Indian Journal of Animal Sciences **85** (8): 897–901, August 2015/Article

Effect of azolla based complete pellet feed on growth, nutrient utilization, blood metabolites and rumen fermentation in Barbari goats

RAVINDRA KUMAR[1], P TRIPATHI[2], U B CHAUDHARY[3], M K TRIPATHI[4] and R K SINGH[5]

ICAR-Central Institute for Research on Goats, Makhdoom, Uttar Pradesh 281 122 India

Received: 24 July 2014; Accepted: 18 March 2015

ABSTRACT

Present study was conducted to examine the effect of replacing concentrate mixture with sun-dried azolla on growth, nutrient utilization, blood and rumen metabolites in growing Barbari goats in completely randomized design. Male Barbari goats (12) were divided into 2 groups, control and experimental, consisting of 6 animals in each group. The control group was fed with complete pellet feed having Bengal gram straw and concentrate mixture in 60:40 ratio, experimental group was fed with complete pellet feed in which 25% of concentrate mixture was replaced with sun-dried azolla. The duration of experimental feeding was 8 weeks. Weekly body weights were recorded to assess growth rate of goats. After 6 weeks of experimental feeding a metabolism trial of 6 days duration was conducted to determine nutrients digestibility and nitrogen balance. Blood and rumen liquor samples were collected at the end of experimental feeding. The average daily gain (g) of control group (56.60) and experimental group (78.12) was statistically similar. The goat of control and experimental group had statistically similar daily dry matter intake. The digestibility (%) of dry matter, organic matter, ether extract and total carbohydrate was statistically similar to control and experimental group of goat (52.41, 55.46, 69.72 and 57.46 respectively). Crude protein digestibility was significantly higher in treatment group (77.43) as compared to control (71.14) group of goats. The digestibility (%) of different fibre fractions (neutral detergent fibre, acid detergent fibre), cellulose and hemicellulose were similar in treatment group. Animals of both the groups were in positive balance of nitrogen. There was no statistically significant effect replacement of concentrate mixture with sun-dried azolla on hematology and blood biochemical metabolites (hemoglobin, total protein, albumin, globulin, triglycerides and total cholesterol) of goats. The pH of rumen fluid was 6.30 for control group and 6.25 for experimental group of goats. Total volatile fatty acids (mmol/100ml) were statistically similar in control (15.46) and treatment (16.70) groups of goats. There was no significant difference in nitrogenous fractions (mg/dl), total nitrogen, TCA-ppt nitrogen and non protein nitrogen of rumen fluid collected at the end of experimental feeding of control and treatment group of goats. From present study it can be concluded that sun-dried azolla can replace 25% of concentrate mixture in the complete pellet feed of growing Barbari goats without any adverse effect on growth, nutrient utilization, blood and rumen fermentation parameters.

Key words*:* Azolla, Barbari goats, Blood metabolites, Nutrient utilization, Rumen metabolites

Majority of goats in India are reared in extensive system and nutrient needs are met by grazing alone or grazing with supplementation. But, grazing lands are shrinking due to increased demand of food and forage under the increasing population pressure, urbanization and industrialization. Therefore, goat production is transforming from extensive system to intensive system in urban and peri urban area. The cost of feed ingredients like groundnut cake and soybean have more than doubled over the past few years. The high cost of feed is largely due to the exorbitant price and scarcity of conventional feed ingredients. In the light of this, it is very much important to search and adopt some new feed resources, which can help in overcoming the feed shortage with enhancing goat productivity for the food security of huge population of the country. Azolla *(Azolla microphylla)* a free-floating aquatic fern seems to hold the solution for this gloomy situation. Azolla fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*, making it an excellent source of protein for livestock. This can be used as a part of complete pellet feed in the ration of goats. Azolla in the complete feed can reduce the cost of feed of goat there by increasing the profits from goat rearing to the farmers. Azolla is used as a feed for pigs and ducks in South-East Asia, for cattle, fish and poultry in Vietnam and for pigs in Singapore and Taiwan (FAO 2011). With this background in view, the present study was conducted to evaluate the effect of partial replacement of concentrate mixture with azolla meal on growth, nutrient utilization, rumen fermentation pattern and blood

Present address: [1,2]Senior Scientist (ravindra.srivastava @gmail.com, prabhat@cirg.res.in), [3,4]Principal Scientist (UBC@cirg.res.in, mktripathi@cirg.res.in), [5]Senior Technical officer (rksinghCIRG14@gmail.com), Division of Nutrition, Feed Resources & Product Technology.

biochemical parameters in goats.

