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Full Length Research Paper

A Study on Rumen Enzymes of Camel *(Camelus dromedaries)* Maintained on Different Diets

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Abstract
The camel Camelus dromedarius, is an important livestock species uniquely adapted to hot and arid environment. Their choicest feed includes dicotyledons. In developing countries like India where economy is growing, common keepers most often do not feed concentrate to their camels unless they become rundown. Due to introduction of new feed resources, this study was an attempt to investigate the rumen enzymes of camels maintained on different diets. Three experimental groups of camel viz., Group 1 camels were given guar phalgati (Cyamopsis tetragonaloba) and ground nut (Arachis hypogaea) chara in 1:1 ratio. Group 2 camels were given ground nut chara alone while in Group 3 camels jaggery 50%w/v was administrated as a single dose orally @15g/kg body weight apart from feeding of ground nut chara were studied for their rumen enzymes. It could be concluded that in Group 3 camels which were given jaggery in addition to groundnut chara showed a significant change in the digestive pattern and it could therefore be envisaged that there exists a significant role of nutrition on digestive pattern.

Key words: *Camel, Rumen enzymes, Diet, Rajasthan*

Introduction

The camel belongs to genus *Camelus* which is probably among the last of the major domestic species to be put to regular use by man. The digestive system and digestive processes seems to be the adaptations of the arid environments in which the camel is usually found. The family Camelidae is found to be much more efficient in digesting dry matter, fiber and crude protein than other ruminants. The way in which the stomach contents are turned over rapidly and frequently is probably the reason for this better efficiency.

Camels are well adapted to diets that are low in protein due to their ability to recycle very effectively one of the end products of digestion. The size of particles tends to increase as the amount of fiber in the diet increases. Digestion therefore takes longer if high roughage diets are fed. In Rajasthan camels mainly feed *Leptadenia spartium* (ver. khimp), *Calligonum polygonoides* (ver. phog), *Prosopis spicigera* (ver. khejri), *Azadirachta indica* (ver. neem), *Phaseolus mungo* (green gram) straw (locally tremed as bhoosa) and *Cyamopsis tetragonaloba* (ver. guar) bhoosa, *Momordica dioica* (ver. kakoda), *Tribulus terrestris* (ver. gokhru). Camels are foregut fermenters. The process of fermentation in the stomach is mainly influenced by the enzymatic microbial digestion.

With above mentioned background the present study was undertaken with the objective of finding out the enzymatic changes in three groups of camels fed different diets or maintained on different planes of nutrition. The rumen fluid profiles can be considered important in evaluating the health status of animals. These have been used widely to identify problem and to indicate dietary causes of diseases or low production. Due to introduction of new feed resources, this study was an attempt to investigate the effect on certain rumen enzymes of camels maintained on different diets.

