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Rumen Fermentation and Microbial Nitrogen Supply in Native Sheep Fed Crop Residue based Densified Feed Blocks Varying in Particle Size of Roughage

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ABSTRACT

Background: Roughages with an appropriate particle size are required to maintain the optimum rumen health, fermentation and nutrient digestibility. In this regard, the present study was conducted in sheep to determine the optimum particle size for preparation of complete feed blocks.

Methods: Twenty-one adult Deccani rams (19.45±0.80 kg) of 2-3 yrs age were used in a complete randomized design to assess the effect of particle size (PS) of sorghum stover (SS) on rumen fermentation characteristics and microbial nitrogen supply. Rams were divided into 3 groups consisting of 7 animals each and fed complete feed blocks containing 8 mm (SSCFB-8), 12 mm (SSCFB-12) and 16 mm (SSCFB-16) particle sizes of the stover. Rumen liquor was collected using stomach tube from each animal at 0, 2, 4 and 6 h after feeding to assess rumen fermentation pattern.

Result: Mean rumen pH was increased (P<0.001) with increased PS of the SS in the CFBs and the rams fed SSCFB-12 and SSCFB-16 diets were shown highest (P<0.001) pH values. Average ammonia nitrogen concentrations were lower (P<0.01) in rams fed SSCFB-16 diet compared to those fed SSCFB-12 or SSCFB-8 diets. The average total volatile fatty acid (TVFA) concentration was decreased with increase in PS of SS in the feed blocks. The rams fed SSCFB-8 diet had shown higher (P<0.05) TVFA mean values than those fed SSCFB-12 or SSCFB-16 diets. Microbialnitrogen (g/day, g/kg $W^{0.75}$) and protein supply (g/day, g/kg $W^{0.75}$ and g/kg DOMI) was not affected by the PS of sorghum stover in complete feed blocks.Based on the results of the current study the SS ground to 8 mm can be considered as optimum for sheep fed SS based diets.

Key words: Particle size, Rumen, Sheep, Stover.

INTRODUCTION

Tropical countries like India rely heavily on fibrous feedstuffs such as crop residues (CR) to feed ruminants. However, these CR are characterized by low digestibility, low nutritional value and are bulky in nature. Attempts have been made to improve the utilization of nutrients from these roughage sources in many ways, densified complete feed block technology being one of them. Densified complete feed block (DCFB) consists of forage, concentrate and other nutritional supplements in desired proportions capable of meeting the nutritional needs of livestock. DCFB is a ready-to-eat, convenient, economic, multi-nutrient corrected complete feed for ruminants providing a more ideal and stable environment for rumen fermentation to boost the productivity of animals (Salem and Nefzaoui 2003). Processing of CR into complete feed blocks (CFBs) improves dry matter intake and nutrient utilization in animals, which in turn leads to high yields and sustainable animal reproduction.

The PS of the forage component of the complete feed block (CFB) is an important criterion in the utilization of fibrous feedstuffs by animals, as the PS of the forage plays an important role in maintaining rumen function, which is related to fiber digestibility and pH optimization for cellulose-degrading microorganisms (Gunun *et al.,* 2013). In sheep, the size and shape of the forage particles ingested have been reported to affect voluntary feed intake and also the effect of PS in sheep is more pronounced than in dairy cows.

The PS of forage in sheep's diets can vary widely and it is not clear which measure of forage physical properties best predicts an animal's response. Therefore, the aim of this experiment was to evaluate the effect of using three particle sizes of sorghum stover in the CFBs on ruminal fermentation characteristics in Deccani sheep.

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MATERIALS AND METHODS

Animals, experimental design and feeding management

Twenty one adult Deccani rams of 2-3 yrs of age (19.45±0.80 kg) were randomly divided into 3 groups consisting of 7 animals in each group in a completely randomized design. SS was grinded into three different particle sizes of 8, 12 and 16 mm in low density cutter cum grinder. SS based complete diet was formulated with roughage to concentrate ratio (R:C) of 50:50 so as to meet the nutrient requirements of rams as recommended by ICAR (2013). The CFBs were prepared by compacting the feed materials (mash) in an iron mould into sizes of 15 × 15 × 10 cm using a specially designed semi-automatic hydraulic press equipped with a manual ejection system at a pressure of 1500 psi. The ingredient composition of experimental diets is presented in Table 1.