MATERIALS AND METHODS

Animal management and rations: Barbari male goats (12), approximately 3–4 months of age and weighing between 9–13 kg (Avg b. wt. 11.31 kg), were divided into 2 groups (Control, Gr A and treatment Gr B) consisting of 6 goats in each group. All the goats were kept under uniform management conditions by housing them in well ventilated sheds. All the goats were fed with complete pellet feed having concentrate mixture and Bengal gram straw in 40:60 ratio to meet their nutrient requirement for the body weight gain of 50–100 g/day. Two types of complete pellet feed was formulated, control (C) and treatment (T).Composition of control pellet was gram straw 60%, barley 22.8%, ground nut cake 12%, wheat bran 4%, mineral mixture 0.8% and salt 0.4%. In case of experimental group, 25% of the ingredients used in the concentrate mixture were replaced with azolla meal on weight basis.

Experimental procedure: An experimental feeding on both the groups of goats was carried out for 8 weeks. Weighed quantities of respective pellet were offered to different group of goats at 08:00AM and 2.00 PM, daily in a completely randomized design (CRD). *Ad lib.* water was provided and was changed twice daily throughout the experimental period. Pellet offered and residues were sampled weekly for subsequent analysis of DM to determine DM intake. Body weight of each goat before feeding was recorded at weekly intervals to observe live weight changes and average growth rate throughout the experimental period. Blood was collected from the jugular vein of all experimental animals in last week of experimental feeding. Whole blood was used for hematology and serum was extracted for estimation of different metabolites like protein, its fractions, triglycerides and cholesterol. The serum was preserved at –4° C till further analysis. Rumen fluid was also collected in last week of experimental feeding from each animal using stomach tube to study the effect of feeding different types of pellet on rumen metabolites.

To determine the digestibility of nutrients and nitrogen utilization, a metabolism trial was conducted after 6 weeks of experimental feeding for 6 days. Metabolism trial was conducted in metabolic cages having facility for individual feeding and separate collection of faeces and urine. Representative samples of pellet offered and remnants were taken daily in previously tared trays and kept in a hot air oven at 100±2°C overnight for DM estimation. The dried material obtained during the trial period was pooled, ground and stored for proximate and fibre analysis.

The faeces voided in 24 h by the individual animal were collected in a previously weighed container. After weighing the faecal container, the faeces were thoroughly mixed and a representative sample from each animal was taken in a previously labeled polythene bags. From the sample a suitable aliquot (10%) of faeces was kept for drying at 100±2°C in a hot air oven for dry matter determination. The dried faecal sample obtained daily was pooled, ground and used for chemical analysis. Another portion of aliquot (1% of fresh faeces) was thoroughly mixed with 10 ml of 1:4 sulphuric acid and kept for nitrogen estimation. Urine collected was measured at the end of the 24 h period in order to assess the daily output. A suitable aliquot (2% v/v) of daily urine from individual animals was kept in Kjeldahl flasks containing 40 ml of laboratory grade sulphuric acid.

Laboratory and statistical analysis: Nutrient composition of feed, residue, urine and faeces were analysed using the protocol described by AOAC (1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HC) were determined as per Robertson and Van Soest (1981).

