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## Effect of Levels of Lucerne Straw in Total Mixed Ration on Rumen Fermentation Pattern and Blood Metabolites in Bullocks

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### Abstract

A study was planned to ascertain effect of 50 (T<sub>1</sub>), 60 (T<sub>2</sub>) and 70 (T<sub>3</sub>) % lucerne straw in total mixed ration (TMR) on rumen fermentation and blood metabolites in Kankrej crossbred bullocks. Two bullocks were assigned in each treatment using 3 x 3 Latin Square design. The rumen and blood metabolites were evaluated on last day of each period. The pH of strained rumen liquor (SRL) was slightly alkaline (7.09-7.16) on account of green forage feeding in morning before TMR. The pH was lowest at 4 hr post-feeding. The total volatile fatty acids (TVFAs) were 75.79-77.67 mEq/litre and a reverse trend was observed for TVFAs as compared to pH. The values for ammonia, total, soluble, non-protein and protein nitrogen in SRL were 15.07-17.52, 86.92-90.07, 39.90-46.55, 35.88-40.69 and 46.23-53.50 mg/100 ml, respectively. All nitrogen fractions peaked at 2 hr post-feeding, except protein nitrogen which peaked at 6 hr, showing maximum activity up to 6 hr post-feeding. The values for serum protein, phosphorus and calcium as well as blood haemoglobin were within physiological range. The differences amongst treatments for rumen and blood metabolites were non-significant. The results suggest that lucerne straw can be incorporated up to 70% in TMR of bovines for maintenance without adverse effect on rumen fermentation and blood constituents.

**Key Words** : Bullock, Total mixed ration, Lucerne straw, Rumen fermentation, Blood metabolites.

### Introduction

India stands first in the world for cattle and buffalo population (FAO, 1997). The geographical area of the country is just 2% of the world, which clearly indicates heavy pressure of livestock per unit area. The green roughage, dry roughage and concentrate are deficit up to 62.76, 23.46 and 63.00% of requirement (Kore, 2014). The deficit feed resources needs efficient use and improvement in feeding system. The TMR is single feed mixture consist of all the dietary ingredients of roughage and concentrate without grinding in predefined ratio. The main roughages used so far are cereal crop residue and very few studies are available on legume straw as sole roughage. After harvesting seeds of lucerne, the remaining part of the crop has low nutritional value. Since no data are available regarding incorporation of lucerne straw, the study was aimed to ascertain effect of different levels of lucerne straw in TMR on rumen fermentation pattern and blood metabolites in bovines.

## Materials and Methods

The present study was conducted on six Kankrej crossbred bullocks at the Animal Nutrition Research Department, Anand (Gujarat) following 3 x 3 Latin square design with each period of six weeks duration. Two bullocks with uniform body weight (299.11 kg-Square-I and 375.33 kg-Square-II) were assigned to each treatment. The bullocks were weighed every week for three consecutive days before feeding and watering in morning. The 2 kg green NB-21 hybrid napier was fed in the morning to meet vitamin A requirement and total mixed ration with 50, 60 and 70% lucerne straw (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) was fed individually in two equal part in morning and afternoon to meet nutrients requirement (ICAR, 1985). The ingredients used in the TMR of different feed were as shown in Table 1 – Bullocks were let loose daily for 2hrs in the morning and evening throughout the experimental period, except during rumen metabolite study. The rumen metabolites study was conducted at the end of each period. During this period, the bullocks had free access to fresh clean water. During rumen metabolites study water was offered 4 times daily, i.e. 9.30 am, 11.30 am, 3.00 pm and 5.00 pm.

**Table 1: Ingredients composition (%) of total mixed ration**

Lucerne straw	50.0	60.0	70.0
Groundnut DOC	5.0	7.0	8.0
Rice polish	8.0	6.0	5.0
Wheat bran	5.5	5.5	5.5
Deoiled rice bran	20.0	10.0	0.0
Molasses	10.0	10.0	10.0
Mineral Mixture	1.0	1.0	1.0
salt	0.5	0.5	0.5
Total	100.0	100.0	100.0

The progressive changes in pH, total volatile fatty acids (TVFAs) production and various nitrogen fractions were studied before feeding (0 hr), and at 2, 4 and 6 hrs post-feeding in SRL. About 250 ml of rumen liquor was collected each time from each bullock using stomach tube and employing suction. The rumen liquor was immediately brought to laboratory and strained through four layers of cheese cloth. The pH was determined immediately using a digital pH meter. The samples of SRL were analyzed for ammonia-N and total-N by Kjeldahl's method (Tiwari *et al.*, 2012 and Gaylean, 2010). Rest of the SRL was stored in glass bottle by adding mercuric chloride @ 0.5 g/100 ml SRL in deep freeze for further analysis. Soluble-N in supernatant of SRL after centrifuging was estimated by Kjeldahl's method and non protein-nitrogen by Trichloro-acetic acid precipitation of SRL and estimating the N content of supernatant by Kjeldahl's method. The concentration of total VFA was determined in SRL by the steam distillation method using Markham micro-distillation apparatus (Tiwari *et al.*, 2012).