Materials and Methods

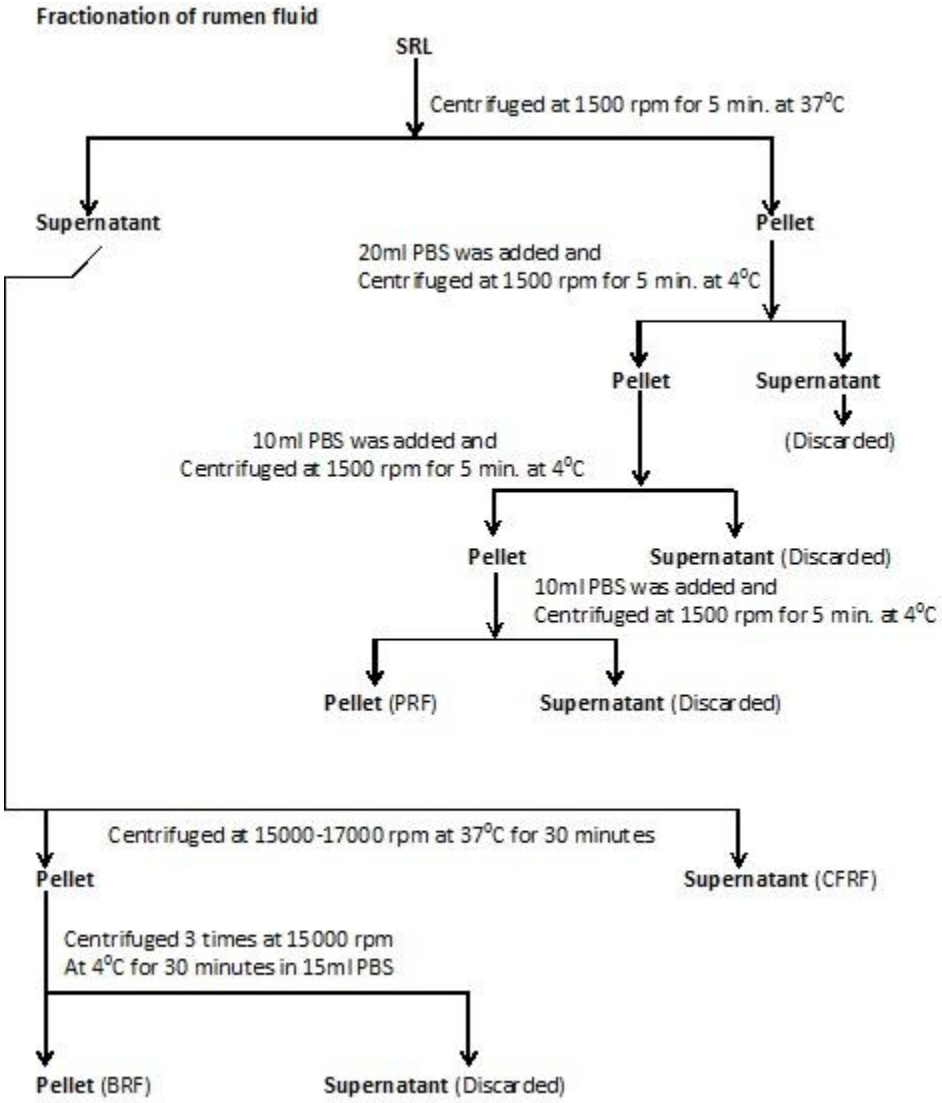
The present investigation was carried out in three groups of four camels each, at National Research Center on Camel, Bikaner, maintained on different diets. Group 1 camels were given guar phalgati (*Cyamopsis tetragonaloba*) and ground nut (*Arachis hypogaea*) chara in 1:1 ratio. Group 2 camels were given ground nut straw (locally termed as chara) alone while in Group 3 camels jaggery 50%w/v was administrated as a single dose orally @15g/kg body weight apart from feeding of ground nut chara. In all the three groups de-worming was done with a broad spectrum anti-helminthes prior to the start of the experiment and all the camels were in clinically healthy condition. All three groups of camels were given ad lib water.

Collection of rumen fluid

Rumen fluid was collected from camels by using rumen fluid extraction unit through suction by using stomach tube specially designed for camels. The camels were tranquilized before rumen fluid collection using 3-4 ml of xylazine. 100 ml of rumen fluid was filtered /strained through 4 layer muslin cloth and then further processed for enzymatic estimations.

Rumen fluid enzyme estimation

For enzymatic estimation in rumen fluid, strained rumen liquid was further processed (Martin *et al*., 1994) to differentiate the Strained Rumen Fluid (SRL) to three different fractions (Flow chart). These fractions are named Cell Free Rumen Fluid (CFRF), Bacterial Rich Fraction (BRF) and Protozoan Rich Fraction (PRF) according to the presence of particular microorganism in them.



Flow chart: Fractionation of rumen fluid

Sonication

The protozoa and bacteria rich fractions were kept in ice bath and were disrupted by sonication at 10µ for 5 min with 30 seconds break. It was followed by centrifugation at 10000 rpm for 30 minutes at 4°C. The supernatant of each fraction was used as the source of protozoan and bacterial enzyme.

