Comparison of MAT and ELISA for Diagnosis of Bovine Leptospirosis in Karnataka*

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ABSTRACT
In the present study, initially an attempt was made to estimate the seroprevalence of *Leptospira hardjo* serovar in bovines of Karnataka state of India based on the antibody detection by Microscopic Agglutination Test (MAT). Serum samples were collected from 582 animals with reproductive disorders (314 cows and 268 buffaloes) from different parts in four divisions of Karnataka state namely Bangalore, Belgaum, Gulbarga and Mysore. Subsequently, the same samples were cross checked with a commercially available Linodee Double sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) kit against the *Leptospira serovar hardjo*. The ELISA has shown 65 (11.12 %) positives when compared to MAT which has shown 56 (9.62 %) positives. All the serum samples which were positive by MAT were positive by ELISA also. Apart from this an additional nine serum samples were positive by ELISA but negative by MAT. On comparison of ELISA with MAT, ELISA has shown 100 % sensitivity and 98.26 % specificity. Thus based on the present findings it could be concluded that for initial screening of large herd or population to study the prevalence of *hardjo* serovar ELISA can be effectively used compared to the MAT.

Key words: MAT, ELISA, hardjo, Linodee. Karnataka.

Leptospirosis is the most wide spread re-emerging zoonosis worldwide and it is present in all continents except Antarctica. *Leptospira* has been found virtually in all mammalian species including domestic animals such as cattle, swine, horses, sheep, goats and dogs and the disease is spread through either direct contact with an infected animal or an indirect contact with contaminated soil or water (Harskeerl and Terpstra, 1996). According to World Health Organization (WHO, 1999), leptospirosis has become an important global human and veterinary health problem especially in countries like India, because of huge livestock, rodent and wildlife populations. Conventionally, laboratory diagnosis of leptospirosis is based on either isolation / identification of leptospires or through detection of antibodies. The isolation and identification of leptospires is time consuming and necessitate specialized reference laboratories. Hence, in diagnosis of leptospirosis serological tests like Microscopic Agglutination Test (MAT) and / or Enzyme-Linked Immunosorbant Assay (ELISA) for antibody detection appears to be the most suitable (OIE, 2004). Presently, MAT is the gold standard reference serological test for diagnosis of leptospirosis upto serogroup / serovar level. However, MAT has some disadvantages such as use of hazardous live organisms which requires good laboratory facilities for the maintenance of live cultures of different serovars.. The ELISA has many advantages over MAT where they use non hazardous killed antigens, are reproducible and can be subjected to stringent quality assurance protocols. This technique has high potential for automation and may be efficiently utilized as a screening test for a large number of animals in a population (Dey *et al.*, 2008). Thus screening of large number of samples in short interval would be helpful in determining the seroprevalence of leptospirosis in bovines.

Keeping above facts in view, a comparison was made in the present study for the seroprevalence of *Leptospira hardjo* serovar using the commercially available double sandwich Linodee ELISA kit with MAT.

MATERIALS AND METHODS
Serum samples from cattle and buffaloes with clinical signs suggestive of leptospirosis which included abortion, repeat breeders, still birth, retention of placenta, were collected irrespective of...
age and breed from four divisions of Karnataka state, namely Bangalore, Belgaum, Gulbarga and Mysore. In all, 582 serum samples were collected from these divisions, out of which 314 were from cows and 268 from buffaloes.

**Microscopic Agglutination test:** This test was conducted as per OIE (2004) in 96 well ‘U’ bottom titration plates (Laxbro, India) using the *Leptospira hardjo* reference strain procured from National Reference Laboratory, ICMR, Andaman and Nicobar islands. The hyper immune serum available at SRDDL Bangalore was used. Serum sample which had shown a titre of 1:100 was considered as positive. The serum samples were initially diluted to 1 in 50 in PBS and then doubling dilutions (25 µl) were made in the rows containing 25 µl of PBS in ‘U’ bottom micro titration plates. Equal volume (25 µl) of the antigen was added to all serum dilutions. The last column was maintained as antigen control without addition of serum. Then, the plates were closed with lids and incubated at 37°C for two hours. A drop (5 µl) all final dilution mixtures (100, 200, 400, 800, 1,600, 3,200, 6,400 and 12,800) was placed on grease free slide and the wet preparation without cover slip was examined at 100X and 200X magnification (10x X 10x and 20x) of the dark field microscope (M/s.Olympus, CX31) for the presence of agglutination and / or reduction in number of organism in comparison with the respective antigen control. All final dilution mixtures (100, 200, 400, 800, 1,600, 3,200, 6,400 and 12,800) were observed under Dark field microscope and the results were recorded. The reciprocal of the highest dilution which shows 50 per cent reduction in the number of free leptospires comparable to the antigen control with or without agglutination was recorded as the respective titres.

**Enzyme Linked Immuno Sorbent Assay (ELISA):** All the 582 sera samples subjected to MAT were retested with commercially available OIE approved Linnodee bovine *L hardjo* ELISA Kit, which was a double sandwich ELISA for detection of *L hardjo* specific antibodies in bovine serum. The reagents were diluted as per the instructions given by the manufacturer and ready to use solutions were prepared just before conducting the test. The washing steps were followed as per the directions given in the kit. For each washing step, the test wells were washed using at least 200 µl of diluted wash buffer for each well. After the final wash, the residual contents of the wash buffer in the wells were removed by inverting the plate and blotting firmly on absorbent paper.

**Test procedure**

1. Added 100 µl of serum sample to each well and sealed the plate and incubated for 40 min at 37ºC with shaking.
2. After incubation plate was washed four times as mentioned above.
3. Added 100 µl of diluted conjugate to each well and plate sealed and incubated in dark for 30 min at 37°C with shaking.
4. After incubation, plate washed four times as above.
5. Added 100 µl of substrate to each well and plate incubated in the dark at room temperature for 10 min.
6. Added 50 µl of stop reagent to each well and read the plate at 450 nm with a correction of 630 nm.

**Calculations**

The positivity of a sample is determined using the following calculation:

\[
\text{Ratio} = \frac{\text{Mean sample OD} - \text{Mean negative control OD}}{\text{Mean positive control OD} - \text{Mean negative control OD}}
\]

**Statistical Analysis**

<table>
<thead>
<tr>
<th>Results</th>
<th>Sera Sample</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>≤ 0.05</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt; 0.05</td>
<td>Exposed to infection</td>
</tr>
</tbody>
</table>

To compare the sensitivity, specificity and overall agreement between the various tests, the statistical formula given by Thrusfield (2007) was used as described below.

\[
\begin{array}{|c|c|c|}
\hline
\text{Test} & \text{Standard test} & \text{Total} \\
& \text{Positive} & \text{Negative} \\
\hline
\text{Test to be Compared} & \text{Positive} & a & b & a+b \\
& \text{Negative} & c & d & c+d \\
\hline
\text{Total} & a+c & b+d & a+b+c+d=N \\
\hline
\end{array}
\]

The notations used above are defined as below:

\[
a = \text{Number of samples positive to both conventional and the gold standard tests}
\]
Table 2: Comparison of ELISA and MAT for L. hardjo serovar

<table>
<thead>
<tr>
<th>ELISA</th>
<th>MAT</th>
<th>Total</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Overall agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>56</td>
<td>9</td>
<td>65</td>
<td>56</td>
<td>56 x 100</td>
</tr>
<tr>
<td>Negative</td>
<td>--</td>
<td>517</td>
<td>517</td>
<td>56</td>
<td>517 x 100</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>526</td>
<td>582</td>
<td>56</td>
<td>526 x 100</td>
</tr>
</tbody>
</table>

Seroprevalence of leptospirosis by Double Sandwich ELISA among cows and buffaloes in Bangalore, Belgaum, Gulbarga and Mysore divisions of Karnataka, revealed 65 (11.12 %) positives with Leptospira hardjo antigen tested, out of total 582 sera tested. Among the divisions, the highest seroprevalence against Leptospira hardjo was recorded in Belgaum with 19 (29.23 %), followed by Mysore 18 (27.69 %) and Bangalore and Gulbarga each with 14 (21.53 %) (Table 1).

Table 1. Division wise comparative results of MAT and ELISA against L. hardjo serovar

<table>
<thead>
<tr>
<th>Division</th>
<th>MAT</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangalore</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Belgaum</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Gulbarga</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Mysore</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>65</td>
</tr>
</tbody>
</table>

Comparative results of MAT and ELISA: Out of the 582 serum samples subjected to MAT and ELISA for detection of leptospiral antibodies against Leptospira hardjo serovar, 56 (9.62 %) were positive by MAT and 65 (11.17 %) were positive by ELISA. All the MAT positive samples were positive by ELISA, however in addition nine samples which were negative by MAT were found to be positive by ELISA.

Table 2: Comparison of ELISA and MAT for L. hardjo serovar

b = Number of samples positive to conventional but negative to the gold standard test

c = Number of samples negative to conventional but positive to the gold standard test

d = Number of samples negative to both conventional and the gold standard tests

\[ a + b + c + d = \text{Total number of samples (N)} \]

Definitions and formulae of the indices used for comparing the different assays are described as follows.

Sensitivity: It is the capacity of the test to detect diseased animals, when compared with the gold standard test.

\[ \text{Sensitivity} = \frac{a}{a+c} \times 100 \]

Specificity: It is the capacity of the test to detect non-diseased animals, when compared with the gold standard test.

\[ \text{Specificity} = \frac{d}{b+d} \times 100 \]

Overall agreement: Is the proportional similarity of the results of both the tests.

\[ \text{Overall agreement} = \frac{a+d}{N} \times 100 \]

RESULTS AND DISCUSSION

Division wise seroprevalence: Seroprevalence of leptospirosis by MAT out of total 582 sera tested among cows and buffaloes in Bangalore, Belgaum, Gulbarga and Mysore divisions of Karnataka, revealed 56 (9.62 %) positives with Leptospira hardjo antigen tested. Among the divisions, the highest seroprevalence against Leptospira hardjo was recorded in Mysore with 18 (32.14 %), followed by Belgaum 16 (28.57 %), Gulbarga 13 (23.21 %) and Bangalore 9 (16.07 %) (Table 1).
The prevalence of hardjo serovar was significantly high in Mysore, Gulbarga and Belgaum divisions and moderate in Bangalore division indicating the presence of this serovar throughout the state of Karnataka. Fifty six samples were found positive by MAT and the same samples were found positive by ELISA also.

Nine samples which were negative by MAT were detected as positive by ELISA (Table 1), indicating that ELISA can effectively be utilized for the initial screening. Based on the results, it can be concluded that ELISA can detect the samples with low antibody titres and even during the early stage of infection which were usually not detected by MAT.

Apart from this the ELISA detects the non agglutinating antibodies also which were not detected by MAT, leading to high sero positivity with ELISA when compared to the MAT.

The findings are in agreement with the observations of Aslantas and Ozdemir, 2005; Sakhaee et al., 2007 and El Jalli (2008) as they also found more seropositivity with ELISA compared to MAT. Thus based on the present findings it can be concluded that for initial screening of large herd or populations to know the prevalence of hardjo serovar ELISA can effectively be used compared to the MAT and subsequently to know the titre values for quantification can be employed MAT. Specificity and sensitivity wise both the tests are equally good.

REFERENCES
Assessment of Hepatic Antioxidant Enzymatic Activity with Gymnemasyylvestre and Chromium in Streptozotocininduced Diabetic Female Rats*

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ABSTRACT

The present study was carried out to compare the efficacy of Gymnemasyylvestre leaf extract and/or trivalent chromium by oral supplementation, in reducing the oxidative stress encountered in streptozotocin(STZ) induced diabetes mellitus in Wistar albino rats at different doses, either alone or in combination for a period of 45 days. The levels of hepatic antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase, were estimated by spectrophotometry on day 15, 30 and 45. The anti-oxidant enzymes were significantly reduced (P<0.05) in diabetic rats, whereas the diabetic treated groups showed a progressive increase in the anti-oxidant enzyme levels and were almost normalized by 45th day. The anti-oxidant enzymic activity were found to be significantly higher (P<0.05) in groups treated with herbal extract and chromium, although dose dependent variations were not evident. The results suggest that Gymnemasyylvestre herbal extract and trace element chromium can effectively reduce the oxidative stress caused by STZ induced diabetes and hence could be used as a tool for management of type 2 diabetes mellitus.

Keywords: Diabetes mellitus, antioxidant enzymes, streptozotocin, Gymnemasyylvestre, chromium.

Diabetes mellitus is a chronic clinical syndrome resulting from intolerance to glucose caused by an absolute or relative deficiency of insulin, with an insidious onset of degenerations, subsequent multiple organ failure due to micro and macrovascular changes as a result of hyperglycemia. The long term effects of type 2 diabetes mellitus(T2DM) are due to the gradual onset of oxidative stress, which may be due to several mechanisms such as, the release of free fatty acids from the cellular membranes (Dasgupta et al., 2011), from decrease in production of antioxidant enzymes, due to loss of mitochondrial integrity, ER stress, loss of MAM(mitochondria associated membrane) integrity(Tubbs et al., 2014) or STZ induced nuclear damage (Yamamoto et al., 1981), glucose autoxidation and nonenzymatic glycation (Shailey and Basir, 2012), which have been documented.

Gymnemasyylvestre (Colloquial name: Meshashrungi or Merasinghi), commonly called as “the sugar destroyer”, a native to tropical forests in India, is used in traditional medical practice as an antihyperglycaemic and hypolipidaemic agent effective in management of T2DM (Vaidya., 2010). Several human studies on T2DM and studies on induced diabetes in rats have shown Gymnemasyylvestre to be having pancreatic beta cell regenerative activity, insulin sensitization and subsequent enhancement of the uptake of glucose from peripheral circulation (Persaud et al., 1999; Pragathi, 2011 and Mallikarjuna et al., 2013).

Several studies have indicated a relationship between the deficiency of micronutrient chromium and the pathology of diabetes mellitus in humans (Anderson et al., 1998). Chromium is a micronutrient trace element that stimulates the expression of insulin receptors on cell surfaces, essential for binding of hormone insulin, thereby influencing the glucose assimilation. Chromium is known to stabilize the cellular membranes, enhance cytoskeletal stability, regulate the sterol regulatory element binding protein which can contribute to maintenance of cellular integrity (Chen et al., 2006; Seallset et al., 2011).

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⁶Professor, Department of Veterinary Medicine, Veterinary College, Bengaluru-560024.
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⁸Veterinary Officer, Haveri..Dept of AH&VS, Karnataka.
In experimental induction of diabetes mellitus, the hepatic oxidative injury can be evaluated by estimation of liver tissue antioxidative enzymes like catalase, glutathione peroxidase and superoxide dismutase. The present study was designed to systematically evaluate the potential of *Gymnemasylvestre* herbal leaf extract with trace element chromium, in reducing the oxidative injury in diabetes mellitus in STZ induced diabetic rat model.

**MATERIALS AND METHODS**

The experimental study was undertaken with prior approval of the Institutional Animal Ethics Committee (IAEC) of the institution. A total of eighty female Wistar albino rats were procured from a reputed breeder, acclimatized for 15 days and maintained under optimal managmenal conditions with *ad-libitum* feed (Nutrilab Rodent, Vetcare) and clean drinking water.

**Test substances:** The alcoholic leaf extract of *Gymnemasylvestre* procured from M/s Himalaya Drug Co. Ltd, Bengaluru., was administered at 200 mg/kg, chromium picolinate (CrPic) (MW-418.3) from Qualikems, India., was given at 2 mg/kg and glibenclamide (Daonil 5mg) was purchased from local medical stores, and used in the present study at 600 μg/kg when given alone, and 300 μg/kg in combination with others.

**Induction of diabetes:** Diabetes was induced in rats of Groups II to VIII, with a single intra-peritoneal injection of streptozotocin (Sigma Chemicals, USA) in cold citrate buffer at 45 mg/kg body weight. Those rats that showed serum glucose levels of more than 200 mg/dL after 72 hours of administration of STZ, were considered diabetic and suitable candidates for the study. The rats of Group I (Normal control) received only citrate buffer by the same route.

**Experimental groups:** The rats were randomized into eight groups of ten animals each, based on body weights with an average deviation of not more than 10%. Group I was normal control (NC) comprising healthy rats, Group II were diabetic rats (DC) and the rats of Groups III to VIII comprised of diabetic rats administered with treatments. The treatment groups comprised of Group III diabetic rats treated with glibenclamide(G), Group IV diabetic rats received chromium picolinate(Cr), and diabetic rats of Group V treated with chromium picolinate and glibenclamide (Cr+G), diabetic rats of Group VI treated with *Gymnemasylvestre* (GS-200), Group VII diabetic rats treated with *Gymnemasylvestre* and CrPic (GS-200+Cr), and Group VIII comprised diabetic rats treated with *Gymnemasylvestre*, CrPic and glibenclamide(GS-200+Cr+G).

**Administration of treatment substances:** The STZ diabetic rats were orally gavageddaily with their respective treatments at specified dosage regimen for a period of 45 days, whereas the control group rats were given potable water only.

**Tissue anti-oxidant enzymic assays:** To study the anti-oxidant enzyme profile in different groups, two rats from each group were sacrificed humanely under light ether anaesthesia on 15th and 30th and the remaining rats on 45th day of experimentation, and the liver of sacrificed rats were collected and immediately stored at -20°C.

**Tissue Homogenate preparation:** Liver tissue samples were homogenized with ice cold 0.1 M Tris-HCl buffer of pH 7.4 to make tissue homogenate [0.5 g liver crushed in 10 mL of ice cold 0.1mol/LTris-HCl buffer]. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was collected and used for estimation of superoxide dismutase, and catalase, glutathione peroxidase levels.

**Estimation of superoxide dismutase [SOD]:** Superoxide dismutase activity was determined bythe method described by Marklund and Marklund (1974). The enzyme activity was expressed in terms ofunits per minute per mg of protein. One unit of SOD was defined as the amount of enzyme required to inhibit pyrogallol auto-oxidation reaction by 50 per cent.

**Estimation of catalase [CAT]:** Catalase was estimated by themethod described by Caliborne (1985). Enzyme activity was expressed asmoles of H₂O₂ decomposed / min / mg protein.

**Estimation of glutathione peroxidase [GPx]:** Glutathione peroxidase was determined by themethod described by Rotruck *et al.*(1973) and the
enzyme activity was expressed as μ moles of glutathione utilized / min / mg protein.

**Histopathology:** Following sacrifice, collected liver tissue samples were preserved in 10% neutral buffered formalin [NBF] for 48 hours and processed as per routine procedure. The sections of 4-5μ thickness were cut using semi automatic microtome and stained with hematoxylin and eosin (Luna., 1968) and observed under light microscope.

**Statistical analysis:** Statistical analysis was performed using the statistical software Graph Pad Prism, version 5.0 for Windows. Mean values and standard error were calculated and all values were expressed as Mean [± SE]. The data were analyzed by two-way analysis of variance [ANOVA].

**RESULTS AND DISCUSSION**

The tissue antioxidants play an important role in protection of cells against damage caused by reactive oxygen species (ROS) that are generated in every cell. The endogenous antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) that are responsible for the degradation of deleterious oxygen free radicals.

The endogenous sources of ROS include mitochondrial electron transport chain molecules, cytochrome P450s, NADPH oxidase and xanthine dehydrogenase/ oxidase. The levels of ROS increase when there is an imbalance in the production of ROS and its degradation by the tissue antioxidant machinery. Free radicals are extremely toxic compounds that target biomolecules such as lipids with unsaturated double bonds and cause lipid peroxidation with subsequent tissue damage (Saritheshkar and Subramanian, 2005). The antioxidants breakup the chains formed during the propagation process by providing a hydrogen atom or an electron to free radical and receive the excess energy possessed by the activated molecule.

An increased production of free radicals and oxidative damage are the features of several chronic diseases including diabetes (Bayens and Thorpe, 1999). In diabetes mellitus, it is known that there is damage to the cellular organelles, especially the mitochondria and the ER, whose less than optimal activity reflects as oxidative stress by ROS, with a gradual and drastic decline in the endogenous antioxidative enzymic production. Superoxide dismutase, catalase, and glutathione peroxidase (GPx) are the biological antioxidant enzymes that directly scavenge free radicals and prevent their conversion to toxic products (Shailey and Basir, 2012). Hyperglycaemia of diabetes has been reported to cause production of free radicals that are associated with the development of diabetic complications (Traverso et al., 2004). It is a well known fact that the circulating free fatty acids have been implicated in causing complications like insulin resistance, obesity and metabolic syndromes (Dasgupta et al., 2011).

In the present study antioxidant effect was assessed by estimating levels of antioxidant enzymes such as SOD, CAT and GPx in STZ diabetic model. Superoxide dismutase (SOD) has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H₂O₂ and molecular oxygen. Catalase (CAT) is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Glutathione peroxidase (GPx), an enzyme with selenium and Glutathione-S-transferase (GST) works together with glutathione in the decomposition of H₂O₂ or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione.

In the present study the rats of Group I exhibited normal activity of the superoxide dismutase (Table 1), catalase (Table 2) and glutathione peroxidase (Table 3) enzyme throughout the experiment.

In the diabetic control group (II), the SOD, CAT and GPx activities were reduced in the liver, which were 31.32, 41.87 and 22.56 per cent by 45th day, when compared to normal control. Diabetes is usually accompanied by increased production of free radicals due to hyperglycaemia induced glucose auto oxidation and protein glycosylation (Wolff and Dean, 1987 and Wolff et al., 1991) with reduced activity of antioxidant system. Streptozotocin has been reported to induce both
Table 1: Mean (±SE) liver superoxide dismutase (units / min / mg of protein) values in rats of experimental treatment groups at 15th, 30th and 45th day

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (±SE) liver superoxide dismutase (units / min / mg of protein)</th>
<th>Days post-treatment</th>
<th>% difference from NC</th>
<th>% difference from DC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>I (NC)</td>
<td></td>
<td>41.32±1.65a</td>
<td>42.43±0.84a</td>
<td>42.10±0.89a</td>
</tr>
<tr>
<td>II (DC)</td>
<td></td>
<td>28.93±0.83b</td>
<td>29.45±0.5b</td>
<td>28.91±0.35b</td>
</tr>
<tr>
<td>III (G)</td>
<td></td>
<td>29.42±0.99b</td>
<td>40.79±0.82c</td>
<td>46.08±2.99c</td>
</tr>
<tr>
<td>IV (Cr)</td>
<td></td>
<td>31.24±1.82d</td>
<td>41.61±1.66d</td>
<td>40.64±0.72d</td>
</tr>
<tr>
<td>V (G+Cr)</td>
<td></td>
<td>30.46±0.93bd</td>
<td>42.44±0.16ca</td>
<td>42.55±3.81ca</td>
</tr>
<tr>
<td>VI (GS-200)</td>
<td></td>
<td>29.92±0.49fbdde</td>
<td>39.10±0.83fabde</td>
<td>44.52±1.92fde</td>
</tr>
<tr>
<td>VII (GS-200+Cr)</td>
<td></td>
<td>30.88±2.51gbcdef</td>
<td>40.44±1.84gacdef</td>
<td>41.84±3.37gacdef</td>
</tr>
<tr>
<td>VIII (GS-200+Cr+G)</td>
<td></td>
<td>30.47±5.55hbcdefg</td>
<td>40.76±1.16hacdefg</td>
<td>41.69±2.91hacdefg</td>
</tr>
</tbody>
</table>

The means with at least one common superscript within the columns are not significantly different (P ≥ 0.05).

Table 2: Mean (±SE) liver catalase (nmoles of H₂O₂ decomposed / min / mg protein) values in rats of experimental treatment groups at 15th, 30th and 45th day

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (±SE) liver catalase (nmoles of H₂O₂ decomposed / min / mg protein)</th>
<th>Days post-treatment</th>
<th>Difference from NC (%)</th>
<th>Difference from DC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>I (NC)</td>
<td></td>
<td>13.88±0.93a</td>
<td>13.42±0.56a</td>
<td>13.34±0.72a</td>
</tr>
<tr>
<td>II (DC)</td>
<td></td>
<td>6.02±0.46b</td>
<td>6.62±0.28b</td>
<td>7.75±0.81b</td>
</tr>
<tr>
<td>III (G)</td>
<td></td>
<td>6.2±0.09ca</td>
<td>11.56±0.75ca</td>
<td>12.45±0.74ca</td>
</tr>
<tr>
<td>IV (Cr)</td>
<td></td>
<td>9.72±0.46d</td>
<td>10.63±1.31dac</td>
<td>12.46±0.36db</td>
</tr>
<tr>
<td>V (G+Cr)</td>
<td></td>
<td>9.90±0.64ed</td>
<td>10.99±0.18acdef</td>
<td>12.48±0.49acdef</td>
</tr>
<tr>
<td>VI (GS-200)</td>
<td></td>
<td>9.91±0.84cde</td>
<td>10.16±0.65fcde</td>
<td>11.76±0.98facde</td>
</tr>
<tr>
<td>VII (GS-200+Cr)</td>
<td></td>
<td>9.97±0.95gde</td>
<td>10.44±0.37gacdef</td>
<td>11.22±0.88gacdef</td>
</tr>
<tr>
<td>VIII (GS-200+Cr+G)</td>
<td></td>
<td>9.88±0.63hdefg</td>
<td>10.99±0.11hacdefg</td>
<td>12.01±0.49hacdefg</td>
</tr>
</tbody>
</table>

The means with at least one common superscript within the columns are not significantly different (P ≥ 0.05).
Table 3: Mean (± SE) liver glutathione peroxidase (μ moles of glutathione utilized / min / mg protein) values in rats of experimental treatment groups at 15th, 30th and 45th day

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (± SE) liver glutathione peroxidase (μ moles of glutathione utilized / min / mg protein)</th>
<th>Days post-treatment</th>
<th>Difference from NC (%)</th>
<th>Difference from DC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>I (NC)</td>
<td>179.36±5.07a 180.25±6.79a 184.75±5.16a</td>
<td>0.00</td>
<td>+29.13</td>
<td></td>
</tr>
<tr>
<td>II (DC)</td>
<td>130.74±4.37b 139.44±3.39b 143.07±3.91b</td>
<td>-22.56</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>III (G)</td>
<td>146.95±5.1c 155.01±1.59c 160.49±3.03c</td>
<td>-13.13</td>
<td>+12.17</td>
<td></td>
</tr>
<tr>
<td>IV (Cr)</td>
<td>145.26±3.37dbc 173.51±3.39da 174.40±3.14d</td>
<td>-5.60</td>
<td>+21.90</td>
<td></td>
</tr>
<tr>
<td>V (G+Cr)</td>
<td>140.42±4.46ebc 166.32±3.72ecd 170.03±1.44ed</td>
<td>-7.96</td>
<td>+18.84</td>
<td></td>
</tr>
<tr>
<td>VI (GS-200)</td>
<td>145.80±3.16bcde 161.55±5.11fde 168.03±2.2fde</td>
<td>-9.05</td>
<td>+17.44</td>
<td></td>
</tr>
<tr>
<td>VII (GS-200+Cr)</td>
<td>143.37±4.85abcdef 158.16±5.08gdef 169.88±2.93gdef</td>
<td>-8.05</td>
<td>+18.73</td>
<td></td>
</tr>
<tr>
<td>VIII (GS200+Cr+G)</td>
<td>142.35±2.87hbcdefg 168.32±1.71hacdefg 171.10±2.99hbcdefg</td>
<td>-7.39</td>
<td>+19.59</td>
<td></td>
</tr>
</tbody>
</table>

The means with at least one common superscript within the columns are not significantly different (P≥0.05).

plasma membrane and organellar membrane damage especially that of rough endoplasmic reticulum and mitochondria following its uptake by hepatocytes through GLUT 2 (Mudasir et al., 2013). In the present study, the decline in the activities of antioxidant enzymes in diabetic rats indicated the extent of free radical induced damage due to hyperglycaemia (Kaleem et al., 2006 and Resmi et al., 2006). The observed decrease in SOD activity could be from inactivation by H₂O₂ or by glycation of enzymes while the decrease in CAT activity could be from inactivation by glycation of enzyme (Yan and Harding, 1997) and reduced activities of GPx may be from free radical induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975). The reduction in the antioxidant enzymes might also be due to the mitochondrial dysfunction due to membrane injury caused by free radicals. An altered mitochondrial function (Kong et al., 2013), mitochondria associated membrane (MAM) and ER stress in diabetes and insulin resistance have been well documented (Tubbs et al., 2014).

In all the diabetic groups treated with various substances (Groups III to VIII), it was observed that although the CAT and GPx levels were significantly better than diabetic control (Group II) by 45th day, they were lesser than the normal control group (I) throughout the experiment. Comparatively, a significant elevation in SOD were noted, which was much higher than the normal control group by 45th day of the experiment.

There was a significant improvement in the mean SOD, CAT and GPx activities in the liver of glibenclamide treated diabetic rats (Group III) compared to diabetic control rats in the present study. The diabetic rats of Group III treated with glibenclamide showed an overall elevation of SOD by 59.39, CAT by 60.50 and GPx by 12.17 per cent by 45th day compared to the diabetic control. The improvement could be attributed to the antihyperglycaemic effect of glibenclamide by increasing the release of insulin thereby reducing production of oxygen free radicals by preventing glucose auto oxidation and protein glycosylation (Upadhyay and Pandey, 1984 and Sathishsekhar and Subramanian, 2005).

The SOD, CAT and GPx activities in the liver of chromium picolinate (CrPic) treated diabetic rats (Group IV) revealed a significant
improvement of 40.58, 60.66 and 21.90 per cent respectively, when compared with those of diabetic control rats and were comparable with those of glibenclamide treated rats in the present study. The observed improvement in the chromium treated group could be attributed to antioxidant and free radical scavenging activity of chromium, where in chromium has been reported to reduce the oxidative stress and inhibit the release of pro-inflammatory cytokines from the monocytes, which are exposed to hyperglycemic conditions (Jain and Kannan, 2001), and chromium treatment in addition is known to decrease protein glycosylation and lipid peroxidation in erythrocytes exposed to hyperglycemic conditions (Jain et al., 2006). Chromium lowers cytokines like TNF-alpha, interleukin-6, c-reactive protein, oxidative stress and lipid levels in STZ induced diabetic rats (Jain et al., 2007). Chromium has also been shown to inhibit the lipid peroxidation in-vitro in a dose dependent manner and was found useful for therapy of dyslipidemia (Yang et al., 2006). An increased level of glutathione and antioxidant enzymes like superoxide dismutase and glutathione peroxidase and a reduced impairment of immune functions have been recorded by Liu et al. (2012), in diabetic mice treated with chromium.

The mean values of SOD, CAT and GPx activities in the liver of chromium with glibenclamide treated rats (Group V) were significantly improved with an overall elevation of 47.16, 60.95 and 18.84 per cent respectively, compared to those of diabetic control rats and the mean values were comparable with those of chromium and glibenclamide treated rats (Group III and IV) individually on all the days of study. The improvement in antioxidant enzyme values could be attributed to antihyperglycemic effect of glibenclamide (Sathishsekar and Subramanian, 2005 and Upadhyay and Pandey, 1984), and chromium increased GLUT-4 translocation in myocardial tissues (Penumathsa et al., 2009) and GLUT-2 levels in diabetic rat livers (Tuzcu et al., 2011), which reduce auto oxidation of glucose, induce stabilization of plasma membranes (Chen et al., 2006) and elevation of antioxidant enzymes (Liu et al., 2012). Similar improvements have been recorded by Gurikar, (2014) in his experiment on STZ diabetic rats, fed with CrPic and glibenclamide.

