



|                  |   |
|------------------|---|
| Title            | Antioxidant status, metabolic profile and immune response of lambs supplemented with tannin rich Ficus infectoria leaf meal |
| Author(s)        | Dey, Avijit; Dutta, Narayan; Pattanaik, Ashok Kumar; Sharma, Kusumakar  |
| Citation         | Japanese Journal of Veterinary Research, 63(1), 15-24   |
| Issue Date       | 2015-02   |
| DOI              | 10.14943/jjvr.63.1.15   |
| Doc URL          | <a href="http://hdl.handle.net/2115/58141">http://hdl.handle.net/2115/58141</a>   |
| Type             | bulletin (article)  |
| File Information | JJVR_63.1_02 AvijitDey.pdf  |



[Instructions for use](#)

## Antioxidant status, metabolic profile and immune response of lambs supplemented with tannin rich *Ficus infectoria* leaf meal

Avijit Dey<sup>1\*</sup>, Narayan Dutta<sup>1</sup>, Ashok Kumar Pattanaik<sup>1</sup> and Kusumakar Sharma<sup>1</sup>

<sup>1</sup>Indian Veterinary Research Institute, Izatnagar- 243 122, Uttar Pradesh, India

Received for publication, September 8, 2014; accepted, December 3, 2014

### Abstract

To study the effect of supplementation of tanniferous tree leaves *Ficus infectoria* on antioxidant status and immune response, twenty four lambs were randomly divided into four groups of six each in a completely randomized design and fed either a conventional supplement (CON) or experimental supplements (FILM-I, FILM-II and FILM-III) containing 1.0, 1.5 and 2.0% condensed tannins (CT), respectively by replacement of wheat bran of supplement CON with *Ficus infectoria* leaf meal (FILM). Blood biochemical profile was monitored in all lambs at 0, 45, 90, 135, 180 days of feeding. Although haemato-biochemical parameters remained similar, there was significant ( $p < 0.05$ ) improvement in catalase activity, total thiol and protein thiol groups with reduction in lipid peroxidation (LPO) in lambs fed FILM diet irrespective of levels. However, intracellular status of reduced glutathione, and superoxide dismutase activity was improved ( $p < 0.05$ ) only in FILM-II and FILM-III supplemented lambs. The cell-mediated immune response was significantly ( $p < 0.05$ ) improved in all the lambs fed FILM supplemented diets. Improved antioxidant status and immunity in FILM supplemented lambs increased voluntary feed intake irrespective of level. However, the average daily gain for a period of 180 d showed a significant ( $p < 0.05$ ) increase by the supplementation of FILM-II diet containing 1.5% CT. The present study reveals that the supplementation of *Ficus infectoria* leaf meal up to 21.2% in the concentrate mixture could improve the antioxidant status and immunity in lambs. However, as feed efficiency was reduced at higher levels due to presence of CT, 15.9% supplementation containing 1.5% condensed tannins in concentrate mixture is suggested to improve the health and production performance of lambs.

Key Words: Antioxidant, *Ficus infectoria*, Immunity, Condensed tannins, Lambs

\*Corresponding author: Avijit Dey (Senior Scientist), Central Institute for Research on Buffaloes, Sirsa Road, Hisar- 125 001, Haryana, India

Phone: +91-9728-334810. Fax: +01662-275004. E-mail: avijitcirb@gmail.com

doi: 10.14943/jjvr.63.1.15

## Introduction

The use of plant bio-actives for improvement of animal health is an area of increasing research importance due to public concern regarding the use of pharmaceuticals in the animal industry. A particular area of criticism has been in the use of antibiotics as growth promoters and the associated risk of developing antibiotic resistance in human pathogens<sup>4</sup>. This increasing trend has led to a closer examination of plants for animal health. The banning of feed antibiotics by European Union in 2006 has pushed to search antibiotics from plants; however, reports are scanty on their effects on ruminant health. The focus of bioactive effect of plants is mainly concentrated on ruminal microflora for modulation of rumen fermentation. Very petite study has been done for their consequence on health parameters, including antioxidative and immune stimulating effects. Type of bioactive compounds of a plant and their quantity also varies with the source, maturity and season.

Phenolic compounds have been known to possess free radical scavenging and anti-inflammatory activities in addition to their association with reduced risk of certain types of diseases. Tannins are naturally occurring plant poly phenolic compounds of high molecular weight containing sufficient phenolic hydroxyl groups. The potential of condensed tannins (CT) as biological antioxidants have been indicated in many *in vitro* studies<sup>12,16,19</sup>. *Ficus infectoria* is an evergreen tree hugely grown in northern parts of India. They are generally planted for shed and not used as fodder tree. The main bioactive compound in the leaves is condensed tannins<sup>7</sup> with the presence of alkaloids, phytosterol and flavonoids<sup>3</sup>. However, no investigation has been carried out into the effect of supplementation of *F. infectoria* leaves to antioxidant protection and immune response. This study, therefore, sought to determine the effect of supplementing *F. infectoria* leaf meal on antioxidant status, metabolic profile, immune response and growth

rate in lambs.

