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Investigation of rumen fermentation parameters and some blood metabolites of dromedary camels fed with C_3 and C_4 forages

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Abstract

The aim of this experiment was to investigate rumen fermentation and some blood parameters of dromedary camels fed with C_3 and C_4 forage. Four fistulated dromedary adult camels were fed with diets as a changeover design, 30 days for each period. The diets included alfalfa hay + wheat straw (C_3 forage) and atriplex+ suaeda + seidlitzia (C_4 forage). At the end of the experiment, rumen and blood parameters, gas production of wheat straw and atriplex as a 2 × 2 factorial experiment were determined. The highest blood glucose and urea nitrogen levels were found for camels fed with C_3 forage, 2 hr after feeding ($p < 0.05$). The maximum NH_3-N concentration in the rumen was for diets C_3 and C_4, 2 and 4 hr after feeding ($p < 0.05$). The lowest rumen pH was observed for C_3 diet at 2 and 4 hr and for C_4 diet at 4 and 8 hr after feeding. The activity of rumen carboxymethylcellulase (CMCase) and microcrystalline cellulase (MCCase) enzymes was the highest for C_3 and C_4 diets, 8 hr after feeding, however, during feeding the enzyme activity in C_4 was higher than that of 2 hr ($p < 0.05$). The rumen volatile fatty acid (VFA) concentrations were significantly higher in camels fed C_3 forage in comparison with C_4 ($p < 0.05$). The results showed that the gas production potential was significantly higher in treatments containing atriplex, however, the gas production rate was higher in treatment containing wheat straw ($p < 0.05$). The results suggested that for camels maintained in closed systems, the replacement of C_3 forages instead of C_4 could be possible and useful.

Introduction

The dromedary camel is a good source of meat and milk production, especially in areas where the climate adversely affects the performance of other animals. This is because of its unique physiological characteristics, including great tolerance to high temperatures, solar radiation, water scarcity, rough topography, and poor vegetation.[1] Knowledge of the quality of feeds selected by the camel, its behavioral activities and feed preferences are important for the understanding of forage–camel relationship.[2] The pre-stomachs of the camels are characterized by the presence of only three compartments in comparison with true ruminants. The nutritional adaptation efficacy of the dromedary is due to several mechanisms such as more efficient fermentation in pre-stomach and high intestinal absorption, high gluconeogenesis, low ketogenesis, and a high lipid mobilization and great urea recycling for protein synthesis.[3,4]

Plants can be classified to C_3 and C_4 types according to the photosynthetic pathway. The C_4 plants that generally are consumed by camels, are found in all tropical forage-lands and are dominant in warm-season temperate forage-lands.[5] The C_4 forage equates to higher cell wall content that decrease feed digestibility compared to C_3 forages.[6]

It has been reported that forage quality influences feeding patterns of camels, where the time available for grazing under adverse pasture conditions would be a limiting factor to their dry matter (DM) and nutrient intake.[7] Therefore, feed shortage is an important constraint to camel production in arid and semi-arid regions in a harsh climate.[8] However, slowly being

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replaced by sedentary systems that feeding camels in these systems should be considered properly.[9]

Alternatively, in these regions, the herbaceous species are often grown in saline soils of desert areas that may contain a large amount of lignocellulosic material, high ash, and various anti-nutrients. As the maturation of these plants in arid and semi-arid regions usually occurs during autumn and winter seasons, camels are forced to use poor food sources and cannot get the nutrients to meet their physiological needs.[8]

Camel nutrition under intensive farming systems has been poorly investigated in the past, however, researches undertaken by others[10] concluded that camels require less comparative energy for maintenance than sheep or cattle whilst another researcher concluded that camel protein requirements are at least 30.00% less than that of dairy cattle, sheep or goats.[11]

This experiment was conducted to compare the nutritive value of the C_3 and C_4 forage hays with similar protein and fiber contents in dromedary camel and effect of the diets on rumen fermentation parameters, kinetic and some blood parameters.

