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Assessment of rumen fermentation pattern in buffaloes fed with roasted guar korma

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Abstract
This study was conducted to evaluate the effect of feeding roasted guar korma by replacing groundnut cake (C) at 50 per cent (T1) and 100 per cent level (T2), on protein basis, on rumen fermentation pattern in buffaloes. Roasted guar korma is a by-product of guar gum manufacturing and is a rich source of vegetable protein (58.8% CP). The rumen fermentation parameters were studied for the three treatments in three rumen fistulated male buffaloes using 3X3 latin square design. The bulls were kept on maintenance diet containing 2.5 kg concentrate mixture and wheat straw ad. lib. Nitrogen fractions, TVFAs and pH in rumen liquor of adult male buffaloes as affected by different treatments and time of sampling were analyzed. The total nitrogen rose at 3 hrs post feeding and again started declining at subsequent intervals for C and T_1 treatment groups but, it was at its highest level at 6 hours post feeding in T_2 treatment group. It was significantly higher for C than T_2 at 3 hrs post-feeding but the mean values of total nitrogen in SRL for different time intervals were statistically non-significant. Similar trend was seen for the NH_3-N level in SRL at different time intervals. The mean values of TCA-ppt.-N and NPN of SRL at different time intervals were also non-significant for different treatments. The difference in mean level of total volatile fatty acids in SRL for different time intervals in C, T_1 and T_2 was significant, the values being highest for T_2 and lowest for C. The differences among treatments were non-significant at 0 and 9 hours post-feeding, but, at 3 hour post-feeding T_1 and T_2 were significantly higher than C and at 6 hour post-feeding there was significant difference among all the treatments, the values being highest for T_2 and lowest for C. The mean level of pH did not vary significantly among the three treatment groups. Thus, from the present study, it can be concluded that roasted guar korma can successfully replace groundnut cake in concentrate mixture at 50 and 100 per cent level in buffalo diet without adversely effecting rumen fermentation pattern and enhancing protein and fiber utilization.

Keywords: rumen fermentation pattern, buffaloes fed, roasted guar korma

1. Introduction
Livestock rearing is one of the most important economic activities in the rural areas of the country contributing significantly to the national economy. It provides supplementary income to most of the family dependent on agriculture and for many landless families, the income generated through the livestock rearing activities has been the mainstay (DADH). Price ratio of concentrate feeds to animal products has narrowed down and thus in order to economize the cost of production of animal products, there is a need for substitution of traditional concentrates by some cheap but nutrient rich agro industrial by-products.

Guar (*Cyamopsis tetragonoloba*) is an important cash crop in rain fed, especially in semi-arid and arid regions of India. It is a drought-tolerant annual legume mostly grown in India and Pakistan (Mishra *et al*., 2013) [14]. It is an industrially important legume, as guar gum of high export value is extracted from it. The average production of guar seed in India is 7-8 lakh tones and it fluctuates largely based on rainfall pattern (APEDA) Guar seed has three parts: the seed coat (14–17%), the endosperm (35–42%) and the germ (43–47%) (Lee *et al*., 2004) [13]. Guar gum extraction results in the production of protein-rich by products, Churi Korma (guar meal), which are the germ and hull portions of the seed (Sharma & Gummagolmath, 2012) [17]. Extracts from Guar seed include Guar Split/Gum (29%), Korma (30-35%) and Churi (35-40%) (APEDA, 2014) [2]. the processed guar korma is usually rich in proteins and carbohydrates and thus forms a high protein feed for ruminants and other animals. It is used mainly to feed the milking animals to increase the milk and milk fat percentage, besides being a good feed for beef animals (Etman *et al*., 2014a) [5]. The CP content of guar korma is 56-58 per cent, Saeed *et al*., (2017); 55.8 per cent, Soliman *et al.*, (2014) [18]; 52.7 per cent, Nidhina and Muthukumar