The hepatic antioxidant enzymes in Groups VI treated with GS-200, were significantly improved as the time progressed indicating a reduction in oxidative stress caused by STZ induced diabetes in the treatment groups. The improvements in levels of SOD, CAT and GPX levels were 53.98, 51.67 and 17.44 per cent respectively by 45th day when compared to Group II. Kang et al. (2012) who studied the Gymnemasisvestre extract by LC-MS, indicated that the antihyperglycemic compounds like gymnemagenin and gymnemic acids in the extract were responsible for antioxidant effects. Further, Kang et al. (2012) in an investigation determined the antioxidant activity of Gymnemaisleaf extract using ethanolic extracts by antioxidant assays like thiobarbituric acid (TBA) assay, superoxide dismutase like activity assay, and 2, 2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (involved in electron or radical scavenging), and recorded significant antioxidant activity of the ethanolic extract and recorded that the level of lipid peroxidation reduced by 31.7% in serum, 9.9% in liver, and 9.1% in kidney in the extract fed diabetic rats (Tiwariet al., 2014). Pragathi (2011) and Mallikarjunaet al. (2013), also recorded similar improvements in antioxidant activities in Gymnemasisvestre supplemented groups.

The antioxidant levels in Groups VII rats treated with GS-200 and CrPicsignificantly improved compared to those of diabetic control following treatment over a period of 45 days, with mean per cent improvement of 44.74, 44.63 and 18.73 for SOD, CAT and GPX levels in liver. The mean antioxidant values were comparable with those of Groups V and VI, treated with CrPic and GS-200 respectively. As discussed under these groups, the improvement in antioxidant levels could be attributed to the alleviation of diabetic effects with an improvement in insulin sensitivity brought about by individual treatments. There was no additive or dose dependent effect in normalization of mean antioxidant enzyme levels and also no significant difference was also observed between
Similarly, a betterment in antioxidant enzyme levels was also recorded by Pragathi (2011), Mallikarjuna et al. (2013) and Kang et al. (2012) with Gymnemasylyvestrein STZ diabetic model and attributed this effect to flavonoids and triterpenoids of the herb which inhibits DPPH, scavenge superoxide, hydrogen peroxide and lipid peroxidation and enhance cellular antioxidant defense. In addition, chromium supplementation along with Gymnemasylyvestre might have reduced loss of fatty acids from membranes, controlled cholesterol levels by membrane stabilization (Yang et al., 2006), enhanced sterol regulatory binding proteins (SREBP) (Chen et al., 2006), reduced the sterol efflux (Pattaret al., 2006), and elevated antioxidant enzymes (Liuet al., 2012).

The combination treatment group (Group VIII) with Gymnemasylyvestre along with chromium picolinate, and half the dose of glibenclamide showed improvement in the levels of SOD by 44.21, CAT by 54.93 and GPx by 19.59 per cent by 45th day in liver, when compared to diabetic rats of Group II. The improvement in the antioxidant enzyme levels almost to normalcy was comparable with other groups treated either individually or in combination with Gymnemasylyvestre, chromium and glibenclamide. As discussed earlier, the normalization of antioxidant enzymes could be attributed to the alleviation of hypoinsulinemia, hyperglycemia and hyperlipidaemia and quenching of reactive oxygen radicals in diabetic rats by the combination treatment (Persaud et al., 1999; Sathishsekar and Subramanian, 2005; Chen et al., 2006; Pattaret al., 2006; Pragathi., 2011; Liu et al., 2012; Mallikarjuna et al., 2013 and Tiwari et al., 2014). However, no synergistic or additive effects were observed with combination of treatment and it was difficult to attribute the antioxidant effect specifically to any of the constituents of the combined treatment.

**Pathology of liver:** Grossly, in diabetic rats (Group II), the liver appeared pale, soft and friable from 15th day of the treatment which could be attributed to fatty liver due to hyperlipidemia in streptozotocin induced diabetic rats (Ohno et al., 2000; Mallikarjuna et al., 2013).

**Illustrations**

Fig 1. Section of liver of normal control rat showing regular hepatic cords and normal architecture of hepatocytes. H&E x 40

Fig 2. Section of liver of diabetic control rat showing extensive cell swelling, vacuolar degeneration, obliteration of sinusoidal spaces and congestion by 30th day. H&E x 40

Fig 3. Section of liver of Group IV treated rat with chromium picolinate, showing an improvement in the architecture of liver by 45th day. H&E x 40
On histopathological assessment, the liver parenchyma showed swelling of hepatocytes with highly vacuolated and granular cytoplasm, obliteration of the sinusoidal space and moderate to severe congestion (Fig 2), when compared to healthy rat liver (Fig 1). Increased number of apoptotic cells was observed and there was a mild bile duct hyperplasia also. The vacuolar appearance of the hepatocytes indicated fatty change and could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein from liver resulted from a deficiency of apolipoprotein B synthesis (Ohno et al., 2000). This finding of fatty liver formation is in agreement with the findings of Pragathi, 2011; Mallikarjuna, et al., 2013; Mudasiy et al., 2013). Liver, being a major organ of metabolism, is a target in streptozotocin induced damage as they have Glut-2 receptors. Streptozotocin has affinity for bonding with organs expressing Glut-2, similar to that of pancreas, and hence hepatic injury is inevitable due to subsequent DNA fragmentation with generation of free radicals resulting in the increased number of apoptotic cells (Mir et al., 2008). Ohkuwa et al. (1995) has reported increased formation of hydroxyl radicals in the liver of STZ-induced diabetic rats which causes lipid peroxidation and cell damage.

Microscopically, in the present study, there was an improvement in the architecture of liver in treated groups (Group III to VIII) compared to diabetic control group (II) from 15th day onwards with appreciable reduction in lesions such as cell swelling, cytoplasmic vacuolation and granularity (Fig 3).

Overall, it could be deciphered that STZ induced diabetic rats experienced hepatic injury as evidenced by significant depression in the production of antioxidant enzymes, also seen as a marked cellular degeneration and apoptosis. The administration of Gymnemasmylestre significantly and gradually elevated the levels of antioxidant enzymes over a period of 45 days. Although chromium picolinate was found beneficial, the levels of improvement observed were much lesser than that of Gymnemasmylestre, as was also the case with combination treatment with glibenclamide. Synergistic effects were not evident in the combination treatment groups in the present study in STZ induced diabetic rat model.

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Studies On Hypolipidemic Effect of Methanolic Extract of Murraya koenigii Spreng Leaves in Alloxan Induced Diabetic Rats*

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ABSTRACT

The leaves of Murraya koenigii Spreng, commonly called as Curry leaves, are traditionally used as dietary ingredient in different parts of India and other Asian countries to prevent the occurrence of diabetes. One of the complications of diabetes is hyperlipidemia leading to atherosclerosis. The present study was designed to evaluate the role of methanolic extracts of the leaves of Murraya koenigii Spreng on the plasma lipid profile in alloxan induced diabetic sprague dawley rats. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate (100 mg kg⁻¹ body weight). Oral administration of methanolic extract of Murraya koenigii Spreng leaves (200 mg kg⁻¹ body weight) for a period of eight weeks in diabetic induced rats resulted in a significant (P<0.05) reduction in the components of plasma lipid profile such as total cholesterol, triglycerides and phospholipids. This hypolipidemic effect of methanolic extracts of Murraya koenigii Spreng leaves was attributed to insulin secretogogue property of the extract.

Key words: Alloxan, Diabetes, Hypolipidemic, Methanolic extract, Murraya koenigii

‘Ayurveda’, is an indigenous and ancient form of Indian medicine, which deals with usage of plants and plant products or their active ingredients for either preventing the occurrence of diseases or treating diseases (Aiman, 1970). Many herbal products including several metals and minerals have been described for the care and management of diabetes mellitus (Nadkarni, 1992). Hyperlipidemia is an associated complication of diabetes mellitus (Biosca et al., 1992).

Hypoglycaemic action of several herbs and spices has been established in humans and animals (Brinker, 1998). Among the spices the leaves of Murraya koenigii Spreng (curry leaves) belonging to the family Rutaceae is one of the most commonly used dietary ingredients of human food in India from the time immemorial to man to add good aroma to the food and also for its preventive role in diabetogenesis (Yadav et al., 2002). It is a small tree growing up to six meters in height and 15-40 centimeters in diameter cultivated for its aromatic and ornamental properties throughout India (Pruthi, 1979). Murraya koenigii is widely used in ayurvedic medicine for the treatment of rheumatism, influenza, leprosy and epilepsy (Joseph and Peter, 1985), antihelmintic, anti-inflammatory and antidysegnetic properties (Drury, 1978) and hypoglycaemic effect (Khan et al., 1995; Vinuthan et al, 2003). In this investigation, we made an attempt to evaluate the hypolipidemic effect of methanolic extracts of Murraya koenigii Spreng leaves in alloxan induced diabetic male sprague dawley rats.

MATERIAL AND METHODS

Plant material: Murraya koenigii Spreng leaves were obtained from plants grown in University of Agricultural Sciences campus, Bangalore, Karnataka, India. The plant was identified and ascertained by plant scientists of University of Agricultural Sciences, Bangalore. The leaves were air-dried at room temperature of 28 °C for four days and finely powdered in an electrical mill. Until the time of use the powdered material was kept separately in airtight plastic containers maintained at –20 °C.

Phytochemistry: Leaves of Murraya koenigii are aromatic and contain proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid and vitamin C. It is rich in vitamin A and calcium. The leaves contain high amount of oxalic acid, leaves also contains crystalline glycosides, carbazole alkaloids, koenigin, resin, fresh leaves contain yellow color 2.5 % volatile oil. It also contains girinimbim, iso-
mahaninbin, koenine, koenigine, koenidine and koenimbine. Mahanimbicine and bicyclomahanimbicine, phebalosin, coumarine as Murrayone imperatoxin etc isolated from leaves (Bonde et al., 2011)

**Preparation of methanolic extract:** The powdered leaves were extracted in a Soxhlet apparatus with methanol at a temperature of 50 °C for 48 h. The resultant extract was filtered. The filtered extract was then allowed for drying in an evaporator to evaporate methanol completely. The dried mass was stored in a refrigerator and considered as the extract. The yield of the extract was 4.18% (w/w, on dry matter basis). This was diluted in dimethyl sulfoxide (DMSO) to give a final concentration of 50 mg ml⁻¹.

**Phytochemical Screening:** The different qualitative phytochemical tests were performed for establishing the phytoconstituents present in the methanolic extract using standard methodologies.

**Condition and preparation of the animals:** A total of 24 male healthy Sprague Dawley rats weighing between 200 – 300 g were obtained from the Experimental Laboratory Animal House, University of Agricultural Sciences, Bangalore. Institutional Animal Ethics Committee of Veterinary College, Bangalore accorded the permission to carry out the experiment. All animals were kept in polypropylene cages under controlled condition of 12-hrs light and dark cycles and at an ambient temperature of 21-28 °C. The rats were provided with ad libitum balanced pellet diet (Amrut Laboratory Animal Feed, Nav Maharashtra Chakan Oil Mill Ltd, Pune, India) and water throughout the experimental period.

**Experimental induction of diabetes mellitus in rats:** Diabetes was induced in 18 male Sprague Dawley rats by single intraperitoneal injection of alloxan monohydrate (100 mg kg⁻¹ body weight) (IOBA Chemie, Bombay) that was freshly dissolved in sterile normal saline. The clinical signs of diabetes mellitus such as polydypsia, polyuria and polyphagia were observed within 48 hrs of alloxan injection. Rats with a blood glucose level beyond 200 mg dl⁻¹ were considered diabetic. Blood was collected from intraorbital sinus for plasma glucose estimation with sodium fluoride as anticoagulant on day ‘0’, 14, 28, 43 and 58. Before induction of diabetes mellitus and on the third day of acclimatization the blood glucose level in all the 18 rats was in the range of 80 – 115 mg dl⁻¹.

**Experimental design:** A total of 24 male Sprague Dawley rats divided into four groups. Group I consisted of six non-diabetic rats served as normal untreated control. Remaining 18 alloxan diabetic sprague dawley male rats were divided into three groups each consisting of six rats. Group II, control diabetic rats, given 1 ml of saline daily. Group III, diabetic rats given 1 ml methanolic extract (100 mg dl⁻¹ suspended in DMSO) daily and Group IV, diabetic rats administered with 1 ml DMSO daily, to serve as DMSO control. Saline, methanolic extract and DMSO were administered using an intragastric tube for 8 weeks.

**Blood collection:** Blood samples were collected on day 0, 14, 28, 43 and 58 and processed for blood glucose and plasma lipids. Prior to sample collection, the animals were kept for overnight fasting. They were thereafter anaesthetized with ether and blood samples were collected retro-orbitally from inner canthus of eyes using micro hematocrit capillaries.

**Determination of blood glucose:** Fasting blood glucose (FBS) was estimated by ortho-toluidine method (Dubowskii, 1962).

**Determination of total cholesterol (TC), triglycerides (TG) and phospholipids (PL):** Fasting plasma TC and TG were estimated by commercially available diagnostic kits (Accurex Biomedical Pvt. Ltd., Bombay, India). The PL concentration in plasma was estimated according to the method of Ackermann and Toro (1963).

**Statistical analysis:** Results were analyzed using ANOVA, using Bonferroni test as per the methodology of Snedecor and Cochran (1989), and a significance was accepted at \( p<0.05 \). All results are expressed as mean ± S.E. of six rats in each group.

**RESULTS AND DISCUSSION** Alterations in the levels of blood glucose and urine sugar on treatment of diabetic rats with methanolic extract are given in Table I. Daily administration of
methanolic extract to diabetic rats produced a statistically significant decrease in blood glucose levels ($P<0.05$) compared to control diabetic rats.

Table I. Effect of administration of methanolic extract of Murraya koenigii leaves on blood glucose and urine sugar in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>87.42 ± 5.72</td>
<td>-</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>308.30 ± 11.95</td>
<td>+++</td>
</tr>
<tr>
<td>Diabetes + Methanolic extract.(200 mg dl$^1$)</td>
<td>153.30 ± 7.61*</td>
<td>+</td>
</tr>
<tr>
<td>DMSO control</td>
<td>135.00 ± 6.45*</td>
<td>+</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE for six rats in each group

Effect of feeding methanolic extract to diabetic rats on plasma total cholesterol (TC), triglycerides (TG) and phospholipids (PL) are presented in Table II. The TC and PL were significantly ($P<0.05$) decreased in the plasma of treated rats compared to diabetic control rats on day 28, 43 and 58. The TG levels in methanolic extract administered groups were significantly lowered on day 14, 28, 43 and 58 of the experimental period compared to saline control group and DMSO control group. All the lipid components (the range being TC = 69.40±1.78-74.60±1.08, TG = 74.40±2.11-84.40±2.62 and PL = 72.80±6.02-80.00±7.00) in the normal group remained unaltered throughout the experimental period.

Alloxan induces diabetes by damaging the insulin secreting β-cells of the pancreas leading to hyperglycaemia in a wide variety of animal species. (Chattopadhyay et al., 1997). This experimental animal model of diabetes mellitus is similar to type II diabetes, in which insulin secretion is defective. An observation in this study correlates with the previous research findings (Khan et al., 1995 and Stanley Mainzen Prince et al., 1998), in that the blood glucose levels were elevated significantly in alloxan diabetic rats. Administration of methanolic extract of Murraya koenigii to diabetic rats significantly reduces the blood glucose levels. The possible mechanism by which Murraya koenigii leaves elicits its hypoglycaemic action was postulated as it potentiate the actions of insulin, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhan’s or its release from bound insulin (Vinuthan et al., 2004).

Table II. Effect of administration of methanolic extracts of Murraya koenigii leaves on plasma total cholesterol (TC), triglycerides (TG) and phospholipids (PL) in diabetic rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>0 day</th>
<th>14th day</th>
<th>28th day</th>
<th>43rd day</th>
<th>58th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>I</td>
<td>69.40±1.78</td>
<td>69.80±2.18</td>
<td>72.60±1.63</td>
<td>73.20±1.56</td>
<td>74.60±1.08</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>87.00±2.35</td>
<td>91.83±3.23</td>
<td>97.17±4.10</td>
<td>99.67±3.53</td>
<td>103.67±2.69</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>93.33±2.79</td>
<td>86.17±2.64</td>
<td>78.40±2.88*</td>
<td>74.00±2.85*</td>
<td>71.00±2.59*</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>85.33±1.75</td>
<td>87.67±1.57</td>
<td>90.00±1.63</td>
<td>92.33±1.93</td>
<td>96.17±2.24</td>
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<tr>
<td>TG</td>
<td>I</td>
<td>74.40±2.11</td>
<td>76.40±2.69</td>
<td>79.40±2.64</td>
<td>81.60±2.66</td>
<td>84.40±2.62</td>
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<tr>
<td></td>
<td>II</td>
<td>146.17±5.56</td>
<td>153.17±5.75</td>
<td>158.33±4.91</td>
<td>160.33±4.33</td>
<td>162.00±4.86</td>
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<tr>
<td></td>
<td>III</td>
<td>139.83±5.91</td>
<td>127.17±6.75*</td>
<td>109.40±6.86*</td>
<td>98.20±5.93*</td>
<td>84.50±7.01*</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>145.33±5.12</td>
<td>150.67±4.87</td>
<td>156.17±4.90</td>
<td>158.50±4.81</td>
<td>161.17±4.81</td>
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<tr>
<td>PL</td>
<td>I</td>
<td>72.80±0.02</td>
<td>75.00±0.00</td>
<td>75.00±5.24</td>
<td>79.00±6.04</td>
<td>80.00±7.00</td>
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<td></td>
<td>II</td>
<td>118.30±5.78</td>
<td>123.33±7.08</td>
<td>128.33±6.72</td>
<td>131.67±6.14</td>
<td>133.33±5.76</td>
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<tr>
<td></td>
<td>III</td>
<td>114.80±6.43</td>
<td>102.80±3.53</td>
<td>96.00±5.45*</td>
<td>91.60±7.17*</td>
<td>85.75±5.00*</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>113.33±5.06</td>
<td>118.30±6.15</td>
<td>125.00±3.65</td>
<td>126.67±5.06</td>
<td>128.33±3.20</td>
</tr>
</tbody>
</table>

(Group I = Normal, Group II = Diabetic control, Group III = Diabetic + methanolic extract, Group IV = DMSO control)

Values are given as mean ± SE for six rats in each group

Experimental groups are compared with diabetic control

Values are statistically significant at *$P<0.05$ as compared with diabetic control
Lipids play an important role in the pathogenesis of diabetes mellitus. The levels of plasma lipids are usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease (Chatterjea and Shinde, 1994). Lowering of plasma lipid levels through dietary inclusion of herbal or plant materials or drug therapy seems to be associated with a decrease in the risk of vascular disease (Kannel and McGee, 1979 and Scott and Grundy, 1999). The abnormally high concentration of plasma lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the unlimited actions of lipolytic hormones on the fat depot (Murray et al., 2000).

In the present study we noticed higher levels of TC, TG and PL in alloxan diabetic rats. Similar results on alloxan diabetic rats was reported by our earlier studies and also by other workers (Khosla, 1995; Kumari and Mathew, 1995, Vinuthan M.K. et al., 2007). The studies on the hypolipidemic effect of methanolic extract of Murraya koenigii Spreng leaves were not available. The results of this study revealed that continuous supplementation of methanolic extract daily for 8 weeks prevents elevation of plasma lipids, the complication arising out of diabetic state. The hypolipidemic effect of Murraya koenigii Spreng leaves extract can be explained as a result of direct reduction in the blood glucose concentration. This effect was attributed to insulin secretogogue property of the extracts of the leaves, which may aid in increased storage of lipids in various body tissues and which decrease the lipid levels in blood.

Our findings indicated that methanolic extracts of Murraya koenigii Spreng leaves can lower the blood glucose and plasma lipids in alloxan induced diabetic rats. It can be concluded that the methanolic extract of Murraya koenigii can prevent the rise in plasma lipid concentrations that occur as a secondary complication in diabetic individuals.

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Isolation and Characterization of Salmonellae from Backyard Poultry

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ABSTRACT

Salmonellosis is one of the important food-borne infections that could be transmitted to the human beings by the consumption of contaminated/infected poultry products. In the present study, characterization of Salmonellae isolated from the backyard poultry was carried out. A total of 54 cloacal swabs (swabs from ten birds were considered as one pooled sample), collected from different locations of Hassan, Chikkamagaluru and Shivamogga districts of Karnataka, were analyzed for Salmonella species using standard method (ISO 6579:2002). Detailed cultural analysis of the samples revealed one Salmonella isolate (prevalence 1.85%). The isolate was confirmed based on the biochemical reactions and amplification of virulence associated invasion A (invA) gene using polymerase chain reaction (284 bp amplicon). The isolate was identified as Salmonella enterica subsp. enterica serotype Typhimurium and its antibiogram showed sensitivity against commonly used antibiotics (amikacin, amoxicillin/ sulbactum, ampicillin, chloramphenicol, cefadroxil, cefotaxime, ciprofloxacin, cotrimoxazole, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfadiazine and trimethoprim); however, the isolate was found resistant to four antibiotics (tetracycline, colistin, cephalaxin and polymyxin-B). This study established the occurrence of Salmonella among backyard poultry in the study area. In view of the potential transmission of food-borne Salmonellosis, need for regular surveillance and monitoring is advised.

Key words: Chicken, backyard poultry, Salmonella, PCR, invA gene, antibiogram

Backyard poultry contributes to nearly 30% of the national egg production. However, this sector is neglected in spite of the fact that the eggs and meat fetch better price than that of the commercial poultry. Uncontrolled movement of the birds in the open rearing system exposes them to a variety of infectious agents and could potentially act as vehicles of transmissions of pathogens to humans. Food-borne Salmonellosis could be acquired through poultry or contaminated poultry products by the way of handling of raw poultry carcasses and products or through consumption of undercooked egg and poultry meat (Bailey and Cosby, 2003; Kimura et al., 2004). Salmonellosis causes heavy economic loss to the poultry sector in terms of mortality or reduced productivity among the affected birds (Khan et al., 1998). Hence, poultry must be periodically checked for the presence of Salmonellae so that suitable preventive and control measures could be adapted by farmers in order to minimize the economic loss and also protect the public health.

MATERIALS AND METHODS

Sample collection: Cloacal swab samples were collected from selected backyard poultry farms located in and around Hassan (Javagal, n=2), Chikkamagaluru (AK Colony & Kote, n=2 each) and Shivamogga (Veterinary College farm, n=7; Sominkoppa, n=13; Padmenahalli, n=3; Kashipura, n=3 and Koteganguru, n=22) districts of Karnataka. Sterile swabs moistened with buffered peptone water (BPW) were inserted into the cloaca and placed into sterile polythene bag containing 10 ml of BPW. Samples were brought to the laboratory under chilled conditions; cloacal swabs collected from 10 birds were pooled and considered as one sample and a total of 54 pooled cloacal samples were subjected for analyses.
Detection of Salmonellae: Standard method ISO 6579:2002 was followed for the isolation and identification of Salmonellae from the poultry. The cloacal swabs kept in BPW were incubated for 24 hours at 37°C (non-selective pre-enrichment). After incubation, 0.1 mL of inoculum was transferred to selective enrichment i.e. 10 mL of Rappaport-Vassiliadis (RV) broth and incubated at 37°C for 24 hours. A loopful of culture was then streaked onto Xylose lysine desoxycholate (XLD) as well as MacConkey agars and incubated at 37°C for 24 hours. Typical colonies suggestive of Salmonella were brought in pure cultures and maintained on nutrient agar slants. Putative colonies were then subjected for biochemical tests viz., triple sugar iron agar (TSI), urease, methyl red, Voges-Proskauer and indole as per the standard method.

Polymerase chain reaction (PCR): in vitro DNA amplification through PCR has been considered as a powerful tool for the diagnosis of Salmonella (Malorny et al., 2003). Several genes have been targeted for the detection of Salmonellae isolated from environment, food and faecal samples, albeit chromosomal genes like invA, invE, himA, phoP have been most widely used as targets (Jamshidi et al., 2009). Of these targets, invA gene of Salmonella has been one of the most widely used owing to its unique sequences specific to the genus and suitability for PCR as a target for diagnosis (Rahn et al., 1992). Therefore, invA gene was used as a diagnostic target for PCR amplification in the present study. PCR was performed for invA gene as per the protocol of Rahn et al., (1992) with minor modifications as standardized by Nagappa et al., (2007).

Antibiotic susceptibility testing: Antimicrobial tests were carried out on Muller-Hinton agar using disc diffusion method (Bauer et al., 1966). Antimicrobials used and their concentrations were chloramphenicol (30 mcg), ampicillin (10 mcg), cefotaxime (30 mcg), amikacin (30 mcg), cefotaxime (30 mcg). Zones of inhibition were measured using digital vernier calipers and sensitivity/resistance was recorded according to CLSI (2012).

RESULTS AND DISCUSSION
Out of 54 samples of cloacal swabs of backyard poultry analyzed for Salmonella spp., one isolate was obtained and confirmed as Salmonella enterica subsp. enterica serotype Typhimurium (S.Typhimurium) at national Salmonella typing centre, Kasauli (H.P.). The Salmonella isolate revealed diagnostic amplicon of size 284 bp specific to the genus Salmonella (Fig); and detection of this PCR product was indicative of carriage of pathogenicity associated invA gene specific to the Salmonella genus. This study confirmed the presence of Salmonella among the backyard poultry. Antibiogram of the isolate recovered from the Padmenahalli village of Shivamogga district showed resistance to tetracycline, colistin, cephalaxin and polymixin-B.

Salmonellosis is one of the major causes of enteric diseases in the developing countries and poultry acts as a major source of human transmission. Pathogenic Salmonellae get transmitted through the faecal-oral route and cause a myriad of disease conditions among the affected individuals. Therefore, control of such food-borne pathogens must be a priority not only by the authorities but even the producers (Buzby et al., 1996).

Fig: PCR amplification of Salmonella genus specific invasion gene

Lane 1: 100 bp DNA Marker
Lane 2: Sample showing 284 bp PCR product specific to Salmonella
Lane 3: No Template Control
CONCLUSION
Owing to the regional paucity of the data on the occurrence of Salmonellae in the backyard poultry, the present study was planned for isolation, identification and characterization of Salmonellae from the backyard poultry. The Salmonella isolate was identified as S. Typhimurium and was found pathogenic as evidenced by the PCR amplification of invasion gene. Further, the isolate was found resistant to four commonly used antibiotics. This study showed presence of Salmonella in the backyard poultry; hence, keeping in view the foodborne and zoonotic transmission potential of Salmonellae, implementation of good poultry husbandry practices; periodic testing of the poultry for Salmonellae and public health education is advocated.

ACKNOWLEDGEMENT
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Isolation and Characterization of Salmonellae from Commercial Poultry

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ABSTRACT

Salmonellosis is one of the common infectious diseases of poultry that causes substantial economic loss to the poultry farmers in terms of mortality and decreased production among survivors. Salmonellae are potentially zoonotic and even could reach consumers by the way of contaminated poultry products. In the present study, 81 pooled fecal samples (10 cloacal swabs from 10 birds was considered as one composite sample), collected from seven different broiler and layer poultry farms located in and around Chikkamagaluru, Shivamogga and Davanagere districts of Karnataka were analyzed for Salmonellae following the standard method (ISO 6579:2002). Of the total of 81 samples analyzed, Salmonellae were detected in two samples collected from the broiler chicken farms located in Chikkamagaluru. Culturally and biochemically confirmed isolates were characterized for invA gene using polymerase chain reaction and amplification of 284 bp product was evident in both the isolates indicating their virulent nature.

Both the isolates belonged to the serotype Salmonella enterica subsp. enterica serotype Enteritidis. Antiibiogram of the isolates revealed sensitivity to chloramphenicol, cotrimaxazole, ciprofloxacin, sulfadiazine, trimethoprim, colistin, cephalexin, streptomycin, cefadroxil, amikacin and cefotaxime; however, the isolates were to polymyxin-B. Both the isolates showed variable sensitivity to ampicillin, kanamycin, amoxycillin/ sulbactum, tetracycline, nalidixic acid and gentamicin. In view of the public health and food safety significance of Salmonellae, strict adherence to good poultry avian husbandry practices and periodic screening of flocks for Salmonellae is emphasized.

Key words: Chicken, commercial poultry, Salmonella, PCR, invA gene, antibiogram

Poultry sector appreciably contributes to the food basket as evidenced by its increased consumption. Commercial poultry requires intensive planning and healthcare for the optimum production; nevertheless, at times diseases affect productivity leading to the economic loss among farmers (Omwandho and Kubota, 2010). Of the several diseases of poultry, Salmonellosis has been one of the most commonly encountered diseases of infectious origin (Rabsch et al., 2001). Salmonellae occur worldwide with their wide host range and also as food-borne pathogens they get transmitted to the humans (Orji et al., 2005). Salmonellosis is endemic in India; apart from its zoonotic potential, it causes heavy economic loss to the poultry sector every year (Humphrey, 2000; Rahman, 2002) by the way of mortality of affected birds and decreased production among survivors (Omwandho and Kubota, 2010). Salmonellae get transmitted through contaminated food such as undercooked products or through cross-contamination occurring at various stages across the chicken egg/meat supply chain (Tauxe, 1991). Since Salmonellosis is an important poultry disease impeding commercial poultry production in developing countries (Asia and Africa), a cross sectional survey was carried out to appraise its prevalence among the poultry maintained at commercial farms.

MATERIALS AND METHODS

Sample collection: Fecal samples were collected from the commercial broiler and layer poultry farms located in and around Chikkamagaluru, Shivamogga and Davanagere districts of Karnataka. Sterile swab dipped in buffered peptone water (BPW) was inserted individually into the cloaca of chicken and a pool of such swabs collected from 10

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birds was considered as one composite sample. Layer and broiler cloacal swab samples (81 samples from 810 birds) were brought to the Department of Veterinary Public Health & Epidemiology Laboratory under chilled conditions and subjected for *Salmonella* isolation.

**Isolation of Salmonellae:** Aseptically collected samples were subjected for *Salmonella* isolation using ISO 6579:2002 method. Briefly, after pre-enrichment in buffered peptone water, the inoculum was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated at 37°C for 24 hours. Selective plating was then undertaken by streaking onto the Xylose lysine desoxycholate agar followed by incubation at 37°C for 24 hours. Pink or red colonies with or without black centres were further streaked onto MacConkey agar and incubated at 37°C for 24 hours. Non-lactose fermenting colorless translucent colonies were obtained in pure culture and stored over nutrient agar slants for further use.

Colonies suggestive of *Salmonella* on the specific culture media were subjected to biochemical characterization viz. TSI, urease, indole, methyl red, Voges-Proskauer tests as per the ISO 6579:2002 method. Further, molecular characterization was undertaken using polymerase chain reaction (PCR).

**Isolation of DNA:** The DNA was isolated by snap-chill method. Briefly, a loop full of pure culture was emulsified in 200 µl of sterile water in a microfuge tube and kept in boiling water bath for 10 minutes followed by freezing. After centrifugation at 6,000 rpm for 10 minutes at 4°C, supernatant (2 µL) was taken as template for the PCR amplification of the target DNA.

**Amplification of invA gene by PCR:** *Salmonella* genus specific associated invasion gene (*invA*) was amplified following the method of Rahn *et al.*, (1992) using primers 5’-GTG AAA TTA TCG CGT CCA CGT TCG GGC AA-3’ and 5’-TCA TCG CAC CGT CAA AGG AAC C-3’ as standardized by Nagappa *et al.*, (2007).

