milk from these animals which affects the farm economy of Indian dairy farmers. Hence, to meet daily nutritional requirement

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[1]Associate Professor and HOD, Department of Livestock Production and Management.

of dairy animals, we need to look for newer, cheaper, locally available and nutrient rich feed sources like, Wet distillers grains with soluble (WDGS) and other fermented products which are traditionally not used by Indian dairy farmers to improve milk production and farm economy.

The Wet distiller's grains with solubles (WDGS) are by-products from ethanol production industry. The WDGS is palatable, excellent source of energy and has fairly high protein content (20% or and above crude protein in dry matter) of good quality with approximately 3.9g lysine/16g N. The crude fiber, crude protein and fat are concentrated approximately 3-fold in distiller's grains (DG) when the starch is fermented to produce ethanol. It also contains relatively high amounts of biologically available phosphorous and other minerals. Wet Distillers Grain with Soluble (WDGS) has a feeding value 30 to 40 per cent greater than maize when included at 10 to 40 per cent of diet DM. The feeding of WDGS results in improved cattle performance (Erickson and Klopfenstein, 2009). The feeding of WDGS to adult producing and working cattle can help to reduce the Green House Gases (GHG) emissions due to improved cattle performance and decreased energy costs at ethanol plant. In India, only few research trials have been done by using WDGS and DDGS in dairy animals because of its scanty data, knowledge of implementation and usefulness at farm level. However, the unconventional feed ressources like WDGS, brewer waste and DDGS could play a key role in reducing the cost of feed as well as improving the performance of animals.

MATERIALS AND METHODS

The experiment was conducted at Department of Animal Nutrition, Krantisingh Nana Patil College of Veterinary Science, MAFSU, Shirwal- 412801; Dist-Satara (Maharashtra) on twelve Holstein Friesian (HF) crossbred lactating cows for a period of thirteen weeks. The selected cows were randomly divided into three groups of four. All the standard managemental practices are followed during the entire trial period. The experimental groups T_0 served as control and received concentrate mixture and roughage as per the practice of farm. In treatment group T_1 and T_2 the concentrate mixture was replaced with WDGS by 10 and 20 per cent on dry matter basis, respectively. The fresh and moist wet distillers grain with solubles was procured from local vender. The feedstuffs including concentrate feed, silage, WDGS were analyzed for proximate principles as per AOAC (2005). The farm practice of feeding commercial concentrates and maize silage separately was followed throughout the experiment. The concentrate feed was offered twice a day just before milking at morning and evening hours. During the experimental period, the observations pertaining to feed intake recorded daily. During the last week of experiment, a digestibility trial of seven days duration was conducted by adapting total collection method. The representative sample of concentrate mixture, maize silage and WDGS collected daily for proximate analysis. The chemical composition of concentrate feed, maize silage and WDGS is presented in Table 1. The observations regarding various parameters recorded during experimental period were tabulated and the experimental data was analyzed as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The weekly average dry matter intake (kg) per cow is presented in Table 2. The dry matter intake (DMI) of cow was recorded as 14.014, 14.076 and 14.089 in $T_{0,}$ T_1 and T_2 groups, respectively. It was observed that the daily DMI of experimental animals receiving 10 and 20 per cent of WDGS was non-significantly higher than control group suggested that the palatability of feed in which concentrate mixture replaced with WDGS was comparable with control group. The similar observations to the present study were recorded by Penner *et al*. (2009) and Yeotikar (2011). However, Murdock *et al*. (1981) and Gehman and Konoff (2010) recorded significantly ($P<0.01$) higher daily DMI in treatment groups which were supplemented with WDGS than control group.

The observations showed that the average DCP intake/day/cow in T_0, T_1 and T_2 groups recorded as 1.370, 1.434 and 1.476 kg, respectively. The average daily DCP intake in treatment groups was significantly

($P \leq 0.01$) higher than control group and the per cent increase of the DCP intake/day/cow of treatment groups was 4.56 and 7.45 per cent for T_1 and T_2 groups, respectively, which was higher than control groups. Thus, results indicate that, the cows receiving WDGS had higher DCP intake which could be correlated to higher crude protein content in WDGS than commercial concentrate mixture. Similar findings has been made by Murdock *et al*. (1981) Yeotikar (2011), who reported considerable improvement in DCP intake in treatment groups when supplemented with wet distillers grain with soluble in the diet.

The average daily TDN intake by cows from T_0, T_1 and T_2 groups was recorded as 9.974, 10.295 and 10.404 kg, respectively. The average daily TDN intake by cows from treatment groups was found significantly ($P<0.01$) higher and it was about 3.13 (T_1) and 4.22 (T_2) per cent higher than control group. The results thus indicated positive effect of supplementation on the average daily TDN intake of cows in treatment groups. The findings in present experiment are in tune with Gehman and Konoff (2010) and Yeotikar (2011) who recorded higher TDN intake (kg) per day during lactation period with supplementation of WDGS in the diet of lactating cows.

The efficiency of feed utilization in terms of DMI, DCP and TDN required per kg of FCM produced is presented in Table 2 and graphically represented in Fig. 2. The average DM intake per kg FCM production in cows from control and treatment groups was recorded as 0.858, 0.845 and 0.828 kg, respectively. The control group required 1.52 and 3.55 per cent higher DMI / kg of FCM than T_1 and T_2 group respectively, while T_1 group required 2.03 per cent more DM than the T_2 group and treatment groups had significantly lower ($P \leq 0.01$) average DMI per kg FCM. Thus, efficiency of utilization of DM for milk production was significantly better in groups that received WDGS to partially replace in concentrate DM. The findings of present study were in agreement with Anderson *et al*. (2006) and Yeotikar (2011), who also recorded similar findings. The cows from T_0, T_1 and T_2 groups required 0.609, 0.616 and 0.613 kg TDN per kg FCM, respectively. It is also observed that partial replacement of concentrate mixture by WDGS on dry matter basis @ 10 and 20 per cent had non-significant effect on the TDN required per kg of FCM production in crossbred lactating cows. The average DCP intake per kg FCM was recorded as 0.089, 0.088 and 0.086 kg for T_0, T_1 and T_2 groups respectively. The difference in average DCP intake per kg FCM among all groups was non-significant. Thus, the substitution of WDGS for concentrate dry matter could not influence the efficiency of DCP utilization per kg of FCM in crossbred lactating cows.

The dry matter digestibility in T_2 was non-significantly higher than that in T_0 or T_1. Gehman and Konoff (2010) and Birkelo *et al*. (2004) also found non-significant effect on digestibility of dry matter and organic matter in cows due to inclusion of WDGS in the ration, while Yeotikar (2011) reported considerable improvement in total digestibility of DM and OM on inclusion of brewers grain in diet of lactating buffalo, hence present findings can be compared with findings of these workers. The average digestibility coefficients for crude protein were 1.88 and 1.81 per cent higher in T_1 and T_2 group supplemented with WDGS @ 10 and 20% respectively; it could be due to higher Crude protein (CP) per cent in WDGS. The similar findings made by Yeotikar (2011) who noticed increased digestibility by some units and Gehman and Konoff (2010) who recorded non-significant effect on CP digestibility in distillers grain supplemented group. However, Birkilo *et al*. (2004) noticed significantly ($P \leq 0.05$) increased digestibility of CP after inclusion of distiller's grain. The average digestibility coefficients for ether extract were 1.81 and 4.99 per cent higher for these nutrients as compared to control group; it could be due to higher EE present in WDGS. Similar findings made by Gehman and Konoff (2010), Birkilo *et al*. (2004) and Yeotikar (2011) who reported significantly increased digestibility of ether extract. The average digestibility coefficient of crude fiber was improved by 2.43 and 5.82 per cent for T_1 and T_2 groups, respectively over the control group. Thus, it was found that inclusion of WDGS in ration of lactating cows helped in improving CF digestibility and could be due to fewer amounts of acid detergent fire (ADF) and lignin

per cent in WDGS. The findings made in the present study could be corroborated with Gehman and Konoff (2010) and Birkilo *et al.* (2004) who reported improvement in ADF and NDF (neutral detergent fibre) digestion in WDGS included diet. The average digestibility coefficient of NFE (nitrogen free extract) indicating improved digestibility by 2.45 and 3.76 per cent. Thus, in general, it was observed that the overall digestibility of nutrients was comparable among all groups.

CONCLUSION

The replacement of concentrate mixture by WDGS proved beneficial in improving the overall dry matter intake, FCM yield, nutrient digestibility, feed efficiency in treatment groups supplemented with WDGS without adverse effect on DMI, nutrient digestibility and feed efficiency.

Table 1: Average chemical composition (% DMB) of concentrate, maize silage and WDGS of ration

Sl.No.	Nutrients	Concentrate Mixture	Maize silage	WDGS
1	Dry matter	89.09	31.10	29.60
2	Organic matter	94.75	95.96	92.00
3	Crude protein	22.35	7.55	31.48
4	Ether extract	3.24	2.5	8.78
5	Crude fibre	6.59	28.94	8.20
6	N.F.E.	62.65	57.37	44.11
7	Total ash	5.25	4.04	8.00
8	AIA	1.75	3.41	1.55
9	Calcium	1.42	0.46	1.64
10	Phosphorous	0.62	0.22	0.98

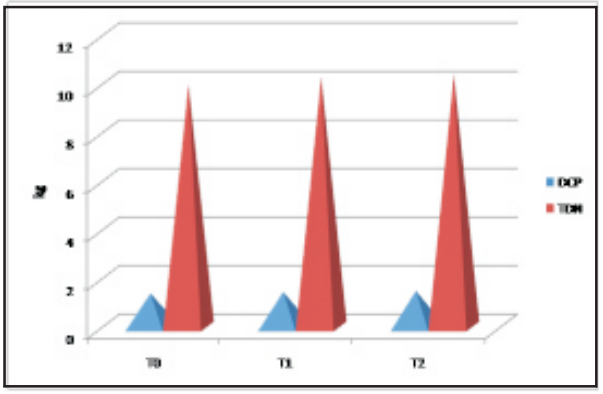

Fig. 1: Weekly average daily TDN & DCP intake of experimental cows

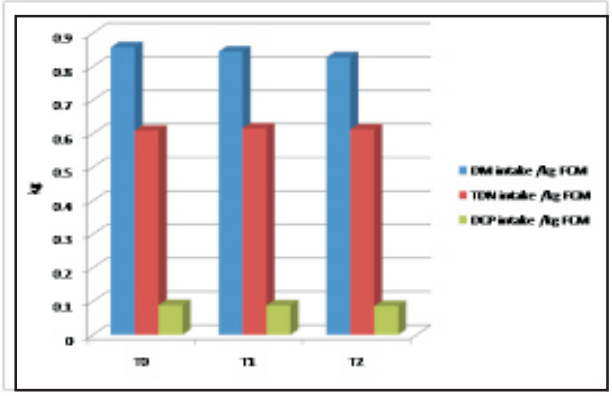

Fig. 2: Weekly average of daily DM, DCP & TDN intake (kg) /kg FCM yield

Table 2: Weekly Average Nutrient Intake and Digestibility Coefficients of Nutrient in Experimental Cows

Parameters *Test feed details*	**T_0 Roughage + Concentrate Mixture**	**T_1 Roughage+ 10% Conc. Mixture replaced by WDGS**	**T_2 Roughage+ 20% Conc. Mixture replaced by WDGS**
Nutrient intake by experimental cows			
Weekly average DMI (Kg)	14.014±0.08^{NS}	14.076±0.04^{NS}	14.089±0.04^{NS}
Weekly average daily DCP intake (Kg)	1.370±0.042^{c}	1.434±0.045^{b}	1.476±0.053^{a}
Weekly average daily TDN intake (Kg)	9.974±0.042^{c}	10.295±0.045^{b}	10.404±0.053^{a}
Weekly average daily milk yield (kg)	16.290±0.136^{b}	16.414±0.135^{b}	16.632±0.160^{a}
Weekly fat corrected milk yield (kg)	16.423±0.111^{NS}	16.691±0.128^{NS}	16.826±0.154^{NS}
Weekly average of DMI (kg) / kg FCM yield	0.858±0.008^{a}	0.845±0.002^{b}	0.828±0.006^{c}
Weekly average of TDN intake (kg)/kg FCM yield	0.609±0.008^{NS}	0.616±0.009^{NS}	0.613±0.004^{NS}
Weekly average of DCP intake (kg)/kg FCM yield	0.089±0.006^{NS}	0.088±0.009^{NS}	0.086±0.007^{NS}
Digestibility coefficients of nutrient in experimental cows			
Dry matter (%)	71.34±0.31	70.90±0.46	71.62±0.42
Organic matter (%)	69.26±0.35	70.20±0.36	70.42±0.48
Crude protein (%)	70.26±0.36	71.55±0.41	72.60±0.49
Ether extract (%)	70.95±0.60	71.58±0.53	73.86±0.46
Crude fiber (%)	68.53±0.45	70.22±0.64	72.64±0.57
Nitrogen free extract (%)	73.97±0.31	75.81±0.42	76.63±0.37
TDN (%)	68.78±0.22	70.19±0.24	72.66±0.28
DCP (%)	8.58±0.01	8.72±0.21	9.01±0.041

