Ruminal microbial fermentation of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages

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Abstract   In this experiment eight 1,600 mL dual-flow continuous culture fermenters were used to measure the fermentation pattern of cultivable and pasture forages in camel. Two adult camels were used as rumen inocula donors. Fermenters were fed with 120 g of DM/d in 3 equal portions at 08:00, 16:00, and 24:00 h. The trial contained two treatments of four replicate each in a completely randomized design. The treatments consisted (DM basis) of alfalfa hay (40%) and wheat straw (60%) as the cultivable forages, and *Atriplex L.* (80%), *Suaeda F.* (10%), and *Seidlitzia R.* (10%) as the pasture forages. Digestibility coefficients of DM, NDF and ADF were significantly smaller (P<0.05) in cultivable than pasture forages. Digestibility of OM for cultivable forages was significantly larger (P<0.05) than pasture forages. Total VFA, acetic, propionic, butyric, valeric and iso-valeric acids concentrations were significantly lower in (P<0.01) pasture forages than cultivable forages. Endoglucanase and exoglucanase activities were higher (P<0.01) in cultivable forages than pasture forages. Maximum NH_3-N concentration for cultivable and pasture forages were observed at 2 and 4 h after feeding, respectively. The NH_3-N concentration at 0 and 8 h after feeding was higher (P<0.05) for pasture forages than cultivable forages. The pH of the fermenter content containing cultivable forages was lower (P<0.05) than that for pasture forages. It seems that the presence of pasture forges in the diet, probably as a result of anti-nutrients in the forages, delayed the normal ruminal fermentation but may have been compensated by the decomposition of anti-nutritional substances by the microorganisms during the final hours of incubation.

Keywords: ammonia nitrogen, atriplex, one-humped camel, dual flow fermenter, rumen

Introduction

Knowledge of the quality of feed selected by camels, their behavioral activities and feed preferences are important to the understanding of the forage–camel relationship (Dereje and Uden, 2005). Several studies have suggested that camelids have superior digestive efficiency compared to pecoran ruminants (San Martin and Bryant, 1989), while others have reported no difference (Hintz et al., 1976; Warmington et al., 1989). Some of these contradictory results may stem from differences in feed quality (Sponheimer et al., 2003). Some researchers suggested that camelids are better adapted to the digestion of poor-quality forage than pecoran ruminants living under the same conditions (Robinson et al., 2006). Plants are classified by the photosynthetic pathway they use (C_3 and C_4). Most temperate forages utilize the C_3 photosynthetic pathway, and C_4 plants are found in tropical grasslands and are dominant in warm-season temperate grasslands (Heckathorn et al., 1999). The C_4 plants generally have higher cellulose and lignin contents, resulting in decreased digestibility compared to C_3 plants (Minson, 1971). Feed shortage is an important constraint to raising camels under harsh climatic conditions in the in arid and semi-arid regions; however, the nomadic system is gradually replaced by the more sedentary systems (Eisa and Mustafa, 2011). In some areas, the long term impact of natural disasters has aggravated the situation and forced many camel herders to settle in the nearby cities (Abbas and Omer, 2005). Moreover, to keep pace with the alarming nutritional crisis and make the ration economical for sustainable camel production, attempts were made to formulate rations for camels' intake of conventional and non-conventional feed resources (Gupta et al., 2012). There is limited data on feed digestibility and ruminal fermentation parameters in the camel using the *in vitro* methods (Riasi et al., 2012). Dual flow continuous culture fermenters that si-

mulate the ruminal environment can be used to measure feed digestion and microbial metabolism (Stern et al., 1997). Therefore, the objective of the present experiment was to determine the effect of cultivable and pasture forages on ruminal microbial fermentation of camel using a dual flow continuous culture system.