## Materials and methods

*Location of Experiment:* The experiment was conducted at the Animal Nutrition Research Shed of Indian Veterinary Research Institute (IVRI), Izatnagar in Uttar Pradesh Province of India. It is located at 170 m above sea level (28°22' latitude north and 79°24' longitudes east) in the Northern Upper Gangetic Plain, having an annual rainfall of 900–1200 mm. It is the region of deepest soil in India with hardly any variation in relief and suitable for growing various types of subtropical crops and trees. Sugarcane, wheat and rice being the main cultivated crops, cereal straws form the basal diet of the ruminants in this area.

*Experimental animals and diets:* Twenty four, 6 months old non-descript lambs ( $11.73 \pm 0.22$  kg) were divided into four groups of six each and allocated to four dietary treatments in a completely randomized design. The lambs were treated with broad-spectrum anthelmintic (Albendazole suspension @10 mg/ kg body weight) at the onset of the study and thereafter at three months intervals. After shearing, the lambs were also treated for ectoparasites and penned individually with free access to fresh water in well ventilated shed. Four iso-nitrogenous supplements (CON, FILM-I, FILM-II and FILM-III) were formulated containing 0, 1.0, 1.5 and 2.0% CT, respectively supplied through dried and ground *Ficus infectoria* leaf meal (FILM) and fed to the lambs of respective group with a basal diet of wheat straw to meet their requirements for maintenance and growth (50 g per day) as recommended by Kears<sup>14</sup>. A small quantity of green fodder (100 g green oats/ maize) was also offered to take care of vitamin A requirement of lambs.

*Ficus infectoria* leaves were harvested in one lot in the month of July from the IVRI campus. The leaves were dried and ground in an electric

grinder before mixing in the supplements. Dried and ground leaves of *Ficus infectoria* were incorporated in graded proportion to the concentrate mixture by replacing wheat bran to bring CT content to 0, 1.0, 1.5 and 2.0% of supplements on dry matter (DM) basis (Table 1). The amount of supplements was adjusted fortnightly as per the body weight changes of each animal.

**Blood sample collection and processing:** The blood samples were collected via jugular vein puncture in the morning before feeding and watering of each animal at 0, 45, 90, 135 and 180 days of post feeding. Serum was separated and preserved at  $-20^{\circ}\text{C}$  for analyses of blood biochemical parameters. Another 2 ml blood sample was collected in vials containing ethylene diamine tetra-acetate @ 1 mg/ ml blood, for haemoglobin (Hb) and packed cell volume (PCV) estimation.

Antioxidant profile was measured following collection of blood samples in 15 ml calibrated tube containing anticoagulant, acid citrate dextrose (1.5 ml/10 ml blood) and centrifuged at 2000 rpm for 15 min at  $4^{\circ}\text{C}$  with separation of plasma and buffy coat. The erythrocyte pellet (packed RBC) was washed with phosphate buffer saline (PBS) solution (NaCl, 137 mM; KCl, 2.7 mM;  $\text{Na}_2\text{HPO}_4$ , 10 mM and  $\text{KH}_2\text{PO}_4$ , 1.8 mM, pH adjusted to 7.4). The packed RBC was mixed with an equal volume of PBS to form RBC suspension. Haemolysate (1 : 20 dilution) was prepared<sup>40)</sup> by mixing 0.5 ml RBC suspension with 4.5 ml of stabilizing solution (EDTA, 2.7 mM and 0.7 mM, 2-marcaptoethanol).

**Immune response:** The effect of dietary treatments on the cell mediated immune (CMI)

**Table 1. Ingredients, chemical composition and tannin fractions of supplements, *Ficus infectoria* leaves and wheat straw**

| Attributes                                   | Supplements* |        |         |          | <i>Ficus infectoria</i> | Wheat straw |
|--|--------------|--------|---------|----------|-------------------------|-------------|
|  | CON          | FILM-I | FILM-II | FILM-III |                         |             |
| <i>Ingredients (kg/100 kg)</i>               |              |        |         |          |                         |             |
| Maize grain                                  | 25.0         | 25.0   | 25.0    | 25.0     | —                       | —           |
| Deoiled groundnut cake                       | 35.0         | 35.0   | 35.0    | 35.0     | —                       | —           |
| Wheat bran                                   | 38.0         | 27.4   | 22.1    | 16.8     | —                       | —           |
| <i>F. infectoria</i> leaves                  | —            | 10.6   | 15.9    | 21.2     | —                       | —           |
| Mineral mixture**                            | 1.0          | 1.0    | 1.0     | 1.0      | —                       | —           |
| Common salt                                  | 1.0          | 1.0    | 1.0     | 1.0      | —                       | —           |
| <i>Chemical composition (% DM)</i>           |              |        |         |          |                         |             |
| Organic Matter                               | 93.4         | 93.4   | 93.4    | 93.5     | 89.7                    | 94.0        |
| Crude Protein                                | 25.1         | 25.5   | 25.2    | 25.0     | 13.4                    | 3.4         |
| Ether Extracts                               | 2.4          | 2.5    | 2.5     | 2.6      | 4.1                     | 1.2         |
| Total Ash                                    | 6.6          | 6.6    | 6.6     | 6.5      | 10.3                    | 6.0         |
| Neutral Detergent Fibre                      | 27.6         | 31.2   | 32.7    | 33.2     | 45.9                    | 81.1        |
| Acid Detergent Fibre                         | 12.0         | 15.7   | 16.0    | 16.8     | 37.1                    | 53.2        |
| <i>Phenolics and tannin fractions (% DM)</i> |              |        |         |          |                         |             |
| TPh <sup>λ</sup>                             | —            | 2.1    | 3.1     | 4.1      | 19.4                    | —           |
| NTPh <sup>λ</sup>                            | —            | 0.6    | 0.9     | 1.2      | 5.5                     | —           |
| TTPh <sup>λ</sup>                            | —            | 1.5    | 2.2     | 3.0      | 13.9                    | —           |
| CT <sup>¶</sup>                              | —            | 1.0    | 1.5     | 2.0      | 9.4                     | —           |

