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Effect of Processing of Sorghum Stover Based Diets on Performance, Values of Haematological and Biochemical Parameters and Immune Response in Growing Nellore Lamb

Research Article

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ABSTRACT

A complete diet (Total Mixed ration) with sorghum stover (40%) as sole roughage source was prepared and offered to animals under two forms: as mash or as expanded-extruded pellets (EEP). These two complete diets were compared with a conventional diet (*ad libitum* chopped sorghum stover fed separately with concentrate mixture to meet 80% of protein requirements). These three diets were randomly allotted to 30 male lambs for a period of 150 days. The lambs on conventional, mash and pelleted diet grew with an average daily gain of 62.5g, 78.5g and 101.3g, respectively, with superiority ($P<0.01$) of EEP diet in relation to the others. The lambs fed complete diets were more efficient in utilizing the dry matter (DM) than those fed conventional diet despite lower DMI. The haematological (total erythrocyte and leucocyte counts, and percentage of neutrophil and lymphocyte) and serum biochemical constituents (total protein, albumin, globulin and urea nitrogen concentration) at 0, 75 and 150 d of trial were not affected by diets, except haemoglobin concentration which was significantly higher in lambs fed EEP diet ($P<0.001$). The lipid peroxidase activity was lower ($P<0.05$) and the RBC catalase activity ($P<0.01$) was higher in both groups of lambs fed with complete diet. The humoral immune response against *Brucella abortus* was higher in lambs fed with EEP diet compared to the lambs fed with mash or conventional diets. The total Ig, IgM or IgG titres against 20% chicken red blood cells were higher in lambs fed complete diets and were not affected by expander extruder processing. The cell mediated immune response of lambs assessed as *in vitro* lymphocyte assay at 150 d of feeding was higher ($P<0.05$) in EEP diet followed by those in mash complete diet and lastly by those fed conventional diet.

KEY WORDS expander extruder pelleting, immune response, lambs, performance, sorghum stover.

INTRODUCTION

Scarcity of feeds is one of the biggest reasons for lower livestock productivity in many of the developing countries including India.

Traditionally small ruminants are managed on grazing, but due to continuous depletion of grazing lands, intensive system of rearing needs attention. The intensive system of feeding can be economized by utilizing various agro-industrial by-products and agricultural crop residues, but these crop residues are low in palatability, digestibility and nutritive value.

Methods to improve the utilization of crop residues could bridge the gap between availability and requirements of feedstuffs and could be achieved by blending crop residues with concentrates in the ration to form complete feed. Increased dry matter intake and nutrient utilization in animals was observed on complete diet system compared to conven tional feeding (Lailer *et al.* 2005).

Pelleting of complete diets containing crop residues like sorghum stover, cotton stalks, sugarcane bagasse, maize cobs with expander extruder has improved nutrient digestibilites in small and large ruminants (Reddy and Nagalakshmi, 2004; Nagalakshmi *et al.* 2010). Reddy and Reddy (1999) reported improved nutritive value of complete diet containing 28.5% sorghum stover with expander extruder pelleting (EEP).

Thus a detailed study was undertaken to evaluate the effect of incorporating sorghum stover as sole roughage source in conventional feeding system and complete diets processed in mash and with expander extruder on performance and health of lambs reared under intensive system.

MATERIALS AND METHODS

Animals and feeding management

Two complete diets of same ingredient composition were formulated with sorghum stover as sole roughage source in a roughage concentrate ratio of 40:60 to meet the nutrient requirements (ICAR, 1998) of lambs. One complete diet was processed in mash form and the second was processed as expanded extruded pellets. These two complete diets were compared with a conventional diet consisting of concentrate mixture and chopped sorghum stover offered separately (Table 1).

These three diets were randomly allotted to 30, five month old Nellore male lambs of uniform body weight (15.5+0.33 kg) in a completely randomized design, with 10 lambs in each group. The lambs were fed the respective diets *ad libitum* thrice a day throughout the experimental period of 150 days.

The diets were analysed for proximate constituents (AOAC, 2000) and fibre fractions (Van Soest *et al.* 1991). All the lambs throughout the experimental period were monitored for disease symptoms.

Growth performance

The body weight of individual lambs was recorded at the onset of experiment and thereafter at 15 days intervals in morning before feeding and watering in order to assess the changes in body and average daily gain. The lambs were offered daily weighed quantity of feed and the residues were weighed the next day before offering of feed to estimate feed intake.

Blood collection and analysis

Blood samples were collected from jugular vein of all lambs at beginning and thereafter at 75 and 150 days and analyzed for various hematological and biochemical constituents.