Materials and Methods

Apparatus and diets

In this experiment, two adult camels (200 ± 50 kg, BW) were used as rumen inocula donors. They were housed in individual pens with free access to drinking water, and were fed a total mixed ration at the maintenance level (Wilson, 1998). The diet consisted of 30% alfalfa hay, 35% wheat straw and, 30% *Atriplex L.*, 5% *Suaeda F.* and *Seidlitzia R.* The animals were handled according to the Iranian Council of Animal Care Guidelines (1995). The inoculum was obtained through a rumen cannula, then mixed and strained through four layers of cheesecloth under anaerobic conditions. Eight dual-flow continuous culture fermenters with 1,600 mL capacity were used for 9 d (6 d of adaptation and 3 d of sampling) according to Hannah et al. (1986). Fermenters were inoculated with the ruminal fluid. Temperature was kept constant at 38.5°C, and the liquid and solid phase rates were 10%/h and 5%/h, respectively. The anaerobic condition was maintained by the infusion of N_2 at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks, and contained 0.4 g/L of urea to simulate N_2 recycling. The fermenters were fed 120 g of DM/day in three equal portions at 08:00, 16:00, and 24:00 h. The trial contained two treatments of four replicates each in a completely randomized design. The diets (Table 1) consisted of:

Table 1. Ingredients and chemical composition (% of DM basis) of the diets containing cultivated or pasture forages

Ingredients	Cultivable forage diet	Pasture forage diet
Atriplex L.	0	80
Suaeda F.	0	10
Seidlitzia R.	0	10
Alfalfa hay	40	0
Wheat straw	60	0
Chemical composition		
Dry matter	89.3	83
Crude protein	7.24	7.07
Neutral detergent fiber	68.1	61.78
Acid detergent fiber	43.45	38.73
Ash	8.19	18.8
Organic matter	91.81	81.2

alfalfa hay and wheat straw (cultivable forages) and *Atriplex L.*, *Suaeda F.* and *Seidlitzia R.* (pasture forages).

Sample collection and processing

During the three d of sampling, 10 mL of filtered fermenter fluid was taken at 10:00 to determine the volatile fatty acid (VFA) concentration, and 25 mL for enzyme activity determination. Five mL of this fluid was taken at 08:00, 10:00, 12:00, 14:00, and 16:00 to determine NH_3-N concentration and pH. During the sampling period, liquid and solid effluent collection vessels were maintained at 4°C to prevent microbial activity; their contents were mixed and homogenized for 1 min, and a 500 mL sample was removed via aspiration. The effluents from three sampling days were then composited and mixed within the fermenter and homogenized for 2 min. The remainder of the sample was oven dried (103°C) (Hannah et al., 1986).

Chemical analyses

The effluent dry matter (DM) was determined by lyophilizing 250-mL aliquots in triplicate, and a subsample was dried at 103°C in an oven for 48 h to determine the final DM content. Dried samples were ashed overnight at 550°C in a muffle furnace and then neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the diets and effluents were determined by the detergent system (Van Soest et al., 1991). The samples were analyzed for NH3-N with phenol-hypoclorite as the main reagent (Broderick and Kang, 1980). The VFAs were analyzed by GC (Model PU4410, PHILIPS; column 10PEG and detector FID) as described by Ottenstein and Bartley (1971). Enzyme activity was measured by the dinitrosalicylic acid (DNS) method which is based on the measurement of reducing sugars released during the enzymatic reaction with a defined substrate (Colombatto and Beauchemin, 2003).

Statistical analysis

Data were analyzed using the GLM procedure of SAS (2005), as a completely randomized design with the statistical model: $Y_i = \mu + T_i + e_i$, where Y_i = dependent variable; μ = population mean; T_i = mean effect of treatment; and e_i = experimental error. The Tukey's test was used to compare the means at $P < 0.05$.