Note: ^{a,b,c} Means with different superscript in a row differ significantly (P<0.01), **NS:** Non-significant

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Molecular Genetic Analysis of Different Strains of Chicken*

Rashmi, S., Nagaraja, C.S., Jayashankar, M.R., Murthy, H.N.N[1]., Jayashree, R[2]. and Umakanth, B[3].
Department of AGB, Veterinary College, Hebbal, Bengaluru- 24
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ABSTRACT

The molecular genetic analysis of different strains of chicken was carried out with the objective of identifying the primers for RAPD analysis of different strains of chicken, to develop the RAPD pattern for differentiating the different strains of chicken and to evaluate genetic diversity and relatedness among different strains of chicken. Genomic DNA was isolated from blood samples of seven strains of chicken namely Naked Neck (NN), Indian Red-3 (IR3), Punjab Brown-2 (PB2), Indian Cornish-3 (IC3), Swarnadhara, Giriraja and Aseel and analysis was carried out by RAPD-PCR technique using five random primers. All the five primers used revealed polymorphism with a total of 55 bands and 38 polymorphic bands. The primer OPP-11produced the maximum scorable polymorphic bands and Primer-4 produced least polymorphic bands. Maximum genetic similarity was found between Giriraja and NN (83%) and minimum genetic similarity was found between NN and Aseel (60%). The within strain genetic similarity was maximum in Aseel (91%) and minimum in PB2 strain. The Mean Average Percentage Difference values ranged from 17.92 ± 4.67 between IC3 and Swarnadhara to 40.35 ± 5.58 between NN and Aseel. The dendrogram constructed revealed that the Aseel strain appeared to be most distant from other strains, followed by PB2 strain. The other strains NN, Swarnadhara and giriraja, IC3 and IR3 were closely related.

Keywords: RAPD, PCR, Chicken, Genetic diversity

Biodiversity can be described at several levels from phenotypic observations to molecular data. Insight into the relationships between breeds can be obtained by examining difference between phenotypic traits. Protein polymorphism is one such phenotype used as marker to estimate genetic variation within and across chicken populations (Mina *et al*., 1991). However, these markers show low degree of polymorphism reflected by a high degree of similarity of gene frequencies among populations and lines, especially when these are closely related. Another class of polymorphic markers, immunogenetic markers such as blood groups, is characterized by genotyping complications and requires accurate locus and allele identification (Gintovt *et al*., 1983). Thus, genetic variation analysis based on the above markers limits chicken biodiversity studies.

Randomly Amplified Polymorphic DNA (RAPD) technique was developed by Williams *et al*. (1990) and Welsh and McClelland *et al*. (1990). This technique is based on polymerase chain reaction using primers homologous to random target sites in the genome. The main advantages of RAPD assays are that it is simple, less labor intensive, comparatively less expensive and safe. The RAPD has been used for various applications including species identification (Kemp and Teale, 1994), establishing genetic relationship (Smith *et al*., 1996), estimating genetic diversity (Sharma *et al*., 2001) and genome mapping (Levin *et al*., 1993) in various livestock species including poultry. These studies reflected the effectiveness of RAPD as potential genetic marker. Molecular genetic studies in chickens, especially using RAPD technique, are very few. Hence the present

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[1]Dean, Veterinary College, Bengaluru.
[2]Associate Professor, Veterinary College, Shivamogga.
[3]Associate Professor (Rtd.), Department of Poultry Science, Veterinary College, Hebbal, Bengaluru.

project attempted to study the intra and inter-population genetic variability in various chicken strains and to establish the genetic relationship between them by using RAPDs marker.

MATERIALS AND METHODS

***Genetic Stock*:** The present study comprised a total 70 birds 10 each of Naked Neck, Indian Red 3, Punjab Brown 2, Indian Cornish 3, Swarnadhara, Giriraja and Aseel. The birds were chosen from University Poultry Farm, Veterinary College, Bangalore, except for Aseel breed, which were selected from Central Poultry Development Organization, Hessarghatta, Bangalore.

***DNA isolation*:** about 2-3ml of blood was collected via brachial vein in vacutainers (3ml) with EDTA (1mg/ml) as anticoagulant agent from of seven strains containing 10 birds each and stored at -20^{0}C. The high molecular weight DNA was isolated using the protocol adopted by checking optical density at 260nm on U.V Spectrophotometer (Smith *et al*., 1996).

***RAPD analysis*:** the 5-decamer primers (Table 1) of arbitrary sequences with high GC (60-70%) content were used. The amplification reaction was carried out in final volume of 20ml reaction mix comprising of 25ng (1ml) of template DNA, 2ml of primer (40p.mol/ml), 200mM of dNTP mix, 1 unit of Taq DNA polymerase, 2.0ml of 10x TE buffer, with the distilled water volume made up to 20ml. The content was mixed thoroughly by spinning for five seconds at 5000 rpm. The DNA was denatured initially at 94 ^{0}C for two minutes. The amplification was carried out for 40 cycles with following thermal cycles. One final extension at 72 ^{0}C for ten minutes was included in the programme. About 8ml of PCR product was used for electrophoresis in 2% at 85V and RAPD bands were visualized and documented in gel documentation unit 2000 (Bio Rad).

Statistical analysis: the RAPD patterns were scored for the presence or absence of bands and the data entered into a binary character matrix. The genetic similarity within and between strains was calculated using the following measures.

a. Band sharing: The band sharing between the individuals was calculated as

$$BS_{ab} = 2 (Bab) / (Ba + Bb)$$

Where, Bab is the number of bands shared by individual a and b,

Ba is the total number of bands for individual a and Bb is the total number of bands for individual b.

b. Mean average percentage difference (MAPD) between species

Mean average percentage difference (MAPD) was calculated as an expression of interstrain dissimilarities. This value was calculated on RAPD bands obtained with the primers using the following three formulae (Gwakisa *et al*., 1994).

1. Percentage difference (PD) = [Nab / (Na + Nb)] x100
2. Average percentage difference (APD) = 1/C S PD_i
3. Mean average percentage difference (MAPD) = 1 / R $SAPD_i$

Where Nab is the number of fragments that differed between two individuals for a single primer.

Table 1: Details of RAPD primers used for analysis of poultry DNA samples

Sl.No.	Primers	Sequence (5'-3')	Length	Source
1	OPP-11	AAC GCG TCG G	10 mer	BioServe Pvt. Ltd.
2	BG-06	CTG AGA CGG A	10 mer	"
3	OPA-16	AGC CAG CGA A	10 mer	"
4	OPP-17	TGA CCC GCC T	10 mer	"
5	Primer-4	ACC GCC GAA G	10 mer	"

Na is the number of fragments resolved in individual a. Nb is the number of fragments resolved in individual b. C is the number of interbreed pair-wise comparisons and R is the number of random primers used.

Dendrogram: Unweighted Pair Group Average Method (UPGAM) of analysis was done using *Statistica* software and a dendrogram was constructed to show the phylogenetic relationship among the seven strains of chicken studied.

RESULTS AND DISCUSSION

The five primers amplified a total of 55 bands, of which 38 bands were polymorphic (69.09%). The total number of bands ranged from seven to 15. The amplified fragments ranged in molecular weights from 120 to 2600bp (Table 2). The primer OPP-11 produced the maximum scorable as well as polymorphic amplified DNA fragments (Fig. 1). In contrast, Primer-4 produced comparatively less polymorphic bands (57.1%). The proportion of primers capable of detecting the polymorphism among the strains evaluated depends upon the genetic background of the strains, genetic distance between them and complexity of the genome. Earlier works reported the values ranging from 4 to 13 per cent (Smith *et al.*, 1996; Wei *et al.,* 1997), 18 per cent (Shivaraman *et al*., 2001), 24 per cent (Sharma and Singh, 2001), and 40 per cent (Ahlawat *et al*., 2004) to 43.6 per cent (Khosravinia *et al*., 2005) proportion of polymorphism in chicken. The present study indicated that the selected primers were efficient in producing polymorphic patterns among the different chicken strains. RAPD markers are dominant markers in that the presence or absence of a particular allele is detected as heterozygote having at least one dominant

Table 2: Number of Randomly Amplified Polymorphic DNA bands amplified and number of polymorphic bands for different primers

Primers	No. of Bands Amplified	Number of Polymorphic Bands	Size Range of Fragments(~bp)	% Polymorhism
OPP-11	13	11	200-2600	84.6
BG-06	15	11	200-2300	73.3
OPA-16	10	6	200-2600	60.0
OPP-17	10	6	200-1500	60.0
Poultry-4	7	4	300-2300	57.1

Table 3: Overall mean average percentage difference values between Strains

Strains	IR3	PB2	IC3	Swarnadhara	Giriraja	Aseel
NN	24.0 ±4.95	20.79 ±4.27	20.23 ±4.10	25.49 ± 1.39	24.33 ±1.81	40.35 ±5.58
IR3	-	20.94 ±4.58	22.55 ±4.58	21.27 ± 4.18	28.72 ± 4.62	37.88 ± 7.22
PB2	-	-	22.82 ± 5.10	22.82 ± 5.10	26.1 ±3.95	37.28 ±1.63
IC3	-	-	-	17.92 ± 4.67	23.56 ±4.50	30.38 ±4.52
Swarnadhara	-	-	-	-	20.73 ±3.91	30.38 ±4.52
Giriraja	-	-	-	-	-	35.81 ±3.51

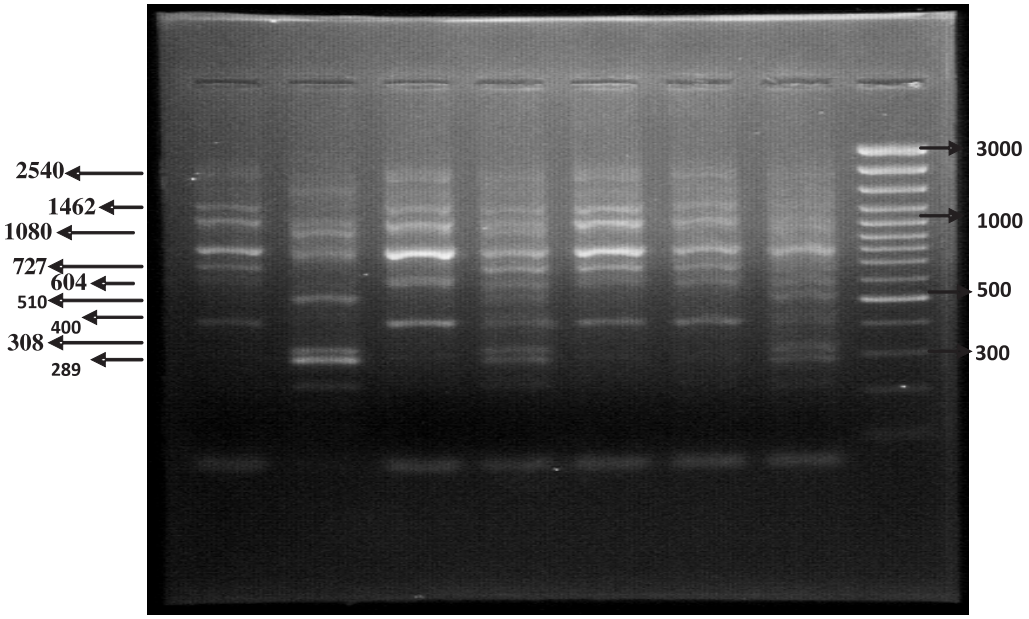

Fig. 1: Randomly Amplified Polymorphic DNA profile generated by primer OPP-11 from seven strains of chicken *viz.,* Naked Neck (NN), Indian Red-3 (IR3), Punjab Brown-2 (PB2), Indian Cornish-3 (IC3), Swarnadhara, Giriraja and Aseel. Lane M is molecular size marker (3kb DNA ladder)