The following Non Protein Nitrogen (NPN) and protein degrading enzymes were estimated in the three different fractions of rumen fluid and their activity was expressed as units/100 ml SRL.

Urease enzyme activity

The activity of urease enzyme was determined by Weatherburn (1967) method and calculated as:
Change in absorbance "A" for sample = O.D. of test-O.D. of control.

A was recorded on calibration curve to get the amount (µg) ammonium nitrogen released.

Enzyme activity (units) = µg ammonia nitrogen/min/ml = $\frac{\text{µg ammonia nitrogen}}{\text{T X S X 14}}$

where, T= Time of incubation (15 minutes), S= Volume of sample (0.1ml), 14= Molecular weight of nitrogen.

Protease enzyme activity

Change in optical density A =O.D. of test-O.D. of control.

A was read on calibration curve to get the µg hydrolyzed protein released.
Enzyme activity (units) =µg hydrolyzed protein/min/ml= $\frac{\text{µg hydrolyzed protein}}{\text{T X S}}$

where, T =time of incubation (10minutes), S = Volume of the sample (0.25ml).

Transaminases enzymes activity

Transaminases included two different but related enzymes *viz*.,
(1) Aspartate aminotrnsaminase (AST)
(2) Alanine aminotrnsaminase (ALT)

(i) Aspartate Aminotransferase enzyme activity

The pyruvic acid which is produced during the reaction was estimated by Reitman & Frankel (1957) method.

Enzyme activity is expressed as µmol pyruvate produced per liter sample per minute: $\frac{\text{T-C x 67µmol}}{\text{S-B}}$

Where, T=optical density of test, C= optical density of control, S= optical density of pyruvate standard, B= optical density of blank.

(ii) Alanine aminotransferase enzyme activity

The pyruvic acid which is produced during the reaction was estimated by Reitman and Frankel (1957) method.

Enzyme activity is expressed as µmol pyruvate produced per liter sample per minute= $\frac{\text{T-C}}{\text{S-B}}$ x 133µmol

Where, T= optical density of test, C=optical density of control, S= optical density of standard, B= optical density of blank.

Glutamate dehydrogenase enzyme activity

Glutamate dehydrogenase activity was determined by measuring the rate of change in absorbance at 340nm following Bergmeyer (1974) method.

E1-E2=A
E3-E4=B

Where, E1= Initial optical density of blank, E2= Optical density of blank after 5 minutes, E3= Initial optical density of test, E4= Optical density of test after 5 minutes.

Change in optical density "A" per 5 minutes= B-A

IU/liter or Mu/ml = $\frac{\text{A x V}}{\text{e x d x v x t}} \times 10^6$

Where, e=Molar extinction coefficient of NADPH at 340 nm, d=Diameter of cuvette in cm=1cm, V= Total volume=1.5 ml, v=Sample volume=0.10 ml, t= Time=5 minutes.

Glutamate Synthetase enzyme activity

The enzyme activity was estimated by measuring the rate of changes in absorbance at 340 nm following the method given by Meers *et al*., (1970).
Change in optical density "A" / 3 minutes= B-A

"A" was calculated as follows

E1-E2= A

E3-E4=B

Where, E1=Initial optical density of blank, E2= Optical density of blank after 5 minutes, E3= Initial optical density of test, E4= Optical density of test after 5 minutes, "A"=B-A.

$$\text{IU/liter or MU/ml} = \frac{\text{"A" x V x } 10^6}{\text{e x d x v x t}}$$

Where, e= Molar extinction coefficient of NADPH at 340nm, d=Diameter of cuvette =1cm, V= Total volume of solution in cuvette =1.5 ml, v= Volume of rumen fluid=0.1.ml, t= Time=5 min.

Results

The mean values of urease in CFRF, PRF and BRF of Group 1, Group 2 and Group 3 animals are presented in Table 1. The mean levels of urease activity were higher in BRF followed by PRF and CFRF in all the three groups. The results of these three groups have been presented in Fig.1.