Results

The nutrient digestibility as affected by the type of forage in the diet is shown in Table 2. Digestibility of DM, NDF, and ADF was lower (P<0.05) in the diets contain-

Table 2. Ruminal nutrient digestibility (%) of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages

	Dry matter	Organic matter	NDF	ADF	Crude protein
Cultivable forage	45.4	80.63	32.79	35.24	63.24
Pasture forage	60.7	71.53	57.93	55.13	59.51
SEM	1.40	1.53	1.52	1.10	3.21
P value	0.049	0.042	0.037	0.027	0.64

ing cultivable forages than pasture forages.

Digestibility of organic matter (OM) for the diet containing cultivable forage (80.63%) was higher (P<0.05) compared to pasture forages (71.53%). Digestibility of crude protein (CP) was not significantly different between treatments, but was lower in the diet containing pasture forages than cultivable forages (63.24% and 59.51% for cultivable and pasture forage, respectively).

Concentrations of total VFA, acetic, propionic, butyric, valeric and iso-valeric acids were lower (P < 0.01) for the diets containing pasture forages (Table 3).

The effect of treatments on enzyme activity is shown in Table 4. Endoglucanase and exoglucanase activities were higher in cultivable forage compared with pasture forage (P < 0.01). Although filter paperease activity was higher in cultivable forages than pasture forages, this difference was not significant.

The effect of treatments on NH_3-N concentration and pH is shown in Figures 1 and 2, respectively. Maximum NH_3-N concentration for cultivable and pasture forages was observed at 2 and 4 h after feeding, respectively. Ammonia nitrogen concentrations at 0 and 8 h after feeding were significantly higher for pasture forages (P< 0.05). Figure 2 shows considerable variations in the rumen pH in dual flow continuous culture fermenters. Results showed that pH for the diet containing cultivable forages was lower than for those containing pasture

forages. The minimum pH values were observed for cultivable forages at 2 h to 4 h and at 4 h to 8 h after feeding for pasture forages. Maximum pH values partly coincided with minimum NH_3-N concentration.

Discussion

The two kinds of forage hays; alfalfa and wheat straw hays were chosen for the C_3 photosynthetic pathway. The three tropical forages using the C_4 photosynthetic pathway were chosen because they had similar crude protein and fiber contents, though C_4 plants contain high level of salt (sodium chloride) and anti-nutritional factors (Munoz et al., 1996). Some researchers reported that the secondary metabolites in arid and semi-arid plants such as oxalates, tannins, and saponins may decrease the microbial activity in the ruminal fluid (Shawket and Ahmed, 2009; Abu-Zanat and Tabbaa, 2005).

As shown in Table 2, dM digestibility was higher in the fermenter fed with pasture forages which suggests that the microbial activity in the treatment with pasture forage was higher. Numerous studies have been conducted on anti-nutritional substances of pasture plants, suggesting that microbial activity of the treatment groups fed with forages was lower; however, the findings are not consistent with the current study. Probably adaptation of microorganisms to anti-nutritional factors

Table 3. Ruminal VFA concentration (mEq/100mL) of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages

	Total VFA	Acetic	Propionic+Isobutyric	Butyric	Valeric	Iso-valeric
Cultivable forage	97.50	57.82	23.37	14.97	0.85	0.47
Pasture forage	45.02	29.42	8.15	6.97	0.22	0.25
SEM	1.777	2.47	0.62	0.625	0.017	0.054
P value	0.000	0.005	0.000	0.004	0.001	0.084

Table 4. Ruminal enzyme activity ($\mu mol_{glucose}$/mL/h) of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages

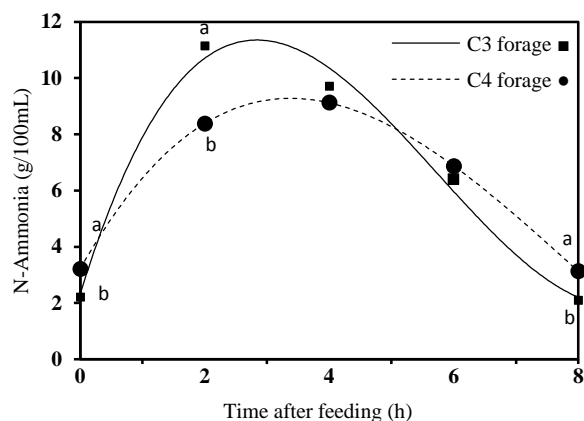| Enzyme	Endoglucanase	Exoglucanase	Filter paperase
Cultivable forage	7.71	4.67	6.09
Pasture forage	4.87	3.33	4.64
SEM	0.163	0.144	0.437
P value	0.001	0.01	0.25