The blood samples of each bullock were collected in the morning on last day of trial from jugular vein under all aseptic precautions. Haemoglobin was measured using Sahli's haemoglobinometer and serum protein estimated as per AOAC (1990). The serum calcium and phosphorus were estimated as per Chauhan and Chandra (2003). The data generated were analyzed statistically (Snedecor and Cochran, 1994).

## Results and Discussion

### Rumen Fermentation:

The periodical and average values for rumen fermentation parameters are given in Table 2. The average SRL pH was 7.09, 7.16 and 7.13 in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively, with non-significant (P>0.05)

difference amongst treatments. The SRL pH values were highest at prefeeding state, decreased after feeding and were lowest at 4 hr post-feeding followed by gradual increase at 6 hr post-feeding. Similar trend was observed in all the treatments. The higher (alkaline) pH might be due first feeding green hybrid napier in morning, which form the mat in rumen and buffered the pH both due to forage & the increased salivation associated with forage consumption (NRC, 2001). The similar ruminal pH (7.04-7.18) of growing male swamp buffaloes was reported on feeding diet having rice straw from 65.39 to 66.22% and time study revealed lower ruminal pH at 4 hr post-feeding (Tatsapong *et al.*, 2010). The feeding of complete diet with 40% rice straw and 40% corn silage to Friesian cows resulted in slightly alkaline (7.10) and slightly acidic (6.79) ruminal pH, respectively and lower pH at 4 hr post-feeding followed by gradual rise as reported by Bassiouni *et al.* (2010). Pinos-Rodriguez *et al.* (2008) also reported that the rumen pH values were unaffected by the roughage to concentrate ratio in lamb. The findings of the present study are in line with these reports. However, the SRL pH (6.90) was higher ( $P<0.01$ ) in buffalo bulls fed TMR with higher level of roughage (70% corn stover) in comparison to bulls fed TMR with 60% corn stover, whereas SRL pH was significantly lower (6.74) at 4 hr post-feeding and again increased at 6 hr post-feeding as reported by Rajamma *et al.* (2014). Similarly Gaafar *et al.* (2010) also reported that the rumen pH value of buffaloes were increased significantly ( $P<0.05$ ) with increasing dietary fiber content in TMR.

The average SRL total volatile fatty acid (TVFAs) was 75.79, 77.67 and 76.00 mEq/litre under  $T_1$ ,  $T_2$  and  $T_3$  respectively. The TVFAs peaked at 4 hr and then decreased at 6 hr post-feeding. This pattern on time study was reverse as compared to that observed for pH. The similar mean ruminal TVFAs as 72.39 mmol/litre was revealed by Tatsapong *et al.* (2010) on feeding diet having 66.22% rice straw to growing male swamp buffaloes. Bilik and Łopuszańska-Rusek (2010) revealed similar rumen fermentation pattern in Red-and-White Polish Holstein-Friesian dry cows as 51.60 mmol/litre at pre-feeding and 94.00 mmol/litre ruminal TVFAs at 3 hr post-feeding on feeding diet with 70.7% medium quality meadow hay as roughage and 29.3% concentrate. The similar pattern of rumen fermentation has also been documented in Friesian cows (Bassiouni *et al.*, 2010), buffalo bulls (Poonooru *et al.*, 2015 and Rajamma *et al.*, 2014) and buffalo (Gaafar *et al.*, 2010).

The present results revealed non-significant difference of average ammonia nitrogen of SRL under  $T_1$ ,  $T_2$  and  $T_3$ , respectively (Table 2). The ammonia nitrogen concentration increased after feeding and peaked at 2 hr post-feeding and decreased gradually thereafter under all three treatments. The ruminal ammonia nitrogen was 12.42 and 16.73 mg%, respectively when diet having 41.51% rice straw and 51.26% urea treated rice straw as roughage was fed to buffalo bulls (Yuangklang *et al.*, 2013). Feeding of TMR with 70% groundnut haulm (roughage) to Murrah bulls resulted mean ammonia nitrogen as 10.12 mg/100 ml SRL and ammonia nitrogen peaked at 4 hr post-feeding (Poonooru *et al.*, 2015). Similar ammonia nitrogen pattern were also revealed by Srinivas kumar *et al.* (2015).

The average SRL total nitrogen of crossbred bullocks was within normal range with the values of 90.07, 89.37 and 86.92 mg/100 ml under  $T_1$ ,  $T_2$  and  $T_3$  respectively, the differences were non-significant. The periodical trends of total nitrogen content of SRL under all the treatments were at peak at 2 hr post-feeding and gradually declined thereafter. The mean total ruminal nitrogen was reported as 75.75 mg/100 ml when Murrah bulls were fed TMR containing 70% groundnut haulm (Poonooru *et al.*, 2015). Raj Kiran and Srinivas Kumar (2013) also revealed mean total nitrogen as 75.90 and 70.71 mg/100 ml SRL, respectively on feeding complete diets with 60% maize stover and 60% sorghum stover to Murrah bulls, which peaked at 4 hr post-feeding.