All the rams used in the experiment were housed individually in a separate pen with adequate ventilation, lighting and fresh water supply. The rams were dewormed with praziquantel and vaccinated against enterotoxaemia at the beginning of the experiment, then gradually acclimatized to the experimental diets for 10 days before starting the experiment. The respective diets were offered twice a day, *i.e*. at 9:30 a.m. and 3:30 p.m. in equal proportions during the preliminary 21 days. Then the rams were transferred to metabolic cages for feeding experimental diets. Metabolic trial was performed for 5 days after acclimatizingthe rams in cages for two days. The feed was offered to the adult rams at 3% of their body weight (BW) to meet the maintenance requirement as recommended by ICAR (2013). During the collection period of 7 d, daily feed offered, leftover as well as feces and urine voided were recorded.

Sample collection and preparation

At the end of metabolic trial, rumen liquor of about 100 ml was collected using stomach tube for two consecutive days from each animal at 0, 2, 4 and 6 h after feeding to assess rumen fermentation pattern. Rumen liquor pH was estimated immediately after collection using a digital pH meter. After determination of pH, rumen liquor was strained through four layered muslin cloth. Two aliquots of 50 ml of strained rumen liquor (SRL) were taken in separate plastic containers and stored in deep freezer for ammonia nitrogen (NH_3-N) and total volatile fatty acid (TVFA) estimation. The aliquot stored for TVFA was added with 0.5 ml of 5% saturated mercuric chloride before storage.

Table 1: Ingredient composition of experimental diets.

Ingredient	Parts
Sorghum stover	50.0
Maize	7.4
Groundnut cake	9.0
Deoiled rice bran	25.0
Molasses	8.0
Mineral mixture	0.2
Salt	0.4
Total	100.0

Chemical analysis and calculations

Feed samples were analysed for proximate principles by AOAC (1997) method. The pH of rumen liquor was measured immediately after collection using the Corning electronic pH electrode. The NH_3-N was estimated by the method of Makkar and Becker (1996). The TVFA in rumen liquor samples was determined as per the method of Barnett and Reid (1957).

Microbial nitrogen flow

The daily intestinal flow of microbial nitrogen (g/d) from total urinary purine derivatives (PD)(mmol/d) was calculated (IAEA-TECDOC-945, 1997) using the PD work software of IAEA (2001). The equation used to relate absorption of microbial purines (X, mmol/l) and excretion of purine derivatives in urine (mmol/l) was Y = 0.84 + (0.150W0.75 e-0.25X). The calculation of X from Y was performed by Newton–Raphton iteration process.

Site of study and ethical statement

The experiment was carried out at the College of Veterinary Science, PVNR Telangana Veterinary University, Rajendranagar, Hyderabad, India. The experimental protocols were done with the approval of Institute's Animal Ethics Committee (IAEC) of PVNR Telangana Veterinary University, Hyderabad, 500 030, Telangana, India.

Statistical analysis

The data of experiment was subjected to analysis through software (Version 15.0; SPSS) by applying two way multivariant analysis of variance through generalized linear model. The treatment means were ranked using Duncan's multiple range test with significance at 5% level (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of experimental diets

The chemical composition (% DMB) of sorghum stover based complete feed blocks (SSCFBs) with different particle sizes of SS is presented in Table 2. The per cent DM, CP, NDF and ADF of feed blocks ranged from 86.54 to 88.39, 10.27 to 10.36, 53.63 to 54.86 and 33.52 to 34.72, respectively. The PS of SS did not affect the chemical composition of complete diets. Non-significant difference in chemical composition of the experimental diets was obvious, as it had similar ingredient composition. No effect of PS of paddy straw grounded through 8, 12 and 16 mm sieves was reported by Nagi *et al.* (2012) which was in line with present findings. Moharrery (2010) and Maulfair *et al.* (2011) observed that, the PS had no effect on the chemical composition of the diets prepared with grass hay, which were similar to the present results.