(2015) [15]; 50 per cent, Etman et al., (2014a) [5] and 46.9 per cent, Grewal *et al*., (2014) [9]. The CP content of guar korma varies according to the type of germ fraction and heat treatment in the final product. Guar korma is generally cheaper feed ingredient than soyabean meal, dried distiller grains, cotton seed cake and groundnut cake and therefore used as a substitute for those traditional meals in feeding animals (Etman *et al.,* 2014a) [5]. Beta-galactomannan gum residue and trypsin inhibitor are the major antinutritional factors present in guar meal. Beta-galactomannan gum residue acts as a growth depressing agent in poultry, but this effect can be overcome by the inclusion of certain enzymes, such as pectinase and cellulase as they are capable of hydrolysing the galactomannan gum (Gheisari *et al.,* 2011) [7]. Several researchers were of the opinion that trypsin inhibitor is the primary antinutritional factor that limits the use of guar meal in feed (Couch *et al.,* 1967) [3] but, according to the reports of Lee *et al*., 2003 [12], guar meal contains negligible amounts of trypsin inhibitor. Saponins decrease the palatability of the feed and impair the digestion of proteins and absorption of minerals and vitamins in the gut (Fransis *et al.,* 2013). Effects of different heat treatment on the antinutritional factors of industrial guar meal showed a significant reduction of trypsin inhibitor and phytate level (Nidhina & Muthukumar, 2015) [15]. Etman *et al*., (2014a) [5] concluded that total and daily gains increased with increasing guar korma levels in experimental rations of growing buffalo calves. When groundnut cake was replaced by guar meal in crossbred calves ration at 0, 50 and 100% levels, daily gains, feed efficiency and digestibility of DM improved as level of guar meal increased in ration (Sagar and Pradhan, 1977).

2. Location of experiment
The experiment was conducted at the animal farm of Animal Nutrition & Feed Technology Division, Central Institute of Research on Buffaloes, Hisar. Hisar city is situated in semi–arid region and climatic conditions are subtropical in nature.

3. Animals and experimental design
Three fistulated bulls of similar age, body weight and dry matter intake were used in a 3x3 latin square design, so that each animal receives every dietary treatment at different time interval, to study the effect of replacing groundnut cake with roasted guar korma on rumen fermentation pattern.

4. Housing and feeding of fistulated animals
The animals were housed separately in a spacious and ventilated shed with provision of individual feeding and rumen liquor collection. The bulls were fed maintenance ration containing 2.5 kg concentrate mixture and wheat straw *ad.lib*. Water was also freely available. The animals were fed concentrate mixture separately every morning at 8.00 am. The concentrate mixture viz. Control, T_1 and T_2 were fed to different animals for a adaptation period of 21 days followed by two days of rumen liquor collection period. Chemical composition of feed offered to experimental animals is presented in Table 1.

Table 1: Chemical composition of concentrate mixture of different experimental rations and wheat straw (% on DM basis)

Attributes	Treatments (Concentrate)			Wheat straw
	C	T_1	T_2	
DM	90.30	90.35	90.64	92.00
OM	90.15	89.82	89.50	89.02
CP	22.96	23.46	23.88	4.30
EE	4.65	4.35	3.95	0.80
CF	12.30	12.05	11.50	39.35
Total Ash	9.85	10.18	10.50	10.98
NFE	50.24	49.96	50.17	44.57
NDF	42.00	42.80	43.40	77.50
ADF	18.80	18.50	18.00	51.60

Each figure is an average of three observations

5. Collection of rumen liquor
Five days before collection the animals were tied separately and the concentrate mixture was fed at 5.00 am and wheat straw given at 2:30 pm. Rumen liquor samples were collected at 0, 3, 6- and 9-hours intervals for two consecutive days.
Samples were collected through rumen cannula with the help of 250 ml plastic bottle. The rumen liquor was collected from four different sites in the rumen to get representative samples and pH was measured immediately. The rumen liquor was strained through four layers of muslin cloth. The strained rumen liquor was immediately used for estimation of TVFAs, NH_3-N, Total-N and TCA precipitated N. The left-over strained rumen liquor was stored in 100 ml plastic bottles containing few drops of saturated mercuric chloride solution and stored at -20 ^oC.

6. Statistical analysis
Data was analysed statistically as described by Snedecor and Cochran, (1994). Analysis of variance was used to study the difference among treatment means and they were compared by using Duncan's multiple range test as modified by Kramer, (1956).

7. Result and discussion
Nitrogen fraction in rumen liquor
Nitrogen fraction in rumen liquor of adult male buffaloes as affected by different treatments and time of sampling are presented in Table 1.