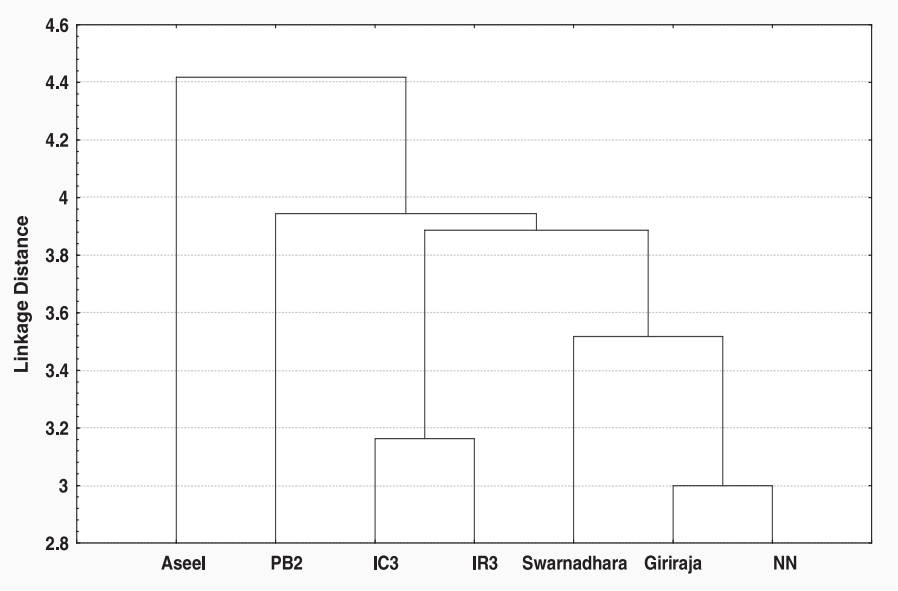

Fig. 2: Unweighted Pair Group Average Method (UPGAM) Phylogenic Tree

allele of RAPD marker when a band is present or as homozygous recessive in the absence of specific band (Levin *et al*., 1993; Wei *et al*., 1997), which may be the reason for absence of band in certain strains. This necessarily takes the complete pedigree reports pertaining to selected individuals for conclusive observation. The average band sharing within the strains NN, IR3, PB2, IC3, Swarnadhara, Giriraja and Aseel were 0.89, 0.88, 0.79, 0.81, 0.89, 0.82 and 0.91 respectively. The maximum within strain genetic similarity (or minimum genetic distance) was found in Aseel (91%) strain. In contrast, minimum genetic similarity (maximum genetic distance) was found in PB2 strain (79%). The average band sharing between different strains of chicken for primers OPP-11, BG-06, OPA-16, OPP-17 and Primer-4 were 0.73, 0.73, 0.69, 0.75 and 0.83, respectively. The maximum between strain genetic similarity (or minimum genetic

distance) was found between Giriraja and NN (83%) and minimum genetic similarity (maximum genetic distance) was found between NN and Aseel (60%). Higher within strain genetic similarity may be due to long term intra population selection and inbreeding and genetic variability may be attributed to genetic factors like population size, selection factors, evolutionary difference etc. Between-population genetic variation may reflect the different sources of origin of the strains and their subsequent propagation. Therefore, the strains NN, IR3, PB2, IC3, Swarnadhara, Giriraja and Aseel, which were expected to be evolved from different sources, even though they were, subjected to similar selection regimes, their response to selection could vary due to their differential reproductive and productive potentials. Hence, some diversity between them is to be expected. The results of RAPD analysis also revealed very low intra-population genetic variability as reflected from high estimates of within strain genetic similarity in these strains. The MAPD values ranged from 17.92 ± 4.67 between IC3 and Swarnadhara to 40.35 ± 5.58 between NN and Aseel (Table 3). Phylogenetic relationship among seven strains was constructed using Unweighted Pair Group Average Method (UPGAM) based on RAPD data, and a dendrogram was constructed (Fig. 2). The Aseel strain appeared to be more distant from other strains, followed by PB2. Strains NN, Giriraja, Swarnadhara, IC3 and IR3 were comparatively closely related.

CONCLUSION

The present study revealed that RAPD markers are effective in detecting polymorphism between different strains of chicken and provide a potential tool for evaluating intra as well as inter strain genetic variations and for establishing genetic relationship. Primer OPP-11 is the primer of choice for differentiating the chicken strains. Aseel can be included in breeding programme based on necessity to create variation and to develop new genetic group.

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Biochemical Traits in Serum and Muscle of Different Breeds of Birds Influencing Taste Quality of Meat*

Hemashree, P., Girish Kumar, V., Arangaswamy, A[1]., Sunil Kumar, K.K., Divya, V. and Nandi, S[1]
Department of Veterinary Biochemistry, Veterinary College, Hebbal, KVAFSU, Bengaluru
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ABSTRACT

The present study was conducted to assess various qualitative traits such as tenderness, juiciness, taste and flavour of meat as influenced by biochemical constituents such as fat, glutamate and lactate (LDH activity) in four different breeds of both sex. A total of 48 birds, 12 from each group (six male and six female birds) were selected. The muscle and blood samples were collected at their respective age of slaughter, *i.e.,* at 8, 6, 12 and 24 weeks for Punjab broiler-1, commercial broiler, Giriraja and native chicken breed respectively. The results for muscle crude fat content revealed no significant difference when observed between sex within a breed or between breeds within a sex. However, the muscle crude fat content of the pooled samples (irrespective of sex within a breed) revealed significantly higher level of muscle crude fat in the native breed when compared to other breeds, The results for serum glutamate content revealed no significant difference when observed between sex within a breed or between breeds within a sex. However, the serum glutamate content of the pooled samples (irrespective of sex between breed) revealed significantly higher level of serum glutamate in the native breed when compared to other breeds, Likewise the muscle glutamate content was found to be lower in the male commercial broiler when compared to other three breeds with highest value being recorded in the native breeds, however no significant change was observed between breeds in the female bird. So also the serum and muscle LDH activity was found to be similar in all the four breeds when observed irrespective of sex between breeds and / or between sex within a breed. The variations in the results of muscle crude fat and glutamate content between breeds indicate that existence of variation in taste quality being due to variations in fat and glutamate content.

Keywords: Taste quality, Biochemical parameters, Poultry, Meat quality

This increase in demand for poultry meat is attributed to relatively low price, no religious taboo (Jaturasitha, 2004) and low fat/ cholesterol (Petracci and Cavani, 2012), rendering large population of consumers for poultry meat with good eating qualities like taste, tenderness, juiciness and flavor (Mullen and Troy, 2005). The taste quality of meat is influenced by its biochemical composition such as lactate (Olaoye, 2011), glutamate (Fuke and Shimizu, 1993), fat (Miller, 1994) and proteins (Bongioni *et al*., 2004), which may be the reason for the meat of desi/native birds being superior in quality when compared to that of commercial broilers (Wattanachant, 2008), As this is not authenticated the comparative study of the biochemical characters influencing taste in Punjab broiler-1, commercial broiler, Giriraja and native chicken breed was undertaken.

MATERIALS AND METHODS

The present study was conducted in four different chicken breeds. The serum and muscle samples from Punjab broiler-1, Giriraja and native chicken breeds

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[1]National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru.

were obtained from birds reared in Poultry Farm, Veterinary College, Bengaluru and the same for commercial broiler chicken was obtained from birds belonging to a private integrated broiler poultry farm. A total of 48 birds, 12 from each group (six male and six female birds) were selected for present study. The muscle and blood samples were collected at their respective age of slaughter, *i.e.* at 8, 6, 12 and 24 weeks for Punjab broiler-1, commercial broiler, Giriraja and native chicken breed respectively.

Collection and Processing of Serum and Muscle Sample

The blood and approximately 100g of muscle (breast muscle) sample were collected from all birds belonging to the present study at the time of slaughter. The serum was separated and stored under -80°C in labeled vials until further analysis. The muscle homogenate was prepared as per the method described by Heinova *et al*. (1999). 100 mg of tissue was manually homogenised using liquid nitrogen in a pestle and mortar followed by addition of 1.0 ml of 0.05M tris hydrochloride buffer (Tris HCl) pH 7.3 and 20 ìl of 100 mM phenyl methyl sulfonyl fluoride (PMSF) and protease inhibitor. Later the homogenate was transferred into 1.5ml eppendorff tubes for homogenisation using polytron homogenizer-PT2100 until the tissue was homogenized uniformly and centrifuged at 13000 rpm for one hour at 4°C, the supernatant so obtained was transferred into labeled sterile 1.5 ml eppendorf tube and stored at -80°C until further analysis.

The assay of biochemical parameters in all the stored serum and muscle samples were as follows. The crude fat content in the muscle was estimated by method of Soxhlet (1995). The glutamate content in serum and muscle sample was assayed as per the method of Beutler (1990), by employing Megazyme L-glutamic acid assay kit (Catalogue No.K-GLUT 07/12) supplied by Megazyme, Ireland. The total LDH enzyme activity in serum and muscle sample was analysed as per modified method of Kornberg (1995).

The results muscle crude fat, glutamate and total LDH activity as well as serum glutamate and total LDH activity were subjected for statistical analysis by ANOVA and t-test using computerized prism software (GraphPad prism 5). The statistical analysis was done to compare the mean value of crude fat, glutamate and total LDH activity between different breeds (*i.e.*, Punjab broiler-1, commercial broiler, Giriraja and native birds), between sex within a breed and for pooled data (*i.e.,* irrespective of sex between different breed).

RESULTS AND DISCUSSION

The mean crude fat in muscle of Punjab broiler-1, commercial broiler, Giriraja and native breed in male, female and pooled (male and female) birds is as shown in Table 1. The result of the pooled samples revealed for significantly higher level in the native bird, Punjab broiler and Giriraja birds when compared to that in commercial broiler, with highest concentration in native birds. Likewise, the results of serum and muscle glutamate content (as shown in Table 2) in the pooled samples revealed for significantly higher level in the native bird, Punjab broiler and Giriraja birds when compared to the that in commercial broiler, with highest concentration in native birds. Further, results of total LDH activity revealed no significant difference when compared between different breeds of birds within a sex as well as when compared between sex within a breed (Table 3).

The higher values of muscle crude fat in native breed when compared to other breeds as observed in the present study could be due to species variation, type of growth, feeding behaviour as opined by Fanatico *et al*. (2007) and Wattanachant (2008). Further, the samples from all breeds collected revealed for absence of significant difference between sex, the same could be due to the fact that oestrogen in female and testosterone in the male birds influence in a similar rate for the uptake of fat from plasma by muscle and synthesis of fat in the muscle (Leclereq, 1984 and Hulan *et al*., 1989) and the increase in the value in females when compared to male birds could be due to oestrogenic effect as opined by Tumova and Teimouri (2010). Estrogen increases the adiposity in the poultry and testosterone has inhibitory effect on lipid accumulation in the poultry.

Table 1: Mean crude fat (%) content in muscle of different breeds of birds

Sex	Punjab broiler-1	Commercial broiler	Giriraja	Native breed
Male	2.7 ± 0.19	2.47 ± 0.3	2.83 ± 0.19	3.08 ± 0.07
Female	2.98 ± 0.25	2.64 ± 0.16	3.3 ± 0.17	3.3 ± 0.18
Pooled	2.82 ±0.15^{ab}	2.55 ± 0.16^{a}	3.06 ± 0.14^{ab}	3.16 ± 0.09^{b}

Superscripts bearing different alphabets within a row indicates significant difference between the means

Table 2: Mean glutamate in serum (g/L) and muscle (g/100g) of different breeds of birds

Sample	Sex	Punjab broiler-1	Commercial broiler	Giriraja	Native breed
Serum	Male	0.56 ± 0.03	0.5 ± 0.04	0.52 ± 0.05	0.70 ± 0.1
	Female	0.51 ± 0.06	0.43 ± 0.05	0.50 ± 0.09	0.64 ± 0.09
	Pooled	1.33 ±0. 8^{ab}	1.20 ± 0.06^{a}	1.52 ± 0.11^{ab}	1.69 ± 0.13^{b}
Muscle	Male	3.00 ± 0.26^{a}	1.85 ± 0.09^{b}	2.94 ± 0.26^{a}	3.09 ± 0.32^{a}
	Female	2.25 ± 0.31	1.65 ± 0.12	2.55 ± 0.27	2.65 ± 0.41
	Pooled	2.62 ± 0.22^{ab}	1.74 ± 0.07^{a}	2.74 ± 0.18^{ab}	2.87 ± 0.25^{b}

Superscripts bearing different alphabets within a row indicates significant difference between the means

Table 3: Mean total LDH activity in serum (IU/L) and muscle (IU/mg) of different breeds of birds

Sample	Sex	Punjab broiler-1	Commercial broiler	Giriraja	Native breed
Serum	Male	2.00 ± 0.22	2.43 ± 0.08	2.09 ± 0.22	2.44 ± 0.03
	Female	2.30 ± 0.19	2.33 ± 0.1	2.32 ± 0.13	2.36 ± 0.26
	Pooled	2.15 ± 0.14	2.38 ± 0.06	2.20 ± 0.12	2.20 ± 0.14
Muscle	Male	9.63 ± 0.60	9.33 ± 1.07	9.68 ± 0.97	10.25 ± 0.30
	Female	9.60 ± 0.81	7.51 ± 1.20	8.43 ± 1.21	9.81 ± 0.72
	Pooled	9.61 ± 0.47	8.42 ± 0.81	9.05 ± 0.76	10.03 ± 0.37

Superscripts bearing different alphabets within a row indicates significant difference between the means

The glutamate content in the serum when compared between different breeds within a sex and between sex within a breed revealed no significant difference, the significantly increased total serum glutamate content in the pooled sample of the native bird when compared to other breeds could be due to their genotype and innate scavenging type of feeding behavior which leads to increased absorption of glutamate in native birds as has been opined by Wattanachant *et al*. (2004). Further the serum glutamate content in the male birds was found to be greater than that in the females in all four breeds could be due to the large muscle mass in males when compare to females (Wattanachant, 2008). The significant increase in muscle glutamate content of male Punjab broiler-1, male Giriraja and male native bird when compared to male commercial broiler as well as the value of the muscle glutamate content being greater in both male and female of native breed when compared to other breeds could be due to the reasons as put forth by Wattanachant *et al*. (2004), who opined that the glutamate content in pectoralis and biceps femoris of Thai indigenous to be greater than in broilers to be due to its genotypes, rearing system and feeds.