Materials and Methods

Animals and diets. This experiment was conducted with four fistulated dromedary camels of Arabian breed (12-18 months old) weighing an average of 200.00 ± 50.00 kg live weight. The animals were allocated to two diets as a changeover design with two periods. Camels were housed in individual pens in a sheltered, cemented-floor, open-side barn, well-ventilated and equipped with adequate feeding and watering facilities. Each period was 30 days that the first 25 days were for adaptation, and the last five days were for sampling. The camels were fed twice a day (at 07:00 and 19:00) at a level of 40 g kg^{-1} metabolic weight.[11] Fresh clean water was available *ad libitum*. In the present study included two treatments and two repeats for each period. The experimental diets consisted of alfalfa hay + wheat straw (C_3 forage) and *Atriplex leucoclada + Suaeda fruticusa* + *Seidlitzia rosmarinus* (C_4 forage), (Table 1).

The experimental protocols regarding the care and handling of camels were approved by the Ethics Committee of Agricultural Sciences and Natural Resources University of Khuzestan, Iran.

Sample collection and processing. During the five days of sampling, rumen liquor was collected at 0, 2, 4, 8 and 12 hr after morning feeding, and then was strained by two layers of cheesecloth. 10.00 mL of filtered rumen fluid was taken to determine volatile fatty acid (VFA), 25.00 mL for enzymes activity and 5.00 mL to determine ammonia nitrogen (NH_3-N) concentration. The pH was recorded immediately by pH meter (Metrohm, Herisau, Switzerland). At the end of each period, the blood samples were taken and the tubes were placed on ice and then centrifuged at 3,000 *g* for 15 min for collecting serum. At the end of the period, rumen fluid was collected at 2 hr after morning feeding. Then rumen contents were strained by two layers of cheesecloth into pre-warmed thermo flasks to transport to the laboratory for running gas production test.

Table 1. Ingredients and chemical composition of the experimental diets (%).

Diet	C_3 forage	C_4 forage
Ingredients		
Atriplex leucoclada	0.00	80.00
Suaeda fruticusa	0.00	10.00
Seidlitzia rosmarinus	0.00	10.00
Alfalfa hay	40.00	0.00
Wheat straw	60.00	0.00
Chemical composition		
Dry matter	89.30	83.00
Crude protein	7.24	7.07
Natural detergent fiber	68.10	61.78
Acid detergent fiber	43.45	38.73
Ash	8.19	18.80
Organic matter	91.81	81.20

Gas production test. *In vitro* gas production (GP) was determined by a modified method as described by Blümmel *et al*.[12] Samples (200 mg) of the oven-dry feed-stuffs (C_3 and C_4 forage) and the respective mixtures were weighed into 100-mL glass syringes fitted with plungers.

This test contained two factors 1: Camel's diets were containing C_3 and C_4 forage (Table 1) and 2: Media substrate that involved wheat straw and atriplex. Therefore, treatments in the gas production test included:

1. Rumen fluid of camels fed C_3 forage with wheat straw as a substrate
2. Rumen fluid of camels fed C_3 forage with *Atriplex L.* as a substrate
3. Rumen fluid of camels fed C_4 forage with wheat straw as a substrate
4. Rumen fluid of camels fed C_4 forage with *Atriplex L.* as a substrate

In this experiment, we attempted to investigate the effects of source of rumen fluid, media substrate and interaction effects between them.

Gas production was manually measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hr using a digital pressure gauge fitted with a 21 mm gauge needle.[13] The samples were incubated in triplicate together with three vials containing only incubation medium (as blank). After 96 hr incubation, mean gas production data of blanks were subtracted from the recorded gas production of the standard and of all the substrates to get the net gas production values. *In vitro* gas production values (mL per g organic matter) were fitted to the following non-linear model Orskov and McDonald:[14] $Y = b\ (1 - e^{-ct})$ where, b is the gas production (mL) from the fermentable fraction, c is the rate constant of gas production (mL per hr), t is the incubation time (hr) and Y is the volume of gas produced at a time.