7.1. Total nitrogen
The total nitrogen content at 0-hour pre-feeding and 6 hours post-feeding were 53.2±2.04, 54.60±2.82 and 55.42±3.03 mg/dl and 63.7±2.39, 67.90±2.89 and 70.23±3.51 mg/dl in treatments C, T_1 and T_2, respectively. The difference among the treatments was non-significant. Total nitrogen (mg/dl) content of the rumen liquor in treatment C, T_1 and T_2 was 79.80±2.26, 73.03±2.3 and 69.38±3.3 mg/dl at 3 hours post-feeding, respectively. The values were significantly higher for C than T_2, however the differences between C and T_1 and T_1 and T_2 were non-significant, because rumen degradable protein was higher in GNC than in roasted guar korma. The results are similar to the findings of Mahesh *et al*. (2017) who

reported the rumen degradable nitrogen and per cent of rumen degradable nitrogen that degraded rapidly in rumen were 75.03 and 80.57; 69.13 and 45.97%, for GNC and guar korma, respectively. Similar findings were given by (Mondal *et al.*, 2008). Moreover, roasting would have also reduced the degradability of protein of roasted guar korma up to certain extent. Total nitrogen (mg/dl) content of the rumen liquor in treatment C, T_1 and T_2 were 56.23±1.79, 62.30±3.6 and 67.67±3.59 mg/dl at 9 hours post-feeding, respectively. The values were significantly higher for T_2 than C. The mean values of total nitrogen in SRL for different time intervals in treatments C, T_1 and T_2 were 63.23±2.03, 64.45±2.85 and 65.67±3.31 mg/dl, respectively, which were statistically non-significant.

The total nitrogen rose at 3 hours post feeding and again started declining at subsequent intervals for C and T_1 treatment groups but, it was at its highest level at 6 hours post feeding in T_2 treatment group. This is also attributed to the rapidly rumen degradable nitrogen of GNC which cause a rapid hike and fall at 3 and 6 hours, respectively for C and T_1 group. The results were in agreement to El-Monayer *et al.* (2015) [5] in buffaloes and soliman *et al.* (2014) [18] in sheep where total N raised by replacing cottonseed cake and soyabean meal in concentrate mixture with guar korma. The rise of total N content of SRL in guar korma was because rumen degradable nitrogen content of cottonseed cake (48.3) and soyabean meal (68.27) was less than guar korma (69.13) (Mondal *et al.*, 2008). Hossein *et al.* (2010) reported that applying heat treatment during processing of guar korma meal led to decrease the effective degradability of DM when compared with cotton meal.

7.2. Ammonia nitrogen

The ammonia nitrogen of the rumen liquor of animals in treatments C, T_1 and T_2 was 9.19 ±0.25, 9.43±0.12 and 10.03±0.09 mg/dl and 11.36±0.44, 11.81±0.13 and 13.23±0.52 mg/dl at 0 and 9 hours post-feeding, respectively. At 3 hours post-feeding the values of ammonia nitrogen in treatment C, T_1 and T_2 were 20.16±0.74, 18.13±0.67 and 15.7±0.77 mg/dl, respectively. At 3 hours post feeding the values (mg/dl) for ammonia nitrogen were statistically higher in C (20.16) and T_1 (18.13) as compared to T_2 (15.70) treatment group. But, at 0 hours (pre-feeding) and 9 hours post feeding the value for ammonia nitrogen of group T_2 was statistically ($P<0.05$) higher than that of C and T_1 The sharp rise in C at 3 hours was followed by a rapid decline in values at 6 hours post-feeding. The value of ammonia nitrogen was statistically non-significant at 6 hours post-feeding for C, T_1 and T_2, which was, 13.98±0.61, 13.44±0.56 and 13.93±0.64, respectively. The mean level of ammonia nitrogen in SRL for different time intervals in treatments C, T_1 and T_2 was 13.67±0.43, 13.20±0.30 and 13.22±0.45 mg/dl, respectively, which was statistically non-significant.

The ammonia nitrogen values of SRL depend on the soluble nitrogen, rumen degradable nitrogen and per cent of rumen degradable nitrogen that degrade rapidly of a feed. The protein degrading bacteria utilize the soluble and freely available N and rapidly convert it into NH_3N. The turnover of this NH_3N into microbial protein is slow and thus NH_3N level rises for few hours after feeding and this rise is proportional to amount of the soluble nitrogen and rumen degradable nitrogen content of the feed. Hence, The values of ammonia nitrogen almost doubled at 3 hours post feeding in C and T_1 treatment groups whereas in T_2 it increased to only about one and a half times to its values at 0 hour (pre-feeding) time interval, which is attributed to the rapidly rumen degradable nitrogen of GNC which cause a rapid hike and fall at 3 and 6 hours respectively for T_1 group. This rapid rise and fall in NH_3N of C was because GNC was degraded completely within few hours and the excess NH_3N formed was absorbed into the blood and converted into urea by liver and excreted in urine. The results were in agreement to Goswami *et al*. (2012) [8] where NH_3N decreased in vitro when GNC was replaced with guar meal. But, El-Monayer *et al*. (2015) [5] reported higher NH_3 in guar korma group than cottonseed cake and soyabean meal fed group in buffaloes because cotton seed cake and soyabean meal were less degradable than guar korma (Mahesh *et al*., 2017).