The whole blood was tested for hematological parameters using hematology analyzer standardized for goats. Total protein content of serum was determined by Biuret method (Wotton 1964). Albumin level in serum was quantified by using bromocresol green dye (BCG), as per Doumas *et al.* (1971). Globulin values were obtained by subtracting the value of albumin from that of total protein. Triglycerides and cholesterol were analyzed by GPO-PAP End point Assay and CHOD-PAP enzymatic method respectively. All the blood metabolites were estimated using diagnostic kits.

The pH of rumen liquor was determined immediately after collection, with an electronic pH meter calibrated against standard buffer solution. The ammonia nitrogen (NH_3-N) in rumen liquor was estimated by distillation method. Total nitrogen and trichloro acetic acid (TCA) precipitated nitrogen was estimated by micro Kjeldahl's method as described by AOAC (1995). Non-protein nitrogen of rumen liquor was calculated by subtracting the TCA precipitated nitrogen from total nitrogen content of rumen liquor.

The data of growth, metabolic trial and other estimated metabolites were analyzed by independent sample t-test as described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Body weight changes: Weekly body weight changes in different group of goats are presented in Fig 1. The initial body weight (kg) in control group and treatment group was 11.19 and 11.42 which finally became 14.44 kg and 15.80 kg, respectively, after experimental feeding. Average daily gain (g) was 56.60 and 78.12 in control and treatment groups, respectively, and showed no significant difference ($P > 0.05$). However, body weight gain was higher in treatment group as compared to control group indicating more nutrient retention in treatment group. The average daily gain of goats was in agreement with 50–100 g / day reported by Ranjhan (1998). Samanta and Tamang (1995) studied the effect of replacement of concentrate mixture at 10, 20 and 50% level with sun-dried azolla in black Bengal goats. They reported similar body weight gain in all the groups. Group with 50% level of replacement was discarded as they suffered from profuse diarrhea and became sick. Ghodake *et al*. (2012) studied the effect of replacement of

Table 1. Chemical composition of pellet used in experimental feeding (on DM basis)

Attributes	Control pellet	Treatment pellet	Azolla
Organic matter	91.54	90.01	79.69
Ash	8.46	9.99	20.31
Crude protein	12.12	12.01	17.30
Ether extract	5.01	4.61	2.71
NDF	40.02	42.39	46.90
ADF	28.27	30.96	31.73
Cellulose	22.57	24.24	15.17
Total carbohydrates	66.23	66.93	59.68
Hemicellulose	11.75	11.43	12.43

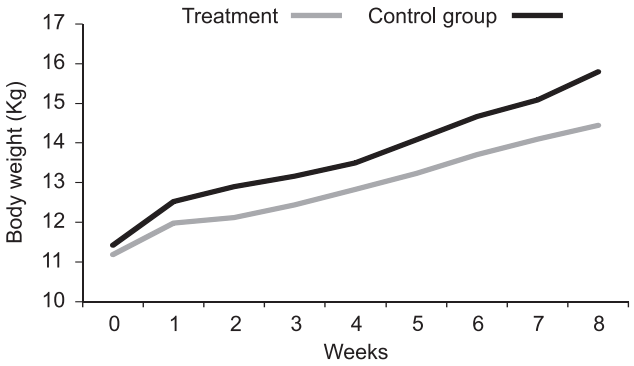

Fig. 1. Weekly body weight changes of goats during experimental feeding.

concentrate mixture with azolla meal in Osmanabadi kids. They have 3 treatments, T_1 (control), T_2 (15% concentrate was replaced with azolla meal), T_3 (25% concentrate was replaced with azolla meal). They observed significantly ($P<0.05$) higher dry matter intake and body weight in T2 group. Earlier reports by Ndemanisho *el al.* (1998) and Mahanta *et al.* (1999) and Pal *et al*. (2010) also found similar body weight gain in goats where, concentrate mixture was replaced with different combinations of leaf meal mixture.