Table 1 Ingredient composition (kg/100 kg) of the experimental diets

Ingredient	Concentrate mixture[1]	Complete diets
Sorghum stover	-	40.0
Maize	40.0	25.0
Groundnut cake	20.0	-
Sunflower cake	15.0	25.0
Wheat bran	22.0	-
Molasses	-	8.0
Urea	-	0.5
Salt	1.0	0.5
Limestone powder	-	0.6
Dicalcium phosphate	-	0.191
Trace mineral and vitamin mixture[2]	-	0.055
Mineral mixture	2.0	-

[1] Concentrate mixture of the conventional diet.
[2] Trace mineral premix provided (mg/kg diet): Iron: 30; Copper: 10; Manganese: 40; Zinc: 30; Cobalt: 0.2; Molybdenum: 0.5; Iodine: 0.25; Selenium: 0.2. Vitamin A and E was provided to supply 940 IU and 20 IU per kg diet, respectively.

Haemoglobin (Hb; Benjamin, 1985) and total erythrocyte (TEC) and leucocyte (TLC) counts (Jain, 1986) and oxidative enzymes (90 d of feeding) by preparing the haemolysate were estimated from the blood collected with anticoagulant (EDTA@ 1 mg/mL). For preparing the haemolysate in whole blood, the blood was centrifuged at 1500 rpm for 10 minutes and the red blood cells (RBC) were washed with phosphate buffer saline (PBS) for 3 times then 1ml of RBC were added with 4ml distilled water to prepare 5% erythrocyte lysate.

In haemolysate, the lipid peroxidase (Placer *et al.* 1966), RBC catalase (Bergmeyer, 1983), glutathione peroxidase (Paglia and Valantine, 1967) and glutathione reductase (Moron *et al.* 1979) were estimated. The biochemical constituents analysed in serum were total proteins (Hiller *et al.* 1927), albumin (Gustafsson, 1976) and urea (Rahmatullah and Boyde, 1980). The serum globulin was determined as the difference between total protein and albumin concentration.

Immunological assay

Heat killed *Brucella abortus* S_{99} and chicken erythrocytes were used as immunogens to study the humoral immune response.

After 75d of experimental feeding, all lambs were sensitized with heat killed *Brucella abortus* S_{99} and 20% chicken RBC (CRBC) suspension, administered I/M and a booster dose of antigen was given after 15 days. Prior to administration of antigen, lambs were screened for Brucellosis antibody with Rose Bengal Plate Test (RBPT; Alton *et al.* 1975).

Serum was collected from the sensitized lambs on 7, 14, 21, 28 and 35th d of post sensitization to assess humoral immune response. The antibody against *Brucella abortus* antigen was measured by indirect enzyme linked immunosorbant assay (ELISA; Perlman and Engvall, 1971).

The total immunoglobulins (Ig), IgM and IgG against CRBC were measured by direct haemagglutination assay (DHA; Wegmann and Smithies, 1966). The IgG was measured by destroying IgM using 2-mercaptoethanol and the IgG titer was measured by difference between total Ig and IgG concentration. The cell mediated immune response was estimated by *in vitro* lymphocyte proliferation assay (LPA) against mitogen Concanavalin A (Con. A) in the blood collected in heparinized vacutainers at 150 d of experimental feeding following the procedure described by Bounous *et al*. (1992).

Cost economics

The total cost of diets was calculated by summing the cost of feed ingredients per 100kg plus processing cost. The processing of diets was worked out taking into considerations of fixed charges (depreciation on building and machinery, interest on block investment and maintenance) and direct charges (cost of power, labour, operators, etc). The cost of production was calculated by taking into account the cost of feed consumed per day and daily gain.

Statistical analysis

The data on performance and antioxidant enzymes were analysed using one-way ANOVA (Snedecor and Cochran, 1980). Hematology, serum biochemical analysis and immunological trial data were subjected to two way factorial analyses with dietary treatments and period of blood/serum collection as factors.

When the interaction was not found significant, the effects of individual factors were considered. Comparisons among multiple means were made by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Expander extruder pelleting of the complete diet did not affect the nutrient composition of the diet. The complete diets contained 13.20 and 12.80% crude protein (CP) in mash and pellet form, respectively and the neutral detergent fibre content was 49.83% in mash and 50.16% in pellet diet (Table 2).

A linear increase in live body weight was observed in lambs on all the diets throughout 150 days of experimental feeding.