\*CON: Control supplement, FILM-I: 1% CT containing supplement, FILM-II: 1.5% CT containing supplement, FILM-III: 2% CT containing supplement.

\*\*Mineral mixture contained (g/kg): calcium 215, phosphorus 95, sodium chloride 285, potassium iodide 2.5, iron 5.0, copper 0.8, cobalt 1.0, manganese 1.0 and sulphur 1.0

<sup>λ</sup>as tannic acid equivalent, <sup>¶</sup>as leucocyanidine equivalent

TPh-Total phenolics; NTPh- Non tannin phenolics; TTPh-Total tannin phenolics; CT-Condensed tannins

response was assessed through *in vivo* delayed type of hypersensitivity reaction against phytohaemagglutinin-P (PHA-P). A stock solution of PHA-P was prepared in phosphate buffer saline at a concentration of 160 mg/ml and filtered through membrane filter. It was suitably diluted so as to provide 20 mg PHA-P per inoculum in a volume of 125  $\mu$ l (5 units in insulin syringe). The skin area to be tested (both sides of neck region) were cleaned and shaved with the help of a razor 24 h prior, so as to facilitate subsiding of any inflammation due to abrasion. An area of about one square cm was encircled with a marker pen, on both sides of the neck region.

The thickness of the skin was measured with the help of a vernier caliper, which would represent the basal (0 h) value. Following this, the calculated volume of PHA-P was injected intradermally to the marked site. The thickness of the skin was subsequently measured at 24 h interval up to 96 h post-inoculation.

*Live weight changes:* The feeding trial was carried out for 201 days duration including the first 21 days for adaptation and subsequent 180 days for measurement. The daily allowance of the supplements was offered in single meals (at 9:30 am) in the morning; green fodder and wheat straw was then offered *ad libitum*, when all the lambs had consumed the concentrate. The left straw residues were weighed 24 h post-feeding to ascertain daily feed consumption. Daily DM intake and fortnightly live weight of all the lambs were recorded before feeding in the morning throughout the study.

*Chemical and Statistical analysis:* Feeds and residue samples were milled to pass through a 1 mm sieve and analyzed for their proximate constituents<sup>2)</sup>. The fibre fractions, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated according to the methods of Van Soest et al<sup>34)</sup>. The total phenolics, tannin phenolics, and condensed tannins content in *F. infectoria* leaves was estimated by the methods

described by Makkar<sup>18)</sup>. Haemoglobin and PCV were estimated in whole blood immediately after collection by acid haematin method<sup>5)</sup> and Wintrobe's tube<sup>11)</sup>, respectively. The serum glucose<sup>13)</sup>, total protein<sup>39)</sup>, albumin<sup>8)</sup>, aspartate aminotransferase and alanine aminotransferase<sup>28)</sup> were estimated as per standard methods.

The antioxidant enzymes in the RBC were examined to assess the antioxidant status of the animals. The superoxide dismutase (SOD) activity was assessed as mM of MTT {3-(4-5 dimethyl thiazol 2-yl) 2,5 diphenyl tetrazolium bromide} formazon formed<sup>17)</sup> and lipid peroxidation (LPO) was determined by estimating the concentration of malonaldehyde (MDA) in haemoglobin<sup>24)</sup>. The reduced glutathione (GSH) and catalase (CAT) were estimated by Dithio-bis-2 nitro benzoic acid (DTNB) method of Prins and Loos<sup>25)</sup> and Bergmeyer<sup>6)</sup>, respectively. Total thiol (T-SH), non-protein thiol (NP-SH) and protein thiol (P-SH) groups in the erythrocytes was estimated following the method of Sedlak and Lindsay<sup>29)</sup>.

The data obtained were subjected to analysis of variance using the General Linear Models Procedures of the SPSS 17.0 software and treatment means were ranked using Duncan's multiple range tests according to Snedecor and Cochran<sup>32)</sup>.

## Results

### *Chemical composition and tannin fractions of leaves and supplements*

*Ficus infectoria* leaves contained 19.4% total phenolics, out of which tannin phenolics was 13.9% (Table 1). Most of the tannins present in the leaves was CT (9.4%). The leaves are also a good source of biomass (39.2% DM) and protein (13.4% CP).