The increased serum LDH enzyme in the native bird when compared to other breeds of birds could be due to significant muscle development at older ages and due to physiological changes that are normal in different ages (Silva *et al*., 2007 and Pietruszynska *et al*., 2010) and also due to nature of flight in birds as opined by (Rioux and Blier, 2006). Further, the male native breed and commercial broilers show higher serum total LDH activity when compared to the female of same breeds could be due to the presence of large muscle mass and more cardiac work load as opined by Bowes *et al*. (1989) and genetic makeup between sex as opined by Hassaan *et al*. (2009). However, the reason for reverse trend of the same in Punjab broiler-1 and Giriraja birds of the present study is not known. Whereas the higher total muscle LDH activity in the male birds of all breed could be due the reasons mentioned above.

The muscle pH (5.6) is a key factor for water holding capacity and juiciness of the meat and it is the resultant of the amount of lactate, which is acidic in the muscle during the conversion of muscle to meat which is further influenced by the amount and the activity of LDH enzyme (Olaoye, 2011). So the reason of greater total serum and muscle LDH activity in native birds when compared to other breeds as well as in male native birds when compared to female native birds indicating that pH and water holding capacity is more in these birds which probably is the cause for improved texture, juiciness and taste in native birds. Further, it can also be postulated that there is increased glycolytic potential also in the muscle of native birds when compared to other breeds as has been reflected by increase in the value of LDH activity and in turn the lactate production when compared to other breeds of birds in the present study.

From the foregoing, it can be construed that a significantly increased muscle glutamate content in the male Giriraja, native and Punjab broiler-1 with highest content of the same present in male native bird indicates that savoury taste of the native bird being higher.

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Therapeutic Management of Recurrent Haemobartonellosis in a Cat – A Case Report

Ramesh, P.T., Anupama, K. and Chetan Kumar, G.K.
Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru
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ABSTRACT

Haemobartonellosis caused by *Haemobartonella felis*, is one of the most common disease condition in cats. Cat's red blood cells are often parasitised by these organisms. In the present paper a case of recurrent hemobartonellosis and its successful management with Imidocarb dipropionate is discussed.

Keywords: Haemobartonellosis, Feline infectious anemia, *Haemobartonella felis,* Alkaline phosphatase, Alanine amino transferase, Red blood cells

Feline haemobartonellosis is also known as feline infectious anemia or feline hemotropic mycoplasmosis. It is caused by *Haemobartonella felis,* otherwise called as *Mycoplasma hemofelis*. These are Gram negative pleomorphic bacteria, which can be found singly, in pairs, or in chains and they will parasitize red blood cells. Cats can also get infected by *M.haemominutum,* but it is less pathogenic (Foley and Pedersen, 2001). *Haemobartonella felis* is a pathogenic large strain of organism, which causes severe haemolytic anemia (Flint *et al*., 1958 and Foley *et al*., 1998). Infected animals shows the clinical signs such as pale mucous membrane, lethargy, anorexia, weight loss and depression and also haematocrit values will be reduced. (Foley *et al.,* 1998 and VanSteenhouse *et al.,* 1993). The present paper reports the case of recurrent haemobortonellosis and its successful management in a cat.

CASE DETAILS

A one and half year non-descripttom cat was presented to the Department of Veterinary Medicine, Veterinary College, Bengaluru with the case history of anorexia, depression, vomiting and weakness since 4 days. Physical examination of animal revealed high body temperature (104 ^{o}F), increased heart rate (143 beats/minute), respiratory rate (32 breaths /minute) and weak pulse. Conjunctival and oral mucus membranes were pale along with ventral abdomen and ear pinna.

Hematological examination revealed low red blood cell count ($3.1X10^{6}$/µl), hemoglobin concentration (6g%) and decreased packed cell volume (24%). There was an increase in mean corpuscular volume (60fl)and decrease in mean corpuscular haemoglobin concentration (24g/dl) indicating macrocytic hypochromic anemia**.** Leucocyte count (6.3×10^{3}/µL) and platelet counts (400×10^{3}/µL) were within the normal range but alanine amino transferase(126 IU/dl) and alkaline phosphatase levels (72 IU/dl) were increased. Smear stained with Giemsa's method revealed coccoid organisms around the periphery of the erythrocytes, anisocytosis and polychromasia (Butt, 1990). Based on physical, hematobiochemical and blood smear examination case was diagnosed as feline hemobartonellosis.

TREATMENT AND DISCUSSION

Pet was kept on doxycycline tablet @ 10mg/Kg Body weight, s.i.d (Tab. Doxypet**,** Sava Healthcare limited), orally after making it as slurry for 14 days (Tasker and Lappin, 2002), because oral administration of dry pills causes oesophageal strictures in cats. Apparent recovery was noticed after 14 days of therapy with doxycycline. After two months animal was presented to hospital with similar history. Clinical examination of animal revealed, body temperature was subnormal (99.6 ºF), increased heart

(165 beats/minute) and respiratory rates (38/minute), with pale and icteric visible mucous membranes.Blood smear examination revealed *Haemobartonella felis* organisms in the periphery of red blood cells. Haematobiological examination revealed low red blood cell ($3.2x10^6$/µl), hemoglobin (6.8g/dl) and packed cell volume (28%) with marginally increased platelet count(3,20,000),alanine amino transferase (132 IU/dl) and alkaline phosphatase (69I U/dl). Based on history, clinical signs, hematobiological changes and blood smear examination the condition was diagnosed as haemobartonellosis. The animal was treated with imidocarb dipropionate (Imicarb®120 mg/ml, SAVA Vet) @ 5mg/Kg body weight intramuscular (Lappin *et al.*, 2002). To reduce the cholinergic effects of imidocarb dipropionate, the animal was pre-treated with atropine. Inj. Dexamethasone was given @ 1mg/Kg Body weight to reduce immune-mediated hemolytic anemia (Vansteenhouse *et al*., 1993).

Hematobiochemical examination on day 14 revealed normal packed cell volume (35%), hemoglobin (9.9g%) and red blood cells ($5.2x10^6$/µl) but alanine amino transferase and alkaline phosphatase were still on higher side, so the second dose of imidocarb dipropionate was administered. Review hematological examination was carried on day 28 in which packed cell volume, hemoglobin, alanine amino transferase and alkaline phosphatase were within normal rangeand the blood smear examination did not reveal any organisms. Owner was advised to come after a month for review. Month later, when pet was presented to clinics, it was active, appetite was good, hematobiochemical parameters were within the normal limit and blood smear examination was negative for *Haemobartonella felis* organisms indicating complete recovery.

In the present study hematological examination revealed macrocytic hypochromic anemia, because of immune mediated hemolytic anemia caused by *Haemobartonellafelis.* Similar observations were made by Tasker and Lappin (2002), Balazs *et al.* (1961) and Vansteenhouse *et al.* (1993).

Imidocarb dipropionate is approved for use to treat *Babesia canis* infection in dog but the drug may also effective against *Haemobartonella felis* infection in cats. Imidocarb dipropionate acts on the organisms by two mechanisms, by interfering with production and/or utilization of polyamines or prevention of entry of inositol into the erythrocyte containing parasite. Imidocarb dipropionate is known to cause hepatic damage when used at higher dosage. In the present study no such untoward reactions were observed. Thus use of imidocarb dipropionate is safe and effective in treating recurrent *Haemobartonella felis* in cats.

CONCLUSION

Recurrent haemobartonellosis in cat can be successfully managed with imidocarb dipropionate injection at 14 days interval until the desired packed cell volume is reached.

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Trans-Rectal and Trans-Abdominal Ultrasonography for Early Pregnancy Diagnosis in Goats

Chamaraj, P., Markandeya, N.M., Mali, A.B. and Puro, N.A.
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, MAFSU, Parbhani-431402
Received: 01/04/2017 ***Accepted:*** 18/09/2017

ABSTRACT

Ultrasonography provides an efficient tool for early diagnosis of pregnancy in all farm animals. Transrectal and Transabdominal approaches are available for scanning and both have been tried in goats. The present study was carried at semi-intensive non-descript goat farm for the study of comparative efficiency of Trans-rectal (TR) and Trans-abdominal (TA) ultrasonography for early pregnancy diagnosis in goats. Twenty four does bred naturally after synchronizing were examined by ultrasonography on 22 to 25th days of gestation by TR and TA approaches respectively. Repeated examinations were performed on 35th to 38th day of gestation. On scanning 16 goats were found to be pregnant and 8 goats were confirmed as non-pregnant on the basis of embryonic vesicle or embryo proper with heart beats by TR approach. The mean foetal crown rump length CRL by Transrectal approach in 16 goats was 7.3 ± 0.4 mm on 22 to 25th day. By Trans-abdominal scanning only 4 goats were confirmed pregnant with CRL 6.1 ± 0.2mm on 22nd to 25th day but on 35 to 38th day of scanning, all 16 goats were confirmed as a pregnant by Trans-abdominal ultrasonography with proper visualization of amniotic vesicle and embryo with heart beats. CRL was recorded by both approaches on ultrasonography and the mean CRL was found to be 24 ± 0.3 mm and 17.5 ± 0.2 mm on 35 to 38th day by TR and TA approach respectively. It was concluded that use of trans-rectal ultrasonography was found to be very effective for early diagnosis of pregnancy in goats than trans-abdominal approach.

Keywords: Ultrasonography, CRL, Goat, Transrectal, Transabdominal

An accurate and early diagnosis of pregnancy is very important for improving reproductive efficiency in goats. Early identification of pregnancy and non-pregnancy provides breeder with an opportunities to cull the non-pregnant animals, to improve reproductive efficiency by controled breeding programmes. Real time B-mode ultrasonography provides a simple, rapid, accurate and non-invasive technique for pregnancy diagnosis and counting fetal numbers in small ruminants on the farm (Buckrell, 1988). In recent years, real-time ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants. Here, the two approaches for pregnancy diagnosis have been used are the transabdominal (Fowler and Wilkins, 1984; White *et al*., 1984; Taverne *et al*., 1985) and transrectal procedures (Gearhart *et al.,* 1988; Garcia *et al*., 1993; Kahn *et al.*, 1992). Caprine pregnancy (anechoic intrauterine fluid) can be diagnosed by trans-rectal ultrasonography (6-8 MHz) and trans-abdominal ultrasonography (3.5to 5MHz) as early as at days 17-20 and 25-28 of gestation, respectively. While the embryo proper can be imaged between days 22-24 and 27-33 days of gestation (Amer, 2010).The present study was undertaken to study the comparative efficiency of trans-rectal and trans-abdominal ultrasonography for early pregnancy diagnosis in goats.

MATERIALS AND METHODS

Non-descript goats (n=24) were synchronised and bred naturally and date of mating of each doe was noted. A real time B-mode, portable

ultrasonography machine (SSD- 500 Aloka Co. Ltd., Japan and Wipro GE logic 100 CL, India) with 7.5 MHz and 3.5 MHz trans-rectal linear rectal probe and trans-cutaneous abdominal sector transducer were used for scanning of uterus.

RESULTS AND DISCUSSION

Pregnancy was confirmed on the day 22 after mating in 16 out of 24 goats examined based on presence of embryonic vesicle, embryo proper with heart beats, foetal crown rump length using B-mode real time trans-rectal ultrasonography. From 22 to 25 day the mean CRL was found to be 7.3 ± 0.4 mm, subsequently trans-abdominal scanning was carried but only 4 goats were confirmed pregnant with CRL of 6.1 ± 0.2mm. Second examination was performed on 35 to 38th day of gestation and CRL was recorded by both approaches on USand the mean CRL was found to be 24 ± 0.3 mm and 17.5 ± 0.2 mm, respectively.

These measured CRL in mm were put in to the formula as explained by Singh *et al*., (2004) (Y=24.42 ± 0.39 X, where Y is gestational age (days) X is crown rump length (CRL in mm) while 24.42 and 0.39 are constant factors), to calculate the age of the foetus, which was compared with recorded gestational age.

CONCLUSION

It is concluded that use of trans-rectal ultrasonography was found to be very effective than trans-abdominal approach in conformation of pregnancy in goats at earlier stage.