Voluntary feed intake

Total dry matter intake (DMI) expressed as g/d in rams was ranged from 585.75 to 597.98. Intake of DM, in terms of g/d or g per $kgW^{0.75}$ was not affected (P>0.05) by PS of the SS (Table 4). The effect of dietary PS on DMI may depend on

forage source; forage to concentrate ratio as well as type of concentrate, especially on its ruminal degradation rate (Tafaj *et al*., 2007). In the present study, the forage source, R:C ratio and concentrate ingredients was similar among the three diets hence no effect of variation in size of SS could be observed. Consistent with these findings, there was no effect of forage PS on DMI for diets containing about 0.40 forage (Beauchemin *et al.,* 2003 and Beauchemin and Yang, 2005). However, dietary PS was reported to affect DMI when diets contain about 0.60 forage (Schwab *et al.,* 2002 and Kononoff *et al.,* 2003). The R:C ratio in the present study was 50:50 and could be also one of the reasons for no effect of PS of roughage on DMI.

Table 2: Nutrient composition (%DM basis) of sorghum stover based complete feed blocks (SSCFB) varying in particle size of sorghum stover.

Constituent	SSCFB-8	SSCFB-12	SSCFB-16
	Proximate constituents		
Dry matter	86.54	87.09	88.39
Organic matter	88.50	88.75	88.52
Crude protein	10.36	10.27	10.28
Ether extract	1.72	1.73	1.76
Crude fiber	22.17	23.62	22.75
Nitrogen free extract	54.23	53.10	53.72
Total ash	11.50	11.25	11.47
	Cell wall constituents		
Neutral detergent fibre	54.65	54.86	53.63
Acid detergent fibre	34.72	34.69	33.52
Hemicellulose	19.92	20.16	20.11
Cellulose	26.26	25.98	25.75
Lignin	5.62	5.66	5.29

Each value is the average of duplicate analysis.

On dry matter basis except for dry matter.

SSCFB-8 @ 8 mm ground sorghum stover in complete feed blocks. SSCFB-12 @ 12 mm ground sorghum stover in complete feed blocks. SSCFB-16 @ 16 mm ground sorghum stover in complete feed blocks.

Rumen fermentation pattern

The rumen fermentation in Deccani rams fed SSCFBs with different PS of SS at four-time intervals (0, 2, 4 and 6 h) is shown in Table 3.

Ruminal pH

Ruminal pH is a very important variable to indicate function of the rumen ecosystem. The incidence of subacute acidosis increased when ruminal pH fell below 5.8 (Tafaj *et al.,* 2007). The ruminal pH range of 6.02 to 6.83 reported in this study was within the normal function of the rumen ecosystem.

The rumen pH values, irrespective of diets, decreased ($P<0.01$) from 0 to 4 h of feeding and increased between 4 and 6 h of feeding. No difference in values was observed between 2 and 4 h value. However, significant ($P<0.001$) difference in rumen pH values was observed among the three dietary groups. Mean pH values increased ($P<0.001$) with increased PS of the SS in the blocks from 8mm to 16mm size and the rams fed SSCFB-12 and SSCFB-16 CFBs are showing highest ($P<0.001$) pH values *i.e*. 6.48 and 6.46, respectively and lowest pH was recorded in animals

Table 3: Effect of feeding sorghum stover based complete feed blocks (SSCFB) with different particle sizes of sorghum stover on rumen fermentation pattern in Deccani rams.

Diet	Time interval				Mean±SE	P value		
	0 h	2 h	4 h	6 h		T	P	TxP
			pH					
SSCFB-8	6.75	6.02	6.19	6.29	6.31^{b}±0.05	0.001	0.001	0.490
SSCFB-12	6.83	6.30	6.35	6.46	6.48^{a}±0.04			
SSCFB-16	6.77	6.30	6.29	6.47	6.46^{a}±0.04			
Mean±SE	6.78^{A}±0.03	6.20^{C}±0.05	6.28^{C}±0.03	6.41^{B}±0.04				
			Ammonia nitrogen (mg/100ml)					
SSCFB-8	14.12	22.46	20.82	16.16	18.39^{a}±0.58	0.002	0.001	0.508
SSCFB-12	14.15	23.16	19.87	16.14	18.33^{a}±0.44			
SSCFB-16	14.05	20.54	18.25	14.22	16.77^{b}±0.63			
Mean±SE	14.11^{D}±0.21	22.05^{A}±0.55	19.65^{B} ±0.50	15.51^{C}±0.38				
			Total volatile fatty acids (mmol/100ml)					
SSCFB-8	7.74	12.28	11.14	9.75	10.23^{a}±0.31	0.035	0.001	0.702
SSCFB-12	7.56	11.02	11.01	8.89	9.62^{ab}±0.31			
SSCFB-16	7.10	10.91	10.03	8.37	9.10^{b}±00.32			
Mean±SE	7.47^{C}±0.17	11.40^{A}±0.37	10.72^{A}±0.28	9.00^{B}±0.25				