**Antimicrobial susceptibility test:** Disc diffusion method was used for testing susceptibility of the *Salmonella* isolates against common antimicrobials (Bauer *et al.*, 1966). Isolates were tested against 22 antibiotics namely chloramphenicol (30 mcg), ampicillin (10 mcg), co-trimoxazole (1.25/23.75 mcg), ciprofloxacin (5 mcg), kanamycin (30 mcg), amoxycillin / sulbactum (10 mcg), tetracycline (30 mcg), sulfadiazine, trimethoprim (5 mcg), colistin (10 mcg), gentamycin (10 mcg), cefalexin (10 mcg), streptomycin (10 mcg), polymixin-B (300 units), cefadroxil (30 mcg), nalidixic acid (30 mcg), amikacin (30 mcg), cefotaxime (30 mcg). Zones of inhibition were measured using digital vernier calipers and sensitivity/ resistance was recorded according to CLSI (2012).

**RESULTS AND DISCUSSION**

A total of 81 cloacal swabs collected from the commercial poultry were tested for *Salmonellae* using ISO 6579:2002 method, of which two samples collected from the broiler farms located at Chikkmagaluru were found positive. Both the *Salmonella* isolates showed PCR amplification of 284 bp specific to the *invA* gene (Fig). Carriage of *invA* gene by the *Salmonella* was indicative of the virulence among the isolates. Confirmed isolates were serotyped as *Salmonella enterica* subsp. *enterica* serotype Enteritidis at national *Salmonella* typing centre (Kasauli, H.P.).

**Fig:** Amplification of 284 bp *Salmonella* specific *invA* gene

Lane 1 : 100 bp DNA Ladder
Lane 2& 3 : Positive samples
Lane 4 : No Template Control (NTC)

In the present study, Salmonellae were detected in 2.46% of the cloacal samples; which was relatively low as compared to Moon *et al.*, (2011), who reported Salmonellae in 28.33% of
samples in Wardha Maharashtra. Similarly, Orji et al., (2005) detected Salmonella Paratyphi A in 12.5% of poultry droppings, followed by other serotypes such as S. Typhimurium (6.7%), S. Gallinarum (6.7%), S. Enteritidis (5%) and S. Pullorum (4.1%). In a similar study, Plummer et al., (1995) reported prevalence of Salmonellae in 16 to 21% of chicken meat samples. Saeed et al., (2013) recorded highest level of Salmonella contamination in India. On the other hand, some studies have also reported less than 2% prevalence of Salmonellae in poultry viz. Kumar and Lakhera (2013) detected Salmonella in 1.5% of poultry samples in Bareilly.

Antibiogram of the isolates showed sensitivity to chloramphenicol, co-trimoxazole, ciprofloxacin, sulfadiazine, trimethoprim, colistin, cephalaxin, streptomycin, cefadroxil, amikacin and cefotaxime; while both the isolates were resistant to polymyxin-B and exhibited variable sensitivity to ampicillin, kanamycin, amoxicillin-sulbactum, tetracycline, nalidixic acid and gentamicin.

Higher level of antimicrobial resistance has been reported among Salmonellae in Indian chicken (Hatha and Lakshmanaperumalsamy, 1995; Suresh et al., 2000 & 2006). Sub-therapeutic and therapeutic use of antimicrobials in the modern poultry production often violating prescriptions, guidelines and withdrawal periods is resulting into the emergence of resistance among such bacteria (Novick, 1981). Keeping in view the public health significance of the antibiotic resistance, serious consumer health concerns associated with the commercial poultry products was underscored.

**CONCLUSION**

In the present study, occurrence of Salmonellae in commercial poultry was at 2.46%; and a single serotype S. Enteritidis was recovered. The isolates showed resistance to common antimicrobials. Since Salmonella is the organism of food safety and public health concern, its recovery from the birds indicated risk associated with the products (chicken egg and meat) derived from the commercial poultry.

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Phylogenetic analysis of Fowl Adenovirus-4 isolate

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ABSTRACT
Liver samples from suspected outbreaks of HPS were subjected to PCR as per the method of Raue and Hess using primers pairs H3/H4, specific for fowl adenovirus. PCR products were gel purified and cloned in pGEM® T Easy vector (Promega). The recombinant plasmids containing 1319 bphexon gene of two FAV-4 isolates, FAV-4 Jammu and FAV-4 Punjab were sequenced and analyzed. The phylogenetic tree of DNA alignment showed very close relationship between FAV4 Jammu and FAV4 Punjab than other isolates whereas the phylogenetic tree of protein alignment showed very close relationship between FAV4 India (I.V.R.I) and FAV4 Jammu than other isolates. The percent DNA identity for fowl adenovirus hexon genes revealed the highest homology (99.7%) between FAV4 Jammu and FAV4 Punjab. Comparision of percent protein identity of hexon protein of FAV4 KR5 and FAV4 Punjab revealed highest homology of 99.5%. Based on the nucleotide sequence and protein alignment, the FAV4 of Jammu, Punjab, India (I.V.R.I) and FAV4 KR5 were closely related. Besides, the DNA and protein sequence also showed a close resemblance to FAV10 hexon gene than FAV1 and FAV8 hexons

Key Words: Fowl Adenovirus, PCR, Cloning, Phylogenetic tree

Hydropericardium syndrome (HPS) outbreaks have been reported from different parts of the world in last few years. The disease is characterized by sudden occurrence with high morbidity and high mortality of upto 80 percent in 3-7 weeks old broilers (Anjum et al 1989, Shane 1996). The studies undertaken at various laboratories have indicated that the causative agent is a fowl adenovirus serotype 4 (FAV-4). There are only few reports on molecular studies with fowl adenoviruses, other than type 1 and type 8. Nucleotide sequencing is a precise and reliable technique for the genomic characterization and taxonomic classification of the pathogens. The nucleotide sequence analysis of published FAVs shows that serotypic variations are present in the hexon gene sequence. In this context, cloning and phylogenetic analysis of hexon gene can be a promising tool, as it could open avenues for further characterization of the hexon gene. Hence the present study was undertaken to know the relationship between different fowl adenovirus isolates.

MATERIAL AND METHODS

Polymerase chain reaction (PCR): Liver samples from suspected outbreaks of HPS from various regions of Northern India were collected in phosphate buffer glycerine for virology work and stored at -20°C until further use. DNA from each liver samples were extracted by using QIAGEN DNA extraction kit by following the procedure supplied along with the kit. QiAamp purified DNA segments were amplified by PCR as per the method of Raue and Hess (1998) using primer pairs H3/H4, specific for fowl adenovirus. The PCR products generated were confirmed for their expected size 1319 bp in 1% agarose gel in 1X TBE buffer using horizontal submarine electrophoresis apparatus.

Molecular cloning of hexon gene: PCR products were purified using the QIAquick gel extraction kit (Qiagen) as per manufacturer’s recommendations. Cloning of 1319 bp purified PCR products were carried out using pGEM®T Easy vector (Promega) as per the manufacturer’s recommendations. The recombinant clones obtained were screened by PCR and Restriction Endonuclease digestion for confirmation of the desired insert.

DNA sequencing and Phylogenetic Analysis of Cloned Hexon Gene Sequence: The recombinant plasmids containing 1319 bphexon gene of two FAV-4 isolates, FAV-4 Jammu and FAV-4 Punjab were subjected to single strand DNA sequencing using T7 and SP6 primers, at the DNA sequencing
facility of University of Delhi South Campus, New Delhi.

Nucleotide sequences of hexon gene of the isolates studied were analyzed and amino acid sequences were deduced by computer using ‘Edit Seq’ programme of Lasergene software. Both nucleotide and amino acid sequences were aligned separately by using clustal method of “Meg Align” programme. For comparison, nucleotide and amino acid sequences of known reference strains were included. These sequences were downloaded from the NCBI, Gen Bank under the following accession numbers: FAV1, U46933, FAV4 (KR5) AJ431719, FAV8, NC_000899, FAV10, U26221, FAV4 AJ459805. A lined nucleotide and amino acid sequences were subjected to phylogenetic analysis using ‘MegAlign’ programme of Lasergene software to derive the ancestral relationship between sequences.

RESULTS AND DISCUSSION

Polymerase chain reaction: Amplification of DNA with H3/H4 primers resulted in a fragment of approximately 1319 bp (fig 1) which is diagnostic for the avian adenoviruses (Raue and Hess 1998). Molecular cloning of hexon gene revealed the presence of single band of appropriate size (1319 bp) in each of the recombinant plasmid. The 1319 bp recombinant plasmids released 1319 bp hexon gene fragment (fig 2). This result showed that the recombinant plasmid was having the correct size of insert and the orientation of the insert was also in correct position.

Phylogenetic Analysis of Cloned Hexon Gene Sequence: The phylogenetic tree of DNA alignment (fig 5) showed very close relationship between FAV4 Jammu and FAV4 Punjab than other isolates whereas the phylogenetic tree of protein alignment (fig 6) showed very close relationship between FAV4 India (I.V.R.I) and FAV4 Jammu than other isolates.

The percent DNA identity for fowl adenovirus hexon genes revealed the highest homology (99.7%) between FAV4 Jammu and FAV4 Punjab (fig 3). Comparison of percent protein identity of hexon protein of FAV4 KR5 and FAV4 Punjab revealed highest homology of 99.5% (fig 4).

Based on the nucleotide sequence and protein alignment, the FAV4 of Jammu, Punjab, India (I.V.R.I) and FAV4 KR5 were closely related. Besides, the DNA and protein sequence also showed a close resemblance to FAV10 hexon gene than FAV1 and FAV8 hexons. Similar findings have been reported by Sheppard et al (1995) and Baruah (2001) upon comparison of the nucleotide sequences of FAV1 and FAV8 hexons.

As the hexon is the major capsid component it could be expected that structural constraints would dictate, to a degree, its amino acid sequence; at the same time the hexon is a major antigenic component of the virion and therefore variation in its amino acid sequence could also arise from immune response selection pressures. Previous studies on the mammalian adenovirus hexons have shown high levels of amino acid sequence conservation (Toogood et al 1989) suggesting that structural constraints do play a part in the amino acid sequence of hexons.

As regards the neighbor joining tree of the hexon genes, among the different FAVs whose sequences were available, the tree revealed that FAV4 was related to FAV10 whereas FAV1 and FAV8 were placed away from the virus. There are some reports that the hypervariable regions are hotspots for both, single base mutation and illegitimate recombination. To prove that FAV 4 might have evolved by a recombination of FAV9 and FAV10 more number of FAV isolates from different geographical regions, over a period of time needs to be studies, not only in the hexon gene region but also other structural proteins viz., penton which take part in the formation of the virus capsid.
Fig. 2: RE digestion of recombinant plasmid with E coR I
Lane M : Molecular weight marker (500 bp)
Lane 1 & 4 : Purified PCR products (1319 bp)
Lane M : Molecular weight marker (500 bp)
Lane 2 & 5 : RE digested recombinant plasmid releasing insert (1319 bp)
Lane 3 : Recominant plasmid

Fig. 3: Percent DNA identity

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Fig. 4: Percent Protein identity

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Fig. 5: Phylogenetic tree of DNA alignment

Fig. 6: Phylogenetic tree of protein alignment

REFERENCES


Detection of Early Pregnancy in Cows

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ABSTRACT

Forty cows were divided into 6 groups viz. Group I (n=9), II (n=7) and remaining group III, IV, V and VI had 6 cows each. These cows were subjected for various early pregnancy diagnostic techniques from day 19 post insemination and later were confirmed by per-rectal palpation method on days 45 and 60 post insemination. The accuracy of pregnancy diagnosis was 100% with bovine pregnancy detection kit–milk on day 19-23 and ultrasonography on days 26 and 33 post insemination in cows. The accuracy of pregnancy diagnosis was higher (75%) in non-return rate, followed by (50%) in bovine pregnancy detection kit - serum on day 19-23 post insemination in cows. The lower accuracy of pregnancy diagnosis was noticed in Punyakoti test (33.3%) on day 21 post insemination in cows. Crystallization pattern of cervical mucus on day 19 post insemination in pregnant cows varied from mixed and negative patterns whereas in non-pregnant cows varied from marked to mixed patterns. Vaginal cytology on day 19 post insemination in pregnant cows revealed, anuclear cells varied from 60-90%, superficial cells varied from 10-20% and 20% intermediate cells were seen in one cow. In non-pregnant cows, the anuclear cells varied from 60-70%, superficial cells 15-25% and intermediate cells 10-15% were recorded. The study concluded that, bovine pregnancy detection kit - Milk on day 19-23 and ultrasonography on days 26 post insemination are the earliest, most accurate, safest method of pregnancy diagnosis in cows.

Key words: Early pregnancy diagnosis, Ultrasonography, Pregnancy detection kit, Vaginal electrical resistance, cows

In dairy cattle, pregnancy diagnosis is an important tool to measure the success of a reproductive management. For any economical dairy farm, cows must calve every year and to maintain this sequence, identifying pregnant animals at an early date seems imperative. Early identification of non pregnant dairy cows and heifers post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between AI services and increasing AI service rate. Thus, new technologies to identify non pregnant dairy cows and heifers early after artificial insemination may play a key role in management strategies to improve reproductive efficiency and profitability on commercial dairy farms (Fricke, 2010). Research comparing the effectiveness of these recently developed diagnostic techniques with previous methods of pregnancy detection is lacking. Hence, the present study was undertaken to detect and compare various methods to diagnose pregnancy like ultrasonography, Punyakoti test and cervical swab, smear and staining with per-rectal examination on days 45 and 60.

MATERIALS AND METHODS

The study was carried out in 40 cows belonging to Department of LPM and farmers of surrounding villages. These cows were randomly divided into 6 groups viz. Group I (n=9), II (n=7), III (n=6), IV (n=6), V (n=6) and VI (n=6). These cows were monitored for estrous cycle and were inseminated in estrus period and were subjected for various early pregnancy diagnostic techniques after insemination. Pregnancy diagnosis was initiated from day 19 post insemination. The various methods of pregnancy diagnosis used in the cows are described as follows.

Group I-Bovine pregnancy detection kits – Milk [Days 19 to 23]: The milk samples from cows (n=9) were collected in a clean vessel after discarding first few strips. The test was done between day 19 and 23 after insemination using bovine pregnancy detection kit. The test cassette was removed from pouch and kept on flat surface. 3 drops of milk is added to the test well (Marked T) using a dropper supplied with the test. Using the fresh dropper 3 drops of reagent 1 is added into the control well [Marked C]. Using the same dropper 1 drop of reagent 1 is added into the test well (Marked T). The milk and reagent was gently mixed by gently tapping the cassette. Care was taken not to splash the liquid in the well. The cassette is kept on flat surface for 20 min. The
cassette is gently tapped at 4 min interval. The cassette was tipped and wells emptied into a sink. Both the wells [C and T] were washed with drinking water by filling and emptying the well 5 to 6 times. The remaining water from wells was shaken off. 6 drops from reagent 2 was added to both the empty wells [C and T]. The test was kept on flat surface. The result was read after 10 minutes till 15 minutes. If ‘T’ well bluer than ‘C’ well, it was considered as non-pregnant. If ‘T’ well same as or lighter than ‘C’ well was considered as pregnant.

**Group II-Bovine pregnancy detection kits – Serum [Days 19 to 23]:** After collection of blood from cows (n=7) between 19 and 23rd day after insemination serum was separated. The Bovine pregnancy detection kit - serum from pouch was removed and kept on flat surface. 2 drops of serum was added to the test well (Marked T) using a dropper supplied with the test. Using the fresh dropper 3 drops of reagent 1 was added into the control well [Marked C]. Using the same dropper 2 drops of reagent 1 was added into the test well (Marked T). The serum and reagent was allowed to mix by gently tapping the cassette. Care was taken not to splash the liquid in the well. The cassette was kept on flat surface for 20 min. The cassette was gently tapped at 4 min interval. The cassette was tipped and wells emptied into a sink. Both the wells [C and T] were washed with drinking water by filling and emptying the well 5 to 6 times. The remaining water from wells was shake off from wells. 6 drops of content from vial reagent 2 was added to both the empty wells [C and T]. The test was kept on flat surface. The results were seen after 10 minutes and were read till 15 minutes. In non-pregnant animals, ‘T’ well was bluer than ‘C’ well and in pregnant animal, ‘T’ well was same as or lighter than ‘C’ well.

**Group III-Cervical swab smear and staining [Days 19 and 20]:** A cervical mucus sample was collected with a sterilized cotton swab and a vaginal speculum from inseminated cows (n=6) on day 19 and 20. The collected mucus was smeared on glass slide and air dried. The microscopic crystallization pattern for each mucus sample was studied directly after preparation of smear and evaluated as per method described by Ghannam and Sorensen (1967). The same smear was subjected for Giemsa staining and cytology is studied. The vaginal resistance reading was taken by passing an estrus detector probe (DRAMINSKI, RL-10-860 Olszyn. Owocows 17) on days 19, 20 and 21.

**Group IV- Non- return Rate: [21 Days]:** All the inseminated cows (n=6) were monitored for estrus exhibition and cows which do not show estrus were considered as pregnant.

**Group V-Punyakoti test: [21 Days]:** About 15 seeds of Wheat were placed in Petri dish containing filter paper with 15 ml diluted urine [1 ml urine + 14 ml water] from the cow whose pregnancy is to be diagnosed. Similarly, a Petri dish containing seeds treated with 15 ml water was kept as control. After 5 days, the cow was diagnosed as pregnant if wheat seeds have not germinated and have turned brown to blackish in colour.

**Group VI-Ultrasonography: [Days 26 and 33]:** All the 6 inseminated cows were scanned trans-rectally using portable ultrasound scanner equipped with probe of 5 MHz (ECOSON 800V, WEST MEDICA WIEN) on days 26 and 33. The inguinal region of inseminated cow was cleaned and wiped with clean towel. The cow was restrained in a trevis in standing position. After back racking, a well lubricated gloved hand along with trans-rectal probe was passed per rectally and uterine horn was scanned for pregnancy by detecting dark amniotic cavity and presence of a fetus.

**Per-rectal palpation: [Days 45 and 60]:** All the inseminated cows (n=40) were screened per rectally on days 45 and 60 for pregnancy confirmation.

**RESULTS AND DISCUSSION**

**Bovine pregnancy detection kits-Milk:** Out of 9 cows subjected for pregnancy diagnosis using milk kits between days 19 to 23 post insemination, four cows confirmed pregnant (44.44%) and 5 cows as non-pregnant (55.55%). The sensitivity and specificity of the test was 100%. The positive and negative predictive value was also 100%. The present findings are in line with earlier workers Lee et al. (1996); England et al. (1997) and Kirsch et al. (2009). Otava et al. (2007) suggested that, pregnancy loss, corpus luteum cysts, hidden infections of the genital system and artificial
insemination of cows not in estrus are the predominant causes of inaccurate diagnosis of pregnancy in cows.

**Bovine pregnancy detection kits-Serum:** Out of 7 cows subjected for pregnancy diagnosis using serum kits between days 19 to 23 post insemination, five cows confirmed pregnant (71.42%) and 2 cows as non-pregnant (28.57%). The sensitivity of the test was 100% and specificity was 50%. The positive and negative predictive value was 60% and 100% respectively. The results of the study are similar with Otva *et al.* (2007) and Wei-Chen (2010). The commercial kit performed well in the diagnosis of non-pregnancy (predictive value negative = 100%) but was less accurate in the prediction of pregnancy (predictive value positive = 60%).

**Punyakoti Test:** The pregnancy diagnosis of cows by using Wheat seeds for germination inhibition technique on day 21 post insemination showed 83.33% of accuracy for pregnancy and 16.66% for non-pregnancy. The sensitivity of test was 100% and specificity 33.33%. The positive and negative predictive value was 60% and 100% respectively. The average germination inhibition percentage for pregnant cows was 94.6% and in non-pregnant cows 62.6%. These values appear to be slightly higher compared to those reported by Swamy *et al.* (2010); Dilrukshi and Perera (2009). With reference to the factor that might be influencing such a differential response in urine treated seeds Veena and Narendranath (1993) have suggested that, plant growth regulators such as auxins are excreted in high concentration in urine during pregnancy in cows which might be causing inhibitory responses to seed germination and shoot growth. But such plant growth regulators as auxins and abscisic acid are likely to be in urine as and when the cows consume plants containing such substances.

**Cervical swab smear and staining:** The crystallization pattern of cervical mucus was studied on day 19 post insemination to diagnose pregnancy in cows. Out of six cows, three showed negative pattern, two showed mixed pattern and one as marked pattern of crystallization. Crystallization pattern in pregnant cows varied from mixed to negative patterns. Similarly, in non-pregnant cows crystallization pattern varied between marked to mixed patterns. In negative pattern, there was 66.66% of pregnancy and 33.33% of non-pregnant cows. Similarly, in mixed pattern there were 50% of both pregnant and non-pregnant cows, whereas in marked pattern 100% of non-pregnant cows were noticed. The above results are consistent with the observations of (Abusinea, 1962; and Ghannam and Sorensen, 1967 and Noonan, 1975). They suggested that, variation in length of estrus in non-pregnant cows and cows with silent estrus were noticed, may me the reason for incorrect diagnosis of pregnancy.

**Vaginal electrical resistance:** The electrical resistance of vaginal mucosa measured on days 19, 20 and 21 post insemination was used to diagnose pregnancy in cows. The mean electrical resistance values of pregnant cows were 208 and in non-pregnant cows 261. T-test was used to compare the mean VER on days 19, 20 and 21 and the level of significance was set at <0.05. There was no significant difference observed on days 19, 20 and 21. The present findings were similar to reports of (Tasal *et al.* 2005; Leidl and Stolla, 1976). The vaginal electrical resistance measured on days 19, 20, 21 did not have significant differences on different days. The findings are similar to those reported by Patil and Pawshe (2011) and Tadesse *et al.* (2011). These differences may have been the result of changes in intra-vaginal probe designs of the earlier studies. Other factors influencing the results could include the depth of probe insertion in the vagina, the position of the probe within the vagina (Foote *et al.*, 1979 and Heckman *et al.*, 1979) and pressure against the mucus membrane and pathological conditions of the reproductive tract (Leidl and Stolla, 1976).

**Vaginal cytology:** The cervical mucus samples were subjected for Giemsa staining to study cytology on day 19-post insemination for early pregnancy diagnosis in cows. In pregnant cows, the anuclear cells varied from 60-90% whereas, superficial cells varied from 10-20% and 20% intermediate cells were seen in only one pregnant cow and no cells in other pregnant cows. In non-pregnant cows, the anuclear cells varied from 60-
70%, superficial cells 15-25% and intermediate cells 10-15% were recorded. The present results are slightly lower than reported by Ribeiro et al. (2012). These differences may be due to variation in length of estrous cycle and infections of genital tract.

**Ultrasonography on days 26 and 33 post insemination:** The pregnancy was diagnosed by using 5 MHz trans-rectal probe on day 26 post insemination by observing dark amniotic cavity and on day 33 presence of echogenic foetus in anechoic gestational sac. Out of six cows subjected for ultrasonography, 2 were confirmed pregnant (33.33%) and 4 cows as non-pregnant (66.66%) on days 26 and 33 post insemination. The sensitivity and specificity of the test was 100%. The positive and negative predictive value was also 100%. The present results are in line with Bhoraniya et al. (2011) and Pawshe et al. (2011).

**Comparative accuracies of pregnancy diagnostic techniques:** The bovine pregnancy detection kit - Milk on day 19-23 and ultrasonography on day 26 and 33 post insemination are highly accurate methods of pregnancy diagnosis in cows. The accuracy of pregnancy diagnosis was higher (75%) in non-return rate than in bovine pregnancy detection kit - serum (50%) on day 19-23 post insemination in cows. The lower accuracy of pregnancy diagnosis was noticed in Punyakoti test (33.3%) on day 21 post artificial insemination in cows.

It can be concluded that, Bovine pregnancy detection kit-Milk is an accurate, easy, rapid and safe method of early pregnancy diagnosis (day 19-23) in cows than Bovine pregnancy detection kit-Serum and Non-return rate and Punyakoti test.

**REFERENCES**


Age Related Gross Morphological Study of Humerus in Kenguri Sheep

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ABSTRACT
The study was undertaken on the humerus bone of Kenguri sheep to know the gross morphology and its development at different age levels in Kenguri sheep. The left and right humerus bones from 24 animals of both sexes were divided into four groups (Group-I: up to 6 months, Group-II: above 6 months to 2 years, Group-III: above 2 years to 4 years and Group-IV: above 4 years to 6 years of age). Humerus showed slender, twisted shaft. The anterior and lateral surfaces were separated by crest of humerus. The deltoid tuberosity was ridge like rough triangular structure at 1.5 months of age, located at proximal end of the crest of humerus and later well developed. Teres tubercle noticed as small blunt area at the proximal to the middle on the medial surface at 2 years of age and later well appreciated. Posterior surface was rounded, smooth and blends with lateral surface. Proximal extremity consisted of head, neck, lateral and medial tuberosities and bicpital groove. Lateral tuberosity was undivided till 4 months of age, later it divided into cranial and caudal parts. Distal extremity was divided into lateral and medial condyles. Both the condyles were equal in length up to 4 months of age, later the lateral condyle was placed lower in level than the medial condyle, which gave oblique appearance to the distal extremity.

Key words: gross morphology, humerus, Kenguri sheep

Sheep as species is one of the major contributors to meat production in India. Kenguri breed of sheep, which is also known as Tenguri sheep, is native to the hilly tracts of the Koppal, Raichur and parts of Gulbarga districts in Karnataka. Kenguri sheep has larger body size and has dark brown color. Most of the animals have white color on its forehead and sometime on the legs and other parts of the body. Some animals have black belly. Males are horned while the females are generally polled and have high potential for quality mutton production.

The developmental morphology of animals shows considerable variation with respect to breed, age, sex, nutritional situation and environmental factors. Examination of long bones provides rather significant insights into intra-species as well as inter-species differences Kaymakci et al. (2001). There has been limited scientific work carried on the gross morphology of long bones in kenguri sheep hence, the present study was undertaken to elucidate the various features of humerus bone.

MATERIALS AND METHODS
The left and right humerus bones were collected from 24 animals of both sexes, immediately after the death from the Department of LPM Veterinary College, Bidar. During the collection of the material, age of the animals was noted and 24 animals were classified into four groups. Group-I included up to 6 months of age, Group-II included above 6 months to 2 years, Group-III included above 2 years to 4 years and Group-IV above 4 years to 6 years.

The collected bones were cleaned and macerated as per the standard anatomical technique (Tompsett, 1970 and Culling, 1974) methods. Following maceration, gross structures of the humerus of the Kenguri sheep were observed.

RESULTS AND DISCUSSION
Humerus of Kenguri sheep consisted of shaft, proximal and distal extremities (Fig.1). Shaft was slender, irregularly cylindrical and twisted in appearance. Shaft showed four surfaces viz., anterior, lateral, posterior and medial. Anterior surface was triangular wide above and narrow below and separated from lateral surface by crest of the humerus. A ridge like rough triangular deltoid tuberosity was differentiated from 1.5 months onwards (Fig.1) in group-I animals and well developed tuberosity was located at proximal end of the crest of humerus in group-III and group-IV animals (Fig.2 and 3). this was in contrary to findings of Sisson and Grossman (1953) which says the humerus of sheep was relatively longer and
deltoid tuberosity was near to the proximal end and is less prominent. But Nickel et al. (1986) reported that the shaft of the humerus in small ruminants was round or oval and deltoid tuberosity appeared as roughened prominence. In the present study it was found that Nutrient foramen was situated in distal third area between the lateral and posterior surfaces and lateral surface was smooth and spirally curved forming the musculo-spiral groove (Fig.1 and 2) in all groups of animals. Posterior surface was rounded, smooth and blends with lateral surface and concave in its proximal half, slightly convex in distal half in all groups of animals. Medial surface was nearly straight, rounded from side to side and blends with the anterior and posterior surfaces. Teres tubercle was noticed as small blunt area at the proximal to the middle on the medial surface in group-II animals and well appreciated in group-III and group-IV animals (Fig.3) but not distinct in group-I animals.

**Fig.1 Gross photograph of left humerus of 1.5 months Kenguri sheep -Lateral view showing**


**Fig.2 Gross photograph of proximal extremity of the left humerus of 3 years Kenguri sheep - Lateral view showing**


**Fig.3 Gross photograph of proximal extremity of right humerus of 5.5 years of Kenguri sheep- Medial view showing**


**Fig.4 Gross photograph of right humerus of 6 years Kenguri sheep-Distal extremity showing**


Proximal extremity consisted of head, neck, lateral and medial tuberosities. Both the tuberosities were divided into cranial and caudal parts and are separated by bicipital groove (Fig. 2 and 3) Similar observations were reported by Mohammad Hussain (2010) in goat humerus. Head was circular with convex articular surface and neck was the constricted area below the head.

The levels of lateral tuberosity and head were equal up to 1.5 months of age and not completely fused with the shaft in group-I animals (Fig.1) and group-II animals, later the lateral tuberosity raised from 1.9 cm to 2.3 cm above the head level and fused completely with the shaft in group-III and group-IV animals (Fig.2 and 3). Whereas, Nickel et al.
(1986) reported that greater tubercle raised above the head level in small ruminants and young cattle. The lateral tuberosity was undivided till 4 months of age in group-I animals and later it divided into cranial and caudal parts in other three groups of animals (Fig. 2 and 3), while the medial tuberosity was much smaller, undivided in group-I and group-II animals, later it divided into cranial and caudal parts.

Distal extremity consisted of lateral condyle, medial condyle, caudal deep olecranon fossa and shallow Coronoid fossa placed cranially above the condyles, which was in agreement with the report of Mohammad Hussain (2010) in goat humerus except that both the fossae were deep and wide. Medial condyle was much larger than the lateral and traversed by an anterio-posterior groove. Both the condyles were equal in length up to 4 months, later the lateral condyle was placed lower in level than the medial condyle, which gave oblique appearance to the distal extremity. Condyles were bounded on either side by lateral and medial epicondyles (Fig.4). Condyloid crest forms the posterior boundary of the musculo-spiral groove in all groups of animals (Fig.1).

CONCLUSION
This study has provided a means of differentiating humerus bone from the remains of Kenguri sheep. All the gross morphologic features of humerus bone indicating the postnatal morphological developmental changes with respect to the advancement of age of the animals.

REFERENCES
**Sub Acute Toxicity Study of *Fusarium Oxysporum* Culture Filtrate From Maize Stalks in Rats**

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**ABSTRACT**

The present study was undertaken to evaluate the toxicity of *Fusarium oxysporum* contaminated maize stalks in rats. Repeated dose 28-day oral toxicity study was conducted in four groups consisting of 6 rats of either sex. The fungal culture filtrate of the isolated fungi *F. oxysporum* was administered to induce the toxicity in rats in the dose range of 0.5, 1, 2 ml/100 g. There was significant change in serum biochemical parameters, when compared with control group rats. The gross and histopathological changes revealed hepatotoxicity, cardiotoxicity and nephrotoxicity of the fungal culture filtrate. It was concluded from the present study that, the mycotoxins found in the *Fusarium oxysporum* culture filtrate caused the toxicity in rats.

**KEY WORDS:** Maize stalks, toxicity, rat and *Fusarium oxysporum*.

*Fusarium oxysporum* is a filamentous, fuzzy or dusty looking fungus that occurs in many feedstuffs including roughages and concentrates. It can infect dairy cattle, especially during stressful periods when they are immuno suppressed, resulting in mycosis. It also produces poisonous substances called as mycotoxins that affect animals when they consume mycotoxin contaminated feeds (Whitlow and Hagler, 2004).