For determination of the partitioning factor (PF) at the end of each incubation period, the content of vials was transferred into an Erlenmeyer flask, mixed with 20 mL neutral detergent fiber solution, boiled for 1 hr, filtered, oven-dried and ashed. The partitioning factor, microbial biomass, and actual digested organic matter were calculated.

Chemical analyses. Ammonia-N of rumen contents of the camels was analyzed in a 5.00-mL subsample of filtered fluid that was acidified with 5.00 mL of 0.20 M HCL by spectrophotometer (Libra S22; Biochrom, Dxford, UK).[15] Rumen contents for VFA were analyzed by GC (Model PU4410; Philips, Amsterdam, The Netherlands); column 10 polyethylene glycol and detector flame-ionization detection (FID) described by Ottenstein and Bartley.[16] About 400 µL of the sample was used and the standard consumption including 4. Methyl valeric acid (Merck, Darmstadt, Germany) was 100 µL. The type of column used was 10 PEG (length 2 m, diameter 45.00 mm). The injection volume was 1.00 µL and the used detector was FID.

Enzyme activity assay of rumen contents of the camels was measured by the dinitrosalicylic acid (DNS) method described by Colombatto and Beauchemin[17] which were based on the measurement of the quantity of reducing sugars released during the enzyme reaction with a defined substrate. For endoglucanase assay, 1.00% (w/v) medium viscosity carboxy methyl cellulose (Merck) was used as the substrate and exoglucanase activity was determined using 1.00% (w/v) solution of microcrystalline cellulose as the substrate. For this assay, 0.10 M citrate-phosphate buffer (pH 6.00; Merck), substrate and distilled water were mixed and incubated for 10 min at 39 ˚C in the water bath for equilibration. The reaction was terminated by adding 3.00 mL of DNS reagent. The absorbance was read at 540 nm using the spectrophotometer (Biochrom). The amount of reducing sugars released was determined using a standard curve made with glucose (Merck). Then the units of activity were expressed as µmoL of glucose equivalents per min mL^{-1} of undiluted enzyme product. Also, concentrations of blood metabolites were measured using standard kits (Sigma, St. Louis, USA).

Statistical analyses. Analysis of variance was done by the GLM procedure (version 6.12; SAS Institute, Cary, USA) to determine statistical differences between treatment diets. Gas production data were analyzed using the model $Y_i = \mu + T_i + e_i$ in a completely randomized design and *in vivo* data were analyzed using the model $Y_{ijkl} = \mu + T_i + \beta_j + sub\ (\beta)_{jk} + D_l + e_{ijkl}$ in a changeover design. Y_{ijkl} = dependent variable; μ = population mean; T_i = mean effect of treatment; β_j = treatment rank effect; $sub\ (\beta)_{jk}$ = animal effect within treatment rank (order); D_l = period effect and e_{ijkl} = residual error. The Tukey test was used to compare means for significance. Effects were considered significant at $p < 0.05$.

Results

The effect of feeding diets on NH_3–N concentration and pH of the rumen several times after feeding is given in Figures 1A and 1B, respectively. Maximum NH_3-N concentration was for C_3 and C_4 forage 2 and 4hr after feeding. NH_3-N concentration was significantly higher for C_4 forage at 0 and 4 hr after feeding ($p < 0.05$). The results showed that the highest blood glucose and urea nitrogen concentration were for camels fed with C_3 forage 2 hr after feeding, then decreased, however, in camels fed with C_4 forage increased to 4 hr after feeding and then decreased ($p < 0.05$), (Fig. 1C). Figure 1B, shows considerable variations in the rumen pH. Minimum pH values were observed for C_3 forage at 2 to 4 hr and for C_4 forage 4 to 8 hr after feeding. In fact, partly the highest pH coincided with minimum NH_3-N concentration ($p < 0.05$). The VFAs concentrations of the rumen were significantly ($p < 0.05$) different between experimental diets (Figs. 2A, to 2D). The most amount of acetic acid production was occurred at 4 hr after feeding in C_3 diet and then decreased. While in C_4 the increment procedure of acetic acid production was increased slowly, however, it was continued up to 8 hr after feeding and then was declined (Fig. 2A). In general, the acetic acid concentrations in rumen fluid of camels fed with C_3 were much higher.