The composition of the supplements with leaves of *F. infectoria* (FILM) and without leaves (CON) was comparable, except higher fibre fractions in FILM as compared to CON.

### Metabolic profile

The Hb and PCV, serum glucose, serum proteins and serum enzyme levels of experimental lambs during entire experiment are shown in Table 2. Mean Hb (g/dl) and PCV (%) levels under different treatments ranged from 11.2–11.7 and 33.9–34.7, respectively without any significant ( $p > 0.05$ ) difference in lambs irrespective of dietary treatments. There was also no significant ( $p > 0.05$ ) difference in concentration of serum glucose level, total proteins, albumin, globulin, albumin-globulin (A : G) ratio, alanine amino transferase (ALT) and aspartate amino transferase (AST) of lambs kept under various dietary treatments.

### Antioxidant status

The levels of GSH, CAT and SOD increased significantly ( $p < 0.05$ ) in FILM supplemented animals as compared to CON (Table 3). The extent of LPO as measured in terms of malondialdehyde (nmol MDA/ g Hb) production was reduced significantly ( $p < 0.05$ ) with increased level of FILM in the diet. The T-SH and P-SH were increased with the supplementation of leaves in the diet; however, there was no effect on NP-SH.

### Immune response

Animals under all the four dietary treatments exhibited an increase in skin thickness following the intradermal injection of PHA-P (Table 3). The mean absolute values for skin thickness were significantly ( $p < 0.05$ ) increased in FILM supplemented animals relative to CON. However, there was no significant difference among the lambs fed graded levels of FILM in the diet.

### Voluntary feed intake and live weight changes

The total dry matter intake (DMI) ( $\text{g/kg W}^{0.75}$ ) by lambs was significantly ( $p < 0.05$ ) higher for animals on dietary treatment FILM-II followed by FILM-I and FILM-III (Table 4). The initial live weight of all the lambs was comparable irrespective of dietary treatments, however, lambs fed FILM-II recorded significantly higher ( $p < 0.05$ ) average final body weight (kg) relative to their counterparts given diet CON and FILM-III. Similarly, the total body weight gain (kg) for the period of 180 days and average daily gain (ADG) for lambs under the treatment FILM-II was significantly ( $p < 0.05$ ) higher (Table 4) as compared to their counterparts kept on other dietary treatments. Feed conversion ratio (FCR) ( $\text{kg DMI/ kg gain}$ ) was comparable irrespective of dietary treatment except for

**Table 2. Effect of graded levels of condensed tannins through *Ficus infectoria* leaf meal (FILM) on metabolic profile in lambs**

| Attributes           | Treatments <sup>¶</sup> |             |             |             |
|----------------------|-------------------------|-------------|-------------|-------------|
|                      | CON                     | FILM-I      | FILM-II     | FILM-III    |
| Hb (g/dl)            | 11.4 ± 0.22             | 11.2 ± 0.26 | 11.7 ± 0.27 | 11.6 ± 0.21 |
| PCV (%)              | 34.2 ± 0.40             | 34.3 ± 0.49 | 33.9 ± 0.47 | 34.7 ± 0.41 |
| Glucose (mg/dl)      | 54.6 ± 0.81             | 55.4 ± 0.74 | 55.6 ± 0.71 | 55.0 ± 0.75 |
| Total protein (g/dl) | 5.6 ± 0.10              | 5.5 ± 0.09  | 5.4 ± 0.10  | 5.5 ± 0.10  |
| Albumin (g/dl)       | 3.1 ± 0.09              | 3.1 ± 0.07  | 3.1 ± 0.09  | 3.0 ± 0.08  |
| Globulins (g/dl)     | 2.4 ± 0.09              | 2.4 ± 0.09  | 2.3 ± 0.08  | 2.5 ± 0.09  |
| A : G ratio          | 1.4 ± 0.7               | 1.3 ± 0.06  | 1.4 ± 0.07  | 1.3 ± 0.06  |
| ALT (IU/ml)          | 15.5 ± 0.29             | 14.9 ± 0.62 | 14.5 ± 0.48 | 15.7 ± 0.57 |
| AST (IU/ml)          | 22.7 ± 0.35             | 21.1 ± 0.60 | 21.0 ± 0.63 | 22.4 ± 0.74 |

CON = Control diet; FILM-I = 1% Condensed Tannins diet; FILM-II = 1.5% Condensed Tannins diet; FILM-III = 2% Condensed Tannins diet; Hb = Haemoglobin; PCV = Packed cell volume; A : G ratio = Albumin globulin ratio; ALT = Alanine amino transferase; AST = Aspartate amino transferase.