*Fusarium oxysporum* can grow and produce mycotoxins during pre harvest or post harvest processing or feeding. *Fusarium oxysporum* growth and mycotoxin production are related to plant stress caused by weather extremes, insect damage, inadequate storage practices and faulty feeding conditions. After harvest, temperature, water and insect activity are the major factors influencing mycotoxin production in feedstuffs (Coulombe, 1993).

Mycotoxins and their impact were under constant research, especially in agricultural and veterinary settings. The present study was undertaken to call the attention and evaluate the problem of mycotoxicosis produced by *Fusarium oxysporum*, that is pertinent in cattle and very often left undiagnosed because of the lack of characteristic clinical signs.

**MATERIALS AND METHOD**

**Isolation and Identification of *Fusarium oxysporum***: Fungal contaminated dry maize stalks were collected from Yellodu and other villages of Gudibande and Bagepalli taluks of Chikkaballapur district, where the cattle were exhibiting signs of toxicity after consuming fungal contaminated maize stalks. Symptoms observed were loss of body condition, ruminal atony, severe anorexia, recumbency, hyperapnoea, abdominal type of respiration, dry muzzle, nasal discharge and abortions in pregnant cows. The representative samples of the maize stalks which had caused above said clinical signs in the cattle were taken randomly for the isolation of the fungi.

Fungal contaminated maize stalk bits of 4-5 mm size were placed on the potato dextrose agar (PDA) medium in Petri-plates after washing in 4% sodium hypochlorite solution for 1-2 min. The inoculated plates were incubated at room temperature for 3-5 days. The fungal growth on the sample was evident in about 5 days. The different fungal colonies were transferred to another plate
containing PDA medium and incubated at 37°C for 5 days. Upon sporulation, after ruling out any contamination, the fungi colony characters were observed and pure culture of each fungus was raised by hyphal tip culture. The pure isolates of the fungi thus obtained were maintained on PDA slants and were periodically used for preparing mass culture. After the growth fungi for 3-4 days at room temperature on PDA slants and petri-plates they subsequently stored in a refrigerator and maintained by sub culturing once in a month.

The PDA slants were sent for identification of the fungi to Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh.

Sub acute toxicity study in laboratory animals:
Sub acute (Repeated 28 days) oral toxicity study was conducted to determine the toxicity of the fungal culture filtrate in rats. Apparently healthy young Wistar albino rats (procured from the Central Animal Facility, IISc, Bangalore) were used in the present study. They were of the age group of eight weeks and the body weight was about 200 ± 10 g. The animals were acclimatized to the experimental laboratory conditions for a week. They were maintained under hygienic laboratory conditions, providing standard laboratory animal feed (Amruth Feeds, Bangalore) and water ad libitum. The rats were grouped (n=6) and housed in polypropylene rat cages during the experiment. The approval of the Institutional Animal Ethics Committee was obtained prior to the start of the experiment (No-92/LPM/IAEC/2011, 17/09/2011).

Four groups of rats were made as follows:

<table>
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<th>Group</th>
<th>Dose (ml/100g)</th>
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<tr>
<td>Control</td>
<td>Potato Dextrose broth</td>
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<tr>
<td>Group IA</td>
<td>Fusarium oxysporum</td>
<td>0.5 (Low dose)</td>
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<tr>
<td>Group IB</td>
<td>Fusarium oxysporum</td>
<td>1 (Medium dose)</td>
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<tr>
<td>Group IC</td>
<td>Fusarium oxysporum</td>
<td>2 (High dose)</td>
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The animals were weighed individually at the beginning of the study and at weekly interval till day 28. The animals of each group were gavaged with the fungal culture filtrate of the respective group once daily. The feed intake was measured daily.

Serum biochemical (SGPT, SGOT, BUN and Creatinine) and haematological (RBC count, WBC count, Platelets count) parameters were estimated on day 0, 14, and 28 in sub acute oral toxicity study by using Semi Automatic Biochemical Analyzer- Microlab 300 (MERCK) and commercially available diagnostic kits (MERCK, Pvt, Ltd. India).

At the end of the study period, all the surviving animals were sacrificed and gross changes in the organs were recorded. Representative tissue samples of liver, kidney, spleen, heart, lung, brain, intestines and stomach were collected in 10 % neutral buffered formalin (NBF) for histopathological study.

Statistical analysis: Mean values and standard error of means were calculated and expressed as mean ± SEM. The data were analyzed by two-way ANOVA with Bonferroni post-tests using GraphPad Prism Trial version 5 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

RESULTS AND DISCUSSION

In the present study, F. oxysporum culture filtrate was administered to induce the toxicity in rats since it was appropriate method to administer the desired dose of the broth culture filtrate containing major secondary metabolites or mycotoxins (Bayman et al, 2002). The dose selected in rats was based on the maximum allowable dose to be administered to these animals as per the standard protocols (OECD, 2001).

Body weight, blood clotting time and serum biochemical parameters such as, Serum alanine aminotransferase (ALT), Serum aspartate aminotransferase (AST), Serum creatinine and Blood urea nitrogen (BUN) were estimated in all the animals on zero day, 14th and 28th day of experiment.

On day 28, body weight (g) of rats was significantly (P<0.001) decreased in group IA, IB, IC compared to control group (Table1). Blood
clotting time of rats was significantly increased (P<0.001) in group IC on day 14 and in group IB, IC (P<0.001) on day 28 (Table 2). On day 28, values of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) were significantly (P<0.001) increased in group IA, IB, IC as compared to control group. Values of serum creatinine and blood urea nitrogen (BUN) were significantly increased in group IC as compared to control and other treated groups on day 28 (Table 3). 

Table 1: The effect of *F. oxysporum* on body weight (g) in rats during repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Type of culture filtrate</th>
<th>0 Day</th>
<th>14th Day</th>
<th>28th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>211.67±6.00</td>
<td>226.67±4.94</td>
<td>248.50±10.47</td>
</tr>
<tr>
<td>Group IA</td>
<td>210.83±6.38</td>
<td>219.50±3.35</td>
<td>202.83±2.49***</td>
</tr>
<tr>
<td>Group IB</td>
<td>210.83±7.03</td>
<td>207.83±4.42**</td>
<td>194.67±3.16***</td>
</tr>
<tr>
<td>Group IC</td>
<td>211.00±5.45</td>
<td>199.00±4.10***</td>
<td>186.00±3.16***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6, *** P < 0.001, ** P < 0.01

The rats gavaged with fungal culture filtrates of *F. oxysporum* showed clinical signs of reduction in feed and water intake, diarrhoea and loss of body weight. Animals were weak and depressed, which might be attributed to reduced feed intake due to the toxic content in culture filtrate. Similar findings were also reported by Vinay *et al.* (2011). On day 28, values of alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) were significantly (P<0.001) increased in group IA, IB, IC as compared to control group. Serum creatinine and Blood urea nitrogen (BUN) were significantly (P<0.001) increased in group IC compared to control group values on day 28. Similar biochemical findings were recorded and attributed to the toxic principles of the culture filtrate which were fed to the rats by Shridhar and Narayana (2003), Veena (2006),Venkanna (2008) and Vinay *et al.* (2011).

Table 2: The effect of *F. oxysporum* on blood clotting time(s) in rats during repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Type of culture filtrate</th>
<th>0 Day</th>
<th>14th Day</th>
<th>28th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.00±2.82</td>
<td>65.83±3.19</td>
<td>65.67±2.52</td>
</tr>
<tr>
<td>Group IA</td>
<td>62.17±3.54</td>
<td>64.83±1.99</td>
<td>82.33±2.62***</td>
</tr>
<tr>
<td>Group IB</td>
<td>65.67±2.70</td>
<td>73.50±2.43</td>
<td>85.67±5.06***</td>
</tr>
<tr>
<td>Group IC</td>
<td>64.83±2.13</td>
<td>85.17±1.19***</td>
<td>91.67±5.17***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6, *** P < 0.001, ** P < 0.01

Table 3: The effect of *F. oxysporum* on biochemical parameters in rats during repeated dose 28 day oral toxicity study.

<table>
<thead>
<tr>
<th>Type of culture filtrate</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.50±1.48</td>
<td>36.83±1.89</td>
<td>37.50±1.23</td>
<td>34.87±3.84</td>
</tr>
<tr>
<td>Group IA</td>
<td>36.67±1.20</td>
<td>46.00±1.75</td>
<td>60.17±1.66***</td>
<td>33.45±2.38</td>
</tr>
<tr>
<td>Group IB</td>
<td>36.33±2.27</td>
<td>45.00±3.28</td>
<td>61.00±3.91***</td>
<td>33.80±2.34</td>
</tr>
<tr>
<td>Group IC</td>
<td>35.00±2.77</td>
<td>59.83±3.081***</td>
<td>79.50±1.94***</td>
<td>32.53±4.38</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 6, *** P < 0.001, ** P < 0.01, * P < 0.05
Microscopically liver showed severe congestion of vessel, degeneration and necrosis of hepatocytes with infiltration of few inflammatory cells (Fig 1). Histologically, spleen revealed presence of haemorrhage, lymphocytolysis and depletion of cells. Intestine showed increased goblet cell activity, degeneration and necrosis of villus epithelial cells with focal areas of inflammatory cell infiltration.

Fig 1. Section of the liver from rat administered with F. oxysporum culture filtrate showing severe congestion of vessel, degeneration and necrosis of hepatocytes with infiltration of few inflammatory cells H&E x100

Microscopically, heart revealed edema, congestion and haemorrhage with presence of red blood cells in between muscle fibers. Wavy appearance of cardiac muscle fibers was seen (Fig 2). Heart damage was related to elevated concentrations of AST (Maxine and Benjamin, 2001) and it was evident in F. oxysporum treated animals. Beauvericin has a negative inotropic effect (decrease in cardiac contraction strength) and a negative chronotropic effect (decrease in the frequency of cardiac spontaneous beating activity) in isolated guinea pig hearts as per the findings of Lemmens-Gruber et al (2000). Thus, the heart damage and also elevated concentrations of AST might be traced to the toxic nature of beauvericin. The beauvericin production by the F. oxysporum fungal spp borrows the support from the study conducted by Logrieco et al (1998) who stated the abilities of different Fusarium isolates to produce beauvericin were determined by analyzing maize kernel fungal cultures.

Fig 2. Section of the heart from rat administered with F. oxysporum culture filtrate showing edema, congestion and haemorrhage with presence of red blood cells in between cardiac muscle fibers H&E x100.

Fig 3. Section of the kidney from rat administered with F. oxysporum culture filtrate showing, degeneration and necrosis of tubular epithelium H&E x100.

The microscopic lesions in the kidney proved mild to moderate congestion and haemorrhage, degeneration and necrosis of tubular epithelium (Fig 3). Increased creatinine and blood urea nitrogen concentration were also suggestive of nephrotoxic nature of the mycotoxins produced by Fusarium spp. Moderate to severe nephrosis and focal tubular necrosis in the renal cortex were reported by Kriek et al (1977) in rats fed with the culture of Fusarium moniliforme var. subglutinans isolated from maize. The gross and histopathological changes revealed hepatotoxicity, cardiotoxicity and nephrotoxicity in rats of both sexes in varying degrees and in all the treated groups.
CONCLUSION
It is concluded from the observations made in the present study that F. oxysporum fungal culture filtrate had shown toxicity in rats, attributed to possible presence of mycotoxins.

ACKNOWLEDGEMENT
The authors thank the Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology (IMTECH) Chandigarh for identification of fungi from maize stalks.

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Histochemical Studies on Forestomach in Blackbuck (*Antelope cervicapra*)

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ABSTRACT

The present histochemical study was conducted on forestomach of six black bucks (*Antelope cervicapra*). The aim of this study is to clarify the histochemical role of the mucosa in each of the three compartments of the forestomach in the blackbucks. A strong positive PAS reaction was observed in the stratum corneum, moderate in basement membrane, tunica muscularis and endothelium of blood vessels while negative (-) PAS reaction was found in lamina propria, tunica submucosa and tunica serosa of forestomach. Glycogen was weakly observed in the lamina epithelium except stratum corneum, and moderately in tunica muscularis of forestomach and lamina muscularis mucosae of reticulum and omasum. A negative reaction for Alcian blue reactions at pH 2.5 and pH 1.0 was observed in forestomach.

Fat globules were found moderately in the stratum granulosum and tunica muscularis while weakly observed in stratum spinosum and tunica serosa. The stratum corneum showed strong reaction for keratin of forestomach. The moderate reaction for iron was found in stratum corneum of rumen while weakly observed in reticulum and omasum. The present results provide additional knowledge to further understand the histophysiological specialization of the different chambers of the ruminant forestomach.

Keywords: Forestomach, Histochemistry, Black buck.

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Keywords: Forestomach, Histochemistry, Black buck.

MATERIALS AND METHODS

Forestomachs of six black bucks were collected from various zoo/national parks of Karnataka state during postmortem. Tissue pieces of 3-5mm length were cut from forestomach and were fixed in 10% neutral buffered formalin. The tissue pieces were routinely processed and embedded in paraffin wax (Luna, 1968). The sections of 5-7µm thickness were used for different staining methods as per Table 1.

Table 1: Staining methods used for histochemical studies

| 1 | Neutral mucopoly saccharides | McManus’s method (PAS) | Singh and Sulochana, 1996 |
| 2 | Glycogen | Best carmine method | Singh and Sulochana, 1996 |
| 3 | Neutral and acid mucopoly saccharides | PAS-alcian blue at pH 2.5 and pH 1.0 | Luna, 1968 |
| 4 | Fat | Oil red O method | Singh and Sulochana, 1996 |
| 5 | Iron | Mallory’s method | Luna, 1968 |
| 6 | Keratin | Ayoub shklar Method Holland’s Trichrome method | Luna, 1968 Gray, 1954 |
| 7 | Proteins | Mercury-bromophenol blue method | Pearse, 1968 |

*Assistant Professor, Dept. of Veterinary Anatomy, Veterinary college, Shivamogga E-mail: drlshree@gmail.com
The photographs were taken with a Nikon digital camera (Nikon, MH 611 COOLPIX P5100, and Japan) attached to CH 20i Olympus trinocular microscope. Depending on the intensity of color, reactions for the different stains were categorized at different layers of forestomach as strong (+++), moderate (++), weak (+), and negative (-).

RESULTS & DISCUSSION

The histochemical observations were recorded qualitatively as shown in table 2. In the present study, a strong positive (+++) PAS reaction was found in the stratum corneum, moderate (++) in basement membrane, tunica muscularis and endothelium of blood vessels of forestomach of blackbuck (Fig1). Basu et al. (1957) also reported PAS positive reaction in the stratum corneum of adult caprine reticulum and the papillary connective tissue of the omasal laminae. However, the reactions for PAS and acidic mucopolysaccharides were negative in the rumen epithelium in sheep (Poonia et al., 2011) and in cattle, sheep and goat (Habel, 1963).

Table 2: Histochemical observations of the forestomach of black buck.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Species/Parameters</th>
<th>Tunica mucosa</th>
<th>LE</th>
<th>LP</th>
<th>SCT/LMm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SC</td>
<td>SG</td>
<td>SS</td>
<td>SB</td>
</tr>
<tr>
<td>1</td>
<td>Rumen: Carbohydrates</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Keratin</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Reticulum: Carbohydrates</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Keratin</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Omasum: Carbohydrates</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Keratin</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Strong: ++++, Moderate: ++, Weak: +, Negative: -


The present study revealed the existence of an exaggerated amount of carbohydrates the mucosa of the various investigated regions of the forestomach. More over, the distribution of mucopolysachrides was slightly different, but in general agreement with some other vertebrate species such as cattle, sheep, and goat. This could conclude that the pattern of mucosubstances distribution throughout the forestomach is not correlated with the different orders of vertebrate species and appeared to be unrelated to diet. A negative (-) PAS reaction was found in lamina propria, tunica submucosa and tunica serosa of the forestomach wall which corroborated with the report in sheep (Poonia et al., 2011) and in cattle, sheep and goat (Habel, 1963).

Glycogen was weakly observed in the lamina epithelium except stratum corneum, and moderately (+++) in tunica muscularis of forestomach (Fig 2) and lamina muscularis mucosae of reticulum and omasum as reported in ruminant forestomach (Habel, 1963), in sheep...
The presence of low glycogen content in the different regions of forestomach of blackbuck is an expected observation, since it does not store these granules, but it is used as a source of energy.

In the present study, negative reactions for Alcian blue reactions at pH 2.5 (Fig. 3) and pH 1.0 was observed in the forestomach of blackbucks as also reported during prenatal development of sheep rumen (Franco et al., 1992). However, stratum corneum of rumen mucosa contained neutral, sulfated acidic and non-sulfated acidic mucosubstances in Hereford cow (Lavker et al., 1969) and in buffaloe (Taluja and Saigal, 1989a). The presence of neutral mucopolysaccharides in the deeper epithelial layers could be directly related to the gradual adaptation of the ruminal mucosa for protection against chemicals in postnatal life, where it acted as a buffer for neutralization of the acid compounds produced during ruminal fermentation (Dellmann and Brown, 1981).

In the present study, weakly positive (+) reaction for protein was found in the tunica mucosa except lamina propria and moderate (++) in tunica muscularis of forestomach of animals studied. No such observations were recorded in the literature reviewed.

Fat globules were found moderately (++) in the stratum granulosum and tunica muscularis (Fig. 4) while weak in stratum spinosum and tunica serosa as reported in ruminant’s forestomach postnatally (Habel, 1959) and epithelium of forestomachs of goat (Shiino, 1962). On the contrary, Dobson et al., (1956) and Basu et al., (1957) observed no lipid in ovine and caprine forestomach epithelium. Ramkrishna and Tiwari (1979) did not observe fat in the omasum of goat foetuses.
The stratum corneum showed strong reaction for keratin of forestomach of animals studied (Fig. 5, 6 & 7) and similar results were also reported in buffaloes (Taluja and Saigal, 1987), in ruminal epithelium of sheep (Poonia et al., 2011) and in rumen wall of red deer (Zitare et al., 2013). Lavker et al. (1969) demonstrated that ruminal mucosa of Hereford cow had the ability to function in the absorption of products of rumen digestion and in the protection of the rumen from the digesta mass by a partially keratinized mucus-covered outer layer. Cells of the stratum spinosum and stratum granulosum could simultaneously produce keratohyalin granules needed for keratinization and function during mucus production. The physical protection against potentially sharp fibers consumed by an animal was offered by the well pronounced keratinized epithelium. The efficiency of nutrient transport across the epithelium also depended to a large extent on the integrity and degree of keratinization of the stratum corneum (Dirksen and Garry, 1987).

Moderate reaction for iron was found in stratum corneum of rumen (Fig 8) while weakly observed in reticulum and omasum of the present study. Taluja and Saigal (1989b); Mahesh (2008) and Poonia et al. (2011) also reported moderated reaction in buffalo, goat and sheep forestomach respectively. Further, Brownlee and Elliot (1961) reported that the darkened epithelium of forestomach of cattle was due to the presence of iron in the keratinized layer while still attached or after shedding. The dark brown colour of papillae in sheep rumen appeared to be a combination of high supply of iron, keratinized tissue, resulted from rapid growth and limited abrasion and an acidic pH (Nockels et al., 1966). Taluja and Saigal (1989b) stated that the presence of iron in buffalo forestomach could be due to the degree of microbial activity, high energy ration and pigmentation. Basal cells were relatively unreactive to all histochemical tests, indicating an undifferentiated cell population probably involved in metabolism and assimilation of absorbed microbial end products (Pelagalli, 2007).
In conclusion, the histochemical characteristics of the forestomach seem to be suitable for helping to regulate absorption and/or secretion. The polysaccharides like glycogen in the forestomach are likely to affect the movement of molecules within the mucosa and to resist local mechanical changes besides the presence of pronounced keratinized epithelium. In addition to the morphological characteristics of the surface structure, the subepithelial capillary networks and the subepithelial connective tissue (Yamamoto et al., 1991), the polysaccharides content of the forestomach is one of its distinctive characteristics, and may be related to the function of the forestomach in processes such as absorption and/or secretion.

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REFERENCES


Antitumor Activity of *Glycyrrhiza glabra* against Ehrlich Ascites Carcinoma (EAC) in Swiss Albino Mice

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**ABSTRACT**

Antitumor activity of hydroalcoholic extract (100 mg/kg) of *Glycyrrhiza glabra* was evaluated against Ehrlich Ascites Carcinoma (EAC) tumor in mice. After 24 h of tumor inoculation, the extract was administered daily for 14 days. After administration of the last dose followed by 18 h fasting, mice were sacrificed for observation of antitumor activity. The effect of *Glycyrrhiza glabra* on the growth of transplantable ascites tumor, body weight of EAC bearing hosts and simultaneous alterations in the hematological profile, serum (ALT, AST, LDH, ALP and glucose) and hepatic biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes) were estimated. The *Glycyrrhiza glabra* showed decrease in abdominal circumference and body weight of EAC tumor bearing mice. Hematological profile reverted towards normal levels in extract treated mice. Treatment with *Glycyrrhiza glabra* restored the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (SOD, CAT and GPx). The hydroalcoholic extract of *Glycyrrhiza glabra* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

**Key Words:** *Glycyrrhiza glabra*, Ehrlich ascites carcinoma, Mice.

Cancer is the most progressive and devastating disease posing a threat of mortality to the entire world despite significant advances in medical technology for its diagnosis and treatment (Graham et al., 2000). The area of anticancer therapeutics has gained enormous attraction from scientists all over the world and cancer is conventionally treated by radiotherapy, surgery, chemotherapy, immunotherapy, molecular targeting or a combination of these methods. Recently there is an increasing interest in the use of complementary and alternative medicine (CAM) for the palliative care of cancer patients worldwide. An extremely promising strategy in CAM for cancer prevention today is chemoprevention, which involves the use of synthetic or natural agents (alone or in combination) to block the development of cancer in humans as well as animals (Leung et al., 2004).

The licorice (*Athimadthuram*, Tamil) shrub is a member of the pea family (Fabaceae). The water-soluble, biologically active complex of licorice is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances (Obolentseva et al., 1999). The flavonoid constituents mainly include flavones, flavonals, isoflavones, chalcones, bihydroflavones and bihydrocalcones which have antioxidant, antibacterial and anti-inflammatory activities (Vaya et al., 1997).

Plants contain abundant quantities active substances which have been shown to be associated with a lower risk of cancers at almost every site (Steinmetz and Potter, 1991). Efforts, therefore, are being made to identify naturally occurring anticarcinogens which would prevent, slow and/or reverse the cancer induction and its subsequent development (Chuang et al., 2000). Keeping the above points in view, the present study was undertaken to evaluate the antitumor activity of hydroalcoholic extract of *Glycyrrhiza glabra* against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.
MATERIALS AND METHODS

Hydroalcoholic extract of *Glycyrrhiza glabra* was obtained from Natural Remedies, Bangalore. 5-fluorouracil (5-FU) was purchased from Himedia, Mumbai. All reagents and chemicals used were of analytical grade. Ehrlich Ascites Carcinoma (EAC) cell line was obtained from National Centre for Cell Sciences, Pune, India. Studies were carried out using Swiss albino mice of both sex weighing 20±2 g which were obtained from the Department of Laboratory Animals Medicine, Madhavaram, Chennai. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee.

Swiss albino mice were divided into 4 groups (n=6). All the groups were injected with EAC cells (0.2 ml of 2x10⁶ cells/mouse) intraperitoneally except the normal group. Drug treatments (extract or 5-FU dissolved in phosphate buffered saline, PBS) were continued for 14 days after inoculation of tumor cells on day ‘0’. From the first day, 5-FU (25mg/kg) and *Glycyrrhiza glabra* extract (100mg/kg) were administered intraperitoneally for 14 days to groups 3 and 4 respectively. After the administration of last dose followed by 18 h fasting, all the mice were sacrificed for the study of antitumor activity, serum biochemical, hematological and hepatic lipid peroxidation and antioxidant parameters. Antitumor effect of *Glycyrrhiza glabra* was assessed by observation of changes with respect to body weight and abdominal circumference.

Haematological studies: Red blood cell (RBC), white blood cell (WBC) counts and haemoglobin levels were determined by routine clinical laboratory techniques. Differential leukocyte count (DLC) was carried out from Leishman stained blood smears (Dacie and Lewis, 1958).

Serum biochemical parameters: Serum glucose was estimated in semiauto analyzer system by glucose oxidase/peroxidase method by using standard kit (Agappe diagnostics). Serum enzymes, ALT, AST, LDH and ALP were estimated in semi auto analyzer system by using standard kits.

Hepatic biochemical parameters: Immediately after sacrificing the animals, the liver was isolated and washed in ice cold normal saline to remove blood and it was blotted dry and stored at -20°C for further analysis. Liver was crushed in tissue homogenizer (Heidolph, Germany) and 10% w/v liver homogenate was prepared in 0.05 M phosphate buffer (pH 7.4) and was used for the estimation of lipid peroxidation (Yagi, 1976) and reduced glutathione (Moron et al., 1979). The rest of the homogenate was centrifuged at 15,000g for one hour at 4°C and the supernatant thus obtained was used for the estimation of superoxide dismutase (Marklund and Marklund, 1974), catalase (Caliborne, 1985) and glutathione peroxidase (Rotruck et al., 1973).

Histopathological examination: A piece of liver, spleen and abdominal muscle samples were fixed in 10% formalin for histopathological examination. The thin sections were cut and then stained by haematoxylin and eosin and observed under light microscope.

Statistical analysis: The results were expressed as mean ± S.E. All analyses were carried out using the SPSS statistical program. The effect of treatments was determined by analyzing the data using one way-ANOVA followed by Duncan’s multiple comparison test.

RESULTS AND DISCUSSION

Effect of *Glycyrrhiza glabra* on body weight of EAC bearing mice: There was a significant (P<0.01) increase in the body weight of EAC-bearing mice from 8th day onwards during a growth period of 14 days as compared to normal group and treatment with *Glycyrrhiza glabra* significantly decreased the body weight of tumor bearing mice from 10th day onwards (Fig.1).
Effect of \textit{Glycyrrhiza glabra} on abdominal circumference of EAC bearing mice: There was a significant (P<0.05) increase in the abdominal circumference of EAC-bearing mice from 6\textsuperscript{th} day onwards during a growth period of 14 days as compared to normal group and treatment with \textit{Glycyrrhiza glabra} significantly decreased the abdominal circumference from 8\textsuperscript{th} day onwards (Fig.2).

Effect of \textit{Glycyrrhiza glabra} on serum glucose level of EAC bearing mice: Inoculation of EAC to mice significantly (P<0.05) decreased the serum glucose level in the EAC group (53.50 \pm 4.97mg %) when compared to normal group (84.5 \pm 2.85mg %) and treatment with \textit{Glycyrrhiza glabra} significantly increased the glucose level as compared to EAC group but not to the level of normal group (Table-1).

Effect of \textit{Glycyrrhiza glabra} on serum biochemical enzymes of EAC bearing mice: There was a significant (P<0.01) increase in serum ALT, AST, LDH and ALP (45.54 \pm 2.75 IU/L), (66.84 \pm 3.55 IU/L), (229.59 \pm 12.64 IU/L) and (45.67 \pm 3.28 IU/L) activity of EAC group as compared to normal group (22.8 \pm 0.87 IU/L), (43.8 \pm 1.51 IU/L), (148.20 \pm 2.73 IU/L) and (21.28 \pm 2.26 IU/L) respectively and treatment with \textit{Glycyrrhiza glabra} significantly decreased the enzyme activity as compared to EAC group (Table-2).

Haematological parameters: Haemoglobin content and RBC count were significantly (P<0.05) decreased while WBC count was significantly (P<0.01) increased in the EAC group as compared to the normal group. Treatment with \textit{Glycyrrhiza glabra} significantly restored the RBC and haemoglobin levels towards the normal.
In the differential count of WBC, the neutrophil count increased, while the lymphocyte count decreased in the EAC group as compared to the normal group. Treatment with *Glycyrrhiza glabra* significantly restored the altered haematological parameters towards the normal values (Table-3).

### Table-3 Effect of drugs on hematological parameters of EAC bearing mice (Mean ± SE, n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC* (x 10^6/µl)</th>
<th>WBC** (x 10^3/µl)</th>
<th>Hb* (g %)</th>
<th>Monocyte* (%)</th>
<th>Neutrophil* (%)</th>
<th>Lymphocyte* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>4.2± 0.2</td>
<td>7.3± 0.2</td>
<td>13.8± 0.3</td>
<td>1.7± 0.3</td>
<td>18.4± 0.4</td>
<td>79.9± 0.3</td>
</tr>
<tr>
<td>EAC</td>
<td>2.9± 0.2</td>
<td>21.0± 0.6</td>
<td>8.2± 0.2</td>
<td>1.1± 0.2</td>
<td>63.8± 2.6</td>
<td>35.0± 2.6</td>
</tr>
<tr>
<td>FU</td>
<td>3.4± 0.2</td>
<td>7.0± 0.2</td>
<td>9.9± 0.1</td>
<td>1.7± 0.4</td>
<td>40.5± 1.9</td>
<td>57.8± 1.8</td>
</tr>
<tr>
<td>GG</td>
<td>4.1± 0.1</td>
<td>8.8± 0.3</td>
<td>11.7± 0.3</td>
<td>1.5± 0.4</td>
<td>44.7± 1.2</td>
<td>53.8± 1.3</td>
</tr>
</tbody>
</table>

Means bearing different superscripts between the drug treatments differ significantly *(P<0.05), ** (P<0.01).

#### Effect of drugs on lipid peroxidation in the liver tissue of EAC bearing mice:*
The level of lipid peroxidation in liver tissue was significantly (P<0.05) increased in the EAC group (515.26 ± 14.7) as compared to the normal group (95.65 ± 3.02). After treatment with *Glycyrrhiza glabra*, the level of lipid peroxidation was significantly (P<0.05) reduced in comparison to the EAC group (Table-4).

#### Effect of drugs on liver antioxidant enzymes (SOD, CAT and GPx):*
There was a significant (P<0.05) decrease in SOD, CAT and GPx activities in EAC group (2.80 ± 0.31, 0.19 ± 0.02 and 4.23 ± 0.32) when compared to normal group (5.27±0.27, 4.67±0.53 and 6.81±0.27) indicating their antimitastatic activity which could be comparable to that of fluorouracil, the standard drug used for comparison.

#### Histopathological examination: *
The metastasis and invasion of neoplastic cells into liver, spleen and abdominal muscle were observed in EAC-bearing mice indicating the metastatic property of tumor. However, treatment with *Glycyrrhiza glabra* exerted a significant inhibition of metastasis.