Table 1. Rumen fluid urease levels (*) in different groups of camels fed with different diets

Group	CFRF**	PRF**	BRF**
Group 1	1.20±0.05[a]	5.94±0.22[a]	**72.44±3.01[a]**
Group 2	1.18±0.05[b]	5.99±0.07[b]	**69.90±1.56[b]**
Group 3	**0.53±0.04[a,b]**	**1.83±0.25[a,b]**	**22.69±1.58[a,b]**

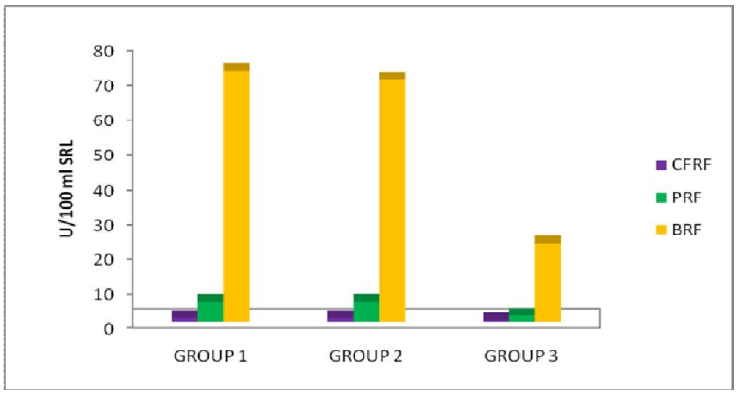


Fig 1. Mean rumen urease enzymes activity (U/100ml) values in different groups of camels fed with different diets

The urease levels were found to be highly significant (P<0.01) among different fractions of different groups. The mean values of protease activity in CFRF, PRF and BRF of Group 1, Group 2 and Group 3 animals are presented in Table 2 and Fig. 2. The mean levels of protease were higher in BRF followed by PRF and CFRF in all the three groups. The protease levels were found to be highly significant (P<0.01) among different fractions of different groups.

Table 2. Rumen fluid protease levels in different groups of camels fed with different diets

Group	CFRF**	PRF**	BRF**
Group 1	0.17±0.01[a]	0.63±0.03[a]	**2.19±0.07[a]**
Group 2	0.20±0.03[b]	0.66±0.04[b]	**2.2±0.05[b]**
Group 3	**0.07±0.00[a,b]**	**0.35±0.04[a,b]**	**1.36±0.10[a,b]**

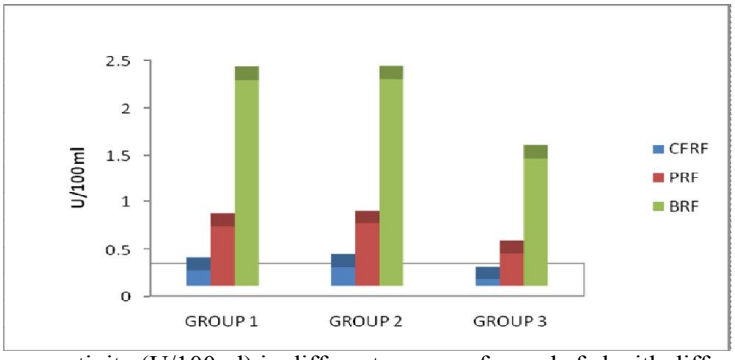


Fig 2. Mean rumen protease enzymes activity (U/100ml) in different groups of camels fed with different diets

The mean values of GPT activity in CFRF, PRF and BRF of Group 1, Group 2 and Group 3 camels were 27.5±2.32, 85.5±3.96 and 16.5±1.84 U/100ml; 28.5±1.32, 84.75±3.35 and 16.75±1.31 U/100ml; 20.5±1.84, 109.5±4.78 and 17.0±1.95 U/100ml respectively. The GPT activity showed a higher level in the PRF followed by CFRF and BRF in all three groups of camels. A significant variation in the GPT activity in PRF and CFRF were noticed among different groups (P<0.05). The results of GPT activity are presented in Table 3. and Fig. 3.