<sup>¶</sup>Values (Mean ± SE) of 6 animals of each group

**Table 3. Erythrocytic antioxidant indices and delayed type of hypersensitivity (DTH) response (%) of lambs fed graded levels of *F. infectoria* leaf meal**

| Attributes  | Treatments <sup>ψ</sup>   |                           |                           |                           |
|---|---------------------------|---------------------------|---------------------------|---------------------------|
|   | CON                       | FILM-I                    | FILM-II                   | FILM-III                  |
| <i>Antioxidant indices</i>                                |                           |                           |                           |                           |
| Catalase (mK/g Hb)  | 9.5 <sup>a</sup> ± 0.24   | 10.1 <sup>b</sup> ± 0.26  | 10.7 <sup>b</sup> ± 0.21  | 10.4 <sup>b</sup> ± 0.23  |
| SOD (mmol MTT formazon formed/g Hb)                       | 1.5 <sup>a</sup> ± 0.15   | 1.7 <sup>ab</sup> ± 0.18  | 2.0 <sup>b</sup> ± 0.22   | 1.9 <sup>b</sup> ± 0.23   |
| LPO (nmol MDA/g Hb)                                       | 73.9 <sup>b</sup> ± 1.81  | 65.2 <sup>a</sup> ± 2.12  | 63.9 <sup>a</sup> ± 2.24  | 60.8 <sup>a</sup> ± 2.43  |
| GSH (µmol/g Hb)   | 9.7 <sup>a</sup> ± 0.53   | 10.8 <sup>a</sup> ± 0.71  | 11.6 <sup>b</sup> ± 0.62  | 11.8 <sup>b</sup> ± 0.74  |
| T-SH (µmol/ml PRBC)                                       | 40.1 <sup>a</sup> ± 1.61  | 46.0 <sup>b</sup> ± 1.23  | 46.1 <sup>b</sup> ± 1.11  | 48.3 <sup>b</sup> ± 1.42  |
| NP-SH (µmol/ml PRBC)                                      | 4.4 ± 0.26                | 4.4 ± 0.26                | 4.6 ± 0.27                | 4.6 ± 0.21                |
| P-SH (µmol/ml PRBC)                                       | 35.6 <sup>a</sup> ± 1.31  | 41.6 <sup>b</sup> ± 1.32  | 41.5 <sup>b</sup> ± 1.01  | 43.7 <sup>b</sup> ± 0.74  |
| <i>DTH response (%) in different hours of inoculation</i> |                           |                           |                           |                           |
| 0   | 100                       | 100                       | 100                       | 100                       |
| 24  | 152.7 ± 1.23              | 196.7 ± 1.12              | 184.2 ± 1.82              | 180.7 ± 1.46              |
| 48  | 133.2 ± 1.92              | 164.4 ± 1.51              | 146.3 ± 1.34              | 162.8 ± 1.62              |
| 72  | 125.6 ± 1.11              | 137.6 ± 1.16              | 135.6 ± 1.24              | 125.3 ± 1.47              |
| 96  | 114.9 ± 1.02              | 129.7 ± 0.94              | 124.5 ± 1.10              | 114.6 ± 1.26              |
| Treatment mean ± SE                                       | 125.3 <sup>a</sup> ± 3.86 | 145.7 <sup>b</sup> ± 6.76 | 138.1 <sup>b</sup> ± 6.63 | 136.7 <sup>b</sup> ± 6.63 |

SOD, Superoxide dismutase; LPO, Lipid per oxidation; GSH, Reduced glutathione; T-SH, Total thiol; NP-SH, Non-protein thiol; P-SH, Protein thiol.

<sup>a, b</sup>Mean bearing different superscript within a row differ significantly ( $p < 0.05$ )

<sup>ψ</sup>Values (Mean ± SE) of 6 animals of each group

significantly ( $p < 0.05$ ) lower FCR in lambs given FILM-II as compared to FILM-III.

## Discussion

The levels of total tannins and CT in *F. infectoria* leaves observed in the present study (Table 1) were within the range, however, the quantity was higher than the autumn collection and lower than the spring collection as reported by Singh et al<sup>31</sup>. The levels of tannins in plants vary greatly between species, within species, maturity, season, stages of development, location<sup>21</sup>. The chemical composition of concentrate mixture and wheat straw offered was within the normal range and comparable to values reported by earlier workers<sup>30,36</sup>. The higher NDF and ADF levels in treatment supplements could be attributed to the high cell wall constituents usually present in leaf meal<sup>1</sup>.

The normal values of haematological parameters<sup>26</sup> like Hb and PCV indicated

erythrocytic normalcy and general well beings of animals. An increased or decreased level of serum glucose level is an indicator of stress to the animals. However, in present study, similar glucose level indicated normal physiological condition of all the experimental animals. In contrary, Wang et al<sup>38</sup>) reported lower plasma glucose level in lactating ewes grazed on tanniferous *Lotus corniculatus* pasture, probably due to increased uptake of glucose from blood for milk lactose synthesis. Total serum proteins, albumin, globulin and A:G ratio remained similar for all the treatments, which clearly indicates that feeding of tanniferous *F. infectoria* leaf meal at low levels did not have any adverse effect on all these three parameters. The comparable ALT and AST levels observed in this study reflect no adverse effect of leaf meal supplementation on liver and muscles.