No significant changes were observed with regard to body weight from start to 45th d body weight, but the body weights measured from 60th d onwards showed significant differences (Table 3). Lambs fed complete diets grew faster (P<0.01) than the conventional diet fed lambs from 60th d onwards up to 120th d. During 135th (P<0.05) and 150th d the lambs on EEP diet had higher body weights than the conventional diet (P<0.01), but lambs on mash diet did not differ from conventional group (P>0.01).

Table 2 Chemical composition of the concentrate mixture and complete diets (% DM basis)

Constituent	Concentrate mixture[1]	Complete diets	
		Mash	Pellet
Organic matter	88.51	91.28	89.63
Ether extract	2.61	1.07	0.91
Crude protein	18.04	13.20	12.80
Crude fibre	10.29	28.35	29.99
Nitrogen free extract	57.57	48.66	45.93
Total ash	11.49	8.72	10.37
Neutral detergent fibre	56.30	49.83	50.16
Acid detergent fibre	23.08	29.38	33.01
Cellulose	13.44	21.78	24.75
Lignin	6.12	6.37	6.20

[1] Concentrate mixture of the conventional diet.

The lambs on conventional, mash and EEP diet grew with an average daily gain of 62.5, 78.5 and 101.3 g, respectively, being significantly (P<0.01) higher in lambs fed EEP diet than the other two groups.

Table 3 Body weights (kg) and feed efficiency (g intake/g gain) in lambs fed sorghum stover based diets[1]

Days	Conventional diet	Complete diets		SEM	P-value
		Mash	Pellet		
0 Start	15.7	15.9	15.0	0.33	0.490
15	15.4	16.1	14.4	0.41	0.235
30	15.6	18.1	17.4	0.47	0.072
45	15.8	19.6	18.9	0.57	0.011
60	16.4^{b}	20.9^{a}	20.0^{a}	0.62	0.004
75	18.1^{b}	23.3^{a}	22.7^{a}	0.70	0.002
90	20.5^{b}	25.4^{a}	25.2^{a}	0.73	0.004
105	20.9^{b}	25.7^{a}	26.7^{a}	0.72	0.001
120	22.9^{b}	26.1^{a}	28.2^{a}	0.70	0.005
135	23.8^{b}	26.0^{ab}	28.6^{a}	0.72	0.018
150	25.1^{b}	27.7^{ab}	30.2^{a}	0.72	0.009
Total gain	9.4^{b}	11.8^{b}	15.2^{a}	0.79	0.006
ADG, g/d[2]	62.5^{b}	78.5^{b}	101.3^{a}	5.24	0.006
DMI, g/d[2]	1021.9^{a}	922.8^{b}	983.2^{b}	7.58	0.001
DMI/kgW$^{0.75}$	113.9^{a}	90.24^{b}	95.81^{b}	2.56	0.001
DMI/ADG	17.7^{a}	11.25^{b}	10.22^{b}	0.875	0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
ADG: average daily gain and DMI: dry matter intake.
SEM: standard error of the means.

Reddy and Reddy (1999) observed higher dry matter, organic matter, CP and nitrogen free extract digestibilities in calves with incorporation of sorghum stover in complete diets and further improvement was observed with EEP complete diet.

The higher growth rates recorded in EEP fed lambs might be a result of higher nutrient availability due to pelleting. Similar beneficial effects on daily weight gains with incorporation of crop residues in complete diets and its expander extruder processing was observed in calves fed expander extruder processed complete diets containing sugarcane bagasse (Reddy *et al.* 2002) or cotton stalks (Kirubanath *et al.* 2003) and in lambs fed expanded-extruded diet containing maize cobs (Nagalakshmi and Reddy, 2003).

The average dry matter (DM) intake by lambs on the conventional diet was higher (P<0.01) than those fed complete diets in either mash or pellet form (Table 3). In conventional diet, the concentrate mixture was provided to each lamb daily to meet the protein requirements and remaining was met by feeding *ad libitum* sorghum stover. So the higher DM intake in conventional diet might be due to higher roughage consumption.

The lambs fed complete diets were more efficient in utilizing the DM than those fed concentrate mixture and chopped sorghum stover separately, while no effect was observed among both the complete diets fed groups which corroborated with the findings of Kirubanath *et al.* (2003) and Nagalakshmi and Reddy (2003). All the haematological constituents analysed were within the normal physiological limits (Ullery, 1965). The Hb concentration in lambs fed the EEP diet was higher (P<0.001) compared to those fed either conventional or complete diet in mash form (Table 4).