Table 3. Rumen fluid GPT levels (*) in different groups of camels fed with different diets

Group	CFRF**	PRF**	BRF
Group 1	27.5±2.32[a]	85.5±3.96[a]	**16.5±1.84**
Group 2	28.5±1.32[b]	84.75±3.35[b]	**16.75±1.31**
Group 3	**20.5±1.84[a,b]**	**109.5±4.78[a,b]**	**17.0±1.95**

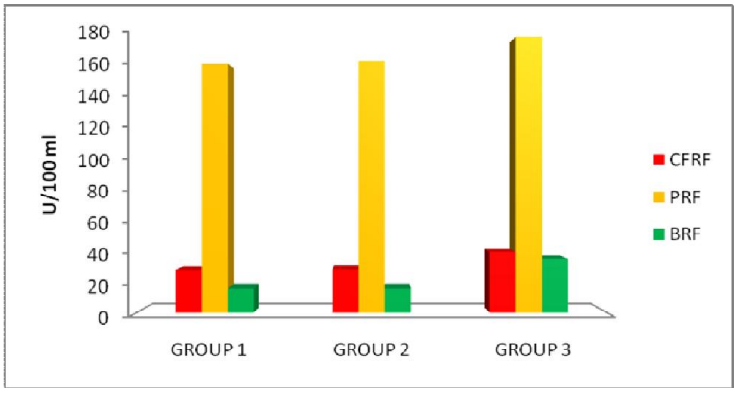


Fig 3. Mean rumen AST enzymes activity (U/100ml) in different groups of camels fed with different diets

The mean values of GOT activity in CFRF, PRF and BRF of Group 1, Group 2 and Group 3 camels were 27.75±2.09, 162.25±3.56 and 15.52±1.04 U/100ml; 28.25±1.43, 163.75±2.56 and 15.47±1.16 U/100ml; 39.5±2.21, 179.75±7.23 and 34.82±3.75 U/100ml respectively. The values are depicted in Table 4. and Fig. 4. The GOT levels were noted to be higher in PRF, followed by CFRF and PRF. A significant variation was noticed among different fractions of all the three groups (P<0.05).

Table 4. Rumen fluid GOT levels (*) in different groups of camels fed with different diets

Group	CFRF**	PRF**	BRF**
Group 1	27.75±2.09[a]	162.25±3.56	**15.52±1.04[a]**
Group 2	28.5±1.32[b]	163.75±2.56	**15.47±1.16[b]**
Group 3	**39.50±2.21[a,b]**	**179.75±7.23**	**34.82±3.75[a,b]**

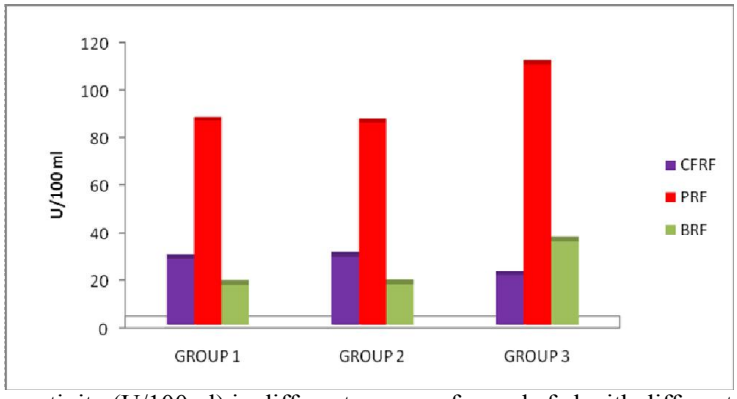


Fig 4. Mean rumen ALT enzymes activity (U/100ml) in different groups of camels fed with different diets

The mean values of GDH activity in CFRF, PRF and BRF of different groups are presented in Table 5. and Fig. 5. The GDH activity showed a higher level in the BRF followed by PRF and CFRF in all three groups of camels. There is no significant variation in the GDH levels among different groups (P>0.05).