Feed intake: There was no significant difference ($P > 0.05$) in the dry matter intake (g/day and g/kg $W^{0.75}$/day) of goats fed on control and treatment pellet feed (Table 2). The pellet feed intake expressed as a percentage of live weight for goats was 4.41 and 4.44 in control and treatment group respectively. Generally the feed intake in the tropical environment ranges from 1.1 to 4.1% in growing goats (NRC 1981, Kearl 1982, Devendra and Bums 1983). Slightly higher intake of dry matter may be attributed to the form of feed which is pellet in nature. The daily total dry matter intake of goats in control and treatment group was 568.03 and 625.81, respectively, showing no statistically significant difference. This clearly indicated that replacement of conventional concentrate mixture with dried azolla meal has no adverse effect on palatability of pellet. Samanta and Tamang (1995) also reported comparable dry matter consumption and feed efficiency in Black Bengal goats with 10 and 20% of concentrate mixture replacement with sun-dried azolla. The feed conversion ratio during whole of feeding trial was lower in treatment group (8.09) as compared to control group (10.45) of goats.

Table 2. Intake and digestibility of nutrients during metabolism trial in experimental goats

Attributes	Group A	Group B
Body wt.(kg)	13.12±1.02	14.07±0.61
Body size (kg)	6.88±0.39	7.26±0.24
Total dry matter intake		
g/animal /day	568.03±16.13	625.81±54.43
g/%live weight	4.41±0.32	4.44±0.34
g/Kg $^{0.75}$/day	83.54±4.94	86.02±6.71
Nutrient digestibility (%)		
Dry matter	55.38±1.87	52.41±3.06
Organic matter	57.89±1.79	55.46±2.78
Crude protein*	71.14±0.71	77.43±2.02
Ether extract	73.55±1.94	69.72±2.04
Total carbohydrate	62.58±2.53	57.46±3.17
Neutral detergent fibre	60.78±3.10	57.26±5.54
Acid detergent fibre	51.27±3.67	52.25±5.06
Cellulose	54.47±2.50	55.86±4.34
Hemicellulose	59.60±6.36	53.13±1.91

*Differ significantly ($P<0.05$).

Nutrient digestibility: The values obtained for digestibility of different nutrients on both the pellet feed are shown in Table 2. The digestibility (%) of dry matter and organic matter was statistically similar ($P > 0.05$) in control (55.38, 57.89) and treatment (52.41, 55.46) group of goats. These findings suggested that replacement of concentrate mixture in pellet feed at 25% level have no significant effect on digestibility. In our lab under *in vitro* condition we found similar result but increasing from this level (>25%) lead to a significant decrease in dry matter and organic matter digestibility. Tamang and Samanta (1993) studied the effect of replacement of concentrate mixture at 10, 20 and 50% level with sun-dried azolla in Black Bengal goats. They reported that digestibility coefficients of all nutrients except NFE, ether extract, cellulose were similar. They also reported a profuse diarrhea at 50% replacement of concentrate mixture with sun-dried azolla. Digestibility of crude protein was significantly higher ($P < 0.05$) in treatment group of goats (77.43%) than control group of goats (71.14%). More balancing of nitrogen from different source might be responsible for the higher digestibility of crude protein. Ether extract (EE) digestibility by the goats was statistically similar in the control (73.55%) and treatment (69.72%) groups. The digestibility of total carbohydrate and different fibre fractions (NDF, ADF, cellulose and hemicellulose) did not differ significantly ($P > 0.05$) between the 2 pellet feeds. However, Tamang and Samanta (1993) reported significantly lower ($P<0.05$) digestibility of nitrogen free extract and cellulose at 20% replacement of concentrate mixture with sun-dried azolla.

Nitrogen balance: Daily intake (g/animal) of nitrogen was comparable among goats irrespective of the pellet feed (Table 3). The excretion of nitrogen (g/d) in faeces was significantly ($P < 0.05$) lower in treatment group (4.49) than the control group (5.35) whereas, in urine it was similar in

Table 3. Intake and utilization of nitrogen in experimental goats

Attributes	Group A	Group B
Nitrogen intake (g/animal/day)	11.11±0.31	12.36±1.05
Nitrogen excretion (g/animal/day)		
Fecal *	5.35±0.25	4.49±0.23
Urinary	1.61±0.10	1.46±0.15
Total*	6.96±0.34	5.96±0.12
Nitrogen absorbed (g/animal/day)	5.75±0.13	7.86±0.98
Balance (g/animal/day)	4.14±0.11	6.40±1.04
Retention		
percent of intake*	37.41±1.49	49.68±5.15
Percent of absorbed	72.00±1.54	78.58±5.26

*Differ significantly (P<0.05).