The propionic acid concentrations in camels fed with C_3 were similar to acetic acid, however, at 2 hr after feeding the propionate concentration was slightly decreased in C_4 diet in comparison with 0 hr (Fig. 2B), however, in resumption, it was continued up to 8 hours after feeding and then was decreased. The propionate reduction at 2 hr after feeding could be due to the reduction of propionic acid produced by microorganisms because of the presence of anti-nutritional components in C_4.

The maximum butyric acid concentrations in camels fed with C_3 were at 4 hr after feeding. Then it was decreased in camels fed with C_4 and the procedure was similar to propionic acid. Two hours after feeding, butyric acid was declined and 8 hr after feeding was reached to the peak and then was decreased. Also, in both diets (C_3 and C_4), the valeric and iso-valeric acids had partly similar procedures with acetic acid, however, after feeding no significant differences for any hours were observed between experimental treatments.

The effect of feeding diets on endoglucanase (CMCase) and exoglucanase (MCCase; Avicelas) activity of the rumen several times after feeding are given in Fig. 1E and F, respectively. Therefore, it seems the procedure of CMCase and MCCase activity changes during 12 hr after feeding is similar. So that in C_3 diet, the mechanism of activity changes for these enzymes up to 8 hr after feeding increased, then decreased. But in C_4 diet before feeding (at 0 hr), the activity of cellulolytic enzymes was slightly

higher, however, 2 hr after feeding it was decreased and then until 8 hr was tended to increase and again was slightly decreased ($p < 0.05$).

The estimated digestion coefficients (b and c) and total gas production are presented in Table 2. The results of this experiment showed that gas production potential (b) ($p < 0.05$) and the fractional fermentation rate (c) ($p < 0.05$) in inoculated media with rumen fluid of C_3 forage was significantly higher. Whereas the total produced gas was not influenced by the type of rumen fluid ($p > 0.05$).

In this study, gas production potential for atriplex was increased; however, the rate of gas production was higher in the wheat straw substrate ($p < 0.05$). The total gas volume was not influenced with the type of substrate.