7.3. TCA precipitated nitrogen

The TCA ppt. nitrogen of the rumen liquor of animals in treatment C, T_1 and T_2 was 33.79±2.72, 33.79±2.72 and 34.47±3.78 mg/dl; 42.08±2.97, 40.78±4.53 and 38.25±3.77 mg/dl; 38.99±2.41, 40.33±4.30 and 43.54±3.16 mg/dl and 37.13±2.7, 37.66±4.27 and 38.68±3.7 mg/dl at 0, 3, 6 and 9 hours post-feeding, respectively. The values for TCA precipitated nitrogen vary in-significantly ($P<0.05$) in different treatment groups at different time intervals. The mean level of TCA ppt. nitrogen (mg/dl) in SRL for different time intervals in treatments C, T_1 and T_2 was 37.12±2.69, 37.66±4.27 and 38.67±3.69 mg/dl, respectively, which was also statistically non-significant.

The values were highest at 3 hours post feeding in C and T_1 but in T_2 treatment group highest value was recorded at 6 hour post feeding. The values for TCA precipitated nitrogen vary in-significantly in different treatment groups at different time intervals. TCA precipitated nitrogen is the true protein N which comprises of both feed and microbial origin. The value was slightly higher in T_2 group indicating more microbial biomass production. The results were in agreement to El-Monayer *et al*. (2015) [5] in buffaloes.

7.4. Non protein nitrogen

The non protein nitrogen of the rumen liquor of animals in treatment C, T_1 and T_2 was 19.41±1.93, 19.79±2.52 and 20.94±1.21mg/dl; 37.72±2.77, 32.25±3.37 and 31.13±2.08 mg/dl; 24.71±2.69, 27.57±2.31and 26.69±2.10 mg/dl and 19.11±1.82, 24.64±2.0 and 28.99±2.26 mg/dl at 0, 3, 6 and 9 hours post-feeding, respectively. There was significant difference between the different treatments at 9 hours post-feeding with values of T_2 higher ($P<0.05$) than C. At other time intervals the difference was non-significant between different treatments. The mean level of non protein nitrogen (mg/dl) in SRL for different time intervals in treatments C, T_1 and T_2 was 25.23±2.19, 26.05±2.45 and 26.93±1.29 mg/dl, respectively, which was also statistically non-significant.

The non protein nitrogen values were higher at 3 hour post feeding in different treatment as compared to other time intervals. The values for non protein nitrogen were statistically similar in different treatment groups at different time intervals. The values were higher for feed having more rumen degradable nitrogen content. It mainly comprises of urea, ammonia, nitrates, nitrites, amino acids, amines etc. At 9 hours post feeding the value was significantly higher for T_2 than C because it contained more amount of slowly rumen degradable nitrogen (Mahesh *et al*., 2017).

Table 2: Mean values of various nitrogen fractions (mg/dl SRL) of the experimental buffalo bulls as affected by different dietary regimes and time of sampling

Time Intervals (hrs)	Treatments		
	C	T_1	T_2
Total-N			
0	53.2±2.04	54.60±2.82	55.42±3.03
3	79.8 ^{b} ±2.26	73.03 ^{ab} ±2.30	69.38 ^{a} ±3.30
6	63.70±2.39	67.90±2.89	70.23±3.51
9	56.23 ^{a} ±1.79	62.30 ^{ab} ±3.6	67.67 ^{b} ±3.59
Mean ± SE	63.23±2.03	64.45±2.85	65.67±3.31
NH3 – N			
0	9.19 ^{a} ±0.25	9.43 ^{a} ±0.12	10.03 ^{b} ±0.09
3	20.16 ^{b} ±0.74	18.13 ^{b}±0.67	15.70 ^{a} ±0.77
6	13.98±0.61	13.44±0.56	13.93±0.64
9	11.36 ^{a} ±0.44	11.81 ^{a}±0.13	13.23 ^{b} ±0.52
Mean ± SE	13.67±0.43	13.20±0.30	13.22±0.45
TCA ppt-N			
0	33.79±2.72	34.81±4.52	34.47±3.78
3	42.08±2.97	40.78±4.53	38.25±3.77
6	38.99±2.41	40.33±4.30	43.54±3.16
9	37.13±2.70	37.66±4.27	38.68±3.70
Mean ± SE	37.12±2.69	37.66±4.27	38.67±3.69
NPN			
0	19.41±1.93	19.79±2.52	20.94±1.21
3	37.72±2.77	32.25±3.37	31.13±2.08
6	24.71±2.69	27.57±2.31	26.69±2.10
9	19.11 ^{a} ±1.82	24.64 ^{ab} ±2.00	28.99 ^{b} ±2.26
Mean ± SE	25.23±2.19	26.05±2.45	26.93±1.29