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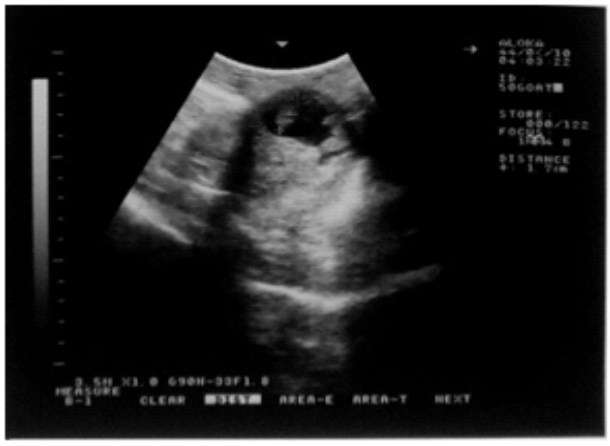

Fig. 1: Transabdominalsonographic image measuring CRL 1.7cm

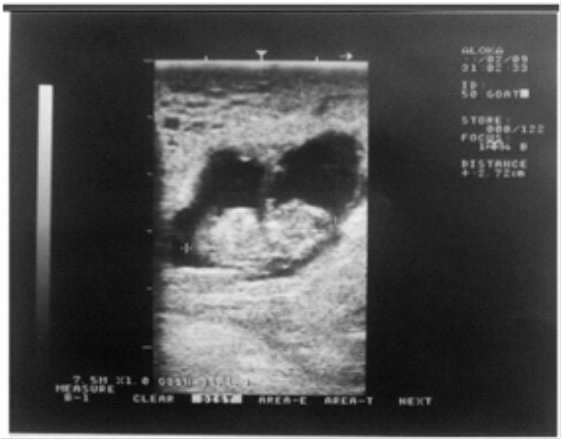

Fig. 2: Transrectalsonographic image measuring CRL 2.7cm

Daysof gestation after mating	USG measurements in mm (mean)		Gestational age in days GA= (24.42+0.39X) X=CRL in mm	
	Trans-rectal	Trans-abdomen	Trans-rectal	Trans-abdomen
22-25	7.3 ± 0.4	6.1 ± 0.2	27.2 ± 0.2	26.7 ± 0.1
35-38	24 ± 0.3	17.5 ± 0.2	33.7 ± 0.1	31.2 ± 0.1

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Management of Sarcoptic Mange in Rabbits with Biweekly Parentral Ivermectin

Ramesh, P.T., Nethravathi, B.T. and Chetan Kumar, G.K.
Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru
Received: 08/07/2017 ***Accepted:*** 15/12/2017

ABSTRACT

Sarcoptic mange caused by *Sarcoptes scabiei* is one of the most common disease in rabbits. Weekly parenteral ivermectin administration is commonly practiced in rabbits for scabies. Present study reveals the therapeutic usage of biweekly ivermectin in scabies affected rabbits. Two non-descriptive rabbits presented with the signs of alopecia, pruritis, lichenification, scab over the upper lip, ear pinnae, periocular and mandibular region. Laboratory examination confirmed the presence of *Sarcoptes scabiei* mite in skin scrapping. The affected rabbits were successfully treated with ivermectin @ 400 µg / kg body weight sub-cutaneously at two week interval about 6 weeks. Uneventful recovery was observed in both the rabbits after 6 weeks of therapy.

Keywords: Rabbit, Mange, *Sarcoptes scabiei*, Ivermectin

In rabbits dermatological diseases are the most common problems when the climate is hot and humid (Aulakh *et al*., 2003). *Sarcoptes scabies* var. *cuniculi* and *Psoroptes cuniculi* are most common mites prevailed in rabbits (Panigrahi and Gupta, 2013). Sarcoptic mange infestation causes major economic losses in commercial rabbit farms in India (Ravindran and Subramanium, 2000; Darzi *et al*., 2007). Overcrowded living conditions and poor hygiene are significant factors for sarcoptic scabies (McCarthy *et al*., 2004).

The eggs from fertilized female mites will be laid in the pseudo-tunnels created by them in the outer skin layer. Young larvae can also be found within the skin while older larvae, nymphs, and males dwell on the skin surface. Mites feed on lymph and sloughed epithelial cells (Hofing and Kraus, 1994). The mites produce their pathological effects by burrowing activity and mechanical damage through excavation, irritation and allergic reactions to some of their extracellular products (Wall and Shearer, 1997). It causes intense itching, pyoderma, crust formation, scale production, thickening and wrinkling on skin of affected areas. The intense pruritus often causes alopecia and dermal abrasions which lead to serous encrustations and secondary bacterial dermatitis. (Scott *et al*., 2001). Clinical signs include scab and crust formation and lichenification on the upper lip, ear pinnae, eyelids, lower jaw and limbs pruritis, alopecia and in prolonged illness the animal was suffered from, emaciated and may even, die due to cachexia (Roy *et al*., 2001).

Diagnosis of *S. scabiei* can be made by identification of the mite by microscopic examination of skin scrapings (Suckow *et al*., 2002). Among several acaricides, ivermectin given either orally or parentrally has been very much effective. However ivermectin was given weekly intervals in most studies (Aulakh *et al.,* 2003; Quesenberry and carpenter, 2004). In this study parenteral administration of ivermectin with increased dose (400 µg / kg) and interval (biweekly) was attempted and reported.

Case History

Two non-descriptive rabbits aged 3 and 10 months were brought to the Veterinary College, Hebbal, Banglore with history of intense itching and hair loss. Physical revealed erythema, alopecia and brownish indurated dry scab like lesions on ears, nose, face, around ears, paws and around genitalia (Fig. 1). Skin scrapings were taken from lesions using a blunt scalpel blade dipped in liquid paraffin. Skin scrapping

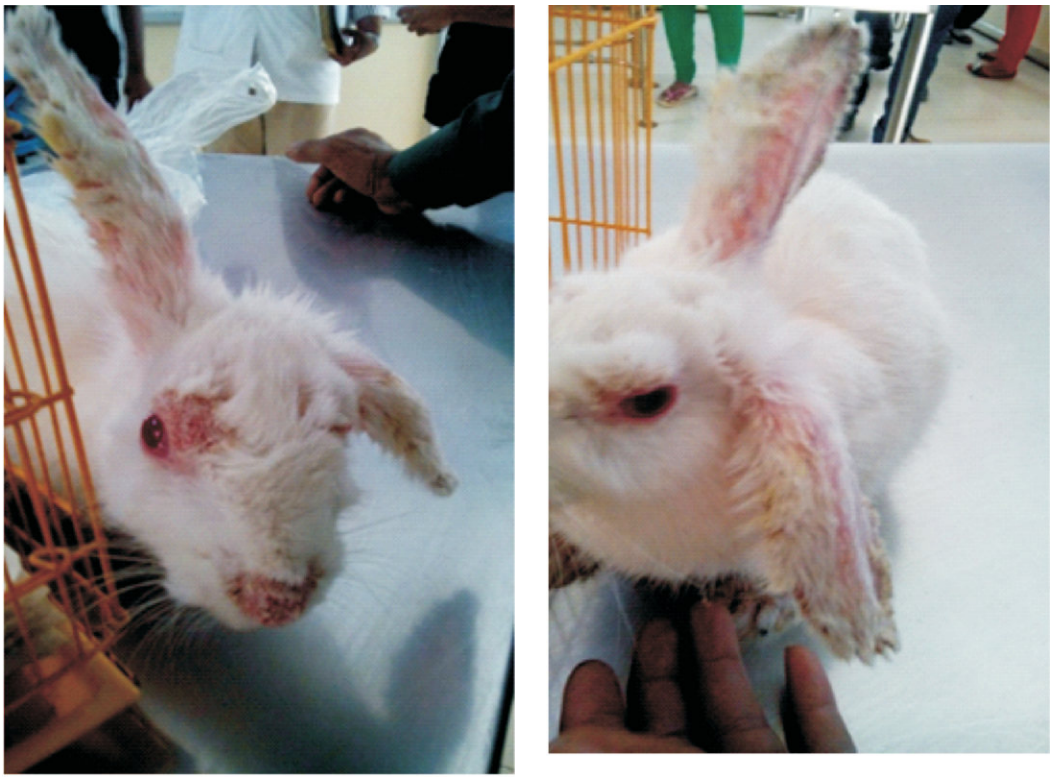

Fig. 1: Erythema and brownish dry crust like lesions on ears, nose, face, around eye before treatment.

Fig. 2: Complete recovery in the above said animal after ivermectin application.

examination revealed round bodied, short legged, adult *Sarcoptes scabiei* organism. Case was confirmed as scabies based on laboratory findings, clinical signs and physical examination.

Treatment and Discussion

The affected rabbits were treated with subcutaneous injection of ivermectin (Trumectin®) @ 400 µg/kg body weight once in 14 days (Plumb's, 2008). At the end first week pruritus was completely reduced. Animals were continuously monitored by skin scraping examination on every two weeks interval. There was marked clinical improvement by fourth week of treatment with reduced lesions and scraping examination found negative for mites. After 3 shots of ivermectin therapy, all the rabbits were recovered completely.

Panigrahi and Gupta (2013) and Bharadwaj *et al*. (2012), reported that subcutaneous injection of ivermectin at weekly interval was effective in rabbits. However in the present study injection of ivermectin @ 400ug/Kg at two weeks interval resulted in complete recovery.

Mellgren and Bergval (2008) reported pain and ataxia after the ivermectin injection in rabbits but in the present study there was no such untoward reactions which indicates that ivermectin can be effectively and safely used in rabbit at two weekly interval for clearance of *Sarcoptes scabiei*.

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Successful Therapeutic Management of Notoedric Mange in a Persian Breed of Cat

Akshatha, G.M., Arpitha, G.M., Anil Kumar, M.C. and Chamaraj, K.P.
Department of Veterinary Medicine, Veterinary College, Hassan
Received: 17/07/2017 ***Accepted:***10/12/2017

ABSTRACT

Feline scabies is a parasitic disease of cats caused by mites usually *Notoedres cati*. These mites cause severe infestation in cats, generally starting on the face and ears and spreading to the rest of the body and are highly contagious. The present abstract describes a case of *Notoedres cati* which was observed in female Persian cat of 8 months old presented to the department of Teaching Veterinary Clinical Complex (TVCC), Veterinary College, Hassan with a history of severe pruritis/ itching, crusts and alopeciasince last one week. On physical examination, ticks, flea and flea dirt were not found. Skin scarping examination (superficial and deep) revealed the presence of both live and dead adult mites of *Notoedres cati*. Based on the history, clinical signs and skin scraping examination it was confirmed the cat was affected with feline scabies. The infected cat was treated with ivermectin 200 µg/kg BW. The skin scrapping examination was carried on day 7 and 14 post treatment to assess the efficacy of the treatment whichrevealed absence of mites. The cat was uneventfully recovered after two weeks of treatment.

Keywords: Cat, Notoedric mange, Ivermectin

Notoedric mange is rare, highly contagious disease of cats and kittens caused by *Notoedres cati*, which can opportunistically infest other animals, including humans (Griffin *et al.,* 1993). Notoedres mites are smaller than Sarcoptes, have 'thumb print'-like dorsal striations, shorter limb stalks and a dorsal anus compared with the terminal anus, dorsal pegs and spines seen on *Sarcoptes* spp. (Scott *et al.,* 2001).The clinical manifestation is, as in scabies, characterised by intense pruritus, hyperkeratosis, peeling skin and lesions, especially on the face andthe ears (Friberg, 2006), extending to the neck, limbs and other body areas in the case of massive infestation. The present report describes a case of Notoedric mange infestation in Persian cat and its successful therapeutic management with ivermectin.

Case History and Observations

An eight month old female Persian cat was presented to the Department of TVCC, Veterinary College, Hassan, with a history of severe pruritis, alopecia and presence of crusts on neck region (Fig.1 & 2). On physical examination, ticks, flea and flea dirt were not found on the external body surface of the cat. Few drops of liquid paraffin was applied and spread over the skin scrapping site and scrapped with a blunt scalpel blade. The material was taken to laboratory and processed by boiling in 10% KOH and centrifuged to concentrate mites. A drop of sediment was spread over a microscopic slide covered with a glass cover slip and examined under the low (10X) and high (40X) power of the microscope which revealed the presence of live and dead adult mites of *Notoedres cati* which was identified based on morphological characteristics such as round body, short legs and long unjointed stalk with a sucker shorter limb stalks and a dorsal anus (Fig. 3 & 4) similar to the reports of Walker (1994). Based on the shape and the presence of dorsal anus in the mite was differentiated as *Notoedres cati* from *Sarcoptes* species.