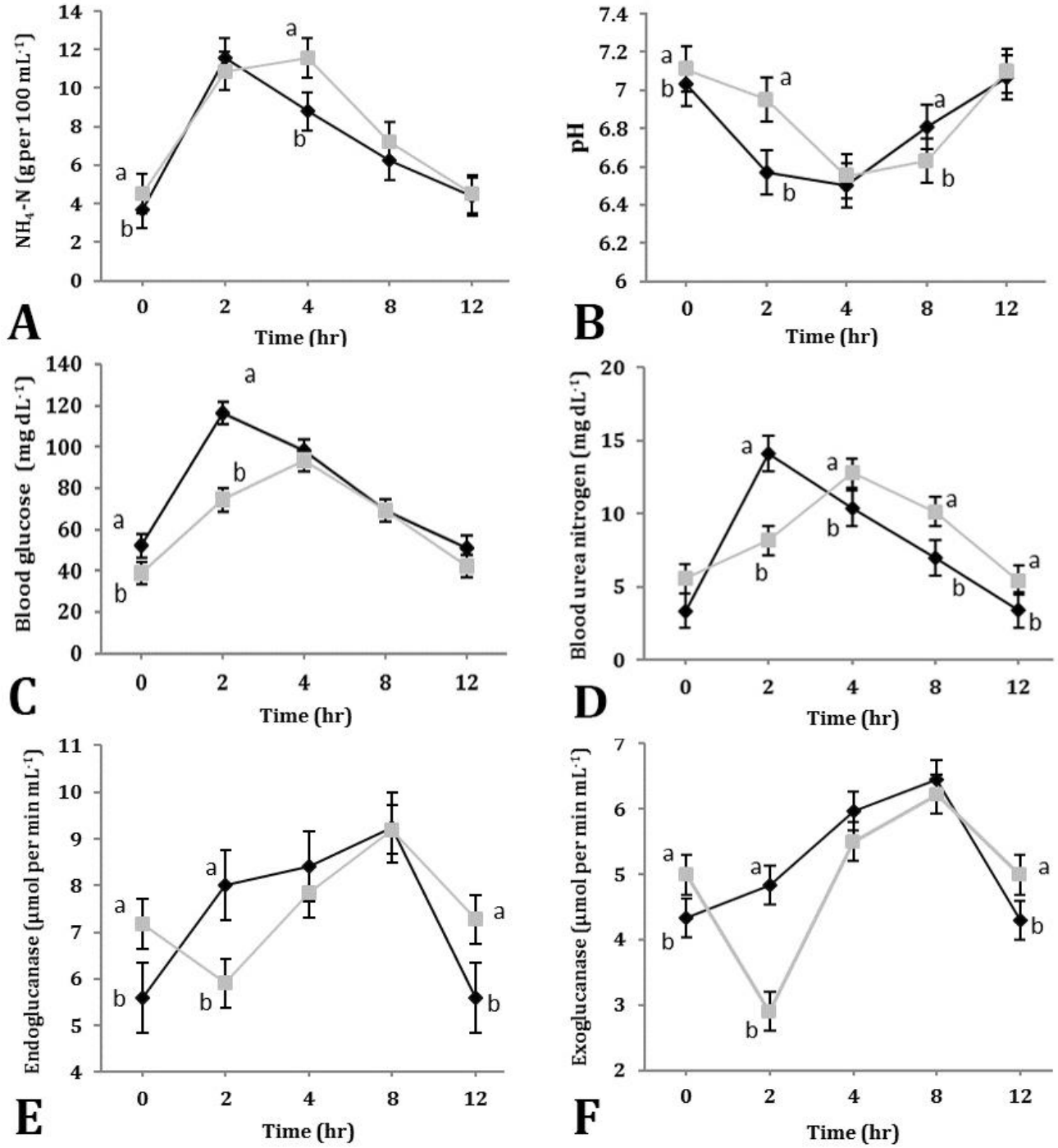

Fig. 1. Diurnal rumen NH_3-N (A) and pH (B), diurnal blood glucose (C) and urea nitrogen (D), diurnal rumen endoglucanase (E) and exoglucanase (F) activity, of camels fed with experimental diets (mean ± SEM; n = 4). ◆ C_3 forage ■ C_4 forage.
[ab] Different letters indicate significant differences ($p < 0.05$).