7.5. Total volatile fatty acids

The total volatile fatty acids (mM/L) of the rumen liquor of animals in treatment C, T_1 and T_2 were 75.33±1.28, 76.67±1.23 and 77.33±1.69; 137.50±2.14, 146.77±1.52 and 150.30±3.44; 91.50±1.12, 97.50±1.09 and 103.83±1.54 and 80.17±1.30, 81.33±1.20 and 83.50±1.54 at 0, 3, 6 and 9 hours post-feeding, respectively (Table 3). The difference among treatments was non-significant at 0 and 9 hours post-feeding but, at 3 hour post-feeding T_1 and T_2 were significantly higher ($P<0.05$) than C and at 6 hour post-feeding there was significant ($P<0.05$) difference among all the treatments, the values being highest for T_2 and lowest for C. The mean level of total volatile fatty acids (mM/L) in SRL for different time intervals in treatments C, T_1 and T_2 was 96.12±0.89, 102.50±0.93 and 107.91±1.54, respectively. There was significant difference among all the treatments, the values being highest for T_2 and lowest for C. The values were higher at 3 hour post feeding in different treatments. The more amounts of TVFAs in T_2 signifies increased digestibility of organic matter (Kholif *et al*., 2005). The results were in agreement to Soliman *et al*. (2014) [18] in sheep fed guar korma by replacing sunflower cake, and Goswami *et al*. (2012) [8] who in an *in-vitro* experiment found high TVFAs in guar meal as compared to GNC. The mean level of total volatile fatty acids (mM/L) in SRL for different time intervals had significant difference among all the treatments, which showed better utilization of energy and protein.

7.6. pH

The pH of strained rumen liquor of buffalo bulls as affected by different dietary treatments and time of sampling is depicted in Table 3. The pH of the rumen liquor of animals in treatments C, T_1 and T_2 was 6.87±0.05, 6.85±0.05 and 6.76±0.03; 6.71±0.03, 6.69±0.06 and 6.62±0.03; 6.69±0.05, 6.69±0.04 and 6.63±0.02 and 6.76±0.05, 6.64±0.11 and 6.72±0.01 at 0, 3, 6 and 9 hours post-feeding, respectively. The difference in pH among different treatment groups at different time intervals was statistically ($P<0.05$) insignificant. The pH decreased at 3 hours post feeding in all the groups because of the carbohydrate fermentation and production of more TVFAs. The pH was slightly lower in T_2 group than other two groups at 3 and 6 hours post feeding because of the more TVFAs and less ammonia produced in T_2 group. These findings are in agreement with the findings of El-Monayer *et al*. (2015) [5] in buffaloes and soliman *et al*. (2014) [18] in sheep.

Table 3: Mean values of TVFAs and pH of strained rumen liquor of buffalo bulls as affected by different dietary treatments and time of sampling

Time Intervals (hrs)	Treatments		
	C	T_1	T_2
TVFA (mmol/L)			
0	75.33±1.28	76.67±1.23	77.33±1.69
3	137.5^{a}±2.14	146.77^{b}±1.52	150.3 ^{b} ±3.44
6	91.5 ^{a} ±1.12	97.50 ^{b} ±1.09	103.83^{c}±1.54
9	80.17±1.3	81.33±1.20	83.5±1.54
Mean ± SE	96.12^{a}±0.89	102.50^{b}±0.93	107.91^{c}±1.54
pH			
0	6.87±0.05	6.85±0.05	6.76±0.03
3	6.71±0.03	6.69±0.06	6.62±0.03
6	6.69±0.05	6.69±0.04	6.63±0.02
9	6.76±0.05	6.64±0.11	6.72±0.01
Mean ± SE	6.75±0.04	6.71±0.05	6.68±0.01

8. Conclusion

It can be concluded that roasted guar korma is a source of by-pass protein and can be fed to high producing animals. By-pass protein content can be attributed to the roasting of the korma, roasted guar korma was found to be better as compared to ground nut cake as it has better nitrogen utilization because soluble protein content is found to be less.

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