Treatment and Discussion

Notoedric mange caused by the mite *Notoedres cati* (Astigmata: Sarcoptidae) is a cutaneous ectoparasitic disease of mammals. This disease often

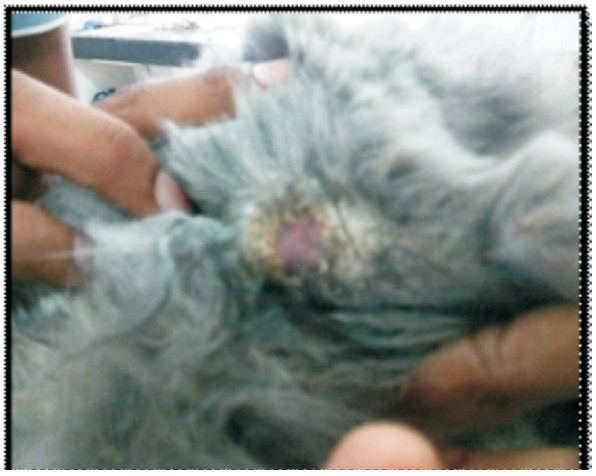

Fig. 1: Crusts and alopetic lesion in the cat

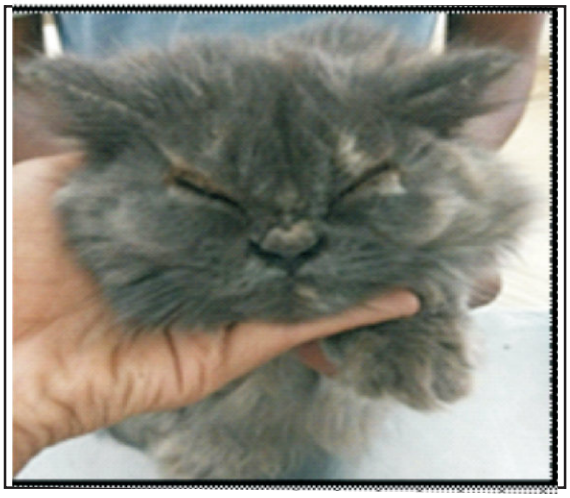

Fig. 2: Crust formation on nose of the cat

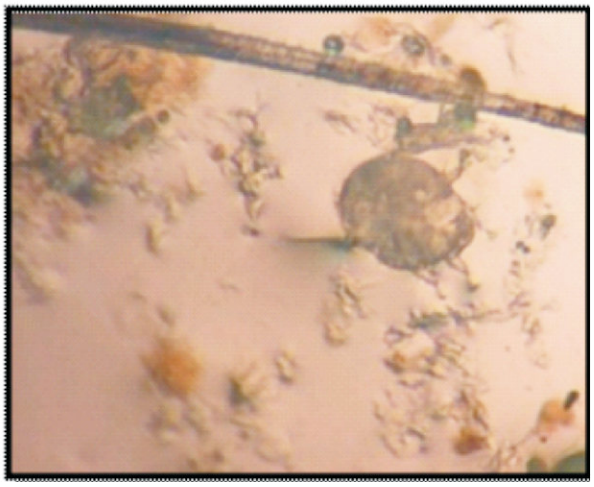

Fig. 3: Microscopic view of *Notoderes cati* mite (100X)

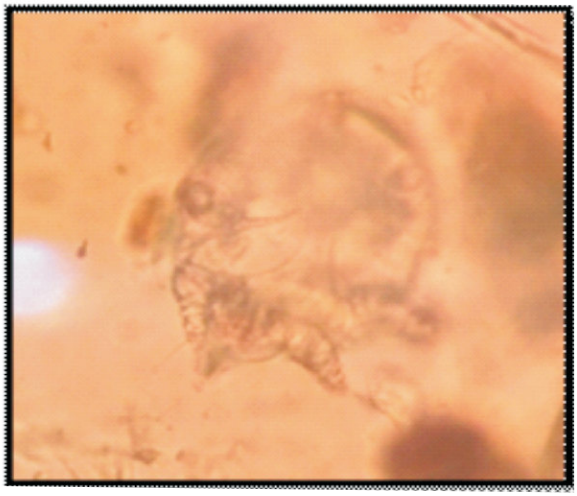

Fig. 4: Microscopic view of *Notoderes cati* mite (400X)

affects domestic cats (*Felis catus*), wild cats and more rarely it has become a major disease among wild animals in captivity and natural reserves (Valenzula *et al.,* 2000). The condition is highly contagious and primarily occurs by direct contact between animals or by contact with infested bedding or sites recently visited by infested animals. The clinical manifestation is, as in scabies, characterised by intense pruritus, hyperkeratosis, peeling skin and lesions, especially on the face and the ears (Friberg, 2006), extending to the neck, limbs and other body areas in the case of massive infestation. The clinical symptoms are often aggravated by secondary bacterial infections, initiated by the excoriations from self-trauma, and the disease can even be lethal. Finally the mite possesses also a zoonotic potential and has been diagnosed in humans after close contact with infested animals (Chakrabarti, 1986; Fujita *et al.,* 1997).

Based on the microscopic examination of skin scraping it was confirmed that the cat was suffering from feline scabies caused by *Notoedres cati*. Treatment was started with subcutaneous administration of ivermectin at the rate of 200 µg per kg body weight (Scott *et al.*, 2001). Thereafter because of the inconvenience of the owner to present the animal to the hospital oral ivernectin tablets @ 400 µg /kg body weight (NEOMEC@10mg) was

prescribed. Clinical recovery was observed along with the absence of mite in skin scraping examination on 7th and 14th day of post treatment.Senthil Kumar *et al.* (2008) reported that higher efficacy with parentral ivermectin at 200 µg/kg body weight, S/C in cats with *Notoderes cati.* The successful recovery of catin the present study confirms the benefit of ivermectin in the treatment of cats suffering from Notoedric mange.

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Atresia Ani Associated With Congenital Recto-Vaginal Fistula in a Malnad Gidda Calf

Naveen, M. and Ravi Raidurg
Department of Veterinary Surgery and Radiology, Veterinary College, Vinobanagara, Shivamogga
Received: 15-05-2017 ***Accepted:*** 29-12-2017

ABSTRACT

Two days old Malnad Gidda calf was presented with a complaint of absence of anal opening and passage of dung and urine through the vulval opening since birth. The condition was diagnosed as atresia ani associated with congenital recto-vaginal fistula and it was successfully treated by surgical intervention. Calf recovered without any complications.

Keywords: Congenital, Atresia, Fistula, Malnad Gidda

Atresia ani is a congenital anomaly observed in calves, lambs and kids. It may also be associated with atresia or agenesis of other parts like atresia recti, rectovaginal fistula, rectocystic fistula, vaginourethral fistula, taillessness, hypospadias, cleft scrota etc. (Tyagi and Jit Singh, 2008). Recto-vaginal fistula is another congenital problem causing direct communication between rectum and vagina in female calves responsible for mixing of faecal matter and the urine (Abdul *et al.,* 2012). A case of atresia ani associated with congenital recto-vaginal fistula in a Malnad Gidda calf and its successful surgical management is reported here.

Case History and Observation

A two day old Malnad Gidda female calf was presented to Department of Veterinary Surgery and Radiology, Veterinary College, Shivamogga with a complaint of passage of urine and faecal matter from the vulval opening. Clinical eaxamination revealed absence of anal opening and a fistula measuring two finger diameter in vagina. Faecal matter and urine were coming from the vulval opening. Calf was active and healthy. The case was diagnosed as "atresia ani associated with rectovaginal fistula.

Surgical site was prepared asceptically. Pre-operatively, Inj Ceftriaxone Tazobactum[a] 562.5 mg IV was administered. Inj Lignocaine HCL[b] 1ml was administered epidurally. Initially anal opening was created as per standard procedure. Skin between anal and vulval opening was incised to expose the fistula and uro-rectal septum (Fig. 1). Transverse incision was made on the uro-rectal septum to separate rectal and vaginal membrane. Vaginal membrane was apposed by simple continuous pattern using polyglactin 910[c] No.1 in a longitudinal manner. Rectal membrane was apposed by simple continuous pattern using polyglactin 910 No.1 in a transverse manner. Finally, skin was sutured by simple interrupted pattern using Monofilament Polyamide Suture[d] No.1 (Fig. 2). Post-operatively, inj Meloxicam[e]@ 0.2mg/kg b.wt IM was administered. Calf was kept on antibiotic for another 6 days. Sutures were removed on 10th post-operative day. Animal recovered without any complications.

RESULTS AND DISCUSSION

Noden and deLahunta (1985) stated that during development of embryo, the urorectal septum grows caudally and separates the cloaca into dorsal and ventral chambers with dorsal portion forming anal

[a]Intacef Tazo, Intas Pharmaceuticals Ltd., Ahmedabad.
[b]Lox 2%, Neon Laboratories Ltd., Mumbai.
[c]Vicryl, Johnson and Johnson Pvt. Ltd., Mumbai.
[d]Trulon, Sutures India Private Ltd., Bengaluru
[e]Melonex, Intas Pharmaceuticals Ltd., Ahmedabad.

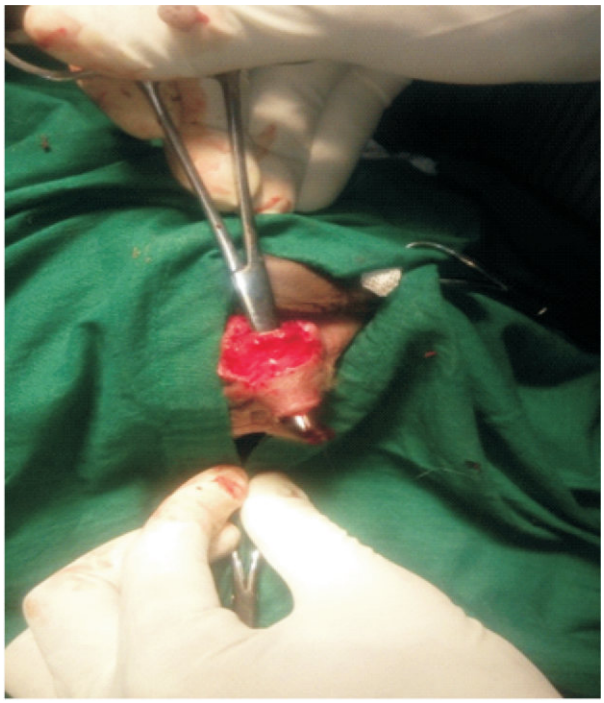

Fig. 1: Figure showing rectovaginal fistula through a created anal opening

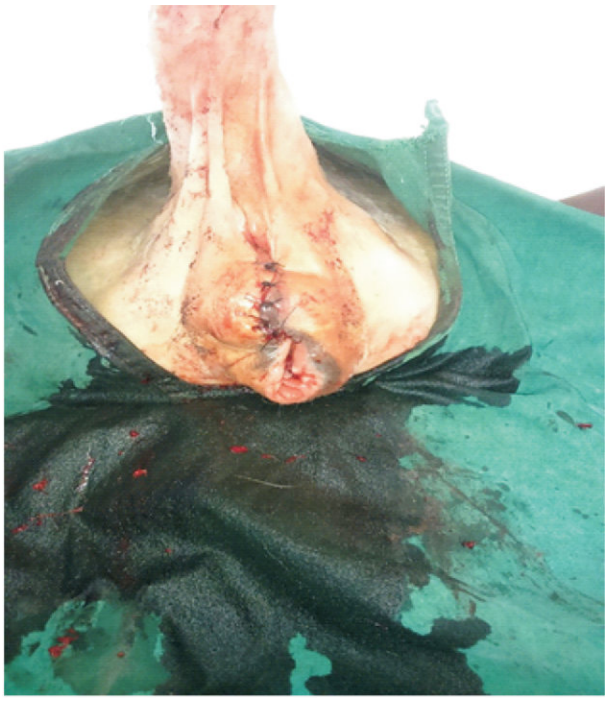

Fig.2: Figure showing post-surgical site

folds while the ventral one forming urogenital folds. Usually cloacal folds differentiate into anal and urogenital folds. Failure in the differentiation results in congenital anamolies like atresia ani, rectovaginal fistula and fused labia. Aslan *et al*. (2009) reported a case of recto-vaginal fistula, imperforate anus and vulvular non–formation in a buffalo calf.

Chaudary *et al.* (2016) surgically repaired a case of congenital atresia of vulva, atresia ani and rectovaginal fistula in a lamb. Mahlar and Williams (2005) and Abdul *et al.* (2012) surgically closed defects of rectum and vulvular lips individually after isolating and transecting the fistula and then reconstructed anal opening. Mahlar and Williams (2005) and Abdul *et al.* (2012) surgically closed defects of rectum and vulvular lips individually after isolating and transecting the fistula and then reconstructed anal opening.

In the present case, anal opening was created first. Skin between the anal and vulval opening was severed. Rectal and vaginal membranes were separated from the urorectal septum and sutured individually. Surgery is the only treatment of choice for correcting this type of congenital abnormalities in calves.