Among the oxygen radicals, hydroxyl radical is the most reactive and induces severe damages to the adjacent biomolecules. Phenolics are good candidates as antioxidants because of their

**Table 4. Effect of *F. infectoria* leaf meal supplementation on growth rate and feed intake in lambs**

| Attributes                                  | Treatments <sup>ψ</sup> |                         |                         |                          |
|---|-------------------------|-------------------------|-------------------------|--------------------------|
|   | CON                     | FILM-I                  | FILM-II                 | FILM-III                 |
| Body weight gain (kg in 180 days)           | 11.2 <sup>a</sup> ± 0.7 | 11.6 <sup>a</sup> ± 0.4 | 13.5 <sup>b</sup> ± 0.7 | 10.1 <sup>a</sup> ± 0.6  |
| Average daily gain (g)                      | 62.4 <sup>a</sup> ± 3.8 | 64.3 <sup>a</sup> ± 2.2 | 75.2 <sup>b</sup> ± 3.6 | 56.1 <sup>a</sup> ± 3.6  |
| Dry matter intake (g/kg W <sup>0.75</sup> ) | 67.0 <sup>a</sup> ± 0.6 | 68.5 <sup>b</sup> ± 0.6 | 70.2 <sup>c</sup> ± 0.5 | 69.3 <sup>bc</sup> ± 0.6 |
| Feed Conversion Ratio                       | 9.3 <sup>ab</sup> ± 0.6 | 9.5 <sup>ab</sup> ± 0.4 | 8.5 <sup>a</sup> ± 0.5  | 10.4 <sup>b</sup> ± 0.5  |

<sup>a, b, c</sup>Mean values with different superscript within a row differ significantly ( $p < 0.05$ )

<sup>ψ</sup>Values (Mean ± SE) of 6 animals of each group

favourable redox potentials and the relative stability of the aryloxy radical. Condensed tannins, because of high molecular weight and the proximity of many aromatic rings and hydroxyl groups, are more potent scavengers and sinks of hydroxyl and superoxide anion radicals in free form and also in complexes with proteins. FILM is rich in total tannin phenolics as well as CT (Table 1). Thus, phenolics of FILM could have improved the biological responses of lambs. Antioxidant enzymes play a significant role in body defense mechanism by scavenging reactive oxygen species. Higher intracellular values of GSH, SOD and CAT in leaf meal supplemented lambs are suggestive of higher endogenous antioxidant status<sup>10</sup>. High concentration of GSH present in mammalian cells protects erythrocytes from oxidative damage. The present study showed a significant ( $p < 0.05$ ) increase GSH level in lambs fed FILM-II and FILM-III diet, indicating higher antioxidant status. The higher ( $p < 0.05$ ) intra-cellular SOD and CAT activity of FILM supplemented lambs indicates that poly phenolic compounds, especially CT of *F. infectoria* leaves had stimulatory effect upon these enzymes. The results get support with the findings that proanthocyanidin A-1 component of tannins from *Vaccinium vitis-idaea* L had strong SOD activity<sup>12</sup>. SOD controls the potentially toxic superoxide radical ( $O_2^-$ ) in cell and dismutate it into hydrogen peroxide and catalase enzymolyzes it into water.

The increased ( $p < 0.05$ ) T-SH and P-SH in all FILM supplemented animals indicates better

antioxidant status. The water soluble thiol group protects biological membrane by scavenging free radicals and through enzymatic reactions within the cell<sup>22</sup>. Lipid peroxidation is known to be one of the reactions set into motion as a consequence of the formation of free radicals in cells and tissues. Membrane lipids are abundant in unsaturated fatty acids. Linoleic acid is especially the target of lipid peroxidation. In oxidative stress, polyunsaturated fatty acids in cells and tissues generate malondialdehyde and 4-hydroxyalkenals, products of lipid peroxidation. The decrease in LPO with increased level of FILM could be due to the poly phenolic compounds of leaf meal in reducing MDA formation. Tannin extract of *Terminalia catappa* was reported to have the ability to prevent LPO and modification of mitomycin C- induced clasto-genicity<sup>16</sup>.

The increased CMI response in all the FILM supplemented lambs could be attributed to the higher availability of essential amino acids (EAA) (viz. cysteine and methionine) at small intestine<sup>20</sup> due to protection of dietary proteins by CT present in leaf meal. A moderate concentration of CT (20–35 g/kg DM) in forage given to sheep has been reported to increase non-ammonia nitrogen flux to the small intestine, to increase the absorption of EAA<sup>35</sup>. In other way, bioactives present in leaf meal especially, CT may have some stimulatory effect to the immune system of the animals. Cellular integrity is very important for receiving and responding to messages needed to coordinate an immune response<sup>15,19</sup>. The increase in total thiol groups and antioxidant

enzymes (CAT, SOD and GSH) and decrease in LPO in FILM supplemented lambs might have improved cellular integrity so that CMI response was increased.

The voluntary feed intake by lambs during entire experimental period was normal<sup>9)</sup>, however; it was higher for FILM supplementation than CON. The present results are in agreement with the earlier observations that moderate levels (1-4%) of CT in the diet from various plant sources exerted stimulatory effect on feed intake<sup>27,33)</sup>. The supplementation of FILM was effective in enhancing ADG (g/d) and better FCR in FILM-II diet containing 1.5% CT. This gives an indication that the binding effects of tannins with feed protein were pronounced only at this level by supplying protein to the lower gastrointestinal tract and subsequent more efficient use for tissue growth<sup>23)</sup>. At appropriate concentration, the CT reduced the degradation of sulphur amino acids in the rumen, decreases the irreversible loss of cystine from plasma and increased the flow of cystine to body synthetic reaction<sup>37)</sup>.