Figure 1. Ruminal NH₃-N concentration of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages (n = 4).
Means denoted with different letters (a and b) are significantly different.

Figure 2. Ruminal fluid pH of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages (n = 4). Means with different letters (a and b) are significantly different (P < 0.05).

of pasture forage has given rise to these results as it has been reported that ruminal microbial population might adapt to the plant metabolites and degrade its component (Teferedegne et al., 2000). These results were in agreement with those reported by Sponheimer et al. (2003). During an experiment, the digestibility of cultivable forage DM (*Bromus inermis*) and pasture forage (*Cynodon dactylon*) in llama was 51% and 63%, respectively, indicating higher pasture forage digestibility. Similar results were obtained for NDF and ADF digestibility. San Martin (1987) showed that llamas were better suited in digesting high-fiber and low-protein forages than sheep, but this advantage was not observed with higher quality diets. The results were in contradiction with those of Robinson et al. (2006) who reported that DM digestibility of cultivable and pasture forages were respectively 61.6% and 59.5% for llama. In contrast to previous studies, the digestibility of OM was higher in the diet containing cultivable forages. Such discrepancy was probably due to the high content of ash in pasture forage (Ben Salem et al*.,* 2005).

The *Atriplex* sp. plants are characterized by their high minerals (ash) but low energy contents in the form of nitrogen free extract (El-Hyatemy et al., 1987). However; OM digestibility reduction in pasture diet might be related to the presence of tannins in atriplex. Accordingly, tannins, as well as other anti-nutrients, can affect the microbial digestion of organic matter through making complexes with carbohydrates, proteins, and polysaccharides (Frutos et al., 2004). Ghadaki et al. (1975) determined the nutritive values of the introduced and native plants grown under natural conditions in Iran, and showed that the shrubs had higher lignin percentages, and there was a negative correlation between lignin con-
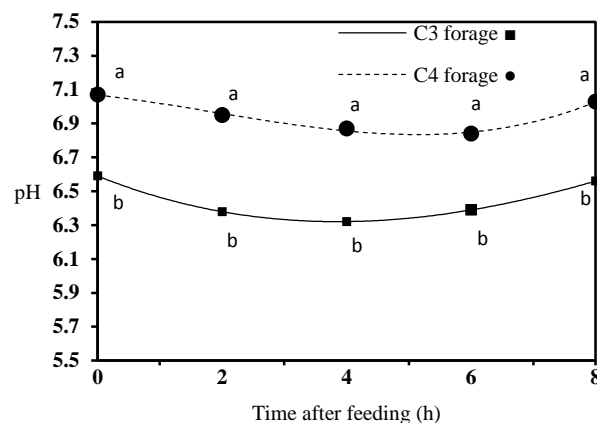
tent and *in vitro* true OM digestibility. Moreover, the diet containing cultivable forages possessed higher protein digestibility, but the difference was not significant. Pasture plants concentrate protein in highly-vascularized bundle sheath cells (Caswell and Reed, 1976) and are resistant to bacterial degradation *in vitro* (Akin et al., 1983). In contrast, protein is dispersed more evenly throughout the highly-digestible mesophyll of cultivable plants. Consequently, even with similar nitrogen and cell wall concentrations, pasture forages are predicted to have lower apparent nitrogen digestibility than cultivable forages (Ehleringer and Monson, 1993). The slight reduction in nitrogen (N) digestibility in pasture forages might be attributed to the tannin content which was found to reduce the protein digestibility (El- Shaer, 2010). These results were similar to those reported by Sponheimer et al. (2003). They reported that protein digestibility of cultivable and pasture forages were the same in llama. But Robinson et al., (2006), comparing alfalfa, cultivable and pasture forage in goat and llama, reported that pasture plant nitrogen was fully protected and unavailable. The fermentation progress is shown in Figures 1 and 2; the cultivable forage fermentation started faster, and ended in 2 h after feeding but was reduced immediately; however, this increase was slower in pasture plants and occurred with a delay, and ended 4 h after feeding and then decreased gradually. Anti-nutritional materials in pasture plants can be the cause of this delay.