No effect of processing was observed on TEC, TLC, lymphocytes and neutrophils. The Hb and TEC was higher (P<0.01) at the start and at the end of feeding trial than the mid period, a particular reason for this trend could not be identified. But no such trend was observed for the other haematological constituents.

None of the biochemical constituents analyzed (total protein, albumin, globulin, albumin globulin ratio, urea and creatinine) were affected by the method of processing the diets (Table 4). The average blood urea concentration was comparable up to 75 d post feeding and later increased (P<0.01) from 75 d to 150d of feeding. The comparable serum proteins, globulin and urea concentration on different feeding regimes in the present study indicated normal balance between anabolism and catabolism of body proteins. Lipid peroxidation increases during stress conditions (Saygili *et al.* 2003) such as vaccination or antigen administration. In the present study, administration of Brucella and CRBC antigens at 75 d of experiment might have caused stress to animals resulting in lipid peroxidation. Feeding of complete diets resulted in lower (P<0.05) lipid peroxidation compared to those fed under the conventional system (Table 5). The enzymatic antioxidants like glutathione peroxidase and catalase play a vital role in scavenging oxidative radicals by converting peroxides to water (Scott *et al.* 1993).

The lower glutathione reductase concentration indicates lower ability to detoxify hydrogen peroxide produced during cellular oxidative stress (Harvey, 1997). The lower lipid peroxidation on feeding of complete diets and higher concentration of glutathione peroxidase and RBC catalase clearly showed that lambs fed complete diets could maintain better oxidant-antioxidant balance to combat the stress conditions and protect immune cells from peroxidation.

Table 4 Haematological, biochemical constituents and lipid peroxidase in lambs fed sorghum stover based diets

Attribute	Diet*					Days post feeding**				
	Conventional	CFM‡	CFP‡	SEM	P-value	0	75	150	SEM	P-value
Haemoglobin (g %)	10.59^b	10.79^b	11.56^a	0.178	0.001	11.13^z	10.42^y	11.38^z	0.176	0.001
TEC ($\times 10^6$/uL)	8.80	8.80	8.77	0.069	0.935	8.92^z	8.64^y	8.80^{yz}	0.061	0.010
TLC ($\times 10^3$/uL)	10.41	10.51	10.42	0.092	0.739	10.49	10.36	10.49	0.369	0.494
Neutrophils %	55.5	54.93	54.74	0.567	0.523	54.56	55.21	55.39	0.564	0.560
Lymphocytes %	41.92	42.11	42.70	0.606	0.642	42.60	42.10	42.03	0.609	0.768
Total protein, g/dL	5.44	5.38	5.54	0.100	0.539	5.48	5.52	5.35	0.100	0.464
Albumin, g/dL	2.03	1.90	1.99	0.065	0.348	2.03	1.97	1.92	0.067	0.438
Globulin, g/dL	3.47	3.48	3.51	0.098	0.948	3.49	3.57	3.40	0.096	0.466
Albumin globulin ratio	0.60	0.56	0.59	0.028	0.562	0.60	0.58	0.57	0.030	0.849
Urea nitrogen, mg/dL	23.40	25.16	25.11	1.196	0.365	21.59^y	22.20^y	29.88^z	0.948	0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
* Means of all three periods for each dietary treatment.
** Means of all three diets for each period.
‡ CFM: complete feed mash and CFP: complete feed pellet.
SEM: standard error of the means.

Table 5 Antioxidative enzyme activity in serum in lambs fed sorghum stover based diets

Attribute*	Conventional diet	Complete diet		SEM	P-value
		Mash	Pellet		
Lipid peroxidase (nmole MDA/mg protein)	5.29^a	4.53^b	4.63^b	0.172	0.015
Glutathione peroxidase (U/mg protein)	6.97^{bc}	12.59^a	11.32^{ab}	0.865	0.027
Glutathione reductase (U/mg protein)	24.65^b	23.62^b	34.96^a	2.012	0.001
RBC Catalase (μmole/min/g Hb)	22.83^c	33.27^b	42.70^a	2.400	0.006

The means within the same row with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
SEM: standard error of the means.

Among the complete diet fed groups, the lambs fed EEP diet had better ability to scavenge oxidative radicals as indicated by higher RBC catalase and glutathione reductase activity.

The better availability of nutrients with EEP could have resulted in higher enzyme activities. No mortality or disease conditions was observed in lambs of either groups, except for respiratory distress in four of ten lambs of conventional group and were recovered after treatment. The antibody titres against *Brucella abortus* antigen were higher (P<0.05) in lambs fed EEP diet compared to mash or conventional diet. The total Ig, IgG and IgM concentration against chicken erythrocytes was higher (P<0.01) in lambs fed complete diets compared to conventional feeding (Table 6). The antibody titres increased (P<0.01) progressively from 7 d to 28 d post sensitization but, later reduced by 35d with peak titres observed on 28^{th} d post sensitization for both antigens.