Each value is the average of the seven observations.

^{abc}Means with different superscripts in a row differ significantly: $P\leq0.05$.

^{ABCD}Means with different superscripts in a column differ significantly: $P\leq0.05$.

SEM: Standard error mean; P: Probability value.

fedSSCFB-8 diet (6.31). One of the most important factors influencing rumen pH is the amount of saliva buffer secretion, which is positively correlated with rumination activity (Zebeli *et al.,* 2008). The underlying mechanism has been proposed that increasing PS increases rumination activity, then increases the salivary secretion flow into the rumen and thus buffers the ruminal pH (Krause *et al.,* 2002 and Beauchemin *et al.,* 2003).

Krause *et al.* (2002) reported that decreasing forage PS from 6.3 to 2.8 mm decreased ruminal pH from 6.02 to 5.81 in dairy cows corroborating with present findings. Kononoff and Heinrichs (2003b) also reported similar pattern of increase in ruminal pH with increase in PS of alfalfa haylage in cows. These results are consistent with Zhao *et al.* (2009) and Gunun *et al.* (2013), who noticed, reduction in the PS of rice straw reduced ruminal pH in steers and goats, respectively. The results of the present study are inconsistent with previous studies which indicated that PS had no significant effect on the rumen pH (Kononoff *et al.* 2003, Yang and Beauchemin 2006 and Zebeli *et al.,* 2008).

Ruminal ammonia nitrogen

In the current study, NH_3-N concentrations were ranged from 14.05 to 23.16 mg/100 ml and were closer to the optimal ruminal NH_3-N range (15 to 30 mg/100ml) (Anantasook *et al.,* 2013) (Table 3). The average concentration of rumen NH_3-N was highest (P<0.001) at 2h after feeding and later decreased by 4h and 6 h after feeding . The NH_3-N decreased with increased PS of SS from 8 to 16 mm. Average NH_3-N concentrations were lower (P<0.01) in rams fed SSCFB-16 (16.77) diet compared to those fed SSCFB-12 or SSCFB-8 diets while no significant difference was observed between the rams fed SSCFB-12 and SSCFB-8 diets. Similar findings were reported by Yang and Beauchemin (2006), who observed linear reduction in ruminal ammonia concentration in dairy cows with increasing PS of corn silage. The results of present experiment are also in agreement with the findings of Zhao *et al.* (2009) who found that increased PS (10 to 80 mm) of rice straw decreased the NH_3-N concentration. These results are inconsistent with previous studies which indicated that PS had no significant effect on ruminal NH_3-N concentration (Yansari *et al*., 2004, Zebeli *et al.,* 2008, Gunun *et al*., 2013 and Kononoff and Heinrichs 2003b).

Total volatile fatty acid

In the present study, TVFA concentrations in all treatments ranged from 7.10 to 12.28 (mmol/100ml) and were found at normal concentrations (70 to 130 mM) (Wanapat *et al*., 2013) (Table 3). The TVFA concentration increased from 0 to 2 h and the levels were maintained at 4 h post feeding and later decreased by 6 h after feeding. The feeding of SSCFB-8 diet had shown higher (P<0.05) average TVFA concentration (10.23) in rams than other groups. In this study, TVFA

Table 4: Effect feeding sorghum stover based complete feed blocks (SSCFB) with different particle sizes of sorghum stover on excretion, absorption of purine derivatives and microbial protein supply in native sheep.