### Table – 4 Effect of drugs on lipid peroxidation, glutathione content and antioxidant enzymes (SOD, CAT and GPx) in the liver of EAC bearing mice (Mean ± SE, n=6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LPO</th>
<th>GSH</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>95.65± 3.02</td>
<td>14.67± 0.77</td>
<td>5.27± 0.27</td>
<td>0.46± 0.02</td>
<td>7.87± 0.35</td>
</tr>
<tr>
<td>TC</td>
<td>515.26± 14.73</td>
<td>7.57± 0.73</td>
<td>2.80± 0.31</td>
<td>0.19± 0.02</td>
<td>4.23± 0.32</td>
</tr>
<tr>
<td>FU</td>
<td>210.73± 17.12</td>
<td>13.52± 0.75</td>
<td>4.67± 0.53</td>
<td>0.35± 0.04</td>
<td>6.81± 0.27</td>
</tr>
<tr>
<td>GG</td>
<td>183.89± 21.33</td>
<td>18.61± 1.27</td>
<td>3.92± 0.26</td>
<td>0.32± 0.03</td>
<td>6.09± 0.36</td>
</tr>
</tbody>
</table>

Means bearing different superscripts between the drug treatments differ significantly P<0.05)

LPO - Lipid peroxidation -µM of MDA/g tissue
GSH - Reduced glutathione -mg of reduced GSH/g tissue
SOD - Superoxide dismutase -Units/mg protein
CAT - Catalase -µM of H_{2}O_{2} utilized/min/mg protein
GPx - Glutathione peroxidase -µM of GSH utilized/min/mg protein

#### Effect of drugs on glutathione content of the liver:*
Inoculation of EAC significantly (P<0.05) decreased the GSH content in the EAC group (7.57 ± 0.73) when compared with the normal group (14.67 ± 0.77). The decreased amount was restored towards the normal levels in *Glycyrrhiza glabra* treated group (Table-4).
the dose of 100 mg/kg inhibited the increase in body weight and abdominal circumference and also brought back the serum biochemical and hematological parameters towards normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD, CAT and GPx in tumor bearing mice to near normal levels. In addition, the extract also exhibited antimetastatic property as evidenced by the histopathological examination of liver, spleen and abdominal muscle (Plate-1-6).

**PLATE 1: TC** – Liver showing multifocal infiltration of neoplastic cells in sinusoids

**PLATE 2: GG** – Liver showing normal architecture with no signs of infiltration

**PLATE 3: TC** – Spleen showing infiltration of multinucleated cells

**PLATE 4: GG** – Spleen showing normal red and white pulp areas

**PLATE 5: TC** – Abdominal muscle showing invasion of neoplastic cells into the muscle

**PLATE 6: GG** – Abdominal muscle showing moderate infiltration of neoplastic cells

Perturbation of haematological parameters in tumor bearing animals is partly responsible for the toxic effects produced in them. In addition, myelosuppression in cancer chemotherapy is a common phenomenon which is responsible for poor prognosis (Donehower, 1990). In the present findings, treatment with *Glycyrrhiza glabra* brought back the hemoglobin content, RBC and...
WBC cell count near to normal values. This indicates that *Glycyrrhiza glabra* possessed protective action on the haematopoietic system.

Several studies have demonstrated that tumor-bearing animals could experience a systemic change of antioxidant enzymes in organs distant from the tumor (Sardar *et al*., 1993). Similar finding was observed in the present study where in tumor group showed a significant increase in the level of lipid peroxidation in liver tissue when compared to the normal group which might be due to the excessive generation of H$_2$O$_2$ (by peritoneal cells) that has been transferred to liver for detoxification along with the sequestration of antioxidants by tumor cells (Devasena *et al*., 2002).

Sinclair *et al*., (1990) reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially in the liver, even when the site of the tumor does not interfere directly with organ function. In the present investigation, a significant decrease in the liver GSH content was noticed in EAC-bearing animals which could be attributed to the increased utilization of GSH for the detoxification of large amount of lipid peroxides produced in the liver of tumor bearing animals (Devasena *et al*., 2002).

SOD and CAT are considered as the primary antioxidant enzymes, since they are involved in the direct elimination of active oxygen species (Kinnula and Crapo, 2004). It has been reported that a decrease in SOD activity in EAC-bearing mice may be due to loss of Mn$^{2+}$-containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver (Sun *et al*., 1989). The inhibition of SOD and CAT activities as a result of tumor growth were also reported (Rushmore and Picket, 1993).

However, treatment with *Glycyrrhiza glabra* restored the altered heptic biochemical parameters to near normal indicating its antioxidant activity which would also explain the possible reason for its anticancer activity against EAC-bearing mice.

**REFERENCES**


Association of Ovine Insulin-Like Factor 3 (Insl3) gene polymorphism with Cryptorchidism in Mandya and Hassan Sheep

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ABSTRACT
The present study was undertaken to study the polymorphism in INSL3 gene and to find its association with cryptorchidism in Mandya and Hassan breed of sheep. Genomic DNA was extracted from sixty cryptorchid and eighty normal unrelated sheep. Three sets of primers were designed to amplify the promoter, exon1, intron1 and exon2 regions of Ovine INSL3 gene. The amplicons were subjected to SSCP and PCR-RFLP to study the genetic polymorphism. The steroidogenic factor sequence “CCAAGGCTC” was observed at 65 bp upstream in the promoter region and first 6 nucleotides was conserved across species. A novel silent mutation at nucleotide 54 G>A position of the exon1 was observed and found not associated with cryptorchidism. Contrary to the earlier report, Ovine INSL3 intron1/ Cac8I mutation(2489C>T) was not associated with cryptorchidism in the population studied. RFLP-PstI (2714C>A) polymorphism was observed in exon2 of Ovine INSL3 and was also not associated with cryptorchidism. The population studied was in Hardy Weinberg equilibrium for all genotypes indicating no selection pressure on the population with respect to INSL3.

Key words: Cryptorchidism, INSL3, Polymorphism, PCR-RFLP, SSCP, Sheep

Cryp orchidism is the failure of one or both testes to be positioned in the scrotum at the time normal for a species. High prevalence of 18 per cent cryptorchidism has been reported in Mandya sheep breed (Naveen kumar et al., 2014). Further, cryptorchidism is said to be the major cause for decline in Mandya sheep population (Bhatia and Arora, 2005). The studies have elucidated that the major regulators of testicular descent are the Leydig cell–derived hormones insulin-like factor 3 (INSL3) and testosterone involved, respectively, in the transabdominal and inguin oscrotal phase of testicular descent. The INSL3 protein is a testicular hormone produced by leydig cells, belonging to relaxin family of protein. The role of INSL3 in testicular descent is mainly related to its effect on gubernaculum differentiation. The homozygous INSL3 knockout mice exhibited complete intra abdominal cryptorchidism confirming its role during the transabdominal phase of the testicular descent. The INSL3 gene structure comprising of two exons and one intron is highly conserved among mammals, including sheep. Several cryptorchid causative mutations have been reported in promoter and exons of human INSL3. Williams et al (2007) identified three single nucleotide polymorphisms (SNP) in Sheep INSL3 gene of which SNP 2489C>T in intron 1 was reported to be significantly associated with cryptorchidism. The present study was undertaken to study the polymorphism in Ovine INSL3 gene and its possible association with cryptorchidism in Mandya and Hassan sheep.

MATERIALS AND METHODS
The blood samples of 35 bilateral cryptorchids and 47 normal unrelated males of Mandya sheep were collected from Livestock Research Information Centre (Mandya Sheep), Nagamangala and farmer’s flock of Mallavalli taluk, Mandya district. Similarly the blood samples of 25 bilateral cryptorchids and 33 normal males of Hassan sheep were collected from farmers flock in Hassan District.

The genomic DNA extracted was carried out adopting the high salt method as described by Miller et al. (1988). Three sets of primers were designed based on the published ovine INSL3 gene

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3Professor, All India Coordinated Research Project on poultry meat, Veterinary college, Bengaluru, India
sequence (Genbank no. DQ473569) using CLCBIO Primer software. The three primers INSL3-I, II, III covered part of promoter sequence and exon1 of INSL3 gene, the intron region covering the SNP reported to be associated with cryptorchidism in sheep, and the part of intron1 and exon 2 of Ovine INSL3 respectively. The sequence details of different primer sets are presented in Table.1.

Table. 1. Details of INSL3 primers used study:

<table>
<thead>
<tr>
<th>Fragment Name</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Tm values</th>
<th>Amplicon size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSL3-I</td>
<td>F</td>
<td>CTATCAGAGTTCACGAGGC</td>
<td>62.46</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AATCCGCTTGACACACCTG</td>
<td>62.67</td>
<td></td>
</tr>
<tr>
<td>INSL3-II</td>
<td>F</td>
<td>GCCACCTTACTGTCTTCTG</td>
<td>60.52</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GAGATGTGTCTTCCACGC</td>
<td>60.88</td>
<td></td>
</tr>
<tr>
<td>INSL3-III</td>
<td>F</td>
<td>TGGGCGCTGTATAGAGGCACTT</td>
<td>67.05</td>
<td>413</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ATCAGGAGGACCAGACTGGACT</td>
<td>66.90</td>
<td></td>
</tr>
</tbody>
</table>

The PCR amplification of fragments of Ovine INSL3 was performed in 25ul reaction. For amplification of ISNL3-II and INSL3-III 2.5% formamide was used in PCR, where 5% glycerol for INSL3-I amplification in addition to the normal ingredients of 2.5 ul of PCR buffer, 100ng of genomic DNA, 1.5 M MgCl₂, 200 μM of each dNTP and 20pm of each forward and reverse primers. The annealing temperature of 62°C and 61°C was used for INSL3-II and III respectively. Touchdown PCR with annealing temperature of 70°C and increment of - 0.5 °C / cycle for 24 cycles was used for INSL3-I. The amplified PCR products were subjected to Single Stranded Conformation Polymorphism and Restriction Fragment Length Polymorphism

The 10 μl of INSL3-II and INSL3-III PCR products of all the samples were subjected to digestion with Cac8I and Pst I restriction enzymes respectively to study the SNP’s previously reported in sheep. The restriction reaction was carried out as per enzyme manufacturer’s protocol. The restriction enzyme digested PCR products were analyzed on 2.4 per cent agarose gel. Single strand conformation polymorphisms of INSL3-I PCR amplicons were analyzed through 12-15 % polyacrlamide gels run in 10X TBE buffer at160 Volts for 16 h followed by silver staining of gels as per Basam et al. (1991).

The PCR amplified gene products showing different banding pattern in RFLP and SSCP were custom sequenced by single pass sequencing method using primers used for amplification of different products. The custom sequencing was done at Chromus Bio, and Amnion inc, Bengaluru.

Statistical analysis was done using SAS 9.2 (2009) software. The genotypes were detected by observing the SSCP / RFLP patterns of each sample in the gels. The gene and genotype frequencies of different fragments were estimated. The expected genotype frequencies were estimated by standard procedure (Falconer and Mackey, 1996) and the Hardy Weinberg equilibrium of the genotypes were tested by chi-square goodness of fit test. The significance of the differences in genotype frequencies between phenotypes (cryptorchid v/s. normal; horned v/s. polled) and breeds (Mandya v/s. Hassan) were analyzed using Wald chi-square analysis of SAS.

RESULTS AND DISCUSSION

Three regions of the ovine INSL3 gene were studied for polymorphism and its possible association with cryptorchidism in sheep. The sequence of ovine pro INSL3 was aligned with Human INSL3 (Fig. 1). The eight substitution mutations reported as associated with cryptorchidism in humans were analyzed. Substitution mutation, one each in signal peptide and B chain regions, Five in C chain region and 1
in A chain region, were observed. The position of the substitution mutation $P\rightarrow S$ in B chain, $N\rightarrow K$ in A chain and three ($W\rightarrow R$, $P\rightarrow L$ and $R\rightarrow H$) in C chain also have conserved amino acids in ovine sequence, hence were considered as probable positions to explore causative mutations involved in cryptorchidism in sheep. One substitution ($V\rightarrow M$) in signal peptide and two substitution ($R\rightarrow X$ and $R\rightarrow C$) in C chain have non conserved amino acids in ovine sequence.

The PCR SSCP analysis revealed a novel mutation at nucleotide 54 $G\rightarrow A$ position of the exon1. The mutation was silent and existed in heterozygous with wild type $G$. The PCR SSCP analysis of the INSL3-I fragment resulted in two types of banding pattern i.e., pattern $GG$ and pattern $GA$. The population studied was in Hardy Weinberg equilibrium. The chi-square test revealed no significant difference between Mandy and Hassan breeds for the SSCP/ INSL3-I genotypes. Similarly there was no significant difference

<table>
<thead>
<tr>
<th>Points of substitution mutations associated with cryptorchidism in Human INLS3 and their relative position in Sheep INSL3.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M\bullet$</td>
</tr>
<tr>
<td>$S$</td>
</tr>
<tr>
<td>$B$ Chain</td>
</tr>
<tr>
<td>$R$</td>
</tr>
<tr>
<td>$C$ Chain</td>
</tr>
<tr>
<td>$L$</td>
</tr>
<tr>
<td>$D-S-N-L-T-L-G-P-G-L-Q-P-L-P-Q-T-S-H-H-R-H-H-R-$</td>
</tr>
<tr>
<td>$S-G-D-P-V-L-V-L-A-P-Q-P-L-P-Q-A-S-R-H-H-H-R-$</td>
</tr>
<tr>
<td>$C$ Chain</td>
</tr>
<tr>
<td>$K$</td>
</tr>
</tbody>
</table>

Fig. 1: Sequence alignment of ovine INSL3 with human INSL3.
between cryptorchid and normal male sheep for the SSCP/INSL3-I genotype. The frequencies of genotypes and their classification are presented in table 2.

<table>
<thead>
<tr>
<th>Breed / Pheno</th>
<th>Total no. of Sheep</th>
<th>INSL3-I /SSCP genotypes (No.)</th>
<th>INSL3-II/RFLP genotypes (No.)</th>
<th>INSL3-III/RFLP genotypes (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
<td>P value for H.W.E</td>
<td>CC</td>
</tr>
<tr>
<td>Mandya</td>
<td>82</td>
<td>0.91 (73)</td>
<td>0.09 (7)</td>
<td>0.805</td>
</tr>
<tr>
<td>Hassan</td>
<td>58</td>
<td>0.92 (55)</td>
<td>0.8 (5)</td>
<td>0.924</td>
</tr>
<tr>
<td>P value for b/w breeds</td>
<td>0.931</td>
<td>0.931</td>
<td>0.749</td>
<td>0.589</td>
</tr>
<tr>
<td>Normal</td>
<td>80</td>
<td>0.90 (74)</td>
<td>0.10 (8)</td>
<td>0.858</td>
</tr>
<tr>
<td>Cryptorchid</td>
<td>60</td>
<td>0.93 (54)</td>
<td>0.97 (4)</td>
<td>0.774</td>
</tr>
<tr>
<td>Total</td>
<td>140</td>
<td>0.91 (128)</td>
<td>0.9 (12)</td>
<td>0.803</td>
</tr>
<tr>
<td>P value for b/w phenotypes</td>
<td>0.554</td>
<td>0.553</td>
<td>0.607</td>
<td>0.805</td>
</tr>
</tbody>
</table>

The PCR-RFLP of the INSL3-II fragment with Cac8I restriction enzyme resulted in three types of banding pattern. The two homozygote types are one with the presence of restriction site (C at 2451 nucleotide position) resulting in two bands of 186 bp and 129 bp (CC) and other without restriction site (T at 2451 nucleotide position) resulting in a single band of 315 bp (TT). The heterozygote CT had three bands of 315 bp, 186 bp and 129 bp.

Chi-square test indicated that the proportion of different genotypes of INSL3-II / Cac8I digestion were in Hardy Weinberg equilibrium with no significant difference between breeds or phenotype.

The PCR amplification of the INSL3-III resulted in a 413 bp product. The sequence was submitted to Genbank with accession no. KF537538.1. PCR RFLP analysis of the INSL3-III fragment by PstI restriction enzyme resulted in three types of banding pattern. The pattern CC showed two bands of 274 bp and 139 bp, and the pattern AA showed only single band of 413 bp. Population studied was in Hardy Weinberg equilibrium. No significant difference between the cryptorchids and normal for genotypes of INSL3-III/PstI was observed. The SSCP and RFLP banding pattern of ovine INSL3 amplicons are shown in Fig.2.
The involvement of Insulin like hormone in the transabdominal phase of testicular descent and thus the deficiency or the defect in the hormone as the cause of cryptorchidism have been reported in mice and human (Nef and Parade, 1999; Krausz et al., 2000). Eight substitution mutations in INSL3 have been reported to be associated with cryptorchidism in human. Comparison of amino acids of these eight regions with ovine sequence revealed conservation at five regions (amino acid P in B chain, N in A chain and three (W, P and R) in C chain), thus these five regions can be probable mutation points even for sheep. The present study on 140 sheep did not show any polymorphism in these regions and thus these regions are not involved in cryptorchidism in Mandya and Hassan sheep breeds.

Other than epigenetic control, the promoter and transcriptional factors are responsible for differential expression of gene. The promoter region revealed lesser conservation with different species compared to exon1 region. The steroidogenic factor sequence “CCAAGGCTC” was observed at 65 bp upstream in the promoter region of the Ovine INSL3. First 6 nucleotides “CCAAGG” were common for all the livestock species compared, indicating its importance in INSL3 promoter. Truong et al. (2003) also have reported that induced mutation in the SF1 sequence caused cessation of promoter activity of canine INSL3. In the present study, no polymorphism in the promoter region of INSL3 was observed in Mandya and Hassan sheep.

Williams et al. (2007) observed two SNPs in the intronic region of the ovine INSL3 gene i.e., 1841T>A and 2489C>T. Further, they reported that the latter SNP was significantly associated with cryptorchidism and the SNP was detectable by digestion with restriction enzymes Cac8I. In the present study, the PCR-RFLP of INSL3-II fragment with Cac8I restriction enzyme in 140 sheep belonging to Hassan and Mandya breed resulted in all the three possible genotypes of 2489C>T SNP. The population studied was in Hardy Weinberg equilibrium indicating no selection pressure on the population with respect to genotype. No significant differences in the distribution of any of the three genotypes between Mandya and Hassan or between cryptorchid and normal males indicated lack of association of SNP with cryptorchidism in the sheep studied.

The exon 2 of INSL3 codes for a part of C chain and A chain of INSL3 protein. Several mutations in the exon2 are known to cause substitution mutation resulting in cryptorchidism in humans. These three mutation regions (P93L, R102H and N110K) are conserved even in sheep genome and no polymorphism was observed in population studied. Williams et al. (2007) have reported presence of only one silent mutation (DQ473569:.2714C>A) which also exist in the population studied and was not significant for cryptorchid phenotype.

CONCLUSION
The Mandya and Hassan sheep population studied exists in Hardy Weinberg equilibrium with respect to genotypes of Ovine INSL3. SNP’s at Ovine INSL3 were not associated with cryptorchidism in the population studied. However, more studies on various regions of INSL3 gene in sheep breeds are necessary to elucidate the problem.
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A Study on Physical Characteristics of Mandya Sheep under Field Conditions

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ABSTRACT
Mandya sheep is considered as one of the excellent mutton type breed among the Indian sheep breeds. In the present study physical characteristics of 939 true to type Mandya sheep of different age groups from the core area of home tract was recorded and documented. The least square means for body weight, face length, tail length, chest girth, body length, wither height, shoulder width, fore leg length, chest depth, distance between hip bones and distance between withers to pin bone were: 27.64±1.08 kg, 15.12±0.31, 13.35±0.33, 67.96±1.03, 49.73±0.83, 50.39±1.26, 23.92±0.48, 39.54±0.70, 15.73±0.55, 57.46±0.70 cm, respectively in adult rams (N=26); 23.84±0.15 kg, 13.64±0.07, 12.70±0.07, 66.25±0.20, 61.47±0.21, 46.24±0.16, 48.20±0.19, 23.15±0.10, 37.63±0.16, 16.69±0.17, 54.18±0.20 cm, respectively in adult ewes (N=608); 13.45±0.78 kg, 12.45±0.42, 11.75±0.38, 58.95±1.26, 55.45±1.13, 45.30±1.07, 44.90±2.12, 22.00±0.64, 37.15±1.44, 14.80±1.69, 49.50±2.21 cm, respectively in 6 months old ram lambs (N=20); 12.68±0.20 kg, 11.98±0.11, 11.62±0.14, 56.90±0.59, 55.19±0.59, 42.31±0.30, 43.61±0.51, 21.44±0.23, 33.96±0.34, 13.41±0.16, 48.56±0.38 cm, respectively in 6 months old ewe lambs (N=124); 7.23±0.34 kg, 10.54±0.10, 10.61±0.28, 48.95±0.72, 48.39±0.70, 37.05±0.72, 39.61±0.73, 20.39±0.93, 31.54±0.81, 11.58±0.23, 43.49±0.68 cm, respectively in 3 month old ram lambs (N=41) and 7.38±0.23 kg, 10.94±0.13, 10.67±0.14, 49.43±0.57, 47.85±0.57, 38.11±0.36, 38.19±0.53, 19.56±0.18, 30.03±0.35, 13.36±0.94, 43.57±0.40 cm, respectively in 3 month old ewe lambs (N=120).

This study highlighted the prevailing scenario on the morphometric features/ physical characteristics of true to type Mandya sheep and rearing practices followed in the core home tract area under field conditions.

Key words: Mandya Sheep, Physical Characteristics, Population status and core home tract.

Majority of the sheep breeds found in Karnataka state are of small to medium sized animals reared predominantly for mutton purpose. These sheep yield low quality coarse hair, which is used for carpet making and other industrial purpose. The Mandya sheep, a native sheep breed of Karnataka is also locally known as Bandur or Bannur sheep, has been recognized as the best mutton type sheep breed and known for its meat quality in terms of taste and aroma (Acharya, 1982). A typical true to type Mandya sheep is relatively small in body built and size. Coat is predominantly white to creamy in colour and in some cases face is covered with light brown shades, which extends up to the neck region. This breed possesses squarely placed short legs, compact and square body with even rump indicative of high fleshiness. From rear, animal’s body resembles a typical reversed U-shaped conformation. Mandya breed is predominantly seen in districts of Mandya and Mysore, but sparingly spread in the adjoining districts of Bengaluru Rural, Ramanagara, Kolar, Chamarajanagar, Hassan and Tumkur district, which also constitutes the breeding tract of Mandya sheep.

In the past although several studies attempted to characterize this breed much of the information generated is of highly varied in nature. This was mainly attributable to the nature of data that was generated on “as and where” basis, where the sampled animals were often mixed type, indiscriminately bred and reared under the vast area of breeding tract, which is spread across several districts and different agro-climatic conditions. Growth potential of meat producing animals can vary and largely dependent on the prevailing management conditions including type and availability of food, water and shelter. In turn livestock management practices are further dependent on the agro-climatic conditions and socioeconomic status of the livestock keepers. In view of the above, this study was carried out to document the prevailing scenario on the

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morphometric features/ physical characteristics of true to type Mandya sheep and rearing practices followed in the core home tract area under field conditions.

**MATERIALS AND METHODS**

This study was undertaken as part of ICAR, New Delhi sponsored Mega Sheep Seed Project on Mandya sheep. The data on various morphometric features was generated from 939 true to type Mandya sheep belonging to different age groups viz., 3 Months, 6 Months and Adult sheep reared in core area of home tract, spreading through the villages of Malavalli taluk, Mandya district, Karnataka. This study was carried during the year 2012 and 2013.

The agro-climatic conditions of Mandya district include subtropical climate with temperatures ranging between 16 °C and 35 °C. April experiences the hottest weather and with the onset of southwest monsoon in June, the temperature drops considerably. December is usually the coldest month. Mandya district receive generally uniform and normal average rainfall of 623 mm. About 50 per cent of the rainfall occurs during southwest monsoon, 20 per cent during northeast monsoon period and 30 per cent during the summer period. The soil of Mandya district range from red sandy loam to red clay loam very thin in ridges and higher in elevations and comparatively thick in valley portions. The soil in Mandya, Malavalli, Maddur and Nagamangala taluks are thin gravelly and underlain with a murom zone containing weathered rock. The soils are highly leached and poor in bases. The water holding capacity is low. On the other hand the soil under the old channel areas of Malavalli, Pandavapura and Srirangapatna are high in clay (Ground water information booklet on Mandya district, Karnataka, July 2008).

True to type Mandya sheep were traced in the core home tract by door to door visit and the owners were convinced about the importance of such data collection and possible benefits in terms of better management practices. The identified farmers were encouraged to participate in the programme by way of periodical extension education programmes, door services on health coverage, onsite demonstration on various aspects of sheep breeding and management and display of true to type Mandya sheep seed flock or breeding flock that was maintained at Farm level. Various linear body measurements and body weights at different age groups were recorded using a measuring tape and a dial type, hanging weighing balance.

The data were analysed by the least square method using the GLM procedure of SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The correlation between body weight and several linear body measurements was based on the Pearson correlation procedure (Ojedapo et al., 2007) and it was estimated by using GraphPad Prism Software version 5.01.

**RESULTS AND DISCUSSION**

In terms of population it was evident that the true to type Mandya Sheep flock strength was alarmingly low and only a fewer number of families (5 per village) were into the sheep rearing activity as a part-time or subsidiary activity of agriculture as main occupation. The flock size was relatively small, this decrease in the flock size is mainly attributable to lack of grazing land and migration of farming communities to the metropolitan cities seeking better jobs and modern lifestyle. Majority of shepherds were either landless or marginal farmers.

The typical management practices under field conditions include grazing for a period about six hours in a day followed by confinement at sheds. Other than grazing and supply of limited green leaves, no defined supplementary feeding regimen were in practice. The animals were housed within the backyard or a corner of dwelling houses of farmers for obvious reasons of security to animals and in some instances, during day light hour they were usually housed in open type sheds.
Most of the personnel, who were the sole care takers of sheep were either elder family members or women folk and some school dropout children. There were no defined methods of disease control measures that were in practice except periodical worm dose or need based homemade or herbal medication often administered by some self designated, experienced shepherds. Governmental services were limited to need based mass immunization programmes during disease outbreaks. There were no defined methods of extension services or awareness programmes that were available to the livestock farmers in field conditions.

Growth usually defined as the increase in body size or body weight at a given age, it is one of the important selection criteria for the improvement of meat animals such as sheep. Body measurements such as Chest Girth, body weight and body length have been used in the definition of adult size, nutritional requirements and physiological maturity of the animals (Rocha et al., 2003). Further, they are helpful in describing the breed characteristics on scientific lines, evaluation of meat production potential and prediction of body weights in the absence of weighing scale under field conditions. These measurements provide important information about the physical development according to sex and category, allowing the establishment of the relation between conformation and functionality of the animal (Araújo Filho et al., 2007).

The linear measurements and body weights recorded in different age groups and sex of Mandya sheep is depicted in Table 1. Average body weights of adult rams was significantly higher than the females (P<0.05) and the body weight recorded in 6 month old ram and ewe lambs were similar to the previous studies (Arora & Acharya, 1976; Acharya, 1982 and Govindaiah et al., 2006). The report of Acharya (1982) indicated higher weight of lambs at 3 month of age i.e., 9.71 kg but in the

### Table 1: Linear body measurements and live weight of Mandya Sheep reared under field condition

<table>
<thead>
<tr>
<th>Particulars (cm)</th>
<th>Adult</th>
<th>6 Month</th>
<th>3 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>27.64 ± 1.08 a (26)</td>
<td>23.84 ± 0.15 b (608)</td>
<td>13.45 ± 0.78 a (20)</td>
</tr>
<tr>
<td>Face Length</td>
<td>15.12 ± 0.31 a (26)</td>
<td>13.64 ± 0.07 a (608)</td>
<td>12.45 ± 0.42 a (20)</td>
</tr>
<tr>
<td>Tail Length</td>
<td>13.35 ± 0.33 a (26)</td>
<td>12.70 ± 0.07 a (608)</td>
<td>11.75 ± 0.38 a (20)</td>
</tr>
<tr>
<td>Chest Girth</td>
<td>67.96 ± 0.59 a (26)</td>
<td>66.25 ± 0.20 a (608)</td>
<td>58.95 ± 1.26 a (20)</td>
</tr>
<tr>
<td>Body Length</td>
<td>63.96 ± 1.03 a (26)</td>
<td>61.47 ± 0.21 a (608)</td>
<td>55.45 ± 1.13 a (20)</td>
</tr>
<tr>
<td>Withershight</td>
<td>49.73 ± 0.83 a (26)</td>
<td>46.24 ± 0.16 a (608)</td>
<td>45.30 ± 1.07 a (20)</td>
</tr>
<tr>
<td>Shoulder Width</td>
<td>50.39 ± 1.26 a (26)</td>
<td>48.20 ± 0.19 a (608)</td>
<td>44.90 ± 2.12 a (20)</td>
</tr>
<tr>
<td>Fore leg length</td>
<td>23.92 ± 0.48 a (26)</td>
<td>23.15 ± 0.10 a (608)</td>
<td>22.00 ± 0.64 a (20)</td>
</tr>
<tr>
<td>Chest Depth</td>
<td>39.54 ± 0.70 a (26)</td>
<td>37.63 ± 0.16 a (608)</td>
<td>37.15 ± 1.44 a (20)</td>
</tr>
<tr>
<td>Distance b/w hip bones</td>
<td>15.73 ± 0.55 a (22)</td>
<td>16.69 ± 0.17 a (489)</td>
<td>14.80 ± 1.69 a (20)</td>
</tr>
<tr>
<td>Distance b/w withers to pin bone</td>
<td>57.46 ± 0.70 a (26)</td>
<td>54.18 ± 0.20 a (608)</td>
<td>49.50 ± 2.21 a (20)</td>
</tr>
</tbody>
</table>

**Note:**
1. Figures in the parenthesis are the number of animals measured.
2. Means carrying same superscript are not significantly different (P<0.05) column-wise in different age group.
present study it was 7.23 and 7.38 kg for ram and ewe lambs, respectively. However, the difference among body weights were non significant. The higher live weight recorded in adult males is probably due to the activity of androgen hormones, which enable better use of nitrogen in protein synthesis and therefore, in growth and muscle development (Pereira et al., 2000).

Adult rams had a body length of 63.96±1.03 cm, which is significantly higher (P<0.05) than the body length of ewes i.e., 61.47±0.21 cm. With respect to 3 and 6 month age group, there was no significant difference between ram and ewe lambs. These results were in agreement with the similar reports from Acharya (1982) and Govindaiah et al. (2006). Ram and ewes showed no significant difference with regard to chest girth. But with regard to chest depth adult rams had significantly higher (P<0.05) values than adult ewes. The chest girth and chest depth recorded in the present study are similar to the reports of previous workers (Acharya 1982 and Govindaiah et al., 2006). As the rams have wider chest or brisket region showing masculinity, obviously the chest depth would be more than the ewes.

Further, adult rams with respect to body measurements viz., Face length, Distance between withers to pin bone and shoulder width scored significantly higher values (P<0.05) than adult ewes. But the body measurements such as Fore leg length, Tail length, and Distance between hip bones showed no significant differences between rams and ewes. There was no significant difference between ram lambs and ewe lambs in body measurements except the chest depth up to 6 months of age. The values observed were similar to the reports of Govindaiah et al. (2006).

The correlation between body weight and several linear body measurements of 3 month, 6 month old and adult sheep was estimated based on the Pearson correlation procedure (Ojedapo et al., 2007) and depicted in Table 2, respectively. The correlation was positive, which was low to moderate although most of the linear body measurements were highly significant (P<0.001). Hence, no attempt was made to derive the prediction equation to estimate body weight with the help of body measurements.

| Table 2: Correlation coefficient between live weight and several body measurements at different ages |
|---------------------------------|-----------------|-----------------|------------------|-----------------|
|                                | Chest Girth     | Body Length     | Height at Withers | Shoulder width  |
| Body weight of 3 months old lamb | 0.452***        | 0.409***        | 0.260***          | 0.384***        |
| Body weight of 6 months old lamb | 0.209*          | 0.058 ns        | 0.267**           | 0.050 ns        |
| Body weight of Adult sheep      | 0.464***        | 0.319***        | 0.278***          | 0.262***        |

* = significant correlation at P< 0.05; ** = significant correlation at P<0.01; *** = significant correlation at P<0.001, NS = Non Significant

The height at withers of adult rams were significantly higher (P<0.05) than adult ewes. However, the height at withers recorded during this study was lesser than the previous reports (Acharya, 1982). This difference may be attributable to the sample data that generated during present study, which was based on vigorous selection of true to type Mandya sheep, within the core area of home tract, which was of more homogenous both in terms management practices, agro-climatic conditions and type of animals existed. Further the agro-climatic conditions were highly variable across the so called breeding tract of Mandya sheep. For example, the core home tract comprising Mandya and Mysore districts fall under irrigation belt with plenty of greenery and moderately less stressful climate compared to the extreme weather conditions in Kolar, Chamarajanagar and Tumkur districts due to scanty rains, limited availability of greenery, dry and more stressful weather conditions.
CONCLUSION
This study documented the prevailing status on the morphometric features/physical characteristics of true to type Mandya sheep distributed in core home tract, which was of more homogenous and an attempt was made to highlight the existing sheep rearing practices. Further, the data generated on body weight and linear body measurements on animals which were reared under more uniform and homogenous environment is not only useful for characterization of breed, but helpful in designing of well defined breeding, nutritional and disease control programmes, and augmentation of growth and mutton production potentialities.