Table 5. Rumen fluid GDH levels (*) in different groups of camels fed with different diets

Group	CFRF	PRF	BRF
Group 1	13.25±1.88	37.25±3.68	**175.75±3.32**
Group 2	13.75±2.17	34.75±4.71	**173.00±5.95**
Group 3	**16.00±2.85**	**35.50±3.30**	**173.50±4.57**

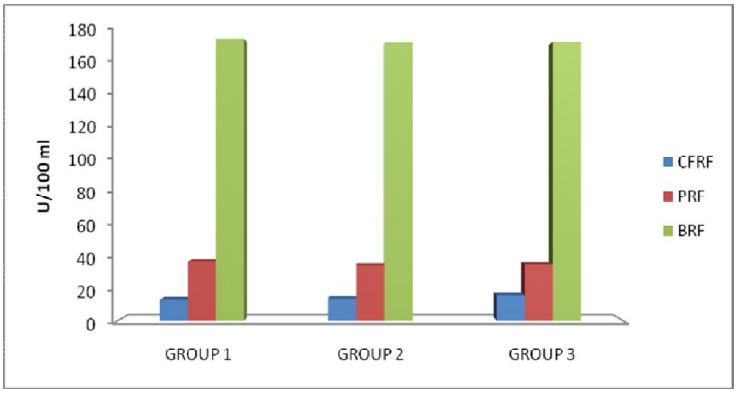


Fig 5. Mean rumen glutamate dehydrogenase enzymes activity (U/100ml) in different groups of camels fed with different diets

The mean values of GS activity in CFRF, PRF and BRF of different groups are presented in Table 6. and Fig. 6. The GS activity showed a higher level in the BRF followed by PRF and CFRF in all three groups of camels. A significant variation in the BRF among different groups (P<0.05) was observed. Highest activity of GS in Group 3 camels as compared to Group 1 and 2 was noticed.

Table 6. Rumen fluid GS levels (*) in different groups of camels fed on different diets

Group	CFRF	PRF	BRF**
Group 1	5.25±1.49	17.75±2.32	**44.00±3.16[a]**
Group 2	6.25±1.18	17.00±3.41	**43.50±2.50[b]**
Group 3	**7.00±2.08**	**18.00±2.58**	**59.00±3.69[a,b]**

**values given are Mean±SE.*
***(P<0.05: significant at 5% level; Figures with similar superscripts reveal significant difference between groups).*
[CFRF: Cell free rumen fluid fraction; PRF: Protozoa rich fraction; BRF: Bacteria rich fraction]

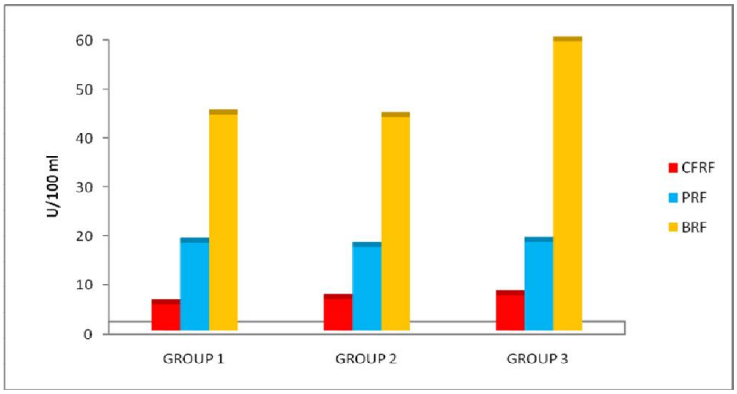


Fig 6. Mean rumen glutamate synthetase enzymes activity (U/100ml) in different groups of camels fed with different diets