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Surgical Management of Coenurosis in a Sheep

Manjunatha, D.R., Nagaraju, N., Basavaraj, B., Veena, M. and Vasanth, M.S.
Department of Veterinary Surgery and Radiology, Veterinary College, Hassan-573202
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ABSTRACT

Three years old ewe was presented with a history of swelling near brisket region which size was increasing day by day. On physical examination the swelling was found to be slightly hard, haemetobiochemical parameters were within the normal range. Ultrasonography reveled clear anechoic structure which appeared like a cyst. The cyst was surgically removed and microscopic examination confirmed *Coenurus gaigeri*. The sheep made uneventful recovery on 10th post operative day.

Keywords: *Coenurus gaigeri*, Cyst, Ultrasonography, Coenurosis

Non cerebral *Coenurus gaigeri*are known to occur in animals and man and are caused by a metacestode of *Taenia gaigeri* (Ing *et al.,* 1998), it is commonly reported in the shoulder, thigh, neck muscle, diaphragm, heart, kidney, uterus, rectum and urinary bladder of domestic goats (Varma and Malviya, 1989; Manjunatha *et al.,* 2010; Naveen and Anjaneya, 2015) and sheep (Oryan *et al.,* 2012; Christodoulopoulos *et al.,* 2013; Saritha *et al*., 2015). This paper presents a case of *Coenurus gaigeri* cyst in the brisket region of a sheep and its successful surgical management.

Case History and Observation

Anewe aged about three years was presented to the Department of Veterinary Surgery and Radiology, Veterinary College Hospital, Hassan, with a history of swelling near the brisket region with increasing in size day by day (Fig. 1). On physical examination the swelling was slightly hard, haemetobiochemical parameters were within the normal range. Ultrasonography revealed clear anechoic structure in a capsule (Fig. 2), on ultrasound guided aspiration reveled clear fluid. Based on the physical and ultrasonographic examination the case was tentatively diagnosed as a cyst and surgical excision was considered as the option.

Treatment and Discussion

The animal was fasted for twenty four hours, sedated with Xylazine I/M at the rate of 0.08 mg/kg body weight (Indian Immunological ltd, Hyderabad – 32) and was secured on the surgical table. Surgical area was prepared aseptically, after local infiltration with lignocaine HCl 2% (Inj Xylocaine, 30ml vial, Astra Zeneca, Bangalore-63). Incision was made on the swelling, subcutaneous tissue was dissected and a sac containing clear fluid with scolices was removed (Fig. 3). Surgical wound was closed in a routine manner. Post-operatively on alternate day the wound was dressed and inj, Enrofloxacin was administered at the rate of 5 mg/kg body weight once a dayfor 5 days. The animal recovered uneventfuly by 10 days without any complication. Further upon examination of the cyst, clear fluid in the sac containing many scolices attached to wall and floating in the fluid was found and it was confirmed as *Coenurus gaigeri* by microscopic examination.

Persual of literature showed *Coenurus gaigeri* known to occur in domestic and wild ruminants in India (Varma *et al.,* 1994) and is significantly higher in goats than sheep (Oryan *et al.,* 2012), where as in the present case it was reported in domestic sheep. Christodoulopoulos *et al.* (2013) reported that there were no specific clinical signs except swelling in *Coenurus gaigeri* affected sheep, similar finding was recorded in the present case also. Saritha *et al*. (2015) reported non cerebral *Coenurus gaigeri* in the quadriceps femoris muscle of male sheep and its successful surgical management. In the present case

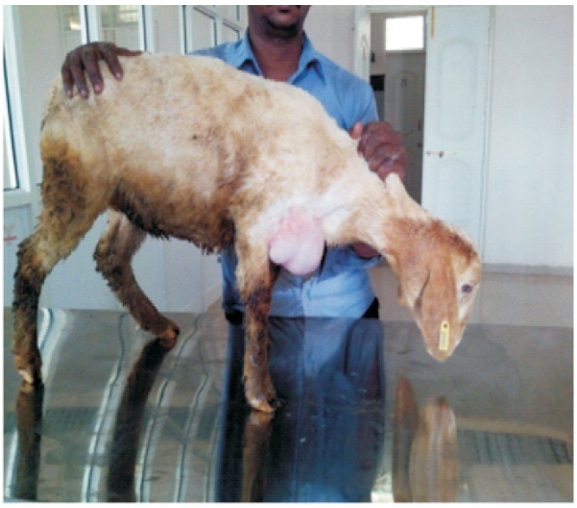

Fig. 1: Swelling near brisket region of sheep

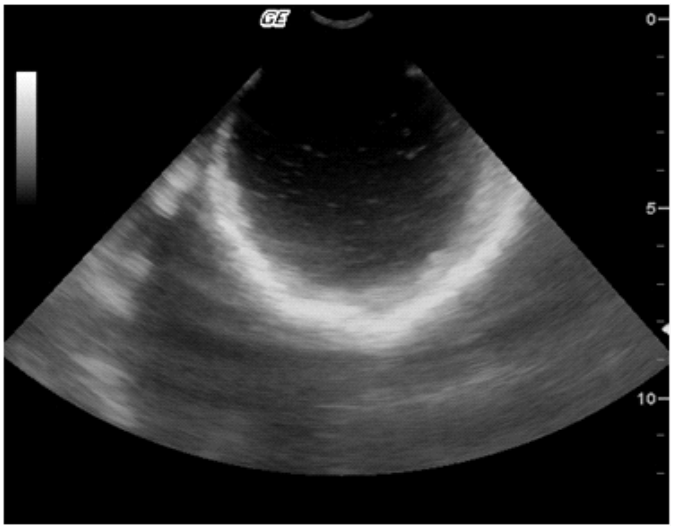

Fig. 2: Ultrasound image of cyst reveled clear anechoic fluid in a capsule

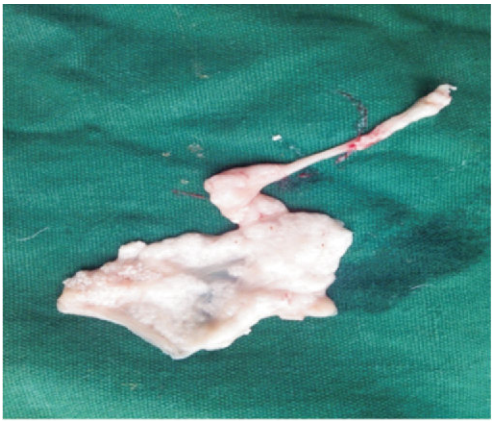

Fig. 3: Excised cyst containing scolices

the *Coenurus gaigeri* cyst was removed from the brisket region of sheep.

CONCLUSION

Coenurus gaigeri cyst from the brisket region of sheep was successfully managed surgically.

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Optimization of Level of Probiotics (*L. acidophilus* LA_5 *and B. bifidum* BB_{12}) with regard to Sensory, Physico- Chemical and Microbiological Quality of Whey Protein Enriched Probiotic Yoghurt*

Sharanagouda B[1] and Venkateshaiah B V[2]
Dept of Dairy Technology, Dairy Science College, Mahagaon Cross, Kalaburagi – 585312

ABSTRACT

The objective of this study was to standardize the level or rate of addition of probiotics in whey protein enriched concentrated yoghurt and to study its effect on the Sensory, Physico- Chemical and Microbiological Quality. Among the different levels of probiotics tried for production of protein enriched concentrated probiotic yoghurt, 3.0% of *L. acidophilus* and *B. bifidum* (along with 3% starters) secured non-significantly (Pd"0.05) higher sensory scores of 7.45, 7.01, 7.10 and 8.00 for colour and appearance, flavour, body and texture and overall acceptability respectively; whereas for control same scores were 8.00, 7.00, 6.90 and 7.80 respectively. As the level of probiotics increased from 0 to 3.0%, all the physico-chemical properties decreased viz., pH: (4.85 to 4.72), acidity (1.69 to 1.26% LA), synersis (1.50 to 0.85 ml), penetration value (125 to 122 mm) and time of setting (360 to 330 min.), whereas the respective log counts of *L. acidophilus* LA_5 and B. bifidum BB_{12} increased significantly (Pd"0.05) from 9.36 to 9.78 and 9.04 to 9.67 respectively.

Key words: Probiotics, starters, WPC_{70}, concentrated yoghurt

Fermented milks have been produced throughout the world, not only due to its therapeutic value but also to preserve milk against spoilage. Yoghurt is one among the most popular fermented milk products in the world, due to various desirable characteristics of the product and its potential health benefits; that are readily accepted by the consumers and also due to the image of the product as 'healthy'. Popularity of yoghurt has been increased significantly in the last few years because of the incorporation of the probiotic organisms into the product that gives an extra nutritional and physiological value. To achieve its therapeutic value, it's suggested that more than 100 g per day yoghurt containing viable probiotic cells of more than 10^6-10^7 cfu/ml should be consumed (FAO/ WHO, 2003). To reach this high microbial number with longer shelf-life of yoghurt, different methods of encapsulation of probiotic organisms with different encapsulating materials such as different stabilizers and whey protein concentrates (WPC) are used. The most popular probiotics that are added to the yoghurt are *Lactobacillus acidophilus*, *Lactobacillus casei, L. rhamnosus* and *Bifidobacterium bifidum* and the product is called as bio-yoghurt (Sarkar, 2010). The production and consumption of the food products supplemented with these gut friendly organisms have increased dramatically in the past two decades and the global probiotics market is growing at a rate of 11.7% every year (Annon, 2009).

WPC_{70} could be used to replace skim milk powder (SMP) in yoghurt without adverse effect on sensory properties. Supplementation of yoghurt with WPC improves viability of the *L. acidophilus* and *B. bifidum* strains without affecting the overall acceptability of probiotic yoghurt. In view of the above, the present study was undertaken with the objective of replacing SMP with WPC_{70} to enhance

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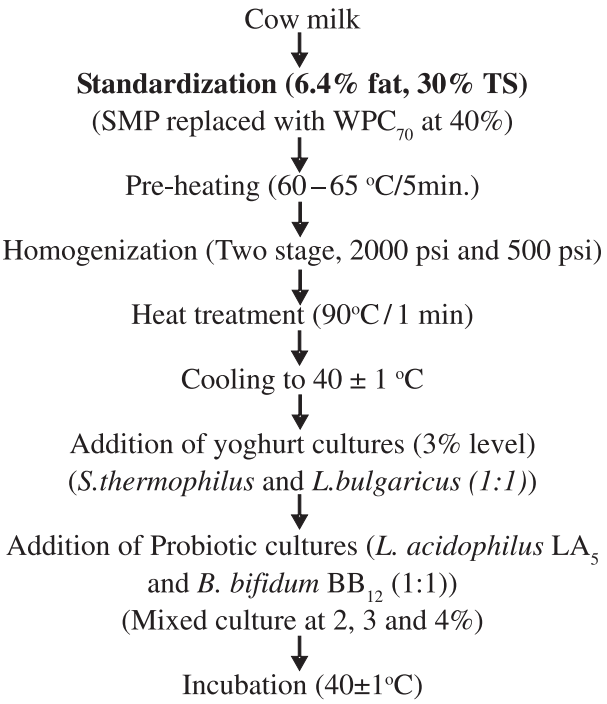
[1.] Corresponding author; Assoc. Prof and Head, Dept of Dairy Tech, DSC, Kalaburagi.
E-mail: sharan_bp@yahoo.co.in
[2.] Former Dean, DSC, Hebbal, Bangalore.

the protein content and to develop concentrated probiotic yoghurt.

MATERIALS AND METHODS

Fresh cow milk procured from Student Experimental Dairy Plant of Dairy Science Collage, KVAFSU, was used for the preparation of yoghurt. Whey proteins concentrate (PROCON 3700 WPC_{70}), procured from M/s. Mahaan Protein Ltd, New Delhi, was used for the protein enrichment of yoghurt. Freeze dried probiotic cultures of *Bifidobacterium bifidum* BB_{12} and *Lactobacillus acidophilus* LA_5 were obtained from National Dairy Research Institute, Karnal. Yoghurt starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were propagated and maintained individually in the Postgraduate Laboratory, Department of Dairy Technology, Dairy Science college, KVAFSU, Bangalore and were mixed in the ratio of 1:1 just before use, for the preparation of yoghurt. The procedure followed by Sharanagouda *et.al.* (2014) was followed for preparation of whey protein enriched concentrated yoghurt.

Fig. 1: Flow diagram for the preparation of protein enriched probiotic yoghurt



Optimization of level of probiotics in protein enriched concentrated yoghurt

To optimize the level of probiotics in the preparation of protein enriched concentrated probiotic yoghurt two probiotic organisms viz., *Bifidobacterium bifidum* BB_{12} and *Lactobacillus acidophilus* LA_5 (1:1 ratio, which were grown separately before adding to yoghurt mix) were used along with yoghurt starters at levels like 2.0, 3.0 and 4.0% by weight of mix in combination. The level of probiotics was decided based on their count in the final product as well as on the basis of sensory evaluation.