The higher antioxidant enzyme activities, increased levels of antibodies against specific (*Brucella abortus*) and non specific (CRBC) antigens and the lower incidences of disease occurrence clearly indicated that lambs fed complete diet had higher disease resistance capacity compared to conventional system of feeding.

The *in vitro* lymphocyte proliferative (LP) response against Con. A mitogen (suppressor of T lymphocyte proliferation) was affected by method of feeding and EEP (Table 6). The lymphocyte proliferative response gradually increased from conventional system of feeding to complete diet in mash form and was highest (P<0.05) with expanded extruded pelleted diet. The higher humoral and cell mediated immune response in lambs with expander extruder processing might be due to protection of immune cells from oxidative damage indicated with higher antioxidative enzyme activities (Table 5).

Each lamb in conventional group on an average consumed 250 g concentrate and 930 g chopped sorghum stover. The lambs fed complete diet in mash or EEP form daily consumed on average 1080 and 1092 g of feed (Table 7).

The daily feed cost per lamb was higher (P<0.01) when fed expanded extruded diet followed by complete diet in mash form and lowest in conventional diet. In spite of higher feed cost, the cost of feed per kg weight gain was lower (P<0.01) when fed complete diets. The higher weight gains and improved efficiency of nutrient utilization by lambs fed complete diets resulted in lower cost of feed per kg weight gain.

Table 6 Humoral and cell mediated immune response in lambs fed sorghum stover based diets

	Humoral immune response				Cell mediated immune response
	Brucella abortus antigen	CRBC antigen			LPSI against Con. A‡
Diets*	A_{492}	Total Ig ($\log_2$)	IgM ($\log_2$)	IgG ($\log_2$)	
Conventional	0.486^{b}	5.38^{b}	4.52^{b}	4.12^{b}	0.297^{b}
Mash	0.483^{b}	6.00^{a}	5.08^{a}	4.87^{a}	0.372^{ab}
Pellet	0.525^{a}	6.04^{a}	5.11^{a}	4.92^{a}	0.420^{a}
P-value	0.026	0.001	0.002	0.001	0.026
Days post sensitization**					-
7	0.212^{v}	4.95^{w}	4.18^{a}	3.56^{w}	-
14	0.294^{w}	5.57^{x}	4.66^{x}	4.41^{x}	-
21	0.579^{x}	6.75^{y}	5.80^{y}	5.67^{y}	-
28	1.028^{z}	7.59^{z}	6.66^{z}	6.48^{z}	-
35	0.775^{y}	6.71^{y}	5.76^{y}	5.63^{y}	-
P-value	0.001	0.001	0.001	0.001	-
SEM	0.0271	0.185	0.198	0.194	0.0197

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
* Means of all three periods for each dietary treatment.
** Means of all three diets for each period.
‡ LPSI: lymphocyte proliferation stimulation index and Con. A: Concanavalin A.
SEM: standard error of the means.

Table 7 Cost economics of sorghum stover based diets fed to lambs

Attribute	Conventional diet	Complete diet		SEM	P-value
		Mash	Pellet		
Cost of feed ingredients in diet (Rs/kg)	846.5*	535.9	535.9	-	-
Cost of processing (Rs/kg)	13.62*	32.09	38.50	-	-
Total cost of feed (Rs/kg)	860.12*	568.05	574.46	-	-
Feed consumed (g/d)	1180^{a}	1080^{b}	1092^{b}	9.126	0.001
Cost of feed consumed/d (Rs)	5.87^{a}	6.14^{b}	6.27^{c}	0.031	0.001
Cost of feed/kg ADG (Rs)	101.67^{a}	80.98^{b}	65.21^{b}	4.708	0.003

The means within the same row with at least one common letter, do not have significant difference (P>0.01).
Of concentrate mixture only. Cost of chopped sorghum stover, Rs. 4/kg.
SEM: standard error of the means.

Among the complete diets, the expanded extruded diet reduced the cost of feed/kg weight gain by 19.5% over its mash form.

CONCLUSION

The study indicated that pelleting of crop residue (sorghum stover) based complete diet with expander extruder enhanced the growth performance in lambs compared to conventional diet or complete diet in mash form at reduced cost of production. The humoral and cell mediated immune response against the antigens / mitogens and disease resistance capacity was higher in lambs fed the EEP diet.

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