ACKNOWLEDGEMENT
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ABSTRACT
A study was conducted to evaluate the effect of Fe- Methionine and Zn- Methionine replacing their inorganic source on the carcass characteristics, organ weights and sensory parameters of Naked neck fowl from 1-8 weeks of age. Totally 192 one week old Naked neck chicks were selected and randomly divided into four dietary groups with four replicates containing 12 chicks in each replicate on completely randomized design. Control diet (T1) was formulated by incorporating inorganic iron and zinc according to BIS, (1992) specifications, i.e., T1 (control) with Fe (120 ppm/kg) and Zn (60 ppm/kg) with inorganic source. T2, T3 and T4 supplemented with 50%, 100% and 150% of the BIS specifications respectively from organic source (methionine salts). The supplementation of Fe and Zn- methionine did not significantly affect dressing percentage, organs weights and overall acceptability of meat. It can be concluded that feeding organic source (T2) of Fe and Zn (60 ppm/kg and 30 ppm/kg) is relatively economical and can be used without any negative effects of mineral utilization on meat yield and quality.

Key words: Naked neck fowl, Fe- Methionine, Zn-Methionine and Carcass

Minerals occur naturally in most feed ingredients but the amount and bioavailability varies considerably. Trace minerals like iron and zinc are essential for broiler growth and are involved in many digestive, physiological and biosynthetic processes and are accomplished with inorganic sources (Bao et al., 2007). Due to increasing concerns nutritionists have focused on reducing mineral excretion without any negative effect on production performance (Devrim et al., 2010). It is because of chelation of metal ions with organic substances such as amino acids or low molecule peptide makes metal ions electrically neutral and chemically stable, thereby allowing easy passage through the small intestinal wall (Wedekind et al., 1992; Aoyagi and Baker, 1993). They are more soluble and mobile to the cell membranes and are more readily absorbed than inorganic oxides and sulfates (Brown and Zeringue, 1994).

Naked neck is a breed of chicken that is naturally devoid of feathers on its neck and vent. Originally from Central Europe, it originated in Hungary and was largely developed in Germany. The trait is dominant and controlled by one gene and is fairly easy to introduce into other breeds (John, 1997). They lay good number of light brown eggs and are considered desirable for meat production, need less plucking. They are very good foragers and are immune to most diseases. The breed is also reasonably hardy despite its lack of feathers and tolerant to hot weather. In the present day context, there is a great need to utilize the organic mineral additives to reduce the gap between cost of production and benefits in successful poultry production. Hence, the present study was aimed at supplementation of organic forms of iron and zinc, their effect on carcass and sensory characteristics of naked neck fowl.

MATERIALS AND METHODS
An experimental study of seven weeks (7-56 days of age) was conducted at the Department of Poultry Science, Veterinary College, Bengaluru, to evaluate the effect of supplementing Fe-Methionine and Zn-Methionine on carcass characteristics, tissue mineral concentration, mineral excretion and sensory evaluation in Naked neck fowl.

*Part of M.V.Sc thesis submitted by first author to KVAFSU, Bidar
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5 Assistant Professor, Department of Livestock Products Technology, Veterinary College, Bengaluru
Experimental birds and their care: One hundred and ninety two one week old straight run Naked neck chicks of uniform body weight were procured from the Department of Poultry Science, Veterinary College, Bengaluru. The chicks were wing banded for identification, weighed and randomly distributed to four treatment groups with 48 birds in each treatment and each treatment having 4 replicates with 12 birds in each replicate. The birds were maintained in cages throughout the experimental period under standard managemental conditions. Birds were vaccinated against New Castle Disease (B₁ strain) on the 7th and 21st days of age and Infectious Bursal Disease (IBD) (Intermediate strain) on 14th and 28th days of age. The diets were provided in linear feeders and potable water was provided in continuous channel to all the birds ad-libitum with free access to feed and water throughout the experimental period. All the methods regarding bird care in this experiment were approved by the Institution of Animal Ethical Committee of the University (KVAFSU, Bidar, Karnataka).

Diets: Basal diets were formulated using maize and soybean meals, (Table 1). Starter mash was fed from 7 to 28 days and finisher from 29 to 56 days. The chicks in the control group (T₁) received the basal diet formulated by incorporating corn, soya, inorganic iron (FeSO₄·7H₂O) and zinc (ZnSO₄·H₂O) as per BIS (1992) specifications (Fe 120ppm/kg and Zn 60ppm/kg) (Table 1). Inorganic Fe and Zn of the basal diet were replaced with Fe-methionine (12% Fe) and Zn-methionine (12% Zn) procured from Pristine Organics Private Ltd, Bengaluru, at 50% (T₂- Fe 60ppm/kg and Zn 30ppm/kg), 100% (T₃- Fe 120ppm/kg and Zn 60ppm/kg) and 150% (T₄- Fe 180ppm/kg and Zn 90ppm/kg).

### Table 1: Composition of basal diet (%)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Broiler starter</th>
<th>Broiler finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow maize</td>
<td>56</td>
<td>63</td>
</tr>
<tr>
<td>Soyabeen meal</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vit AB₂D₃K, **</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Vit B complex***</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Hepatocare</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Albac (Antibiotic)</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Coccidiostat</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Biobantox</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Provided per kg of feed: Calcium-1%, phosphorus-0.5%, copper-12 ppm, cobalt- 60 ppm, iodine-1 mg, Mn-60 mg.
** Vit-AB₂D₃K: Per gram contains Vit A-82, 500 IU, Vit D₃-12,000 IU, Vit B₂-50 mg and Vit K-10 mg.
*** Vit B-complex: Per gram contains Vit B₁-4 mg, Vit B₆-8 mg, Vit B₁₂-40 µg, Vit E-20 mg, Niacin -60 mg and calcium panthothenate-12.5 mg.

Carcass characteristics: At the end of the experimental period eight birds were selected randomly from each treatment and sacrificed on 56th day of the experiment. The selected birds were fasted for 12 hours with access to ad-libitum drinking water. Live weight of the birds was recorded, birds were slaughtered by humane method and bled for 90 seconds by severing jugular vein and carotid artery, scalded at 60°C for two minutes, defeathered mechanically and eviscerated carcass weights were recorded. The fat lining the abdominal cavity, covering the gizzard and bursa was scooped out, weighed and the weight of organs such as heart, liver, gizzard, spleen and bursa were recorded and expressed as per cent of live body weight.

Sensory evaluation: Uniform sized meat samples were collected from thigh and breast portion of the experimental birds and cooked in boiling water with salt (1% w/w) for 15 minutes and then cooled to ambient temperature before subjecting to sensory evaluation. The sensory evaluation was conducted using 5 point Hedonic scale to find out differences in colour, flavor, juiciness, texture and overall acceptability by panelist of the department.
**Fecal sample collection:** A digestion trail was carried out at the end of experiment on same one hundred and ninety two naked neck birds for three days, to estimate the mineral excretion by total collection method. The excreta samples were collected every 24 hours after removing feathers and scattered feed. The samples collected from each group were homogenously mixed and frozen at -20°C. After complete sample collection for three days, samples were homogenously mixed and 1/10th of the samples were dried in a hot air oven at 120°C for 18 hours. The dried samples were well mixed and powered and used for further analysis.

**Analysis of minerals in meat and feces:** At the end of 8th week eight birds per treatment were randomly selected and slaughtered, eviscerated and muscles (breast and thigh) and liver were collected. Dried (60°C for 12 h) and powdered muscle, liver and fecal samples were digested (wet digestion) with concentrated HNO₃ and HCl at the ratio of 2:1 (AOAC, 1990). The extract of muscles, liver and fecal samples were filtered through Whatman filter paper No. 42 and diluted using deionized water to the required volume. Iron and zinc contents were identified by Atomic Absorption Spectrometer (Perkin-Elmer A Analyst 300) at wavelength of 248.3 nm and 214.5 nm (Anonymous, 1982), respectively for Fe and Zn and concentration of iron and zinc were estimated by using Inductively Coupled Plasma spectrometry (ICP).

**Statistical analysis:** Data pertaining to various parameters obtained during the experiment were analyzed statistically according to the methods described by Snedecor and Cochran (1989). The data was analyzed statistically by one way ANOVA using GRAPHPAD PRISM 5.01 statistical software. The significant mean differences between the treatments were determined at P≤0.05 using Tukey's Multiple Comparison Test.

**RESULTS AND DISCUSSION**

**Carcass characteristics:** The carcass characteristics viz dressing percentage, abdominal fat percentage and organs weight are presented in Table. 2.

**Dressing percentage:** The result of present study showed that there was no significant effect of supplementation of inorganic and organic Fe and Zn in different concentrations on dressing percentage. The results of the present study are in agreement with the findings of Vladimir et al. (2010) who found that groups fed with trace elements in proteinated form restricted to 50 per cent Cu, 20 per cent Fe, Zn and Mn and on regular levels of Se had same effect on carcass yield. The present findings are in contrary with that of Ellen et al. (2012) who found that dressing percentage was significantly higher in group fed with 2.5g/ton, 11.25g/ton, 15.0g/ton, and 18.75g/ton of amino acid chelates of Cu, Zn, Mn and Fe, respectively.

**Abdominal fat percentage:** The results on abdominal fat percentage revealed that, replacement of inorganic source of Fe and Zn with that of organic source had significant (P≤0.05) effect. The present study results are in agreement with the findings of Osman and Ragab (2007) who reported that broiler chicks fed with diet supplemented with inorganic Zn had the highest abdominal fat percentage. However, Gheisari et al. (2011) and Vladimir et al. (2010) observed that diets with different levels and sources of zinc, copper and manganese did not have any significant effect on abdominal fat percentage.

**Organ weights:** There was no significant effect of supplementation of inorganic and organic source of Fe and Zn in different concentrations on relative weight of liver, heart, thymus, spleen and bursa, whereas a significant (P≤0.05) increase was observed in gizzard weight compared to that of inorganic sources. Yang et al. (2011) who found that supplementation of graded levels of trace minerals to basal broiler diets had no significant effect on relative weight of spleen. This could have been a result of more nutrients being repartitioned to develop body weight and the immune system needs a relatively small amount of nutrients in relation to what is needed for growth (Bartlett et al. (2003). The finding of this study was in contrary with that of Osman and Ragab (2007), Feng et al. (2009), Moghaddam and Jahanian (2009) and Gheisari et al. (2011) who reported that broiler chicks fed with diet supplemented with organic Fe and Zn caused a significant increase in liver, thymus, gizzard and spleen weights.
Table 2: Effect of supplementing organic iron and zinc on dressing percentage, abdominal fat percentage and organ weights (mean ± SE) in Naked neck birds.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Level (ppm/kg)</th>
<th>Dressing percentage&lt;sub&gt;NS&lt;/sub&gt;</th>
<th>Abdominal fat percentage</th>
<th>Visceral organs (gm/100gm bwt)</th>
<th>Lymphoid organs (gm/100gm bwt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Zn</td>
<td>60 mg/kg Bre</td>
<td>30 mg/kg Bre</td>
<td>120 mg/kg Thigh</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>120</td>
<td>60</td>
<td>67.93±0.18</td>
<td>1.62±0.101</td>
<td>2.69±0.28</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>60</td>
<td>30</td>
<td>67.42±0.53</td>
<td>0.74±0.042</td>
<td>2.55±0.09</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>120</td>
<td>60</td>
<td>66.24±0.99</td>
<td>0.81±0.059</td>
<td>2.47±0.16</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>180</td>
<td>90</td>
<td>67.56±0.24</td>
<td>0.81±0.064</td>
<td>2.68±0.05</td>
</tr>
</tbody>
</table>

Each value is mean of eight observations.
NS= Non-significant. Means bearing different superscripts in a column differ significantly (P≤0.05).

Mineral analysis: The Fe and Zn concentration in tissues viz breast, thigh and liver and mineral excretion i.e., fecal mineral concentration are presented in Table 3.

Table 3: Effect of supplementing organic iron and zinc on mineral concentration (Fe and Zn) in tissues and feces (mean ± SE) in Naked neck birds.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Level (ppm/kg)</th>
<th>Fe(%)</th>
<th>Zn(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Zn</td>
<td>Breast</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>120</td>
<td>60</td>
<td>100±0</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>60</td>
<td>30</td>
<td>140.2±15.06</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>120</td>
<td>60</td>
<td>215±31.91</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>180</td>
<td>90</td>
<td>570±28.10</td>
</tr>
</tbody>
</table>

Each value is mean of eight observations.
NS= Non-significant. Means bearing different superscripts in a column differ significantly (P≤0.05).

Fe and Zn concentration in Breast Muscle: There was significant (P≤0.05) increase in breast muscle iron concentration in the groups fed with higher (100 and 150 per cent) levels of Fe and Zn-methionine when compared with control. There was significant difference in breast muscle zinc concentration between control group and the group fed with lower (50 per cent) level of Fe and Zn-methionine (T<sub>2</sub>). The result of the present study is in accordance with Ma et al. (2012) who found that the enhanced concentration of Fe in breast of chicks fed diet supplemented with 120 and 160mg/kg Fe-treatments as compared to control. The results suggest that the increased tissue Fe and Zn concentration may be due to better bioavailability of organic minerals through selective transport of peptides across intestinal mucosa which has improved the absorption of these minerals and in turn deposition into the muscles. The results were in agreement with Vladimir et al. (2010) who found that groups fed with trace elements had higher concentration of these minerals in the breast muscle of broilers as compared to inorganic mineral supplementation.
**Table 4: Effect of supplementing organic iron and zinc on sensory evaluation (mean ± SE) in Naked neck birds.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Level (ppm/kg)</th>
<th>Colour</th>
<th>Flavour&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Juiciness</th>
<th>Texture&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Overall&lt;sup&gt;NS&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>120</td>
<td>4.85±0.06</td>
<td>4.65±0.18</td>
<td>5.15&lt;sup&gt;ab&lt;/sup&gt;±0.11</td>
<td>5.05±0.13</td>
<td>5.53±0.15</td>
</tr>
<tr>
<td>T2</td>
<td>60</td>
<td>5.30&lt;sup&gt;NS&lt;/sup&gt;±0.20</td>
<td>5.03±0.13</td>
<td>5.50&lt;sup&gt;b&lt;/sup&gt;±0.12</td>
<td>5.23±0.24</td>
<td>5.48±0.16</td>
</tr>
<tr>
<td>T3</td>
<td>120</td>
<td>5.68±0.08</td>
<td>4.68±0.12</td>
<td>5.00&lt;sup&gt;b&lt;/sup&gt;±0.00</td>
<td>4.85±0.15</td>
<td>5.38±0.11</td>
</tr>
<tr>
<td>T4</td>
<td>180</td>
<td>4.98&lt;sup&gt;b&lt;/sup&gt;±0.09</td>
<td>5.15±0.17</td>
<td>4.80&lt;sup&gt;b&lt;/sup&gt;±0.12</td>
<td>5.33±0.12</td>
<td>5.53±0.08</td>
</tr>
</tbody>
</table>

Each value is mean of eight observations.

NS= Non-significant. Means bearing different superscripts in a column differ significantly (P≤0.05).

Fe and Zn concentration in Thigh Muscle: There was a significant (P≤0.05) increase in thigh muscle iron concentration in the groups fed with higher (100 and 150 per cent) levels of Fe and Zn- methionine when compared to that of control. There was non-significant difference in thigh muscle zinc concentration between the groups fed with inorganic and organic Fe and Zn. Similar results have been reported by Vladimir et al. (2010).

Fe and Zn concentration in Liver: The present study result revealed that there was a significant (P≤0.05) increase in liver iron concentration in the groups fed with organic Fe and Zn- methionine when compared with control. There was non-significant decrease in liver zinc concentration between the groups fed with higher levels of organic Fe and Zn (100 per cent and 150 per cent) when compared with group fed with inorganic Fe and Zn. This was in agreement with that of Ao et al. (2006) and Wedekind et al. (1992) who found that liver zinc content increased linearly with increase in the levels of dietary Zn in broiler chicks. Similarly Ma et al. (2012) found that the concentration of liver Fe increased with increasing the dietary Fe- Gly. However, on contrary to the findings of this study, Ellen et al. (2012), Bao et al. (2007) and Devrim et al. (2010) observed that broilers fed with inorganic minerals had a significant increase in the liver Zn and Fe concentration in comparison to those fed organically- complexed minerals.

**Sensory evaluation:** The results on sensory evaluation are presented in Table. 4 and the results revealed that no significant difference is observed among treatment groups. The overall acceptability score was almost similar in all the treatment groups which clearly indicate that dietary supplementation of inorganic and organic source of Fe and Zn had no effect on sensory evaluation score of naked neck fowls. The colour scores revealed that supplementation of Fe increased the redness scores, which might be due to the pigment imparting character of Fe and the ability of Zn to bind to...
myoglobin and increase its oxygenation (Saenmahayak et al. 2010). The findings of the study was in agreement with Salim et al. (2011) who found that dietary organic zinc at different levels (20 ppm, 40 ppm, 80 ppm) did not affect sensory properties of broiler chickens.

CONCLUSION

Among the various dietary treatment groups, there was no significant difference in dressing percentage between control and groups fed with organic source of Fe and Zn. There was significant difference in abdominal fat percentage between control and groups fed with organic source of Fe and Zn (T2, T3 and T4). In the present study, among the various treatment groups organic source showed less fat percentage (T2 0.74 per cent) followed by T3 and T4 (0.81 per cent) and control (1.62 percent). Supplementation of Fe and Zn from either inorganic or organic source did not influence the relative organ weights viz, liver, heart and thymus among various levels of different dietary treatment groups. However, there was significant increase in relative weight of gizzard in groups fed with organic source of Fe and Zn (T2, T3 and T4) compared with groups fed with control diet. No significant effect of either inorganic or organic source of Fe and Zn was observed on overall acceptability and sensory attributes of meat in Naked neck fowl.

REFERENCES


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Effect of Different Starter Feeds on the Growth Performance of Pre-Ruminant Crossbred Calves*

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ABSTRACT

The present study was undertaken to investigate the effect of feeding three different calf starters on the growth performance of Holstein-Friesian crossbred calves up to eight weeks of age. Based on the birth weight, eighteen HF crossbred calves of either sex, after overnight colostrum feeding, were randomly divided into three groups with six calves in each group. Calf starter and lucerne hay were given ad libitum to all the calves from fourth and tenth day, respectively. The diet in T1 group (Control) contained 50% crushed maize grain whereas, T2 and T3 (Test groups) contained 25% and 0% crushed maize grain, respectively. The total dry matter intake at the end of eighth week was almost similar in all the groups (39.26, 39.52 and 38.47 kg in T1, T2 and T3 groups, respectively). The total gain in body weight (kg) was observed to be higher in T2 (20.65) compared to T1 (18.01) and T3 (18.81), but the differences were statistically non-significant. Average daily gain (g) was also found to be non-significantly higher in T2 (368.75) group as compared to T1 (321.73) and T3 (336.01) groups. Similarly, feed to gain ratio was found to be better in T2 (1.91) group as compared to T1 (2.18) and T3 (2.05) groups. The present study indicated that optimum growth performance in crossbred calves could be achieved with calf starter containing limited amount of maize grains (25%) and good quality lucerne hay.

Key words: Calf starter, Average daily gain, Feed:Gain ratio, Crossbred calves

Calves are the future herd at a dairy farm. They must be produced to replace the older and uneconomical individuals at the farm through voluntary culling. In developing countries like India, rearing of crossbred calves is not given much attention. This is largely due to economic compulsion to sell milk for human consumption (Ranjhan, 1992) and perhaps not realizing the potential value of these animals in their adulthood. The preparation and use of milk replacers and starter feeds are generally accepted practices in developed countries but not in developing countries like India (Krishnamoorthy and Moran, 2011). Intake of colostrum by neonatal calves and early transition to calf starter are two important factors in a successful calf rearing programme.

Early consumption of dry feed by young calves is desirable to support rapid rumen development and enable early weaning. Early weaning of calves contributes to early development of ruminal microbial activity because of accelerated intake of dry feed (Anderson et al., 1981). Adequate microbial population seems to be present very early in rumen of calves and subsequent microbial development is stimulated by increased dry feed consumption (Anderson et al., 1987). Calf starter ration improves the health of calves, reduces the stress of weaning and reduces the growth depressing factors. It also reduces the chances of diarrhoea in calves as conventional ration causes digestive problems due to high fiber content. Considerable studies have been conducted in the past on economical calf rearing with more emphasis on sparing milk for human consumption, given the demand of growing human population in India. Hence, the present experiment was undertaken to study the growth rate and feed conversion efficiency in crossbred calves fed calf starters with different levels of grains.

*Part of Ph.D. thesis work of first author
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6Assistant Professor, Department of ILFC, Veterinary College, Bengaluru
MATERIALS AND METHODS

The present experiment was conducted on eighteen Holstein-Friesian (HF) crossbred calves for a period of eight weeks at the Dairy Farm, Department of Instructional Livestock Farm Complex, Veterinary College, Bengaluru. Based on the birth weight, the HF crossbred calves of either sex after overnight colostrum feeding were divided randomly into three groups (T1, T2 and T3) viz., six calves in each group. The experimental calves were housed individually in pens under identical management and milk feeding schedule (Table 1). Deworming was carried out in all the calves at the age of two weeks. Clean drinking water was made available.

Table 1: Feeding schedule followed for experimental calves.

<table>
<thead>
<tr>
<th>Age of calves</th>
<th>Milk (as part of Body weight)</th>
<th>Calf starter (4th day onwards)</th>
<th>Lucerne hay (10th day onwards)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 3 d</td>
<td>1/10</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4 - 35 d</td>
<td>1/10</td>
<td>ad lib.</td>
<td>ad lib.</td>
</tr>
<tr>
<td>36 - 45 d</td>
<td>1/15</td>
<td>ad lib.</td>
<td>ad lib.</td>
</tr>
<tr>
<td>46 - 56 d</td>
<td>1/20</td>
<td>ad lib.</td>
<td>ad lib.</td>
</tr>
</tbody>
</table>

One standard control (T1) and two treatment groups (T2 and T3) as test calf starters were prepared. The experimental calves in T1 group were fed with calf starter containing 50% maize grain whereas, the calf starter fed to T2 and T3 groups had 25% and 0% maize grain, respectively (Table 2.). Milk was fed twice daily, in the morning and evening, as per their body weight. A one-week adjustment period was given to the experimental calves of all the groups to make them accustomed to the new feeding regimes. Calf starter and lucerne hay were introduced on the fourth and tenth day, respectively. The experimental calves were trained to eat calf starter by rubbing a little starter on the muzzle for first 2 to 3 days. The measured quantities of calf starter ration and lucerne hay were offered in the morning and the left over was weighed next day to determine the feed intake. Intake of milk, calf starter and lucerne hay was recorded daily for each experimental calf. Dry matter in feeds and residue samples was determined daily in hot air oven at 100±1°C. Body weights of experimental calves in all the groups were recorded at the beginning of trial and then at weekly intervals till the completion of the trial, which lasted for 8 weeks. Standard equipment was used to record the data. Experimental data was analysed using the data analysis tools in Microsoft Excel 2007 software.

Table 2: Composition (per cent) of calf starter in different treatment groups

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Crushed maize</td>
<td>50</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>2</td>
</tr>
<tr>
<td>Common salt</td>
<td>1</td>
</tr>
<tr>
<td>Nutritive Composition</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>20</td>
</tr>
<tr>
<td>TDN</td>
<td>74</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Initial body weight of calves in T1, T2 and T3 groups was 29.87±3.19, 29.20±2.98 and 29.52±2.97 kg, respectively. The total dry matter intake (kg), final body weight (kg), total gain (kg), average daily gain (g) and feed: gain ratio are presented in Table 3. The total dry matter intake (kg) was observed to be slightly lesser in T3 group (38.47±2.66) compared to T1 (39.26±2.96) and T2 (39.52±3.01) groups. Though, the calves in T2 (20.65±2.04) group showed better gain in body weight (kg) than T1 (18.01±1.02) and T3 (18.81±1.21) groups, the differences were statistically non-significant. Though the calves in T2 (368.75±28.21) group showed higher average daily gain (g) as compared to T3 (336.01±21.61) and T1 (321.73±18.31), no significant difference was observed among the three experimental groups. The feed to gain ratio was observed to be better in T2 group (1.91±0.17) as compared to T3 (2.05±0.21) and T1 (2.18±0.13) groups. No significant difference observed with respect to feed: gain ratio among the three experimental groups.
Table 3: Growth performance, dry matter intake and feed to gain ratio in different groups of experimental calves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Performance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (kg)</td>
<td>29.87±3.19</td>
<td>29.20±2.98</td>
<td>29.52±2.97</td>
<td>0.988</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>47.88±3.17</td>
<td>49.85±3.01</td>
<td>48.33±2.10</td>
<td>0.875</td>
</tr>
<tr>
<td>Total gain (kg)</td>
<td>18.01±1.02</td>
<td>20.65±2.04</td>
<td>18.81±1.21</td>
<td>0.207</td>
</tr>
<tr>
<td>ADG (g)</td>
<td>321.73±18.31</td>
<td>368.75±28.21</td>
<td>336.01±21.61</td>
<td>0.207</td>
</tr>
<tr>
<td><strong>Dry matter intake (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>21.29±0.57</td>
<td>21.10±0.82</td>
<td>21.19±1.00</td>
<td>0.987</td>
</tr>
<tr>
<td>Calf starter</td>
<td>13.20±0.15</td>
<td>13.43±0.39</td>
<td>12.78±0.42</td>
<td>0.430</td>
</tr>
<tr>
<td>Lucerne hay</td>
<td>4.77±0.07</td>
<td>4.99±0.09</td>
<td>4.50±0.09</td>
<td>0.912</td>
</tr>
<tr>
<td>Total DMI</td>
<td>39.26±2.96</td>
<td>39.52±3.01</td>
<td>38.47±2.66</td>
<td>0.821</td>
</tr>
<tr>
<td>Feed: Gain ratio</td>
<td>2.18±0.13</td>
<td>1.91±0.17</td>
<td>2.05±0.21</td>
<td>0.405</td>
</tr>
</tbody>
</table>

Figure 1: Total DMI (kg) and total gain (kg) in different groups of experimental calves

Figure 2: The average daily gain (g) in different groups of experimental calves.

Figure 3: Feed: gain ratio in different groups of experimental calves

Though, the calf starter was variable in TDN contents, the body weight gain was comparable mainly due to higher intake of lucerne hay in T2 group. The results of the present study are in accordance with the study conducted by Mondal et al. (1996) in HF crossbred calves, but ADG and total gain in present experiment were slightly higher than the previous study. Hill et al. (2008) reported the ADG in HF bull calves to be higher (439 g) than the present experiment. Similarly, in another experiment the ADG was 212 g in crossbred calves when fish meal was used as source of protein in calf starter (Sahoo and Pathak, 1998).
Sahoo and Pathak (1994) reported that the source of fodder fed in early life had significant effect on feed intake and growth response of the calves. It has also been suggested that the starter should be relatively high in readily fermentable carbohydrates but adequate in digestible fibre to support ruminal tissue growth (Greenwood et al., 1997). Adoption of dry feed consumption in calves at an early age leads to early weaning because of rapid ruminal metabolic development (Quingly et al., 1991).

The present experiment showed that optimum growth rate in crossbred calves can be achieved by introducing crushed maize grains at the rate of 25 per cent of the calf starter and good quality lucerne hay early in their life.

REFERENCES
Effect of Supplementary Feeding on Growth Performance of Lambs to Improve Livelihood of Shepherds

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ABSTRACT

Two feeding trials one for morning with seven lambs (6 kg initial BW) and evening feeding trial with sixteen lambs (5 kg initial BW) were conducted for 100 d and 104 d respectively to determine whether supplementary feeding affects the performance of lambs and whether time of feeding has any effect on growth rate under Indian grazing small ruminant production system. Treatments were T0 as control i.e. grazing + suckling, T1 graze+ suckle+ 50g ground maize per lamb per day and T2 graze+ suckle+ 50g ground maize+ 2% SRNP 4% mineral mixture per lamb per day for morning feeding trial and T0 as control i.e. grazing + suckling, T1= graze+ suckle+ 50g ground maize+ 2% SRNP+ 4% mineral mixture per lamb per day for evening feeding trial. The morning supplemented lambs improved their performance by 63% (T1) and 89.54 % (T2) over control group whereas evening supplemented lambs improved their performance by 38.46% over control group. In conclusion, supplements such as energy, SRNP and mineral mixture appears promising as a simple means of achieving high ADG under Indian grazing small production system and evening supplementation is better than morning supplementation.

Key words: Lamb, Growth, Supplementation, Indian grazing.

Small ruminant production in India is done by farmers and shepherds by keeping small manageable flock strength of twenty to thirty adult parent stock with one breeding ram and their followers. Shepherds generally, take their sheep for grazing throughout the day in adjoining harvested agriculture field, road sides and forest area and bring them back in the evening time to their home for night sheltering. The lambs born to the ewes are sold in the market at an approximate body weight of 10-12 kg at 3-4 m age and this meets out his regular monthly income. The sale of lambs at an early age serves the dual purpose of meeting monthly house hold income and early weaning of lambs by sale brings the ewe into heat thereby further conception and more lamb production per ewe. The other system of small ruminant production is keeping larger herd generally in thousands managed by a group of people who migrate from place to place in search of pasture and generally stay in agriculture field during night time along with the herd (Dhayani et.al. 2000 and Dorji et. al. 2003).

Generally supplementary feeding in any form is not practiced and shepherds rely solely on grazing resources to meet the nutritional requirement of their flock (Mehta et. al. 1995 and Mishra et. al. 2004). This practice of rearing of small ruminants in India is not meeting nutritional requirement of lambs and hence not getting desired body weight gain when the farmers ultimately go to sell his animals in the market to meet his day to day expenses. Looking into the above method of small ruminant production system in India an attempt was made to study the effect of supplementary feeding on growth performance of lambs. Two feeding trials were conducted at Instructional livestock farm complex (ILFC), Veterinary College Bangalore in order to study the effect of supplementary feeding on growth performance of lambs and time of feeding.

MATERIALS AND METHODS

Trial I: Seven Bannur lambs of three month age were divided into three groups consisting of two, two and three lambs in each group based on comparable body weight and age. The experiment

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was a complete block design and flock feeding was practiced using lamb feeders using enclosures and partitions. The feeding of lambs was done during morning hours at 8.30 AM. The feeding trial was conducted for 100 days. The lambs of T0 group acted as control and were taken for grazing along with their dam for seven hours per day. The lambs of treatment 1 (T1) were provided with 50 g of ground maize along with grazing and suckling. The lambs of treatment 2 (T2) were provided with 50g ground maize+ 2% SRNP+ 4% mineral mixture per lamb per day along with grazing and suckling. The slow release nitrogen product (SRNP) is a commercial product (Optigen II of M/s Alltech Biotechnology Private Limited, Bangalore, India) consisting of 42 per cent nitrogen. Weekly body weight of lambs was recorded using standard electronic Salter spring balance.