Discussion

The ruminal microbes include a large variety of bacterial, protozoan and fungal species making the rumen habitat very complex. Numerous interactions have been observed between these large groups of microorganisms that exist in the rumen (Dehority, 1998).These microbes, through their enzymes (Pathak *et al.,* 1996) play roles in digestive processes. Complete digestion of complex feeds such as hay or grain literally requires hundreds of enzymes (McAllister *et al.,* 2001). In microbial terms, the enzyme activity is not particularly high; it is the high microbial biomass and long residence time of feed material in the digesta that causes the high extent of breakdown of susceptible material (Morgavi *et al.,* 2000). Considerable progress has been made towards the development of quantitative relationships among the chemical composition of ruminant feeds, dynamic aspects of digestion in the rumen, products of digestion absorbed by the ruminant, and most important, how these can be manipulated to improve animal productivity. In the present study the mean levels of urease activity were higher in bacterial rich fraction. A significant variation ($P<0.01$) was noticed in different groups of camels. The urease levels were significantly lower in the animals administered jaggery and was highest in the camels fed guar phalgati and groundnut chara. The mean levels of protease were higher in BRF followed by PRF and CFRF in all the three groups. Significantly ($P<0.01$) lowered protease levels were noticed in Group 3 camels followed by Groups 1 and 2. There was no significant variation in the protease levels of guar phalgati and groundnut chara fed group and groundnut chara alone fed group. A significant variation was noticed among different fractions of all the three groups ($P<0.05$).

Literature on enzyme estimations is meagerly available in other species of animals. The enzyme urease is produced by certain rumen and intestinal bacteria and serves an essential function in the process of urea recycling by catalysing the conversion of urea to ammonia. In ruminant species, urease activity is greatest within the stratified layers of the rumen epithelium (Abdel Rahman & Decker, 1966; Cheng *et al.,* 1979) and has been shown to be associated with the adherent, facultatively anaerobic and strongly proteolytic bacterial population which colonizes this tissue (McCowan *et al.* 1978; Cheng *et al.,* 1979). Urease activity in rumen fluid is thought to result mainly from the sloughing of heavily-colonized distal epithelial cells from the keratinized layers of the epithelium (Cheng *et al.,* 1979; Dinsdale *et al.,* 1980). This urease is responsible for hydrolysis of urea that is recycled through the saliva or present in the diet (Allison, 1970). In spite of this importance, very little is known about the properties of urease isolated from rumen bacteria (rumen urease). Urease is a constitutive enzyme (Jensen & Schroder, 1965) and no quantitative values for ruminal urease activity are available on animals subjected to different feeding conditions. Even though fasted animals had lower ($P < .01$) ruminal ureolytic activity than those fed the mixed ration (Clifford *et al.,* 1968). Since the microbial population concentration of fasting probably decreased, there is a possibility that the decreased urease activity in these animals was associated with a lower dry matter in rumen contents. The findings of the present study also depicts that in Group 3 where the DM was less *i.e.,* administered jaggery there was a lowered urease activity.

Wallace & Cotta, (1988) reported that the role of protozoa in ruminal protein degradation is mostly related to the degradation of feed particle sand bacterial proteins, but their overall effect on protein degradation is still controversial. Most of the protease is cell bound and only a small portion is extracellular. In this regard, protease activities in SRFWP and EABC are higher than in CFRF. Blackburn & Hobson, (1960) reported that the proteolytic activity of whole rumen fluid of sheep did not depend on the diet of the animals. Present data showed that increasing protein could be an enhancing factor for more production of protease, which has shown its effect in the CFRF fraction. Pollock, (1962) showed that this protease may be truly extracellular. In the present study the protease activity was lower in Group 3. In all the cell free rumen fluid fractions the enzyme activity was less which might be attributed to the enzyme inactivation in CFRF.

The highest concentration of tranaminases is present in the protozoal rich fraction but didn't show any significant difference by paired t-test between Groups of 1 and 2. Group 3 showed increased GPT activity which was highly significant in comparison to Groups 1 and

2. The GPT activity showed a higher level in the PRF followed by CFRF and BRF in all three groups of camels. A significant variation in the GPT activity in PRF and CFRF were noticed among different groups ($P<0.05$). Highest rumen ALT activity was noticed in Group 3, followed by Group 2 and 1.