It seems that ammonia nitrogen (NH3-N) concentration mechanism changes at different hours after feeding in the diet containing pasture forages in comparison to cultivable forage and having occurred with a slight delay could be due to pasture anti-nutritional components.

As shown in Figure 1, NH3-N production of the diet containing pasture forages was higher than cultivable forages at the end of the meal intake and therefore microbial fermentation activity was higher. This can compensate the initial delay in the digestion process. The results in Table 2 are taken from the fermenter output of the samples.

A 5 mg/dL of NH3-N concentration has been suggested as the minimum level required to maximize bacterial growth efficiency (Satter and Slyter, 1974). This value is lower than that obtained for cultivable and pasture diets (11.14 and 9.13 mg/dL, respectively) in the present study. Ariza et al. (2001) reported that NH_3-N concentration in the fermenters depended on the extent of CP degradation and N uptake by ruminal bacteria. Ferme et al. (2004) reported that inhibition of major ammonia producing bacteria such as *Prevotella ruminantium* and *Prevotella bryantii* resulted were effective in reducing the NH_3-N concentration in continuous culture fermenters with ruminal microbes. Also Ferme et al. (2004) reported that the fermenters had fewer protozoa; however, *in vivo*, protozoa play a major role in protein degradation.

Changes in pH values (Figure 2) also indicated a delay in the onset of the fermentation process in the presence of pasture forages; the cultivable forage pH was lower than pasture forages at all times after feeding. However, the slow slope of the pH decrease represents the slow and continuous pasture forage fermentation by microorganisms.

VFAs concentrations (Table 3) showed that 2 h after feeding most of the fermentation activity for the cultivable forage diet was higher than for pasture forage diet. This can be due to higher salt and lower energy contents of pasture forages which shorten the rumen turnover time with consequential influences on rumen physiology and metabolism (Konig, 1993) and decrease the VFAs production in the rumen (Shawket and Ahmed, 2009). Such results might be also attributed to the secondary metabolites in pasture plants such as oxalates, tannins, and saponins which might decrease VFA production. Similar results were reported by Shawket and Ahmed (2009) and Abu-Zanat and Tabbaa (2005). Mansfield et al. (1994) noted that in continuous culture fermenters, total VFA production decreased as the dietary rumen degradable protein (RDP) concentration decreased. The major source of variation affecting molar proportions of ruminal VFA is OM digestibility; therefore, feeding forages of higher digestibility is associated with lower proportions of acetate and higher proportions of both propionate and butyrate (Lopez et al., 2000). However, our data showed that in addition to higher OM digestibility of cultivable forages, the VFAs were higher than that for pasture forages. Fayed et al. (2010), in a study on sheep, replacing alfalfa partly by atriplex, concluded that VFA production decreased, because of high salt and low energy contents in atriplex.

Results of cellulolytic enzyme activity (Table 4) showed less activity of microorganisms in the diet containing pasture forages. This reduction was probably due to the presence of microbial inhibitors in pasture forages and accordingly the microorganisms will keep their activity and enzyme production by annihilation. Shawket et al. (2010) reported that tannins in atriplex inhibited cellulolytic and proteolytic enzymes in the rumen. Nonetheless, Makkar et al. (1988) assumed that the inhibitory or stimulatory effect of tannins on enzyme activity may result from a change in the conformation of enzyme leading to substrate variability at the catalytic site of the enzyme.