**Trial II**: Sixteen Bannur lambs of three months age of uniform body weight were divided into two groups (average group body weight was 40 kg) consisting of eight lamb in each group. All other method of feeding were similar to trial one except for the T1 group which was removed here looking at the clear cut advantage received by T2 group over control. The lambs of T0 group acted as control and were taken for grazing along with their dam for seven hours per day. The lambs of treatment 1 (T1) were provided with 50g ground maize+ 2% SRNP+ 4% mineral mixture per lamb per day along with grazing and suckling. The feeding trial was conducted for 104 days and the dosage of ground maize was increased to 75g/d per lamb from 75 day of trial till the completion of trial. The feeding time was changed to evening hours to 4.00 PM

Morning feeding trial (Trial1) consisted of 7 lambs of uniform body weight that were equally divided into three groups ( T0( n=2): Control = Graze+ Suckle, T1( n=2): Maize (50g/d)+ Graze+ Suckle, T2:(n-3): Maize (50g/d)+ Mineral Mixture (4% ) + Slow release nitrogen product (SRNP2%)+ Graze+ Suckle) while Evening feeding trial (Trial 2) consisted of 16 lambs of uniform BW that were equally divided into two groups with 8 lambs in each group (T0 (n=8): Control = Graze+ Suckle, T1 (n=8): Maize (50g/d)+ Mineral Mixture (4% ) + SRNP(2%)+ Graze+ Suckle)

**RESULTS AND DISCUSSION**

The table shows that there was a clear cut enhancement of performance due to morning supplementary feeding of maize alone over control group. T2 showed much better results over control group. An average daily gain of 42.46 g/d was observed in control group whereas it was 69.90 g/d in T1 group and the ADG was 80.46 g/d in T2 group. The performance of T1 group was 63 per cent more than the control group whereas it was 89.54 per cent more than the control group in T2 group. The performance of both supplementary group was quite higher than the control group clearly indicating definite advantage of supplementary feeding. Comparison between T1 and T2 suggest 16.27 per cent increase in performance which is attributed to the mineral mixture and SRNP provided in the feed. Present finding reported match with the findings of Shivasharnappa et. al. (2014) who also reported better ADG due to supplementary feeding.

In order to clear the doubt of gut fill effect or satiety factor and also looking at the clear cut advantage of T2 over T1 evening supplements feeding experiment was carried with only two groups i.e. T0 and T1. The analysis of data revealed that the ADG in control group was 42.69 g/d whereas it was 59.11 g/d in treatment group. There was an increase in performance by 38.46 per cent due to supplementary feeding and was significantly (P <0.05) better than the control group. Looking into the expenses involved in the feeding and extra gain in body weight, an approximate expenditure of Rs 1.00 on feed can gain a net profit of Rs 8.00 in the form of live body weight gain, considering the cost of maize the primary ingredient to be at Rs 14.00 per kg and live BW of lambs to be at Rs 200.00 per kg at existing market prices.
Table 1: Average body weight gain of lambs grams per day (g/d) in morning feeding trial.

<table>
<thead>
<tr>
<th>Days</th>
<th>T0(n=2)</th>
<th>I(n=2)</th>
<th>T2(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7d ADG</td>
<td>18.57</td>
<td>128.57</td>
<td>120.47</td>
</tr>
<tr>
<td>29d CADG</td>
<td>68.10</td>
<td>93.62</td>
<td>100.34</td>
</tr>
<tr>
<td>43d CADG</td>
<td>72</td>
<td>102.45</td>
<td>116.12</td>
</tr>
<tr>
<td>57d CADG</td>
<td>66.67</td>
<td>87.89</td>
<td>107.11</td>
</tr>
<tr>
<td>71d CADG</td>
<td>54.23</td>
<td>77.11</td>
<td>94.55</td>
</tr>
<tr>
<td>86d CADG</td>
<td>51.10</td>
<td>83.25</td>
<td>94.92</td>
</tr>
<tr>
<td>100d CADG</td>
<td>42.46</td>
<td>69.90</td>
<td>80.46</td>
</tr>
</tbody>
</table>

Fig: Graphical representation of growth of lambs in three different groups.

CONCLUSION

Supplementary feeding if incorporated in the Indian system of small ruminant production will give definite advantage over traditional system of sheep rearing. Incorporation of energy source such as maize, mineral mixture and slow release nitrogen product improves the rumen function and provides better microbial protein and volatile fatty acids which is clearly evident in the form of better body weight gain compared to the control group. Overall ADG in 104 d feeding trial for the supplemented group was significantly better than the non supplemented group suggesting potential use of supplements to enhance the performance of small ruminant production. Evening supplementation was found to be better than morning supplementation. Hence supplementation during evening time can be practised among famers and shepherds rearing lambs in order to get increased body weight gain and better price for lambs sold in the market.

ACKNOWLEDGEMENT

Appreciation is expressed to the Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, India staff and officials for enabling the researcher to take up the experiment and for all the financial assistance.

REFERENCES


A Study on Genetic Divergence among the Different Sets of Ongole Cattle.

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Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendranagar

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ABSTRACT

The first lactation production and reproduction data on 622 cows belongs to 5 sets of Ongole bulls tested so far in the Network Project, were analyzed by employing Mahalonobis (1936) $D^2$ statistics as per the procedures described by Singh and Chaudhary (1985) and Basu (1996) to estimate the genetic divergence among the sets. The Mahalonobis genetic distances ($D^2$) between the all combinations of sets were highly significant. The calving interval and total lactation milk yield and lactation length contributed 40%, 40% and 20% to the total genetic diversity among the sets. Two distinct clusters were formed, the first one consisting of sets 1, 2, 3 and 5, while the second cluster had only 4th set. The average $D^2$ values were the maximum between the 1st and 2nd, 3rd and 5th sets. Out of all combinations, the maximum divergence was noticed between the 1st and 3rd set and least intra cluster distance was observed between 2nd and 5th set. The average intra cluster distance of cluster one was 1247.86, while the inter cluster distance between the two clusters was 14044.62.

Key words: Genetic Divergence, Mahalonobis, Ongole bulls

Ongole cattle one of the pride breed of Andhra Pradesh was efficiently used in its breeding tract for both milk and draught purpose. They are usually docile; bullocks are powerful and suitable for heavy work, while females are fair milkers. Ongoles, which won laurels all over the world, not only declining in its homeland over the period of time, but are subjected to genetic erosion which may be attributed to indiscriminate cross breeding and fast changing socio economic levels of farmers. The Indian Council of Agricultural Research (ICAR) has realized the importance of conservation of this unique germplasm and established a Germplasm Centre with elite animals at Lam farm, Guntur and its associate herds for undertaking the progeny testing program to produce the elite bulls and cows for supply to the field. The progeny testing programme was initiated during the year 1986 and so far, five sets of bulls have been progeny tested. Relative superiority or inferiority of different groups based on single trait can’t represent fully the real differences among them. To compare different groups, some important characters have to be combined. Mahalonobis $D^2$ statistics consider more than one trait simultaneously for estimation of divergence among the different groups. The present investigation was undertaken to study the genetic divergence among the different sets of Ongoles based on Mahalonobis $D^2$ Statistics.

MATERIALS AND METHODS

The data for the present study were collected from pedigree and performance records of Ongole cattle maintained at Livestock Research Station, Lam farm, Cattle Breeding farm, Chadalawada, Livestock Research Station, Mahanandi and Composite Livestock farm, Chintaladevi maintained under Network Project for improvement of Ongole cattle where progeny testing programme is going on, Livestock Research Station, Lam farm as a main centre. The data on age at first calving, total lactation milk yield, lactation length, service period, gestation period, dry period and calving interval were utilized for the multivariate analysis after adjusting the data for significant effects of farm, period of calving and season of calving as per Harvey (1976). The adjusted data were used for the estimation of genetic divergence among the five sets of bulls tested so far in the Network Project, by employing Mahalonobis (1936) $D^2$ statistics as per the procedures described by Singh and Chaudhary (1985) and Basu (1996).
Statistical Analysis- Testing the presence of divergence: The estimates of variance and covariance from the various traits considered in the present study were obtained from an analysis of variance and covariance using the following model.

\[ Y_{ij} = \mu + S_i + e_{ij} \]

Where,
- \( Y_{ij} \) is the \( j^{th} \) observation on \( i^{th} \) set
- \( \mu \) = overall mean
- \( S_i \) = effect of \( i^{th} \) set
- \( e_{ij} \) = random error, assumed to be normally and independently distributed with mean zero and variance \( \sigma^2_e \).

Estimation of magnitude of divergence: The transformation of the character mean of the population to uncorrelated variables was done using the pivotal condensation method. \( D^2 \) values were calculated as the sum of the differences of sets over the transformed variables. The \( D^2 \) values between two sets were estimated as

\[ D^2_{ij} = \sum X_{ij} d_i d_j \]

Where,
- \( d_i \) is the difference between the mean values of population for the \( i^{th} \) trait.
- \( d_j \) is the difference between the mean values of population for the \( j^{th} \) trait.

The \( D^2 \) value obtained for a pair of population is taken as the calculated values of \( \chi^2 \) and is tested for its significance against the tabulated values of \( \chi^2 \) for \( p \) degrees of freedom, where \( p \) is the number of characters considered in the multivariate analysis.

Contribution of individual character to overall \( D^2 \) value: Contribution of individual characters to the total divergence was estimated as average \( D^2 \) values for each character over all the comparisons calculated and rank total was calculated. In the present study five sets were under study and only ten combinations were possible. In all the combinations, each character was ranked on the basis of

\[ d_i = Y_{ij} - Y_{ki} \]

Where,
- \( d_i \) is the differences in value of the \( j^{th} \) and \( k^{th} \) set for the \( i^{th} \) character.

Rank one was given to the highest mean difference and rank \( p \) to the lowest mean differences, where \( p \) is the total number of characters. Percent contribution of a character to total divergence was estimated by counting the number of times the character was first ranked and the percent contribution was computed taking \( 10 \times 100 \).

Grouping of various sets into different clusters: For grouping of the sets into various clusters, Tocher’s method was followed. The general criterion used was the sets belonging to the same cluster should have smaller \( D^2 \) value than those belonging to different clusters.

Estimation of Intra and inter cluster distances: The intra cluster \( D^2 \) values were calculated using following formula

\[ \frac{\sum D^2_{i}}{N} \]

Where, \( \Sigma D^2_{i} \) is the sum of inter-cluster distances between all possible combinations of set groups included in the clusters \( N \) is the number of combinations of distances. The inter cluster \( D^2 \) values were computed by the following formula

\[ \frac{\Sigma D^2_{ij}}{n_in_j} \]

Where, \( \Sigma D^2_{ij} \) was the sum of inter-cluster distances between set groups of clusters and \( n_j \) is the number set groups of cluster.
- \( n_i \) was number of set groups in cluster \( i \)
- \( n_j \) was number of set groups in cluster \( j \)

RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed that out of seven traits, namely age at first calving, total lactation milk yield, lactation length, gestation period, service period, dry period and calving interval, the genetic differences among sets were significant for calving interval only. The analysis of dispersion revealed significant genetic differences between the sets when all the traits considered simultaneously (Table 2).

Relative Diversities: The relative diversities (\( D^2 \)) arranged in ascending order, for each set, is presented in Table 3. The test of significance revealed that the \( D^2 \) values were highly significant between all the sets. The relative divergence was the maximum between the first and fourth set of bulls (22797.96), while it was the minimum between 2\textsuperscript{nd} and 5\textsuperscript{th} set (139.28).
Table 1. Analysis of variance for first lactation traits

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f</th>
<th>Mean sum of squares</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFC</td>
<td>4</td>
<td>83777.24</td>
<td></td>
</tr>
<tr>
<td>LMY</td>
<td>4</td>
<td>137026.25</td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>4</td>
<td>5437.16</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>4</td>
<td>44.82</td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>4</td>
<td>5958.20</td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>4</td>
<td>3590.37</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>4</td>
<td>92821.40**</td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>4</td>
<td>83777.24</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at P≤ 0.01

Table 2. Analysis of variance for Dispersion

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean sum of squares</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sets</td>
<td>4</td>
<td>2.09E+27</td>
<td>108998.04**</td>
</tr>
<tr>
<td>Error</td>
<td>617</td>
<td>2.95E+24</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at P≤ 0.01

Table 3. Standardized squared Mahalonobis distances (D^2) between the sets arranged in ascending order

<table>
<thead>
<tr>
<th>Set 1</th>
<th>Set 2</th>
<th>Set 3</th>
<th>Set 4</th>
<th>Set 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2075.99** (5)</td>
<td>139.28** (5)</td>
<td>170.69** (5)</td>
<td>8573.82** (3)</td>
<td>170.69** (3)</td>
</tr>
<tr>
<td>3411.22** (3)</td>
<td>594.91** (3)</td>
<td>594.91** (2)</td>
<td>11121.76** (5)</td>
<td>1158.12** (2)</td>
</tr>
<tr>
<td>13684.95** (2)</td>
<td>1158.12** (1)</td>
<td>3411.22** (1)</td>
<td>13684.95** (2)</td>
<td>2075.99** (1)</td>
</tr>
<tr>
<td>22797.96 **(4)</td>
<td>13684.95** (4)</td>
<td>8573.82** (4)</td>
<td>22797.96 **(1)</td>
<td>11121.76** (4)</td>
</tr>
</tbody>
</table>

Significant at P≤ 0.01 Figures in parentheses are set numbers

Table 4. Contribution of individual traits towards the divergence

<table>
<thead>
<tr>
<th>Age at first calving</th>
<th>Total Lactation milk yield</th>
<th>Lactation length</th>
<th>Gestation period</th>
<th>Service period</th>
<th>Dry period</th>
<th>Calving interval</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of times appearing first in ranking</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Percent contribution</td>
<td>0</td>
<td>40</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>
cluster one was 1247.86, while the inter cluster distance between the two clusters was 14044.62. The intra cluster distance for the cluster 2 could not be estimated since it contains only one set.

REFERENCES


Milk is a highly perishable food and therefore cannot be stored for longer period. Fermentation is among the methods of preserving milk nutrients; thus fermented milk products play a vital role in the human food. Dahi, an Indian fermented milk product has mixed strains of lactic cultures, such as lactococci, streptococci, leuconostoc and lactobacilli. Lactic acid bacteria (LAB) are a group of microaerophillic, gram positive cultures fermenting lactose to produce primarily lactic acid.

According to the FAO/WHO, (2007) probiotics are defined as mono or mixed cultures of “live microorganisms which, when administered in adequate amounts confer a health benefit on the host”. Billions of bacteria inhabit the human digestive tract and these bacteria are referred to as the gut flora. Maity and Misra (2009) have shown that the gut flora consists of more than 400 different species or types of beneficial/harmful bacteria. One way of maintaining balance between the beneficial and harmful bacteria in the gut is to consume a source of probiotics (beneficial bacteria) that can be introduced into the digestive tract through the probiotic food (Maity and Misra, 2009).

The primary role of LAB in dahi is to produce lactic acid followed by development of flavour, body and texture by producing exopolysaccharides and tertiary function of producing various aromatic compounds such as diacetyl, acetaldehyde, alcohols and so on (Tamime and Robinson, 1999). The LAB occurring in dahi varies with unbranded and branded samples and this may be due to the storage conditions or the cultures inoculated. There are very few reports suggesting the presence of probiotic cultures in the market samples of dahi sold in India.

MATERIALS AND METHODS

Dahi samples: Both branded and unbranded (four each) dahi samples were collected aseptically from various markets of Bengaluru and were coded accordingly.

Plating of samples: The samples were serially diluted and the required dilutions were plated using Neutral Red Chalk Lactose agar (NRCLA) for lactococci with incubation at 30°C and MRS agar for lactobacilli and leuconostoc with incubation at 37°C and 30°C respectively for 24 to 48 h.

Further the first dilution samples were subjected to a heat treatment of 63°C for 30 min and then plating on M17 agar to isolate S. thermophilus. Plates were incubated in an anaerobic jar at 37°C for 24 to 48 hours. After enumeration, colonies were selected based on the morphology and transferred as well as maintained in respective broth media.

After purification, acid and bile tolerance tests were carried out on these isolates as per standard procedures (Harrigan, 1998) while genotypic test was carried out as described by Pospiech and Neumann (1995). Probiotic tests namely acid tolerance and bile salt tolerance were
carried out as per the protocol developed by Clark et al. (1994) and Gillin and et al. (1984).

**RESULTS AND DISCUSSION**

**Enumeration of lactic acid bacteria from dahi samples:** The viable log count for lactococci in unbranded dahi samples revealed that the log count of Lactococci, *S. thermophilus*, Leuconostoc and Lactobacilli ranged from 4.80 to 6.00, 5.20 to 5.70, 5.16 to 5.60 and 5.39 to 6.11 respectively; whereas for the branded dahi the same viable log counts of range 5.20 to 6.00; 5.20 to 5.99; 5.14 to 5.70 and 5.14 to 5.60 respectively. It may be seen in Table 1 that log counts of lactobacilli were highest followed by lactococci. It is also observed that the LAB counts obtained in unbranded and branded dahi samples differed significantly (P≤0.05). These results are in agreement with by Mohanan et al. (1983), reported the predominance of lactobacilli ranging from 7x10^1 to 3x10^6 cfu/ml followed by streptococci of 0 to 2x10^6 cfu/ml in 15 domestic dahi samples collected from Bengaluru. While, Pradeep (2007) found predominance of viable lactococci followed by lactobacilli and leuconostoc among four domestic dahi samples collected from Bengaluru. A similar study by Rajasekhar et al. (2013) recorded the predominance of leuconostocs of 4.54 (log_{10} cfu/g) followed by lactobacilli of 4.20, lactococci of 3.71 and streptococci of 3.22 in domestic dahi samples from Bengaluru market.

**Isolation & phenotypic identity of lactic acid bacterial isolates from dahi samples:** A total of 24 LAB isolates (Table 2) were picked from the respective selective agar media. Out of 24 lactic isolates, 8 were having circular, smooth colonies from NRCLA plates incubated at 30°C, they were considered as lactococci; while other 8 isolates looking smooth, creamy from NRCLA plates incubated at 37°C were streptococci. Opaque or transparent, glistening colonies of 2 isolates picked up from MRS (Mann Rogosa Sharpe) plate incubated at 30°C were leuconostoc and subsurface, oval shaped colonies of 6 isolates from MRS agar plates incubated at 37°C were lactobacilli. During the first transfer of colonies from the plates to the respective broths, 3 of lactococcal isolates and 6 of streptococcal isolates showed very faint growth and hence not used in further study. The lactic acid bacterial isolates obtained from dahi samples were coded as LC1 to LC5; ST 1 to ST 2; LEU 1 to LEU2 and LB1 to LB6 for lactococcal, streptococcal, leuconostoc and lactobacilli isolates respectively and were phenotypically characterized as lactococci, *S. thermophilus*, leuconostoc and lactobacilli.

**Growth and acid production of the isolates in milk:** All the isolates were incubated in milk to assess their ability to grow and produce acid. These isolates, 5 of lactococci, 2 of streptococci, 2 of

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**Table 1:** Enumeration of log counts of lactic acid bacteria from Unbranded and branded dahi samples of Bengaluru market

<table>
<thead>
<tr>
<th>Codes of dahi samples</th>
<th>Lactococi</th>
<th><em>S. thermophilus</em></th>
<th>Leuconostoc</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>UD1</td>
<td>6.00</td>
<td>5.70</td>
<td>5.60</td>
<td>6.11</td>
</tr>
<tr>
<td>UD2</td>
<td>4.80</td>
<td>5.70</td>
<td>5.60</td>
<td>5.66</td>
</tr>
<tr>
<td>UD3</td>
<td>5.44</td>
<td>5.20</td>
<td>5.16</td>
<td>5.40</td>
</tr>
<tr>
<td>UD4</td>
<td>5.44</td>
<td>5.49</td>
<td>5.30</td>
<td>5.39</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>6.0000^A</strong></td>
<td><strong>4.8000^C</strong></td>
<td><strong>5.4400^H</strong></td>
<td><strong>5.4400^H</strong></td>
</tr>
<tr>
<td><strong>F-value</strong></td>
<td><strong>35.72</strong></td>
<td><strong>5.56</strong></td>
<td><strong>4.87</strong></td>
<td><strong>16.90</strong></td>
</tr>
<tr>
<td>BD5</td>
<td>6.00</td>
<td>5.80</td>
<td>5.70</td>
<td>5.60</td>
</tr>
<tr>
<td>BD6</td>
<td>5.70</td>
<td>5.99</td>
<td>5.30</td>
<td>5.14</td>
</tr>
<tr>
<td>BD7</td>
<td>5.20</td>
<td>5.60</td>
<td>5.40</td>
<td>5.50</td>
</tr>
<tr>
<td>BD8</td>
<td>5.54</td>
<td>5.20</td>
<td>5.14</td>
<td>5.30</td>
</tr>
<tr>
<td><strong>Mean value</strong></td>
<td><strong>6.0000^H</strong></td>
<td><strong>5.7000^A</strong></td>
<td><strong>5.2000^H</strong></td>
<td><strong>5.5400^A</strong></td>
</tr>
<tr>
<td><strong>F-value</strong></td>
<td><strong>11.07</strong></td>
<td><strong>11.67</strong></td>
<td><strong>5.54</strong></td>
<td><strong>4.21</strong></td>
</tr>
</tbody>
</table>

Note: UD: unbranded; BD: branded; CD: Critical Difference.
leuconostoc and 6 of lactobacilli isolates, curdled the milk within 8 h with titratable acidity of 0.52 to 1.12% LA and Direct Microscopic Count (DMC) ranged between 7.30 and 8.30 log<sub>10</sub> cfu/ml. A study by Deepa (2011) showed that <i>S. thermophilus</i> (6 isolates) set the heat treated whole milk in 8 hrs., produced lactic acid of 0.67 – 0.69% with DMC of 8.67-8.69 log<sub>10</sub>/g; while Rajasekhar <i>et al</i> (2013), found that sterile skim milk inoculated with wild strains of <i>Lactobacillus</i> isolates produced more lactic acid (0.50 – 0.68%) and DMC (7.41 – 8.17 log<sub>10</sub>/g) among the 8 lactic isolates obtained from the samples of unbranded dahi.

**Genotypic identification of Lactic acid bacteria isolates:** After the phenotypic identification of all the four purified LAB isolates were named as <i>L. lactis</i> ssp. <i>lactis</i> (LC1 to LC4) and <i>Lactococcus lactis</i> ssp. <i>diacetylactis</i> LC5; streptococcal isolates of <i>S. thermophilus</i> as ST1 and ST2; Leuconostoc isolates of <i>Leuconostoc mesenteroides</i> ssp. <i>lacits</i> as LEU1 and LEU2; lactobacillus of <i>L. fermentum</i> as LB1 to LB6 and the same identification was confirmed through genotyping as shown in Table 2.

**Acid and bile tolerance of the lactic acid bacterial isolates obtained from dahi samples:**

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Acid tolerance</th>
<th>Bile tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcal isolate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LC2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LC3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LC4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LC5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ST1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ST2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leuconostoc isolates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEU1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LEU2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacilli isolates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LB2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LB3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LB4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LB5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LB6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: ‘+’ Growth of culture; ‘-’ No growth
Among probiotic isolates, LEU1 showed statistically significant difference in acid tolerance when compared with LC1, ST1 and LB4. With respect to bile tolerance, ST1 and LEU1 showed statistically significant difference when compared with LC1 and LB4.

**CONCLUSION**

Dahi samples collected from Bengaluru showed the presence of lactic acid bacteria and some isolates of LAB possessed probiotic property. This study clearly demonstrated that field samples of dahi do possess probiotic strains of lactic cultures; but, their counts in branded and unbranded dahi samples were less than the standards prescribed. Further the study also revealed that these probiotic strains survive under field conditions and their presence in field samples suggest the stability of these strains and consumers may be benefitted by consuming such probiotic dahi samples.

**REFERENCES**


Efficacy of Doramectin in Rabbit Affected with Scabies—A Case Report

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Received: 21 May 2015; Accepted: 01 August 2015

ABSTRACT

*Sarcoptes scabiei* infestation in rabbits results in crusted lesions on nose, ear pinna, lips and legs. Routine treatment schedule involves usage of gamma BHC and benzyl benzoate topically or injecting ivermectin once in a week for 3 weeks. In the present case 6 rabbits were infested with scabies and all were treated with single dose of doramectin. Complete recovery was seen in 4 weeks after the treatment. The present case suggests the usage of doramectin as first line of therapy against scabies in rabbits.

**Keywords:** Scabies, Rabbits, Doramectin and Single dose.

In fur bearing animals like rabbits, dermatological problems are a common occurrence. In adult rabbits as well as in young bunnies ear mite and mange infestation had been reported as one of the common skin diseases (Siegmund, 1979). *Sarcoptic scabiei* infestation is a very common and wide spread problem in rabbit colonies caused by *Sarcoptic scabiei var cuniculis*. Rabbits affected by scabies have crusty lesion on face, ear pinna, legs and they suffer from weight loss. As scabies in rabbitry is an important zoonotic disease, it should be treated immediately (Aiello et al., 1998; Soulsby, 1982). Avermectin group of drugs are indicated for treating scabies. The avermectin drug includes ivermectin, doramectin, eprinomectin and salamectin. Doramectin has been reported as the safe and effective drug for treating scabies in rabbits (Jayakumar et al., 1999). In the present study, six rabbits of a backyard rabbitry suffering from scabies were treated with doramectin. Efficacy of doramectin in treating scabies affecting rabbits under field condition is reported in the present paper.

Six rabbits belonging to a farmer of Beechagondanahalli village, Hassan taluk, Hassan district, Karnataka raised in backyard were presented at Veterinary Dispensary, Anathi with the history of itching since 5-10 days. Clinical examination revealed slight alopecia along with encrusted lesions on nose, lips, face and edges of ear pinna (Fig. 1). Three of them were anorectic since 2 days. Skin scrapings from the lesions were collected and examined under microscope. One to two mites per microscopic field was observed. Based on clinical history, distribution pattern of lesions, clinical observation and detection of sarcoptes mite in skin scrapings, it was confirmed as *Sarcoptic scabiei var cuniculis* infestation.

All the rabbits were administered with single dose of doramectin at the dose rate of 1mg/kg body weight subcutaneously. After 5-7 days, crusts fell off and healthy reddish skin was noted in affected areas. Complete recovery was seen four weeks after the treatment (Fig. 2). Further, absence of mites upon microscopic examination of skin scrapings of recovered rabbits confirmed the recovery. No adverse effects had been noticed.

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Fig. 2: Rabbit after recovery

Single dose of doramectin injection was found convenient to administer under field condition when compared to repeated applications required with topical preparations like gamma Benzenehexachloride and benzyl benzoate. Indiscriminate use of these drugs may cause damage in terms of residual effect (Dakshinkar and Sarode, 2000). Single dose of doramectin was found more efficient in treating scabies over ivermectin which needs repeated administration. Doramectin has longer duration of action than ivermectin (Toutain et al., 1997). Though the approved dose of doramectin is 200µg, dose rate of 1mg/kg body weight (Jayakumar et al., 1999) was found effective without producing any toxic effect.

Since doramectin do not cross blood brain barrier, it has a low mammalian toxicity (Brander et al., 1991). Considering the outcome of the present report, doramectin can be considered as the first line of therapy in treating scabies in rabbits.

REFERENCES
Treatment of Sarcoptic Mange in a Crossbred HF Cow with Ivermectin and Topical Herbal Preparation

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ABSTRACT
An adult crossbred HF cow with severe itching, hairfall, alopecic skin lesion around the eyes, neck, shoulder and head was presented to Teaching Veterinary Clinical Complex, Veterinary College, Hassan. The examination of skin scrapings from the affected areas revealed Sarcoptic scabies mites. The cow was successfully treated with parenteral Ivermectin and application of topical herbal paste of lemon, garlic and turmeric.

Key words: Mange, Sarcoptes scabiei, Ivermectin, Herbal paste

The epidermal mite, Sarcoptes scabiei var bovis, causes sarcoptic mange in cattle and infests other livestock like sheep, pigs etc. and also humans. It is contagious and disease transmission occurs directly from animal-to-animal. Peak outbreaks are observed in late winter and early spring. The affected animals suffer from intense pruritis with initial appearance of erythema and red papules and later followed by vigorous scratching, biting and rubbing the affected parts which results in thinning and broken hairs (Kazmi et al. 2009). Further self inflicted trauma leads to invasion with secondary bacteria. Other skin lesions like keratinization, thickening of skin, exudation and alopecia of the affected areas are also observed (Lavenge and Smith 1983). The disease results in weight loss, reduction in milk production and weakness. Diagnosis is mainly based on examination of skin scrapings for Sarcoptic mites.

A six year old cross bred Holstein Freshen cow was presented to Teaching Veterinary Clinical Complex, Veterinary College, Hassan with a history of severe itching, hair fall and thickened scaly skin. Clinical examination revealed scaly, crusty and focal alopecic skin lesions around the eyes, face, neck, shoulder and head. Skin was also found wrinkled and thickened with plaques of keratinous crusts in the neck region.

Blood sample and deep skin scrapings from edges of skin lesions were collected from the cow and subjected to haemato-biochemical (Jain, 1996) and microscopic examinations respectively as per the standard lab procedures. Based on the observation of mites under microscopy which were small, roughly circular with short legs especially the third and fourth pairs which did not extend beyond the body margin and triangular scales on surface, the cow was diagnosed with Sarcoptes scabiei infestation. The cow was treated with Inj. Ivermectin (Hitek) @ 0.2 mg/kg b wt., SC, weekly for 2 weeks and topical herbal paste consisting of lemon, garlic and turmeric for 15 days.

The clinical findings in the present study are in agreement with Kazmi et al. (2009) and Lavenge and Smith (1983). The haematological evaluation revealed slightly decreased TLC values (7000/cumm) with neutropenia (8 %) and eosinophilia (12%). The eosinophilia might be due to parasitic infestation, allergic based inflammation (Kazmi et al. 2009) and antigen antibody reactions in the skin. The blood biochemical evaluation revealed low total plasma proteins (5.5 gm/dl), albumin (1.8 gm/dl) and higher globulin (3.7 gm/dl) values. The decrease in total protein might be due to dietary protein deficiency, due to reduced feed intake and intense pruritis. The elevation in the globulins might be due to antigenic stimulation observed in chronic inflammatory conditions. The present observations are in agreement with Ramaprabhu et al. (2001).

On treatment with Ivermectin and application of herbal paste of lemon, garlic and turmeric, the pruritis was reduced by 4th day and good clinical recovery of cow on healing of skin
lesions was observed within 15 days. Lavenge and Smith (1983) and Soll et al. (1987) successfully treated sarcoptic mange in cattle on using Ivermectin. Dustur (1960) and Nadkarni (1976) reported acaricidal effect of lemon and turmeric respectively on ticks and mites. Dwivedi and Sharma (1985 and 1986) reported the miticidal properties of garlic and successfully treated sarcoptic mange in pigs using similar topical herbal paste consisting of garlic, onion, lemon extracts, turmeric powder and camphor in kharanj oil.