Table 4. Hematology and blood metabolites of experimental goats

Attributes	Group A	Group B
WBC (m/mm^3)	18.20±0.97	14.42±1.34
RBC (M/mm^3)	13.38±0.79	13.15±0.77
HCT (%)	16.72±0.92	17.86±1.29
MCV (fl)	13.10±0.26	13.50±0.28
Hb (g/dl)	6.18±0.27	6.20±0.42
THR (m/ mm^3)	751.80±50.14	700±25.10
MPV (fl)	4.8±0.13	4.63±0.07
MCHC (g/dl)	37.12±1.38	34.71±0.47
RDW	18.57±0.66	18.50±0.55
Lymphocytes (%)	64.46±1.31	64.91±2.27
Monocytes (%)	12.18±1.24	10.05±0.91
Granulocytes (%)	23.36±1.12	25.03±2.29
Total protein (g/dl)	7.55±0.46	7.39±0.82
Albumin (g/dl)	3.96±0.18	4.09±0.14
Globulin (g/dl)	3.59±0.51	3.29±0.78
AG ratio	1.21±0.18	1.61±0.33
Triglycerides (mg/dl)	17.49±3.09	16.28±4.24
Cholesterol (mg/dl)	33.10±6.39	34.33±12.16

the control (1.61) and treatment (1.46) groups. Net balance of N (g/d) was statistically similar (P > 0.05) in control group (4.14) and treatment group (6.40) diet. Nitrogen retention as % of intake N was significantly (P < 0.05) higher in treatment group (49.68) than that in control group (37.41) whereas nitrogen retention as % of absorbed N was statistically similar (P > 0.05) in control (72.00) and treatment (78.58) groups. All the animals irrespective of the complete pellet fed had positive nitrogen balance indicating adequate nutritional level of all the animals given different diets. Samanta and Tamang (1995) also reported positive nitrogen balance in the Black Bengal kids fed with diet in which concentrate mixture was replaced with sun-dried azolla. In both the group there was a tendency for lower urinary nitrogen excretion, a pointer to the higher efficiency of nitrogen as evidenced by comparatively higher nitrogen retention in these animals probably due to the more balanced amino acid composition and apparent biological value of proteins in oil cake and azolla (McDonald *et al.,* 1995). Nitrogen retention as % of intake N was significantly (P < 0.05) higher in treatment group (49.68) than control group (37.41),whereas no significant difference was found in the nitrogen retention as percentage of absorbed nitrogen (an indicator of availability of amino acid-N at tissue level) between both the groups. The faecal nitrogen comprises of undigested dietary as well as metabolic faecal nitrogen and thus depending upon the microbial N yield, apparent N digestibility may get altered without an actual change in available amino acid at tissue level (McDonald *et al.* 1995).

Blood profile: The effect of replacing concentrate mixture with sun-dried azolla on hematology and blood metabolites is presented in Table 4. Count of red blood cells, white blood cells, thrombocytes and hematocrit values are similar in both the groups. There was no significant difference in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) of control and treatment group of goats indicating no anemic condition in goats. Hemoglobin (Hb) concentration (mg/dl) was 6.18 and 6.20 in control and treatment group, respectively, which was within the normal range (6–12 g dl^{-1}) as reported by Kaneko (1997). Hb is an indicator of erythrocytic normality and general well being of the animal (Radostits *et al.* 1994). Differential counting of white blood cell indicated statistically similar % of lymphocytes, monocytes and granulocytes in both the group. This suggested that the general health of goats given 2 different diets remained similar. The findings are in agreement with Tamang and Samanta (1993) who also reported that diets containing various levels of azolla did not produce any significant effect on haematological and biochemical parameters.