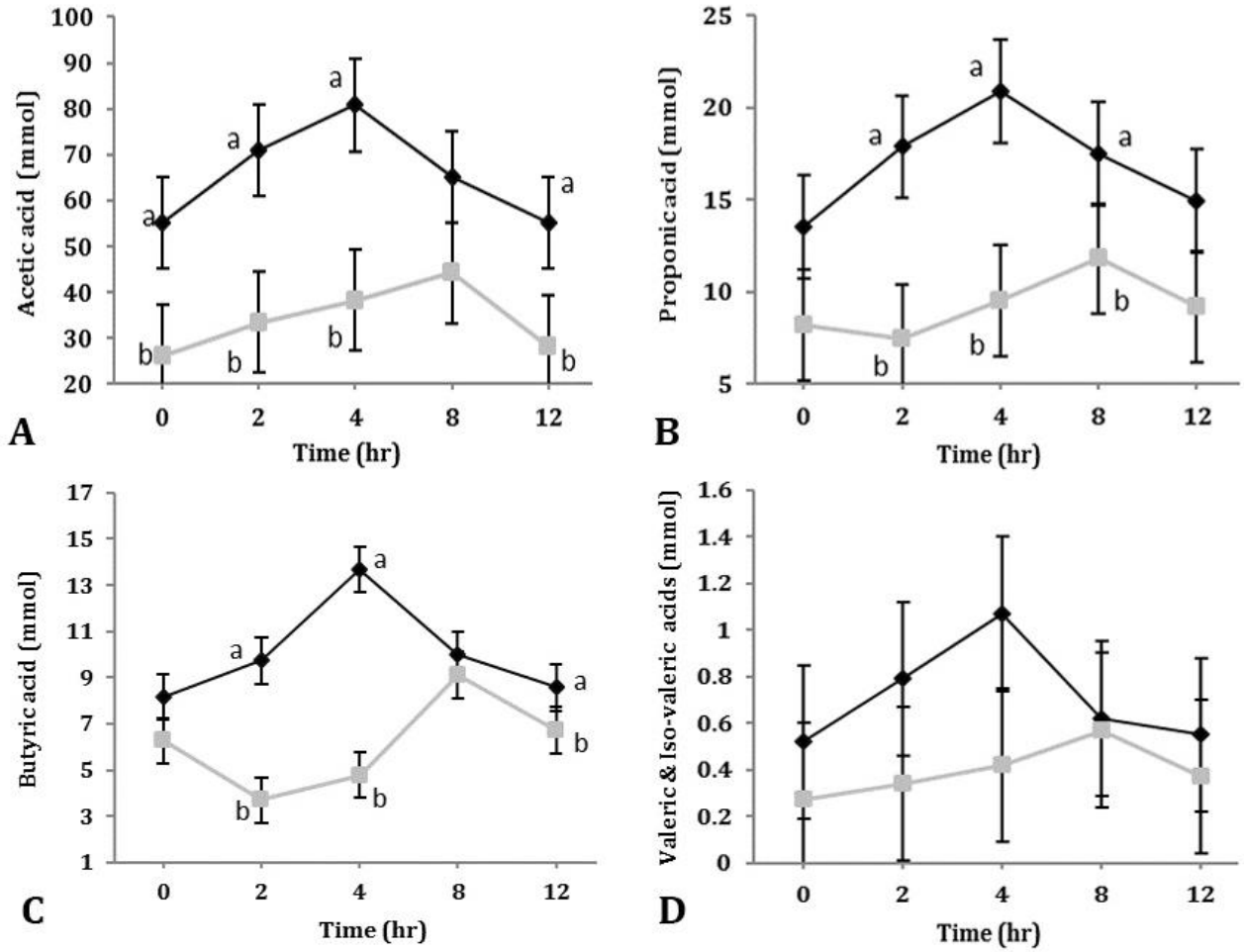

Fig. 2. Diurnal rumen VFAs including acetic (A), propionic (B), butyric (C) and valeric and iso-valeric acids (D) of camels fed with experimental diets (mean ± SEM; n = 4). ◆ C_3 forage ■ C_4 forage. [ab] Different letters indicate significant differences ($p < 0.05$).

Table 2. Gas production coefficients and total GP of C_3 and C_4 forage by rumen fluid of camels fed with experimental diets.

Effects	Gas production from the fermentable fraction (mL)	Rate constant of gas production (mL per hr)	Total gas of 96 hr (mL)
Source of rumen content			
Camel fed C_3 forage	151.60 [a]	0.0062 [a]	39.38
Camel fed C_4 forage	126.89 [b]	0.0041 [b]	36.18
SEM	7.65	0.00044	1.364
Sig.	*	†	NS
Substrate			
Wheat Straw	80.24 [b]	0.0080 [a]	39.01
Atriplex	198.25 [a]	0.0024 [b]	36.55
SEM	7.65	0.00044	1.36
Sig.	†	†	NS
Interactions@			
Treatment 1	83.11[c]	0.0108 [a]	49.77 [a]
Treatment 2	220.09 [a]	0.0016 [c]	28.98 [b]
Treatment 3	77.37[c]	0.0052 [b]	28.23 [b]
Treatment 4	176.41[b]	0.0032 [c]	44.12 [a]
SEM	10.83	0.00062	1.92
Sig.	†	†	†