The average SRL values for soluble nitrogen/100 ml were 43.05, 39.90 and 46.55 mg, NPN/100 ml 38.73, 35.88 and 40.69 mg and protein nitrogen/100 ml as 50.68, 53.50 and 46.23 mg, in crossbred bullocks under  $T_1$ ,  $T_2$  and  $T_3$  respectively. The treatment differences for all three traits were non-significant. The concentrations of soluble and non-protein nitrogen were at peak at 2 hr post feeding and decreased gradually thereafter, while protein nitrogen peaked at 6 hr post-feeding. The

Table 2: Rumen fermentation pattern of bullocks fed lucerne based TMR

	7.62	7.62	7.60
	6.92	7.12	7.20
	6.75	6.85	6.80
	7.07	6.99	6.98
	+		
	53.83	54.17	57.50
	77.83	82.17	71.50
	89.67	91.00	90.83
	81.83	83.83	84.50
	+		
	11.83	11.83	12.89
	21.93	22.52	23.57
	17.03	16.74	20.30
	9.98	11.84	13.30
	+		
	70.17	83.33	71.87
	104.30	102.67	103.37
	93.33	92.17	87.50
	92.17	87.83	84.93
	+		
	38.73	38.33	37.57
	56.70	51.57	60.20
	39.43	40.60	48.53
	37.33	38.33	39.90
	+		
	34.71	38.33	33.83
	49.00	46.38	52.71
	37.63	37.92	43.46
	33.58	38.17	33.25
	+		
	35.76	38.33	38.03
	53.54	56.29	51.16
	55.71	54.25	44.05
	57.68	58.18	51.68
	+		

NS= Non-significant

protein nitrogen showed maximum activity up to 6 hr post-feeding. All nitrogen fractions were peaked at 2 hr post-feeding when crossbred bulls were fed complete diet containing 40% sunflower heads and 50% cotton seed hulls (Reddy and Reddy, 1998). Reddy *et al.* (2001) and Murthy *et al.* (2001) also reported peak ruminal nitrogen fractions at 2 hr post-feeding when Murrah bulls and bullocks were fed complete diet containing 40% sugarcane bagasse and diet with groundnut haulm (40%), green sorghum (20%) and concentrate (40%), respectively. The mean values of rumen ammonia nitrogen, total nitrogen, protein nitrogen, residual nitrogen and food & protozoal nitrogen was reported as 10.12, 75.75, 25.40, 22.41 and 17.81 mg/100 ml, respectively when Murrahs were fed 70% groundnut haulm as roughage in TMR and all nitrogen fraction peaked at 4 hr post-feeding (Poonooru *et al.*, 2015). The higher values of various nitrogen fractions may be due to higher proportion of pulse straw in TMR, but the values were within normal range (Lunagariya, 2016). The lucerne straw can be incorporated up to 70% in TMR without any adverse effect on rumen metabolites.

### Blood Metabolites:

The average values for blood haemoglobin content (Table 3) of crossbred bullocks were 10.84, 10.93 and 10.94 g per cent, for serum protein content 6.07, 6.23 and 5.28 g/dl, for serum phosphorus content 4.60, 4.42 and 4.35 mg/dl and for serum calcium 10.32, 10.05 and 10.12 mg/dl, respectively, under T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. The differences amongst treatments were non-significant for all these traits, and the values were within normal range. Similar observations have been documented by earlier workers also (Radotra *et al.*, 2002; Shinde, 2002, and Merck Veterinary Manual, 2011). The incorporation of lucerne straw up to 70% in TMR has no adverse effect on blood metabolites.

**Table 3: Blood metabolites of bullocks fed lucerne based TMR**

Haemoglobin (g%)	10.84 ± 0.08	10.93 ± 0.14	10.94 ± 0.13	0.49 <sup>NS</sup>
Serum protein (g/dl)	6.07 ± 0.08	6.23 ± 0.12	6.28 ± 0.11	3.35 <sup>NS</sup>
Serum phosphorus (mg/dl)	4.60 ± 0.10	4.42 ± 0.15	4.35 ± 0.14	1.42 <sup>NS</sup>
Serum calcium (mg/dl)	10.32 ± 0.16	10.05 ± 0.12	10.12 ± 0.10	0.92 <sup>NS</sup>

NS=non-significant.

### Conclusion

The rumen metabolites like pH, TVFAs and nitrogen fractions were within normal range and the differences amongst treatments (Lucerne straw @ 50, 60 and 70% in TMR) were non-significant. The blood metabolites like serum protein, phosphorus and calcium as well as blood haemoglobin were also within normal range with non-significant differences amongst treatments. The results suggest that lucerne straw can be incorporated up to 70% in TMR for maintenance of bullocks.

**Conflict of Interest:** All authors declare no conflict of interest.

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