Effect of different levels of Probiotics (*L. acidophilus and B. bifidum)* on the different physico- chemical quality of protein enriched concentrated probiotic yoghurt.

Fat content in Yoghurt was estimated as per the procedure of AOAC (1980). pH was measured using digital pH meter (Elico make). Titratable acidity and total solids of all the samples were estimated as per the method described in IS:SP:18 (Part XI) 1981**.** The penetration value of yoghurt was measured as per the procedure of Shihata and Shah (2002). Syneresis of yoghurt samples were analysed as per Yeganehzad *et al.,* (2007). The time for setting was noted by noting the time of inoculation to the end of yoghurt setting.

Sensory evaluation of the products was conducted by applying 9-point hedonic scale (Peryam and Pilgrim, 1957). Evaluation was carried out by a panel of five expert judges.

Enumeration of yoghurt starters and probiotic cultures

Enriched yoghurt containing mixed cultures stored at 4±1^{0}C was examined for their viability. Bifidobacterium agar with 0.3% lithium chloride was added (by dry weight of media, for selective growth) and Rogosa SL agar (with 1.32 ml/l glacial acetic acid for selective enumeration) were used for enumeration of *B. bifidum* BB_{12} and *L. acidophilus* LA_5 respectively; Dave and Shah (1996) procedure was followed for the estimation of same; whereas yoghurt starters were estimated as per Suk *et al.* (1997). The plates were inverted and incubated in

anaerobic jars under CO_2 atmosphere at 40±1°C for 72 h. The colonies developed were counted; the average counts of duplicate plates were taken and tabulated.

Statistical analysis

The data was analysed using one way ANOVA, Two factor ANOVA depending on the experiment and the number of treatments in question. The results were analysed statistically for test of significance by using three factorial ANOVA as per the procedure of Sundarraj *et al.* (1972) in SAS 9.2 Version.

RESULTS AND DISCUSSION

Effect of levels of probiotics (*L. acidophilus* LA_5 and *B. bifidum* Bb_{12}) *on* sensory quality of protein enriched concentrated probiotic yoghurt.

It was observed from Table 1 that as the level of probiotics increased from 0 to 3.0% each, the flavour and overall acceptability increased non-significantly (P≤ 0.05), this could be attributed to the higher acetaldehyde and other flavouring compounds produced by probiotics and yoghurt starters in whey protein enriched yoghurt utilizing non-protein nitrogenous compounds required for flavour production (Alok and Kanawajia, 2010). Whereas probiotics above 3.0% level, the scores decreased significantly (P≤0.05), may be due to over flavour and acid production which might have imparted harsh flavour and that might have affected the overall acceptability (Hekmat *et al.,* 2004). The colour and appearance scores decreased though non-significantly (P≤0.05) with the increase in the level of probiotics up to 3.0%. This may be due to the slighter dull appearance with little wheying off on the surface of yoghurt; but above that level, wheying off was significantly ((P≤0.05) higher than that was observed in the present study (Table 2). The body texture scores at all levels of probiotics was not significantly (P≤0.05) affected. Based on the sensory scores good quality protein enriched probiotic yoghurt with 3.0% probiotics can be prepared.

Effect of different levels of Probiotics (*L. acidophilus and B. bifidum)* on the physico-chemical quality of protein enriched concentrated probiotic yoghurt

The pH of protein enriched concentrated yoghurts decreased slowly and significantly (Pd≤0.05) from 4.85 to 4.70 (Table 2) as the level of probiotic from 0% to 4% each, this may be due to small quantity of acids produced by probiotics. There could have been more decrease in pH, but the added WPC_{70} might have imparted the buffering capacity to yoghurt (Kailasapathy *et al.,* 1996). After inoculation of starter cultures, the yoghurt was kept for incubation, the time required for setting of protein enriched concentrated probiotic yoghurt decreased from 360 to 300 min (Table 2) may be due increased level of probiotics which might have set the probiotic yoghurt faster than control (Bury *et al.,* 2003; Christopher *et al.,* 2006).

It was also noticed from the Table 2 that there was a gradual significant decrease (P≤0.05) in the acidity of samples from 1.69% to 1.32% LA as the level of probiotics increased from 0 to 4.0%. This could be attributed to the increase in the concentration of probiotic cultures decreased the setting time, which might have affected the acid production due to less time available for the yoghurt starters to produce acid in probiotic yoghurt than control (Sady *et al.,* 2009).

Syneresis (wheying-off) is defined as the expulsion of whey from the network which then becomes visible as surface whey. Wheying-off negatively affects consumer perception of yoghurt as consumers think there is something microbiologically wrong with the product. Yoghurt manufacturers use stabilizers, such as, pectin, gelatin, starch and WPC. And try to prevent wheying-off. Another approach is to increase the total solids content of yoghurt milk, especially the protein content, to reduce wheying-off. There was significant decrease (P≤0.05) in syneresis and increase penetration value from 1.69 to 0.75 ml and 120 to 125 mm with increase in level of probiotic from 0 to 4.0% (Table 2). This could be attributed to the binding of water by the whey protein gel matrix formed, whereas the decrease in penetration value may be due to the smoother body produced by whey

proteins and also due to decrease in the level of casein content (Guzman-Gunzalez *et al.,* 1999). There is no effect on the fat and SNF content as with increasing probiotics levels due to the same level of fat and SNF concentrations maintained at all the levels of probiotics.

Effect of different levels of probiotic (*L. acidophilus and B. bifidum)* on the counts of starter cultures and probiotics in protein enriched concentrated yoghurt

It was evident from Fig. 1 and the Table 3 that the viable log counts of *S. thermophilus* and *L. bulgaricus* decreased non-significantly (P≤0.05) from 9.95 to 9.90 and 9.91 to 9.78 respectively; whereas the counts *L. acidophilus* and *B. bifidum* increased from 9.36 to 9.85 and 9.04 to 9.69 respectively with increase in level of probiotics from 2.0 to 4.0%. But, the increase was non-significant (P≤0.05) between 3.0% and 4.0% level of inoculation. This could be attributed to the WPC which might have acted as prebiotic for the growth of probiotics as reported by Janer *et al.,* (2004), and Christopher *et al.,* (2006). The decrease in the viable log counts of starter cultures may be due to reduced incubation time from 360 min in control to 300 min in 4.0% probiotic yoghurt.

CONCLUSION

Consumers today are highly conscious of nutritionally rich, functional and therapeutic foods. Considerable interest has recently been focused on the development of probiotic and protein enriched fermented dairy products. Yoghurt, the most popular fermented dairy product is used as a vehicle of

Table 1: Effect of levels of probiotics (*L. acidophilus* LA_5 and *B. bifidum* Bb_{12}) *on* sensory quality of protein enriched concentrated probiotic yoghurt

Sensory attributes	Percentage of total probiotics (%)				CD (Pd"0.05)
	0	2.0	3.0	4.0	
	→ Sensory Scores of 9.0 →				
Colour & Appearance	8.00 ± 0.17^{a}	7.50 ± 0.19^{a}	7.45 ± 0.20^{a}	7.00 ± 0.18^{b}	0.632
Flavour	7.00 ± 0.38^{a}	6.85 ± 0.18^{a}	7.01 ± 0.09^{a}	6.50 ± 0.50^{b}	0.487
Body & Texture	6.90 ± 0.39^{a}	7.05 ± 0.08^{a}	7.10 ± 0.06^{a}	7.00 ± 0.15^{a}	0.709
Overall acceptability	7.80 ± 0.25^{a}	7.95 ± 0.10^{a}	8.00 ± 0.20^{a}	7.44 ± 0.30^{b}	0.324

n=5.Treatments bearing different superscripts in row are statistically different (pd"0.05)

Table 2: Effect of different level of probiotics (*L. acidophilus and B. bifidum)* on the physico-chemical properties of protein enriched concentrated probiotic yoghurt.

Physico-chemical attributes	Per cent of probiotics				CD (Pd"0.05)
	0	2.0	3.0	4.0	
Ph	4.85 ± 0.06^{a}	4.78 ±0.09^{ab}	4.72 ±0.05^{bc}	4.70 ± 0.04^{c}	0.071
Acidity (% TA)	1.69 ± 0.10^{a}	1.12 ± 0.10^{b}	1.26 ± 0.08^{c}	1.32 ± 0.05^{d}	0.013
Syneresis (ml)	1.50 ± 0.1^{a}	0.96 ± 0.05^{b}	0.85 ± 0.10^{c}	0.75 ± 0.05^{d}	0.001
Penetration value (mm)	125 ± 10^{a}	124 ± 15^{a}	122 ± 15^{b}	120 ± 10^{c}	0.951
Fat (%)	6.4±0.10^{a}	6.4±0.20^{a}	6.4 ± 0.10^{a}	6.4±0.20^{a}	NS
Total solids (%)	30.0 ± 1.35^{a}	30.0 ± 1.51^{a}	30.0 ± 1.05^{a}	30.0 ± 1.39^{a}	NS
Time for setting (min) at 40±1°C	360 ± 15^{a}	330 ± 15^{b}	330 ± 15^{b}	300 ± 15^{c}	0.009

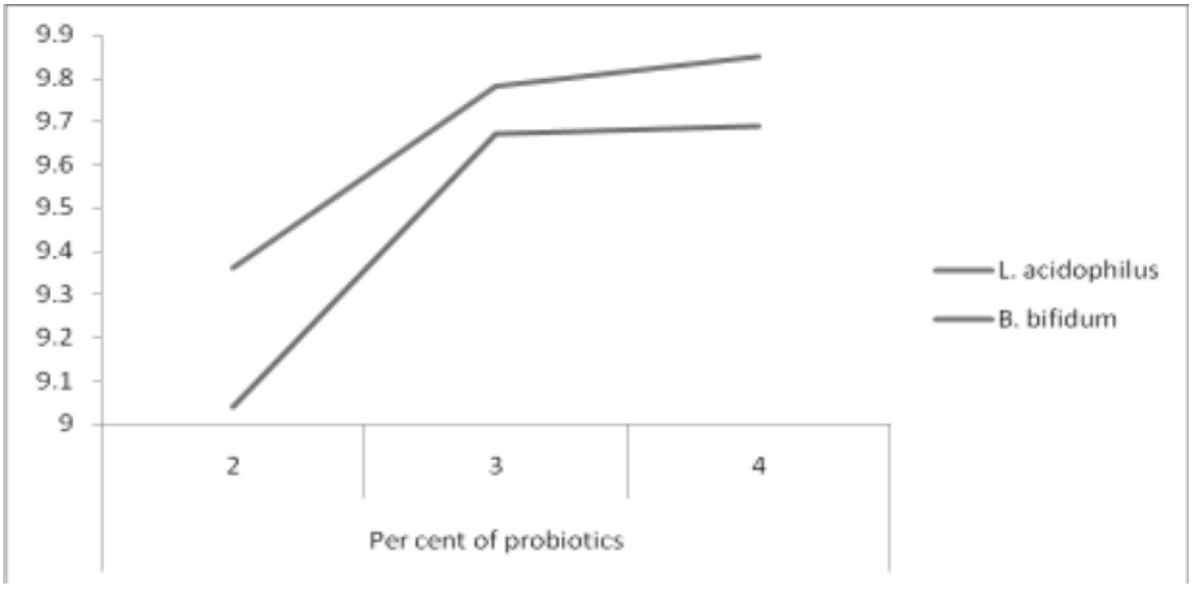
n=5.Treatments bearing different superscripts in row are statistically different (p≤0.05)

Table 3: Effect of different levels of probiotic (*L. acidophilus and B. bifidum)* on the count of starter cultures and probiotics in protein enriched concentrated yoghurt

Starter organisms	Per cent of probiotics			CD (pd≤0.05)
	2.0	3.0	4.0	
S. thermophilus	9.95 ± 0.13[a]	9.93 ± 0.11[a]	9.90 ± 0.17[a]	0.908
L. bulgaricus	9.91 ± 0.20[a]	9.85 ± 0.18[a]	9.78 ± 0.19[a]	0.576
L. acidophilus	9.36 ± 0.15[a]	9.78 ± 0.18[b]	9.85 ± 0.13[b]	0.075
B. bifidum	9.04 ± 0.14[a]	9.67 ± 0.19[b]	9.69 ± 0.16[b]	0.023

n=3.Treatments bearing different superscripts in row are statistically different (pd"0.05)

Fig. 1: Effect of different levels of probiotics (*L. acidophilus* and *B. bifidum*) on the count of probiotics in the protein enriched concentrated probiotic yoghurt



probiotics and protein enrichment. The most popular probiotics that are added to the yoghurt are *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. A good quality protein enriched concentrated probiotic yoghurt can be prepared with 3.0% of *L. acidophilus* and *B. bacterium* (1.5% each) along with yoghurt culture which has secured higher sensory scores than control without much effect on physico-chemical properties.

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