Total serum protein (g/dl) was 7.55 and 7.39 in control and experimental group of goats. There was no significant difference (P > 0.05) in the level of total protein in goats kept on different pellet. Similarly, serum albumin (g/dl), serum globulin (g/dl) and their ratio ranged was 3.96 and 4.09, 3.59 and 3.29, 1.21 and 1.61 in control and treatment group, respectively, and showed no significant difference (P > 0.05) between the 2 pellets. Serum concentration of proteins depends upon a variety of factors including extent, duration and nature of the hepatic disorder; the inflammatory or metabolic hepatic process and the presence of other organ disorders (Kaneko 1997). The observed range of total serum protein in experimental goats was within the normal range of 6 to 7.5 g/dl (Kaneko 1997) showing no adverse effect of replacement. Similarly, albumin, globulin ratio also remained near to normal range (0.63–1.2) of healthy goats (Kaneko 1997). Reddy *et al.* (2010) reported no significant change in the total protein, blood glucose, total cholesterol, BUN and creatinine in Nellore weaners after feeding of azolla under intensive and semi-intensive management systems. They however, found significantly higher albumin level of weaners fed with concentrate having replacement of groundnut cake with azolla. Pal *et al*. (2010) did not report any significant change in total protein and its fractions after replacement of concentrate mixture with leaf meal mixture.Serum triglycerides (mg/dl) and cholesterol (mg/dl) was 17.49 and 33.10 in control group and 16.28

Table 5. Rumen fermentation metabolites in experimental goats

Rumen metabolites	Group A	Group B
pH	6.30±0.07	6.25±0.09
TVFA (mmol/dl)	15.46±0.74	16.70±0.55
Total nitrogen (mg/dl)	100.52±8.92	97.96±7.72
Ammonia nitrogen (mg/dl)	19.32±1.49	16.33±0.69
Non protein nitrogen (mg/dl)	69.44±6.53	76.53±6.73
TCA-ppt nitrogen (mg/dl)	31.08±6.95	21.43±2.65

and 34.33 in treatment group, with no significant difference (P > 0.05) between the 2 groups. The values were within the normal range as suggested by Kaneko (1997).

Rumen fermentation parameters: The value for pH, total volatile fatty acids, ammonia nitrogen, total nitrogen, TCA precipitated nitrogen and non protein nitrogen is presented in Table 5. The pH of the rumen liquor of control group (6.30) was statistically similar to the treatment group (6.25) and was within normal range (6.0–7.0) reflecting no adverse effect of replacement. Total volatile fatty acid (mmol/100ml) was statistically similar in control (15.46) and treatment (16.70) group of goats. The mean ammonia nitrogen was found to be 19.32 and 16.33 mg/100 ml of rumen liquor in control and treatment groups respectively, with no significant difference (P > 0.05) between the dietary groups. The mean values for total nitrogen were 100.52mg/100 ml in control group and 97.96.14mg/100 ml in treatment group. There was no significant difference (P > 0.05) between the dietary groups. The mean TCA precipitated nitrogen was 31.08 and 21.43 mg/100 ml of rumen liquor in control and treatment groups, respectively, which revealed no significant difference (P > 0.05) between the dietary groups. The mean values for non protein nitrogen were 69.44 mg/100 ml in control group and 76.53 mg/100 ml in treatment group, with no significant difference (P > 0.05) between the dietary groups. Pal *et al*. (2010) also reported no difference in pH and various nitrogen fractions of rumen liquor of goats fed with replacement of concentrate mixture by leaf meal mixture. Anbarasu *et al.* (2002) also reported no difference of nitrogen fractions of rumen liquor on supplementation of leaf meal mixture.