Attribute	SSCFB-8	SSCFB-12	SSCFB-16	SEM	P value
Dry matter intake					
g/d	597.98	591.60	585.75	13.19	0.937
g/kg $W^{0.75}$	65.68	65.07	62.98	1.504	0.764
Purine derivatives excretion (mmol/d)					
Allantoin	8.63	8.50	8.10	0.190	0.38
Uric acid	1.55	1.53	1.45	0.290	0.36
Xanthine+Hypoxanthine	0.17	0.17	0.15	0.006	0.26
Total	10.35	10.20	9.70	0.192	0.36
Purine absorbed					
mmol/d	12.24	12.06	11.45	0.231	0.35
mmol/kg $W^{0.75}$	1.35	1.33	1.23	0.034	0.33
Microbial nitrogen supply					
g/d	8.90	8.77	8.32	0.168	0.35
g/kg $W^{0.75}$	0.98	0.97	0.90	0.024	0.33
Microbial protein supply					
g/d	55.62	54.82	52.01	1.049	0.35
g/kg $W^{0.75}$	6.13	6.04	5.60	0.152	0.33
DOMI (kg)	0.347	0.341	0.320	0.008	0.36
g/kg DOMI	160.58	160.81	162.59	0.633	0.38

Each value is an average of seven observations.

SSCFB-8 @ 8 mm ground sorghum stover in complete feed blocks.

SSCFB- 12 @ 12 mm ground sorghum stover in complete feed blocks.

SSCFB-16 @ 16 mm ground sorghum stover in complete feed blocks.

concentration decreased (P<0.001) with increased PS of SS, which agrees with the findings of Krause *et al.* (2002) and Kononoff *et al.* (2003) who found that TVFA concentration increased linearly with reduction in PS of forage in dairy cows. These observations are also consistent with other studies in which the reduction in PS of both alfalfa haylage (Kononoff and Heinrichs 2003b) and barley silage (Soita *et al.,* 2002) resulted in increased TVFA concentration.

Reason for decreased TVFA concentration in rams fed the blocks with 16 mm PS might be attributed to the longer PS of roughage which increases chewing time and saliva flow and lowers the concentration of VFA as saliva flow has a dilution effect and increases the turnover rate of rumen liquid (Halako *et al.,* 2020). Further, reduction of PS increased the surface area of particles and their fermentability, resulting in increased concentration of TVFA in rams fed SSCFB-8 and SSCFB-12 diets compared with rams fed SSCFB-16 diets and same was reported by Yansari *et al.* (2004). However, these results are in contrast with some previous studies (Zhao *et al.,* 2009, Yang and Beauchemin 2006 and Zebeli *et al.*, 2008), who did not find any effect on VFA production with varying levels of PS of roughages.

Excretion and absorption of purine derivative and microbial protein supply

Estimation of urinary PD excretion is an indirect, non-invasive method of estimating the rumen microbial protein production (Moorby *et al.,* 2006). In the present study, PS of SS in CFBs did not show any influence (P>0.05) on the excretion and absorption of purine derivatives (allantoin, xanthine plus hypoxanthine and uric acid) (mmol/day and mmol/kg $W^{0.75}$) (Table 4). Similarly, the microbial nitrogen (g/day, g/kg $W^{0.75}$) and protein supply (g/day, g/kg $W^{0.75}$ and g/kg DOMI) was not effected by PS of SS. In support of this observation, no effect of forage PS on microbial N yield in dairy cattle was reported by Krause *et al.* (2002) and Krause and Combs. (2003). Genovez Chanona *et al.* (2008) also did not find any effect on microbial N yield in cattle fed diets varying in PS (3 and 25 mm) of hay. Though statistically comparable, higher values (P>0.05) of microbial nitrogen supply (g/d) and microbial protein supply (g/d) were recorded in rams fed SSCFB-8 blocks than SSCFB-12 and SSCFB-16 diets. This could possibly be due to matching supply of energy and nitrogen to the microbes and the values obtained in this study were below the mean value (32 g N/kg of RDOM) established by the ARC (1984) for sheep fed different diets since the diets were crop residue based.

CONCLUSION

Based on the results obtained in this study, it was concluded that feed blocks with 8mm PS of SS was optimum for sheep as it resulted in higher concentration of ruminal NH_3-N and TVFA with high microbial protein supply in rumen.

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Declaration of conflict of interest

The authors declare that there are no conflicts of interests.

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