The parenteral Ivermectin and application of topical herbal paste of lemon, garlic and turmeric was found to be effective in the treatment of Sarcoptic mange in cattle.

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Dystocia due to Dicephalus Monster Condition in HF Crossbred Cow -
A Case Report

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ABSTRACT
A case of dicephalus monster reported in a HF crossbred cow was presented to the Veterinary Dispensary, Gorur, Hassan District of Karnataka state. The calf was a fully grown dead male monster with dicephalus condition. The common finding with dicephalus was both the heads were facing in opposite direction and were continued to a single neck leading to single thorax, two forelimbs (dibrachius), two hind limbs and one tail (unicaudatus).

Keywords: Dicephalus monster, dystocia, HF crossbred cow

Dystocia is a major cause of economic loss to the dairy farmer. There are many reasons for dystocia. Fetal anomalies, monstrosities are common cause of dystocia in bovines. The prolonged dystocia is dangerous for the survival of the cow due to complications like rupture and hemorrhages of uterus (Vidya Sagar et al., 2011).

Congenital defect present at birth may affect a single structure or function, or entire system, or part of several systems (Marrow, 1980). Duplication of cranial part of the foetus is more common than the caudal parts (Roberts, 1971). These duplications may arise during the primitive streak elongation or regression (Noden and De Lahunta, 1984). Monstroglia in bovine often leads to dystocia and caesarian section as the most common sequelae (Sharma, 2006). Here is an uncommon case of dicephalus, biaatlanticus, tetraopthalmus, and unicaudatus.

CASE HISTORY AND CLINICAL OBSERVATIONS
A full term HF crossbred cow was presented to the Veterinary Dispensory Gorur, Hassan District of Karnataka state, with a history of labor pain since 24 hours. The cow was alert with moist muzzle and healthy body condition. The rectal temperature was recorded as 100° F. The reddish and slightly foul smelling discharge was noticed from the vulva. Fetal forelimbs protruded from the vulva. Vulva was edematous and vaginal mucus membrane was congested. Per-vaginal examination revealed that the foetus was in anterior longitudinal presentation. The heads were found to be normal in their rostral portion but caudally both heads were attached together (Fig 1). On postmortem examination, the monster calf was found to be attached at the atlanto-occipital joint. A hypertrophy of heads was observed. In the thorax, congested lungs were observed. Diaphragm was fully developed. The stomach, intestine, kidney and gall bladder were observed normal.

DISCUSSION
Considering the severity of the case and lack of space within the vagina and vestibule, the caesarean section was performed for delivery of the foetus. The dead male monster foetus was delivered through the caesarean section. The monster had two heads (dicephalus), each head having separate nostrils, two eyes (tetraopthalmus) and two ears. The heads articulated with two atlas bones (biaatlanticus) free from each other but fused at their caudal part to articulate with a single cervical
vertebra (Fig 2). Similar findings were reported in buffalo by Raju *et al.* (2000).

**Fig.2 Morphologic view of the heads**

Dystocia due to dicephalus monostomus in a crossbred cow has also been reported by Nakhashi *et al.* (2006). In the present case the two heads were continued to a single neck leading to single thorax, two forelimbs (dibrachius), two hind limbs and one tail (unicaudatus).

The genetic and/or environmental factors affect development during different fetal stages. The embryo is resistant to agents that can cause congenital malformations (teratogens) up to 14th day of gestation in cattle i.e. period of attachment which becomes highly susceptible to teratogens. Subsequently, susceptibility decreases as the critical periods of the formation of various organs are passed; the foetus becomes increasingly resistant to teratogenic factors during later stages of development. During the first weeks of embryogenesis, a teratogen can be lethal, but if the embryo survives, it will not necessarily have malformations. After the period of maximum susceptibility, a teratogen can interfere with growth but does not directly affect organogenesis (Niebyl and Simpson, 2008).

**Possible Etiological Factors:** Conjoined twins may be caused by a number of factors, being influenced by genetic and environmental conditions. It is believed that these factors are responsible for the failure of twins to separate after the 13th day after fertilization (Srivastva *et al.*, 2008). Jones and Hunt (1983) stated that many congenital anomalies are essentially unknown; however, the important known causes are prenatal infection with a virus, poisons ingested by mother, vitamin deficiency (Vitamin A and folic acid), genetic factors and/or combination of these factors. Assisted conception techniques such as In Vitro Fertilization and Intracytoplasmic sperm injection (ICSI) may be a factor (Romero *et al*., 1988).

Based on the gross anatomical findings, this malformation was classified as dicephalus. As in this case, embryonic duplications are of great importance since they are usually associated with dystocia and reproductive wastage (Dennis, 1975). Embryonic duplications have been most commonly reported in cattle (1 in every 100,000 births), but less commonly in other ruminants (Roberts, 1986). The specific etiology is usually not known, but genetic factors or environmental factors or both have been implicated. Toxic plants, trace elements, infectious agents and physical agents such as hyperthermia, undue pressure during rectal palpation in early pregnancy and irradiation have also been identified as teratogens in the cow (Saperstein, 2002). Other possible etiological agents include nutritional deficiencies, endocrine disturbances, extremes of temperature during pregnancy, drugs and chemicals (Smolec *et al*., 2010). The formation of a normal foetus is dependent on complex intracellular, intercellular and tissue temporal-spatial interactions. In cases where no teratogenic agent is implicated, abnormal development may be due to failure of gene control, failure of cellular and tissue interactions or local environmental effects on gene expression (Smolec *et al*., 2010). The etiology of the abnormality in present case could not be ascertained, but it is speculated to be due to environmental factors, toxic plants or infectious agents (or a combination of any of these) since the cow was allowed to graze freely before and during pregnancy.

**Embryological Basis:** This is a very rare condition and accounts for 1-2% of monozygotic twins. The embryonic disk starts to differentiate on the 13th day. If the split occurs after day 13, then the twins will share body parts in addition to sharing their chorion and amnion (Finberg, 1994). However, some studies provide convincing evidence that they all result from the secondary union of two
originally separate monovular embryonic discs. This fusion theory seems to be confirmed by the adjustments to union and the pattern and incidence of specific anomalies at the proposed sites of conjunction. No theoretical fission of the vertebrate embryo at any stage of development, in any plane, in any direction can explain the selection of the observed sites of fusion, the details of the union, or the limitation to the specific areas in which the twins are found to be jointly separate monovular embryonic discs. (Fernando, 1993).

The maternal effect genes expressed in the mother's ovaries produce messenger RNAs that encode transcriptional and translational regulatory proteins. Two of these proteins, Bicoid and Hunchback, regulate the patterning of anterior structures head and thorax, while another pair of maternally specified proteins, Nanos and Caudal, regulates the formation of the posterior parts of the embryo. The Bicoid and Hunchback proteins act synergistically at the enhancers of these "head genes" to promote their transcription. Driever et al. (1989) predicted that at least one other anterior gene besides hunchback must be activated by Bicoid. The low levels of Bicoid and absence of Hunchback proteins may lead to malformations of the head.

Siamese twins or double monsters in human have always been a subject of curiosity and mystery for the general public. The present case highlights morphological features of an antenatal undiagnosed Dicephalus Dipus Conjoined Twins, a rare form of conjoined twins. Indirectly emphasizing the importance of careful antenatal sonographic assessment in all monochorionic, monoamniotic twin pregnancies to rule out conjoined twins (Sethi et al., 2004).

Early prenatal diagnosis and precise characterization of conjoined twins are essential for optimal obstetric, interventional and postnatal management as well as to reduce financial burden and psychological trauma to the owners.

Since there is no recorded history about the mother of the calf and due to the inability to detect a causative agent, it is not possible to ascertain the cause of this anomaly. With the limited information available, causes of this sporadic case cannot be determined.

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Mange Infestation in Pigs and its Successful Treatment

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ABSTRACT
Sarcoptic mange is the most common external parasite of pigs world-wide. Around 6 pigs which showed signs of skin infection like frequent head shaking, severe rubbing of the skin against the sides of the pen, presence of thick asbestos like lesions on the ear, along the sides and top of the neck, elbows and front parts of the hocks at Instructional Livestock Farm Complex, Veterinary college, Hassan were examined and diagnosed as Sarcoptic Mange based on skin scrapping examination. There was also decrease in feed intake, restlessness and the affected pigs had poor skin condition. The treatment was initiated with Doramectin injection @ 0.3 mg/kg b.wt twice at an interval of 10 days by i/m route. All the pigs showed drastic improvement in skin condition after 2 injections of doramectin.

Key Words: Sarcoptic Mange, Pigs, Doramectin

Mange is a parasitic disease of skin caused by one of the two mites either Sarcoptes scabiei or Demodex phylloides. Sarcoptic mange is caused by Sarcoptes scabiei var suis which is the most common external parasite of pigs worldwide. It affects pigs of any breed and age. Infestations with mites are technically called as acariosis or acariasis, both in animals and humans. There are species-specific strains that attack cattle, sheep, goats and also humans. As a general rule, pig mange mites are not contagious for cattle, sheep, or humans and vice-versa. As all mite species, pig mange mites also spend their whole life on the host. The mites dig tunnels beneath the skin. Their saliva has potent digestive enzymes that dissolve the skin tissues and feed on the resulting liquids. They do not suck blood. Sarcoptic scabei var suis is the most important because it is irritant and uncomfortable for the pig, causing it to rub and damage the skin. It significantly depresses growth rate and feed efficiency. Mange can result in a reduction of 5 to 10% in feed conversion efficiency and weight gain (Australian Pig Health Monitoring User Guide). Pigs affected with sarcoptic mange at Instructional Livestock Farm Complex, Veterinary College Hassan and its successful treatment is hereby discussed.

CASE HISTORY AND CLINICAL SIGNS
Around six pigs aged about 2-3 years were showing signs of dermatitis at Instructional livestock Farm complex, Veterinary College, Hassan. Upon clinical examination all the affected pigs showed similar symptoms of skin infection like frequent head shaking, severe rubbing of the skin against the sides of the pen, hair are often shed away leaving bare patches and thick asbestos like lesions on the ear, along the sides and top of the neck, elbows and front parts of the hocks. There was also decrease in feed intake, restlessness and the affected pigs had poor skin condition.

DIAGNOSIS
Based on the above clinical signs the disease was diagnosed as Mange infestation in pigs and was confirmed by skin scrapping examination. Skin scrappings were collected from inner aspect of the ear and processed by both direct and indirect method. Sarcoptic mange was clearly visible in both direct and indirect skin scrapping examinations. Smets and Vercruysse (2000) reported that detection of mites in pig ears seems to be useful in diagnosis of scabies. Other diseases that might be confused with mange include greasy pig disease, swine pox and sun burn.

TREATMENT AND DISCUSSION
All affected 6 pigs were injected with Doramectin injection ((Dectomax®, Pfizer inc., New York, USA) @ 0.3 mg/kg b.wt twice at an interval of 10 days by intramuscular route. The affected pigs started showing improvement in the skin lesions after 1\textsuperscript{st} injection of doramectin itself, but not completely recovered. So the 2\textsuperscript{nd} injection of doramectin was given on 10\textsuperscript{th} day. The pigs were re-examined by clinical examination and also by skin scrapping examination on 17\textsuperscript{th} day and on 24\textsuperscript{th} day respectively. All the pigs showed complete
improvement in the skin texture and the skin scrappings were also negative by 24th day post treatment. Two injections of doramectin at 10 days apart have cleared the mites completely in all the six pigs. The recovered animals had better feed intake. Jacobson et al., (2000) reported that it is possible to eliminate Sarcoptes scabieii from the herd with one single injection of doramectin. In our case two injections of doramectin at 10 days interval were given since the lesions were not completely recovered after 1st injection of doramectin. Further Arends et al. (1999) reported that doramectin has longer duration of action than ivermectin. 

Mange is widespread across the countries with up to 60% of national herds affected. The mite spreads directly from pig to pig, either by close skin contact or contact with recently contaminated surfaces. The boar helps to maintain infection in the herd because he is constantly in direct skin contact with breeding females and he remains a chronic carrier. If pigs are housed in groups there is increased opportunity for spread of mange. The mite dies out quickly away from the pig, under most farm conditions, in less than five days. This is an important factor in control. If a herd is free from mange, it is one of the easiest diseases to keep out because it can only be introduced by carrier pigs. However, once it is introduced it tends to become permanently endemic unless control measures are taken. Pigs in poor health and hygienic conditions are more prone to develop the disease. Consequently, a first measure to prevent the appearance of pig mange is to keep the animals well fed and in good health. 

*S scabiei suis* infestation is negatively correlated with daily weight gain and feed conversion in pigs. Davies (1995) found that sarcoptic mange was associated with increased pruritic behaviour and the growth rate tended to be slower in mange infested pigs. The lesions usually start on the head, especially the ears, and then spread over the body, tail and legs. Itching is usually intense and associated with a hypersensitivity reaction to the mites. As the hypersensitivity subsides, usually after several months, the thickened, rough, dry skin is covered with grayish crusts and thrown into large folds. Pigs affected by itching often shake their heads and rub intensively against whatever objects around them, which causes hair loss and sometimes also bleeding injuries that can become infected with secondary bacteria. 

Due to the major economic impact of sarcoptic mange on the pig industry, local, regional, and national eradication programs have been developed. These are very cost effective and typically include 2 injections of ivermectin or doramectin (300 μg/kg,) which give good results. Sarcoptic mites are very host specific, meaning they prefer to live on pigs, and they do not survive away from the host. For this reason, the main method of spread is by direct pig-to-pig contact. Mites can infest pigs all year round but may spread more readily in the winter months when pigs come in close contact to keep warm. On the other hand, signs of irritation may be greater in warmer months because at higher temperatures mites are more active. Mange infestation in pigs is not self-limiting. Older pigs such as breeding sows often have few clinical signs but can still carry mites that provide a source of infestation to younger stock. Doramectin is a pain-free, oil-based formulation. It has longer period of activity means that Doramectin treated pigs are protected for longer duration and the opportunity for spread of infection is greatly reduced. Furthermore, acaricides only kill adult, larval and nymphal stages not eggs. As we know that eggs hatch within 3 to 10 days of being deposited, pigs treated with short-acting acaricides will readily get self-reinfected if these eggs hatch beyond the protection date. Doramectin, however, maintains effective blood levels for longer duration, so that when the eggs hatch the larvae are readily killed. (Arends J.J et al., 1999).
REFERENCES


Foreign bodies in the airways or intestines of dogs or cats are a common problem in companion animal practice. These items may be a pet’s or child’s toy, strings, leashes, clothing and sticks, including human food products like bones or trash. Improved endoscopic techniques, together with their greater availability made often possible to remove the foreign body endoscopically, avoiding unnecessary surgery. Endoscopic foreign body removal is associated with a low complication rate (Gianella et al., 2009). Flexible endoscopy is the initial procedure of choice for removing most types of gastric foreign bodies. Benefits of this approach includes, less invasive and less expensive than a gastrotomy (Tams 1999). There is no pain and no extensive surgical procedure.

CASE HISTORY
A four month old nondescript male dog was presented to Teaching Veterinary Clinical Complex hospital, Veterinary College, Hassan for treatment of skin infection. The pet was treated for skin infection and the owner was advised to present the pet after one week. But about 15 minutes later the owner complained that his pet has swallowed the cap of the vial which was in the hospital premises. Upon palpation of oesophageal region a small mass was felt and the pet also showed some discomfort and started coughing (Fig). The case was tentatively diagnosed as ingestion of foreign body (cap of an injection vial).

DIAGNOSIS AND TREATMENT
After confirming foreign body ingestion based on the history and palpation of a small mass in the oesophageal region, immediately it was decided to induce vomition. For that Xylazine was administered @ 1 mg/kg b.wt i/m. After 3-4 minutes the pet vomited but no cap or any foreign body was detected in the vomitus. Then the pet was subjected for radiography.
of cervical, thoracic and abdominal region which revealed no foreign body either in oesophageal or abdominal region since the ingested material was plastic and radiolucent. Therefore, endoscopic examination was contemplated. The pet was anaesthetized with Profofol @ 6 mg/kg b.wt i/v and positioned in left lateral recumbency and slowly endoscope was inserted via mouth into the oesophagus. In the oesophagus, blue coloured vial cap was clearly visible. The oropharynx and oesophagus were normal and there was no evidence of trauma. Because of lot of mucus in the oesophagus and movement of the probe during endoscopic examination the vial cap has moved further downwards into the stomach. The endoscope was passed into the stomach where the vial cap was present and the cap was held with endoscopic forceps and removed. The pet was prescribed with cimetidine and Sucralfate for 2 days. The owner reported that the pet was taking food and water normally and recovered uneventfully.

**DISCUSSION**

Foreign bodies in the gastro-intestinal tract of dogs or cats are a common problem in companion animal practice. Foreign bodies occur when pets consume items like child’s toy, strings, leashes, clothing, sticks or any other that fails to pass, including human food products like bones or trash. The problems caused by ingested material vary with, the type of material, shape, the duration of stay inside the body and also the location and degree of obstruction. Some ingested items, like older pennies or lead material, can cause systemic toxicities while others may cause regional damage to the gastro-intestinal tract. In the present case, the foreign body was cap of a vial lodged in the oesophagus. Initially, an attempt was made to retrieve the foreign body from the oesophagus endoscopically but as there was lot of mucus in oesophagus and movement of endoscope the foreign body moved into the stomach. The endoscope was passed into the stomach and the foreign body was grasped with the help of endoscopic forceps and slowly removed through oesophagus and mouth. Jankowski et al., (2013) reported the best diagnostic method for detection of foreign bodies in the oesophagus was esophagoscopy. Endoscopy is an essential tool not only for visualization of upper digestive tract but also for retrieval of the foreign body. Leib and Sartor (2008) reported esophageal foreign body obstruction caused by dental chew treats in 31 dogs. They also reported oral endoscopic removal of the foreign bodies was uncommon since most were pushed into the stomach. The foreign bodies located in the stomach may be retrieved with the use of an endoscope; however, many animals require surgical abdominal exploration and removal. In the present case, the endoscopic foreign body removal was attempted immediately because it was just a matter of time before it caused gastrointestinal distress like gastric lacerations, erosions, ulcers and peritonitis due to gastric perforation (Guilford, 1990). Flexible endoscopy is the initial procedure of choice for removing most types of gastric foreign bodies. Benefits of this approach include less invasive and less expensive procedure than a gastrotomy (Tams 1999). There is no pain and no surgical incision. Dogs of any age are susceptible to developing foreign body problems but this is most commonly seen in young dogs less than 2 years of age. These young dogs are naturally curious and enjoy chewing. The best way to prevent gastric foreign bodies is to prevent access to objects that could be swallowed. Thus a case of foreign body ingestion by pup and its removal endoscopically was successful, without giving way for further gastrointestinal damage or surgical interventions.

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Effect of Varying Levels of Metabolizable Energy on the Growth Performance of Giriraja Birds

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ABSTRACT
A study was conducted to evaluate the effect of varying levels of energy on the growth performance of Giriraja birds. A total of 400 day old Giriraja chicks were randomly divided into five dietary groups with four replicates containing 20 chicks in each replicate on completely randomized design. The experiment lasted for 56 days. Five dietary treatments consisted of 2600, 2700, 2800, 2900 and 3000 Kcal ME/kg with constant protein level of 22 per cent during the starter phase and 2700, 2800, 2900, 3000 and 3100 Kcal ME/kg with constant protein level of 20 per cent during the finisher phase keeping BIS standards as control group in both phases. Results showed that varying levels of energy had no significant effect on body weight gain and survivability. The feed consumption progressively and significantly (P≤0.05) decreased as the level of energy increased from 2600 to 3000 Kcal ME/kg during starter period and from 2700 to 3100 Kcal ME/kg during finisher period. The dietary energy levels had significant effect on feed conversion ratio. Hence it can be concluded that the optimum energy requirement for Giriraja is 2800 Kcal ME/kg during starter phase and 2900 Kcal ME/kg during finisher phase which is in confirmation with BIS specifications.

Key words: Metabolizable energy, Giriraja, Protein, Performance, Bodyweight)

Nutrient requirements increase/ decrease proportionately with rate of growth, age of the bird, sex and genotype (breed/strain). These recommendations may not be optimum for scavenging birds such as Giriraja, Swarnadharana, Vanaraja etc. since these birds differ in their growth rate compared to commercial broilers / layers. The present study was conducted to determine the optimum energy requirement of Giriraja chicks reared under intensive system for eight weeks to optimize the level of metabolizable energy requirement for Giriraja birds till 8 weeks of age.

Four hundred day old straight run Giriraja chicks from a single hatch were wing banded for identification, weighed and randomly distributed to five treatment groups with four replicates and 20 birds in each replicate. Basal diet (control) was formulated as per BIS (1992) standards. Treatment group diets were formulated using basal diet with varying levels of metabolizable energy, keeping protein requirement as constant. Data on weekly body weight, feed intake and FCR were analyzed statistically according to the methods described by Snedecor and Cochran (1989) by one way analysis of variance (ANOVA) using GRAPHPAD PRISM 5.01 statistical software.

Body weight gain: The results of the present study indicated that dietary energy levels evaluated in this experiment had no significant (P<0.05) influence on body weight gain of Giriraja chicks during eight weeks of age (Tables I). Energy contents in experimental diets did not significantly affect body weight gain of the chicks. However, body weight gain was slightly improved with higher level of energy. Present observations are in agreement with the findings of Sheikh et al. (2008) in Vanaraja birds who found no difference in body weight gain between groups fed with different energy levels upto six weeks of age. On the contrary,

Feed consumption: The feed intake per week per bird progressively and significantly decreased as the level of energy was increased from 2600 to 3000 Kcal ME/kg during starter period and from 2700 to 3100 Kcal ME/kg during finisher period except on second and seventh week. At the end of eighth week of age, the mean value of cumulative feed consumption of the birds under T1
At the end of eight weeks of age, the mean value of cumulative feed conversion ratio under different treatments ranged from 1.66 in T1 (3100Kcal/kg) to 2.02 in T5 (3100Kcal/kg). There was no significant difference between T1 and T3 and also among control, T4 and T5. In this study, 2600 and 2700 Kcal ME/kg diet had poor feed efficiency while 2800 Kcal ME/kg resulted in better feed efficiency during starter period and 2700 and 2800 Kcal ME/kg diet had poor feed efficiency while 2900 Kcal ME/kg resulted in better feed efficiency during finisher period. The data obtained support the findings of Sheikh et al. (2008) in Vanaraja birds who found significant difference in feed conversion ratio when fed with different levels of energy.

**Survivability**: The results of the study revealed good survivability of birds and had no significant (P<0.05) effect among different energy levels.
CONCLUSIONS
From these result, it can be concluded that the optimum energy requirement for Giriraja bird is 2800 Kcal ME/kg during starter phase and 2900 Kcal ME/kg during finisher phase.

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Immune Status and Carcass Characteristics in Giriraja Birds as Influenced by Different Levels of Metabolizable Energy


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ABSTRACT

A study was conducted to evaluate the effect of varying levels of energy on the growth performance of Giriraja birds. A total of 400 day old Giriraja chicks were randomly divided into five dietary groups with four replicates containing 20 chicks in each replicate on randomized complete block design. Five dietary treatments consisted of 2600, 2700, 2800, 2900 and 3000 Kcal ME/kg with constant protein level of 22 per cent during the starter phase and 2700, 2800, 2900, 3000 and 3100 Kcal ME/kg with constant protein level of 20 per cent during the finisher phase keeping BIS standards as control group in both phases. Results showed that varying levels of energy had no significant effect on proximate composition of breast and thigh meat (moisture, total ash, crude protein and ether extract), cholesterol percentage, immune status (ND and IBD) and carcass characteristics of Giriraja birds. The amount of fat deposition in abdominal area was significantly (P≤0.05) reduced by reducing the dietary energy content.

Key words: Metabolizable energy, Giriraja, Immune status, Cholesterol, Abdominal fat
of Giriraja birds (Table II). Present observations are in agreement with the Mansour and Atena (2010).

**Table I: Effect of varying levels of energy on proximate composition of thigh meat in Giriraja birds**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proximate composition of thigh meat&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Cholesterol&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Abdominal fat percentage&lt;sup&gt;NS&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture (±)</td>
<td>Total ash (±)</td>
<td>Crude protein (±)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>74.09 ± 0.39</td>
<td>2.28 ± 0.06</td>
<td>19.34 ± 0.10</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>74.85 ± 0.54</td>
<td>2.13 ± 0.04</td>
<td>18.73 ± 0.27</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>74.50 ± 0.46</td>
<td>2.19 ± 0.04</td>
<td>19.10 ± 0.03</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>74.07 ± 0.53</td>
<td>2.22 ± 0.04</td>
<td>19.10 ± 0.03</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>73.86 ± 0.46</td>
<td>2.30 ± 0.03</td>
<td>19.22 ± 0.11</td>
</tr>
</tbody>
</table>

NS=Non- significant

**Table II: Effect of varying levels of energy on proximate composition of breast meat and cholesterol percentage in tissues in Giriraja**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proximate composition of breast meat&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Cholesterol&lt;sup&gt;NS&lt;/sup&gt;</th>
<th>Abdominal fat percentage&lt;sup&gt;NS&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture (±)</td>
<td>Total ash (±)</td>
<td>Crude protein (±)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>72.97 ± 0.39</td>
<td>2.41 ± 0.06</td>
<td>22.50 ± 0.31</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>73.47 ± 0.33</td>
<td>2.47 ± 0.04</td>
<td>22.10 ± 0.23</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>72.20 ± 0.55</td>
<td>2.44 ± 0.03</td>
<td>22.34 ± 0.14</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>72.20 ± 0.39</td>
<td>2.45 ± 0.03</td>
<td>22.98 ± 0.30</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>73.13 ± 0.19</td>
<td>2.31 ± 0.03</td>
<td>22.37 ± 0.14</td>
</tr>
</tbody>
</table>

NS=Non- significant

**Immunological parameters- HI titer against ND and Antibody titer against IBD virus:** The results of present study revealed that there was no significant effect of varying levels of energy on HI titer against ND and IBDV antibody titer of Giriraja birds. The results of the present study are in agreement with the findings of Dehury et al. (2007) in Vanaraja birds.

**Carcass characteristics- Dressing percentage and Visceral organs weight (giblets):** The results of the present study showed that there was no significant effect of varying levels of energy on dressing percentage of Giriraja birds. The findings of the present study are in agreement with the findings of Leeson et al. (1996), Zamana et al. (2008) who reported that the abdominal fat pad decreased significantly with a decrease in diet energy level. Raju et al. (2004) also found similar effect in naked neck birds when fed with different energy levels. The results of the present study confirmed the positive relationship between dietary energy and abdominal fat deposition which is in agreement with Yalcin et al. (1998).

**CONCLUSIONS**

From the results observed in the present study, it is concluded that different levels of energy do not have any significant effect on proximate composition of breast and thigh meat, immune status and carcass characteristics of Giriraja birds, whereas the fat deposition in abdominal area was
significantly ($P\leq0.05$) reduced by reducing the dietary energy content.

REFERENCES


Soup is a liquid food made by cooking vegetable, meat etc. together in a stock or water. Soup made from skimmed bone broth is considered as an important convalescent food. The thickeners used in soup would provide the desired consistency/viscosity along with improved nutritive value to soup. Ruales et al. (1988) described the use of maize starch in dry soup mixes. Optionally modified maize starch can be used as a thickener in many foods including tomato sauce (Wurzburg and Fergason, 1984). Lindemann and Reinhold (1980) replaced 75% of the wheat flour used as a thickener by corn starch.

Fresh whey, shanks and other ingredients viz. spice and condiment mixture, common salt, monosodium glutamate were procured. Properly descaled, chopped chicken shank pieces of approximately 2 cm length were pressure cooked along with whey at 1:4 ratio at 15 psi pressure for 30 minutes followed by filtration to get shank-whey extract. The extract was mixed conventionally with other ingredients viz. spice mix, condiment mixture, common salt, monosodium glutamate (MSG) at the rate of 0.2, 2.0, 1.0, and 0.1 per cent w/v of bone extract respectively and then simmered in a stainless steel container for 1.5 to 2 minutes to get the shank-whey soup (Control, C). The extract was additionally thickened with rice flour at 2 (T1), 4 (T2) & 6 (T3) per cent w/v level before cooking. All soup samples are subjected to physico-chemical and sensory properties.

The shank-whey soup was analyzed for content protein, total solids, fat and ash (AOAC, 1995), lactose (IS: 1479, 1961) and titratable acidity (IS: SP: 18, 1981). The pH of soup was measured. Sensory evaluation performed based on 7-point hedonic scale. Wherein 7 denoted “extremely desirable” and 1 denoted “extremely undesirable” (Keeton, 1983). The statistical design of this study was CRD- One way classification with 4 treatments with 3 replicates. The data were analyzed as per the methods described by Snedecor and Cochran (1989).

The mean ± SE values for various physico-chemical and sensory parameters of chicken shank-whey soup are presented in Table.

Rice flour as thickener linearly increased the content of total solids, protein and consistency of soup, whereas decreased the lactose content, meat flavor intensity of soup. The pH, titratable acidity, ash and ether extract content of soup non significantly changed. The shank-whey soup incorporated with 2 per cent (w/v) rice flour found good.

Key words: shank–whey soup, rice, thickener

Quality of Shank-Whey Soup Thickened with Rice Flour

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ABSTRACT
An attempt was made to prepare the chicken shank-whey soup employing rice flour as thickener at the rate of 0, 2, 4 and 6 per cent w/v of shank-whey extract. The increased concentration of thickener linearly increased the content of total solids, protein and consistency of soup, whereas decreased the lactose content, meat flavor intensity of soup. The pH, titratable acidity, ash and ether extract content of soup non significantly changed. The shank-whey soup incorporated with 2 per cent (w/v) rice flour found good.

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2Assistant Prof., Livestock Products Technology, Veterinary College, Vinobhanagar, Shimoga-577 204
Table: Effect of rice flour as thickener on physico-chemical and sensory properties of shank-whey soup

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>Titratable acidity</th>
<th>Lactose</th>
<th>Total Solids</th>
<th>Protein</th>
<th>Ash</th>
<th>Ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.58</td>
<td>± 0.02</td>
<td>0.13</td>
<td>± 0.02</td>
<td>2.52</td>
<td>± 0.29</td>
<td>7.24</td>
</tr>
<tr>
<td>T1</td>
<td>5.62</td>
<td>± 0.04</td>
<td>0.12</td>
<td>± 0.01</td>
<td>2.51</td>
<td>± 0.29</td>
<td>8.99</td>
</tr>
<tr>
<td>T2</td>
<td>5.66</td>
<td>± 0.05</td>
<td>0.12</td>
<td>± 0.01</td>
<td>2.32</td>
<td>± 0.20</td>
<td>9.08</td>
</tr>
<tr>
<td>T3</td>
<td>5.68</td>
<td>± 0.05</td>
<td>0.12</td>
<td>± 0.01</td>
<td>2.25</td>
<td>± 0.23</td>
<td>10.75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensory attributes (Mean±SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour &amp; appearance</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>T1</td>
</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
</tbody>
</table>

*Means with different superscripts in a column differ significantly (P<0.05)

C (Control), T1 (2 per cent rice), T2 (4 per cent rice) and T3 (6 per cent rice).

Chidanandaiah (1999). The overall acceptability has also decreased at 6 per cent (w/v) level thickener incorporation. According to Lacchiramani (1979), chicken soups prepared by using chicken shank in water by pressure cooking method was rich in taste and flavours. It could be concluded that the shank- whey soup with acceptable quality can be prepared by incorporation of rice flour as thickener at the rate of 2 per cent (w/v) level.

REFERENCES