On the basis of this study, it is concluded that sun-dried azolla can replace 25% of concentrate mixture on weight basis in the ration of growing goats without any adverse effect of body weight gain, nutrient utilization, blood and rumen fermentation metabolites. So, economic complete pellet feed can be formulated using azolla meal in the ration of growing goats.

ACKNOWLEDGEMENT

The authors are thankful to Director, ICAR- Central Institute for Research on Goats, for providing necessary facilities to carry out this work.

REFERENCES

Anbarasu C, Dutta N, Sharma K and Uma N. 2002. Blood biochemical profile and rumen fermentation pattern of goats fed leaf meal mixture or conventional cakes as dietary protein supplement. *Asian Australasian Journal of Animal Sciences* **15**: 665–70.

AOAC. 1995. *Official Methods of Analysis*, 16th ed. pp. 4.1–4.17. Association of Official Analytical Chemists, Washington, DC, USA.

Devendra C and Bums M. 1983. *Goat production in the tropics*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, pp.183.

Doumas B T, Watson W A and Biggs H G. 1971. Albumin Standards and the Measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**: 87–96.

FAO. 2011. Successes and failures with animal nutrition practices and technologies in developing countries. *Proceedings of the FAO Electronic Conference*, 1–30 September 2010, Rome, Italy. pp119.

Ghodake S L S, Fernandes A P, Darade R V and Zagade B G. 2012. Effect of different levels of Azolla meal on growth performance of Osmanabadi kids. *Research Journal of Animal Husbandry and Dairy Science* **3** (1): 13–16.

Kaneko J J. 1997. *Clinical Biochemistry of Domestic Animals*. 5th Ed. Academic Press.

Kearl L C. 1982. *Nutrient requirements of ruminants in developing countries*, Utah Agricultural Experimental Station, Utah State University, International Feedstuffs Institute, Logan, UT.

Mahanta S K, Singh S, Kumar A and Pachauri V C. 1999. Subabul leaf meal as a replacement of mustard cake in lamb diets. *Small Ruminant Research* **32**: 37–42.

McDonald P, Edwards R A, Greenhalgh J F D and Morgan C A. 1995. *Animal Nutrition*. Edn. 5, Addison. – Wesley Longman, U.K.

Ndemanisho E E, Mtenga L A, Kimbi E F C, Kimambo A E and Mtengeti E J. 1998. Substitution of dry *Leucaena leucocephala* (DLL) for cotton seed cake (CSC) as a protein supplement to urea treated maize stover fed to dairy weaner goats. *Animal Feed Science and Technology* **73**: 365–74.

NRC. 1981. *Nutrient requirements of goats*. National Academy of Sciences. National Research Council, Washington, DC.

Pal A, Sharma R K, Kumar R. and Barman K. 2010. Effect of replacement of concentrate mixture with isonitrogenous leaf meal mixture on growth, nutrient utilization and rumen fermentation in goats. *Small Ruminant Research* **91**: 132–40.

Radostits O M, Blood D C and Gay C C. 1994. *Veterinary Medicine*. (8th Ed). ELBS, Bailliere Tindall 24–28 Oval Road, London NWI TDX. pp. 1726.

Ranjhan S K. 1998. *Nutrient Requirements of Livestock and Poultry*. ICAR. New Delhi.

Reddy Y R, Rao K S, Sudhakar K, Gupta D R and Prakash M G. 2010. Biochemical profile of nellore sheep on feeding of Azolla and Sheanut cake under different management systems. *Indian Journal of Small Ruminants* **16**: 54–57.

Robertson J B and Van Soest, P J. 1981. *The detergent system of analysis and its application to human foods*. Cornell University, Ithaca, New York.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 7th edn. The Iowa State University, Iowa (USA).

Tamang Y and Samanta G. 1993. Feeding value of azolla (*Azolla pinnata*) an aquatic fern in Black Bengal goats. *Indian Journal of Animal Sciences* **63**: 188–91.

Wotton I D P. 1964. *Microanalysis in Medical Biochemistry*, 4th Ed. J&A. Churchill Ltd., London, pp.138–40.