[@] Treatments containing: 1. Rumen fluid of camels fed with C_3 forage × wheat straw as a substrate; 2. Rumen fluid of camels fed with C_3 forage × *Atriplex L.* as a substrate; 3. Rumen fluid of camels fed with C4 forage × wheat straw as a substrate; 4. Rumen fluid of camels fed with C4 forage × *Atriplex L.* as a substrate. NS = Not significant.

[abc] Means denoted with different letters in a column differ significantly at * = $p < 0.05$ and †= $p < 0.01$.

The results of interaction effects between rumen fluid and substrate denoted that potential of gas production was significantly higher in treatments 2 and 4 that fed with atriplex substrate, however, rate of gas production was higher in treatment 1 ($p < 0.05$). Also after 96 hr, the volume of total gas production for treatments 1 and 4 was significantly higher ($p < 0.05$).

No significant differences on PF, microbial biomass, efficiency of microbial biomass and truly organic matter (OM) degradability between the sources of rumen content were observed (Table 3). However, the microbial biomass and truly OM degradability was significantly affected by the type of substrate and the higher one was observed in wheat straw treatments ($p < 0.05$). The interaction between source of rumen content and substrate demonstrated that only the microbial biomass and truly OM degradability were affected and that in treatment 1 was higher than others ($p < 0.05$). However, little information is known about GP for herbages about camel rumen liquor.

Discussion

The value of blood urea nitrogen (BUN) is very similar to glucose (Fig. 1D). The BUN is correlated with ruminal crude protein and non-protein nitrogen (NPN) degradation. The degradation of NPN in the rumen resulted in an increase in ammonia nitrogen concentration. The researchers believe that in pH 6.70, the NH_3 absorption through the rumen wall will increase. When pH is increased, the NH_4 is converted to NH_3 and its absorption from the rumen wall will be increased.[18] The results of Figures 1A and 1B justify the results of Figure 1D, because with an increase in ammonia nitrogen and decrease in rumen pH, the BUN concentration is increased. The higher BUN in camels fed with C_4 in final hours could be due to the higher passage protein. It has been demonstrated that when the passage protein from the rumen is increased, the rumen NH_3 is decreased, however, BUN is not changed.[19] The others have reported the lower blood urea concentration in salt-tolerant forages mixture diet.[20] Also, similar results have been observed the in ewes fed with atriplex and acacia mixture.[20]

It seems that the mechanism of ammonia nitrogen changes at different hours after feeding in treatment with C_4 in comparison with C_3 has occurred by a slight delay and it could be due to anti-nutritional components in C_4 diet.

This suggested that tropical forage is expected to have lower N availability because C_4 forage concentrates protein in highly vascularized bundle sheath cells, which have proven to be a deterrent to insectivorous and bacterial degradation.[21]Also, higher water consumption consequent to high daily salt intake from atriplex could cause to a faster transit of feed along the digestive tract.[22] This might have depressed the digestibility and nutritive value of atriplex.[23] It seems that there was a low negative correlation between these two parameters. This reduction at 2 hr is probably due to the presence of inhibitor components for microorganism's activity which are available in C_4 that the microorganisms will keep their activity and enzyme production by annihilation.

It is reported that available tannins in atriplex inhibit cellulolytic and proteolytic enzymes and decrease the microbial DNA and RNA in the rumen of ewes.[8] Although it is assumed that the inhibitory or stimulatory effect of

Table 3. Gas production parameters of C_3 and C_4 forage by rumen fluid of camels fed with experimental diets.