The economics of feeding depends on feed intake, cost of feed as well as efficiency of utilization. The cost of FILM supplement was determined excluding the cost of leaves, as the farmers usually collect them free of cost deploying family labor. However, this is a labor intensive process involving collection, drying and grinding which may cost around INR 2/ kg DM (1 US\$ = 60 INR). Based on current market rate of ingredients, per kg cost of formulated supplements; CON, FILM-I, FILM-II and FILM-III were worked out to be INR 21.5, 20.2, 19.5 and 18.8, respectively. The feeding cost per kg live weight gain was 17% less in FILM-II supplement fed group as compared to control.

The present study reveals that the supplementation of *Ficus infectoria* leaf meal up to 21.2% in the concentrate mixture could improve the antioxidant status and immunity in lambs. As the leaf meal contains condensed tannins which could negatively affect the feed

utilization at higher levels, evident by lower feed efficiency, 15.9% supplementation containing 1.5% condensed tannins in concentrate mixture is suggested to improve the health and production performance of lambs.

### Acknowledgement

This study was financially supported by funds provided by the Indian Council of Agricultural Research, New Delhi, India.

### References

- 1) Anbarasu, C., Dutta, N., Sharma, K. and Rawat, M. 2004. Response of goats to partial replacement of dietary protein by a leaf meal mixture containing *Leucaena leucocephala*, *Morus alba* and *Tectona grandis*. *Small Rum. Res.*, **51**: 47-56
- 2) AOAC. 1995. *Official methods of analysis*, 16th ed., Vol. I, Association of Official Analytical Chemists. Washington, D. C.
- 3) Ashok Kumar, Jha, K. K., Dinesh Kumar, Agrawal, A. and Gupta, A. 2012. Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficus Infectoria* plant. *Pharma Innov.*, **1**: 71-76
- 4) Barton, M. D. 2000. Antibiotic use in animal feed and its impact on human health. *Nutr. Res. Rev.*, **13**: 279-299
- 5) Benjamin, M. M. 1985. *Outline of veterinary clinical pathology*, 3rd ed. Kalyani Publishers, New Delhi.
- 6) Bergmeyer, H. U. 1983. UV method of catalase assay. In: *Methods of enzymatic analysis*, vol. 3, Weinheim, Deerfield Beach, Florida, Bansal, pp. 273.
- 7) Dey, A., Dutta, N., Sharma, K. and Pattanaik, A. K. 2006. Evaluation of condensed tannins from tropical tree leaves and its impact on in vitro nitrogen degradability of groundnut cake. *Anim. Nutr. Feed Technol.*, **6**: 215-222.
- 8) Doumas, V. T., Watson, W. A. and Biggs, H. G. 1971. Albumin standards and the measurement of serum albumen with bromocresol green. *Clin. Chem. Acta*, **31**: 87-96.
- 9) Hadjipannayiotou, M., Louca, A. and Lawole, M. J. 1975. A note on the straw intake of