It is suggested that saponins in foliage may exert antiprotozoal activity (Newbold et al., 1997). On the other hand; ruminal protozoa possess high levels of fibrolytic activity (Williams and Coleman, 1992). Therefore, reduced digestibility of the fiber fraction in defaunated ruminants may be due to the stabilization of the ruminal environment favoring development of cellulolytic microbes (Hegarty et al., 1991) and a stimulatory effect of rumen ciliated protozoa over rumen bacteria (Onodera et al., 1988). Therefore, a decrease in protozoal number may have resulted in lower enzyme availability for pasture forage digestion soon after feeding.

Conclusion

In general, the presence of pasture forges in the diet, probably as a result of anti-nutrients in the forages, delayed the normal fermentation process, but this delay was probably compensated by degradadtion of anti-nutritional substances by the microorganisms during the final satges of incubation and fermentation.

Acknowledgments

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تخمیر میکروبی شکمبه شترهای یک کوهانه در فرمنتورهای کشت پیوسته دوجریانی با استفاده از علوفه زراعی و مرتعی

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چکیده   در این آزمایش از هشت محفظه تخمیر ۱۶۰۰ میلی لیتری کشت پیوسته استفاده شد و مایع شکمبه از دو نفر شتر یک کوهانه تأمین گردید. فرمنتورها به طور روزانه با ۱۲۰ گرم ماده خشک، در سه وعده یعنی ساعت ۸:۰۰، ۱۶:۰۰ و ۲۴:۰۰ تغذیه شدند. این آزمایش بر اساس یک طرح کاملاً تصادفی شامل دو تیمار و چهار تکرار بود. تیمارها شامل ۱) یونجه (۴۰ درصد) و کاه گندم (۶۰ درصد) به عنوان علوفه قابل کشت و ۲) سلمکی ساقه سفید (۸۰ درصد)، اِشنان (۱۰ درصد) و سیاه‌شور (۱۰ درصد) به عنوان علوفه مرتعی بودند. نتایج این آزمایش نشان داد که هضم پذیری ماده خشک، NDF و ADF به طور معنی داری در علوفه قابل کشت کمتر از علوفه مرتعی بود (۰/۰۵>P). هضم پذیری ماده آلی در علوفه قابل کشت در مقایسه با علوفه مرتعی به طور معنی داری بالاتر بود (۰/۰۵>P). غلظت کل اسیدهای چرب فرار، اسید استیک، اسید پروپیونیک، اسید بوتیریک، اسید والریک و اسید ایزووالریک به طور معنی‌داری در علوفه مرتعی پایین‌تر از علوفه قابل کشت بود (۰/۰۱>P). فعالیت اندوگلوکاناز و اگزوگلوکاناز در علوفه قابل کشت بیشتر از علوفه مرتعی بود (۰/۰۱>P). بیشترین غلظت نیتروژن آمونیاکی برای علوفه قابل کشت و مرتعی به ترتیب در ۲ و ۴ ساعت بعد از خوراکدهی مشاهده شد. غلظت نیتروژن آمونیاکی قبل از تغذیه و ۸ ساعت پس از تغذیه در علوفه مرتعی بیشتر از تیمارهای با علوفه زراعی بود (۰/۰۵>P). میزان pH فرمنتورها در تیمارهایی که با علوفه قابل کشت تغذیه شده بودند نسبت به علوفه مرتعی کمتر (۰/۰۵>P) بود. به نظر می‌رسد به دلیل حضور علوفه مرتعی در جیره که احتمالا حاوی مواد ضد تغذیه‌ای هستند، تاخیر در فرایند طبیعی تخمیر شکمبه رخ داده است، اما در ساعات پایانی انکوباسیون احتمالا این تاخیر بوسیله تجزیه مواد ضد تغذیه‌ای توسط میکروارگانیسم‌ها جبران می‌شود و تخمیر افزایش می‌یابد.