Effects	Partitioning factor (mg mL^{-1})	Microbial biomass (mg)	Microbial biomass efficiency (%)	Truly organic matter degradability (g kg^{-1})
Source of rumen content				
Camel fed C_3 forage	3.82	42.05	31.55	74.87
Camel fed C_4 forage	3.99	44.19	30.71	70.51
SEM	0.13	1.87	1.51	1.75
Sig.	NS	NS	NS	NS
Substrate				
Wheat Straw	4.07	35.07 ^{a}	45.58	77.98 ^{a}
Atriplex	3.74	27.18 ^{b}	40.67	67.39 ^{b}
SEM	0.13	1.51	1.87	1.75
Sig.	NS	*	NS	*
Interactions@				
Treatment 1	3.81	40.02 ^{a}	42.14	94.78 ^{a}
Treatment 2	3.84	23.08c	41.96	54.96 ^{c}
Treatment 3	4.34	30.12 ^{b}	49.02	61.18 ^{c}
Treatment 4	3.64	31.29 ^{b}	39.37	79.83 ^{b}
SEM	0.18	2.14	2.65	2.47
Sig.	NS	*	NS	*

@Treatments containing: 1. Rumen fluid of camels fed with C_3 forage × wheat straw as a substrate; 2. Rumen fluid of camels fed with C_3 forage × *Atriplex L.* as a substrate; 3. Rumen fluid of camels fed with C4 forage × wheat straw as a substrate; 4. Rumen fluid of camels fed with C4 forage × *Atriplex L.* as a substrate. NS = Not significant.

^{abc} Means denoted with different letters in a column differ significantly at * = $p < 0.01$.

tannins on enzyme activity may result from a change in the conformation of the enzyme in presence of tannins leading to variability of the substrate at the catalytic site of the enzyme. [24]

Tannins present in atriplex (5.20% of DM) have been found to decrease the production of volatile fatty acids in the ewe's rumen.[8] The researchers using atriplex as replacement of alfalfa in the sheep diet has concluded that the highest and the lowest values of ruminal total VFA's was only in alfalfa and atriplex diet, respectively. [25] This result may be due to higher salt and lower energy contents of atriplex which shortened the rumen turnover time with consequential influences on rumen fermentation [26] thus the production of VFA in the rumen is decreased.[8]

The absorbed butyrate during the transformation cycle of volatile fatty acids is directly used by the kidney as an energy source.[27]

Haddi *et al*. found that digestion coefficient of GP (b and c) by camel's rumen content were 207.00 mL g^{-1} and 2.1 per hr, respectively, for *Atriplex halimus* and 284.00 mL g^{-1} and 1.50 per hr for commercial hay.[28] Such results may be also attributed to the secondary metabolites in atriplex which include oxalates, tannins, and saponins which might decrease rumen fermentation that the same results were reported by researchers.[8,22] The studies [13] noted that gas production is inhibited by tannins. Otherwise, another study reported that the high salt level in atriplex limits its intake and digestion by ruminants.[29] Saponins content in atriplex was 0.33% that has been suggested as an anti-protozoal agent.[30,31] Although it is reported that defaunation reduces methane production,[32] the results of this study showed that in treatments 1 and 4, rumen fluid microorganisms had consistency with the substrate so that the gas production was increased, however, in treatments 2 and 3 it was not come off. It has been demonstrated that the GP curve had impacted with the adaptation of rumen microorganisms exposed to the feed samples type.[33]

It means that a more ration of digested feed is used for gas production rather than microbial protein synthesis.[29] It was reported that the presence of sources containing tannin, cause to increase PF, that this order has known as a positive effect on protein feeding by camel. However, our PF results for tannin in atriplex are not in agreement with these researches[34] and no significant difference was observed between experimental treatments. It is maybe because of tannin degradation by rumen microbes that have been adapted before.

Based on the findings of the present study, where camels are maintained in closed systems, the replacement of C_3 diets instead of C_4 for dromedary camel feeding could be possible and useful. The studies in this field are very poor; therefore, more extensive studies in the field and particularly the effect of these diets on camel performance are suggested.

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Conflict of interest

The authors declare that they have no conflict of interest.

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