Nutritional requirements of ruminants are different from those of monogastric animals. Alanine represents the amino acid found in the highest concentration in the intracellular pool of free amino acid rumen bacteria. Rumen bacteria possess effective mechanisms for alanine synthesis from ammonia (e.g., alanine dehydrogenase and alanine aminotransferase). Both alanine aminotransferase and aspartate aminotransferase belong to the most common transaminases in the rumen. The scope of the transamination reaction is broad and virtually all the amino acids participate in enzymatic transamination and in protein synthesis (Meister& Tice, 1950**).** The finding that micro-organisms synthesize amino acids from the corresponding α-keto acids by means of transamination lends strong support to the key role of transamination in amino acid metabolism and protein synthesis in the rumen. Evidence that transaminase activity exists in rumen fluid has already been presented by Goss & Kleiber, (1955).

The GDH activity showed a higher level in the BRF followed by PRF and CFRF in all three groups of camels. There was no significant variation in the GDH levels among different groups ($P>0.05$). The GS activity showed a higher level in the BRF followed by PRF and CFRF in all three groups of camels. There was significant variation in the BRF among different groups ($P<0.05$). There was highest activity of GS in Group 3 camels in comparison to Group 1 and 2. Glutamate concentration by rumen bacteria depends on gamma-glutamyl transferase activity. This enzyme plays an important role in some peptide and amino acid transfers through the rumen wall and in the formation of an intracellular pool of glutamate as well. An inverse relationship between GS and GDH activities was observed. The synthesis of GS and urease are coordinately regulated in *Selenomonas ruminantium* and fluctuate as a function of environmental ammonia levels (Smith *et al.*, 1981). Ammonia is the main nitrogen source used for growth by the numerically important bacterial species of the rumen (Bryant & Robinson, 1963) and by many other anaerobic species inhabiting the mammalian gastrointestinal tract (Bryant, 1974). Ammonia incorporation by enteric bacteria is mediated by two major pathways: the low-affinity glutamate dehydrogenase (GDH) pathways, used when environmental ammonia levels are high, and the high-affinity glutamine synthetase (GS) pathway, is used when the levels are low (Tyler, 1978). The GS activity is subject to a complex regulation system including enzyme synthesis, covalent modification by adenylylation-deadenylylation, and feedback inhibition. Little knowledge is available on the ammonia assimilation pathways (and their regulatory aspects) for the anaerobic bacteria of the rumen and mammalian gastrointestinal tracts. The GS in this gram-negative organism differs substantially from the GS in enteric bacteria, particularly in that an adenylylation-deadenylylation regulatory mechanism is absent. Both GS and GDH have been detected in whole rumen contents (Chalupa *et al.,* 1970), continuous cultures of mixed ruminal bacteria (Erfle et al., 1977), and pure cultures of several ruminal bacteria (Pettipher & Latham, 1979). Rumen microbes can synthesize enough amino acids and peptides from the inorganic nitrogen in ammonia or other nitrogen source and carbon skeletons and sulphur precursors. Ammonia assimilation by rumen microbes depends on rumen pH (Veth *et al.* 1999), rumen ammonia concentration (Mehrez *et al.,* 1977) and ruminal ammonia-assimilating enzyme activity. Enzyme glutamate dehydrogenase plays an important role in maintaining the balance between ammonia- and α-amino-nitrogen of the rumen.

Conclusion

It can therefore be concluded that in Group 3 camels which were given jaggery in addition groundnut chara showed a significant change in the digestive pattern leading towards acid indigestion based on enzymatic profile as compared to Group 1 and Group 2 camels. There was an insignificant variation among the enzymatic profile of Group 1 and Group 2. From the above enzymatic changes noticed in different groups fed with different diets, it can be envisaged that there exists a significant role of diet on digestive pattern in camel.

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