- sheep given supplements of urea-molases, soyabean meal, barley-urea or barley. *Anim. Prod.*, **20**: 429.
- 10) Han, B., Yoon, S., Su, J., Han, H. R., Wang, M., Qu, W. and Zhong, D. 2004. Effects of selenium, copper and magnesium on antioxidant enzymes and lipid per oxidation in bovines fluorosis. *Asian- Aust. J. Anim. Sci.*, **17**: 1695-1699.
  - 11) Hawk, P. B. 1965. *Hawk's Physiological Chemistry*, 14th ed. McGraw Hill Book Company, London.
  - 12) Ho, K. Y., Huang, J. S., Tsai, C. C., Lin, T. C., Hsu, Y. F. and Lin, C. C. 1999. Antioxidant activity of tannin component from vaccinium vitis-idaea L. *J. Pharm. Pharmacol.*, **51**: 1075-1078
  - 13) Hultmann, E. 1959. Rapid specific method for determination aldoheoses (Aldorac chandes) in body fluid. *Nature*, **103**: 108-109.
  - 14) Kearl, L. C. 1982. *Nutrient requirements of ruminants in developing countries*. Int. Feed Stuffs Inst., Utah Agric Exp Station, Utah State University, Logan, Utah- 84322, USA.
  - 15) Latshaw, J. D. 1991. Nutrition-mechanism of immuno suppression. *Vet. Immun. Immunopathol.*, **30**: 111-120
  - 16) Lin, C. C., Hsu, Y. F. and Lin, T. C. 2001. Antioxidant and free radical scavenging effects of the tannins of Terminalia catappa L. *Anticancer Res.*, **21**: 237-243.
  - 17) Madesh, M. and Balasubramanian, K. A. 1998. Micro titre plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian J. Biochem. Biophys.*, **35**: 184-188.
  - 18) Makkar, H. P. S. 2000. *Quantification of tannins in tree foliage*. A laboratory manual for the FAO/IAEA co-ordinated research project on "use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage", Joint FAO/IAEA working document, IAEA, Viena.
  - 19) Middleton, E. 1998. Effect of plant flavanoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.*, **439**: 175-182.
  - 20) Min, B. R., Fernandez, J. M., Barry, T. N., McNabb, W. C. and Kemp, P. D. 2001. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in ewes during autumn. *Anim. Feed Sci. Technol.*, **92**: 185-202.
  - 21) Morris, P. and Robbins, M. P. 1997. Manipulating condensed tannins in forage legumes. In: *Biotechnology and the improvement of forage legumes*, pp. 147-173, McKersie, B. D. and Brown, D. C. W. eds., CAB International.
  - 22) Moscio, A., Bufano, G., Sevi, A., Manchisi, A., Martemucci, G., Casamassima, D., Djajanegara, A. and Sukmawati, A. 1994. Effect of slaughtering age and dietary energy level on productive performance of fattening lambs. Sustainable Animal Production and Environment. Proceedings of the 7th AAAP Animal Science Congress, Bali, Indonesia, 11th to 16th July, 1994, volume 3, pp. 75-76.
  - 23) Ngwa, A. T., Nsahlai, I. V. and Iji, P. A. 2002. Effect of supplementing veld hay with a dry meal or silage from pods of *Acacia sieberiana* with or without wheat bran on voluntary intake, digestibility, excretion of purine derivatives, nitrogen utilization and weight gain in South African Merino sheep. *Livest. Prod. Sci.*, **77**: 253-264.
  - 24) Placer, Z. A., Cushman, L. L. and Johnson, B. 1966. Estimation of product of lipid per oxidation (malonaldehyde) in biochemical system. *Anal. Biochem.*, **16**: 359-364.
  - 25) Prins, A. and Loos, G. 1969. *Biochemical methods in red cell genetics*. Academic Press, pp. 127-129.
  - 26) Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchcliff, K. W. 2003. *Veterinary Medicine—A text book of the diseases of cattle, sheep, pig, goats and horses*. 9th ed. Book Power, China.
  - 27) Ramirez-Restrepo, C. A., Barry, T. N., Lopez-Villalobos, N., Kemp, P. D. and McNabb, W. C. 2004. Use of *Lotus Corniculatus* containing condensed tannins to increase lamb and wool production under commercial dry land and farming conditions without the use of anthelmintics. *Anim. Feed Sci. Technol.*, **117**: 85-105.
  - 28) Reitman, S. and Frankel, S. 1957. Determination of serum glutamic-pyruvate transaminase by colorimetric methods. *Am. J. Clin. Path.*, **28**: 57.
  - 29) Sedlak, J. and Lindsay, R. 1968. Estimation of total protein bound and non-protein sulphhydryl groups in tissue with Ellman's reagent. *Biophys. Acta*, **25**: 92-105.
  - 30) Sharma, K., Dutta, N. and Naulia, U. 2004. An on-farm appraisal of feeding urea treated straw to buffaloes during late pregnancy and lactation in a mixed farming system. *Livst. Res. Rural Dev.*, **16**: 1-7.
  - 31) Singh, B., Chaudhary, L. C., Agarwal, N. and Kamra, D. N. 2011. Effect of feeding *Ficus infectoria* leaves on rumen microbial

- profile and nutrient utilization in goats. *Asian-Aus. J. Anim. Sci.*, **24**: 810-817.
- 32) Snedecor, G. W. and Cochran, W. G. 1994. *Statistical methods*. 8th ed. East West Press Pvt. Ltd., New Delhi.
- 33) Terrill, T. H., Douglas, G. B., Foote, A. G., Purchas, R. W., Wilson, G. F. and Barry, T. N. 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *J. Agric. Sci. Camb.*, **119**: 265-273.
- 34) Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, **74**: 3583-3597.
- 35) Waghorn, G. C., Shelton, I. D., McNabb, W. C. and McCutcheon, S. N. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *J. Agric. Sci. Camb.*, **123**: 109-119.
- 36) Wales, W. J., Doyle, P. T. and Pearce, G. T. 1990. The feeding value of cereal straws for Sheep. I. Wheat straws. *Anim. Feed Sci. Technol.*, **29**: 1-14.
- 37) Wang, Y., Waghorn, G. C., Barry, T. N. and Sheltons, I. D. 1994. The effect of condensed tannins in *Lotus corniculatus* on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. *Br. J. Nutr.*, **72**: 923-935.
- 38) Wang, Y., Douglas, G. B., Waghorn, G. C., Barry, T. N. and Foote, A. G. 1996. Effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. *J. Agric. Sci. Camb.*, **126**: 353-362.
- 39) Wotton, I. D. P. 1964. *Microanalysis in medical biochemistry*. 4th ed. G & A Churchill Ltd, London, pp. 138-140.
- 40) Yagi, Y., Furunchi, S., Takashi, H. and Koyama, H. 1989. Abnormality of osmotic fragility and morphological disorder of bovine erythrocytes infected with *Theileria sargonti*. *Jpn. J. Vet. Sci.*, **51**: 389-395.