IJAS Iranian Journal of Applied Animal Science

In vitro Fiber Digestibility, Gas Production and Enzyme Activity of Cellulolytic Bacteria of Arabian Camels (Dromedary) Fed Cultivable and Pasture Forage

Research Article

P. Dadvar[1], T. Mohammadabadi[1*], M. Sari[1] and J. Fayazi[1]

[1] Department of Animal Science, Faculty of Animal and Food Science, Ramin Agricultural and Natural Resources University, Mollasani, Ahvaz, Iran

Received on: 30 Jan 2018
Revised on: 28 Mar 2018
Accepted on: 15 Apr 2018
Online Published on: Sep 2018

*Correspondence E-mail: mohammadabadi@ramin.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This experiment was conducted to evaluate of rumen cellulolytic bacteria activity of dromedary camels fed cultivable and pasture forage. Four fistulated dromedary camels were fed for 35 days in 2 periods as cross over design with cultivable and pasture forage (4 camels per forage). Then rumen fluid was collected after morning feeding. Inoculant of cellulolytic bacteria was prepared and *in vitro* gas production, digestibility and enzyme activity were measured (6 replicates). Treatments were including: rumen fluid of camels fed cultivable forage × wheat straw and or atriplex as a substrate and rumen fluid of camels fed pasture forage × wheat straw and or atriplex as a substrate. The result showed, after 96 hours incubation, the produced gas and actual digested organic matter for treatments 1 and 4 were higher ($P \leq 0.01$). However, the ammonia-N was affeted by treatments at 24 and 48 h incubation ($P \leq 0.05$). Digestibility of dry matter, organic matter and natural detergent fiber by bacteria were influenced by treatments ($P \leq 0.01$). The results revealed that the activity of endoglucanase and exoglucanase in treatments 1 and 3 at 48 h incubation ($P \leq 0.01$) and values of treatment 4 at 96 h was the highest ($P \leq 0.01$). Therefore, pasture forage such atriplex caused to a silent delay in starting bacteria fermentation activity in camels, then primary delay was compensated by adaptation to the substrate. Also feeding of C_4 pasture forage to camels improved the fiber degradability and enzyme activity of rumen bacteria in compared with C_3 cultivable forage.

KEY WORDS atriplex, dromedary camel, endoglucanase, exoglucanase, *in vitro* digestibility.

INTRODUCTION

Camel as is pseudo ruminant that live in desert ecosystem of Africa, Middle-East and Australia. The proven symbol of adaptation with its unique bio-physiological characteristics has some formidable ways of living on unpleasant situations of wasteland and semi-wasteland regions. The camel has many unique qualities to survive and serve under harsh climate and unpleasant situations of wasteland and semi-wasteland regions. Also they utilize low quality feed resources which other species cannot consume. Despite of differences in anatomical structures, camels are the same with true ruminants, functionally and obtain all their nutritional needs from pasture forages. Plants can be classified to C_3 and C_4 plants that the first photosynthetic products in C_3 plants are 3-carbon compounds while the first products of C_4 plants are 4-carbon. Pasture C_4 plants are dominant in tropical and warm-season temperate grasslands and have higher cellulose and lignin content that cause to decrease of digestibility in compared with cultivable C_3 plants (Haddi *et al.* 2009). The camels diet with mixed feeding behavior can be varied extraordinarily in traditional long-range no-

madic systems (Dereje and Uden, 2005). This system that has been used by camel herders since long times ago, is strongly efficient. The shortage of feed resources in arid and semi-arid regions is the most important constraint to raising camels.

However, nomadic system has being slowly replaced by sedentary systems due to frequent droughts, resulted in appropriation of traditional grazing lands for marginal cultivation or grazing lots for trade herds (Yagil, 1994).

As a result, the once desirable mixed exposure and feed intake in the sedentary systems are lost. In sedentary camel raising methods, many factors lead to the low productivity, that may be the quantity and quality of feed deficiency is the most important factor.

In view of the trend to sedentary systems, there is an immediate need to study ways for improving the nutritional conditions of the camels for improving of pastoral societies life. Researcher suggest that under the same conditions, camelids are the most adapted animals to the digestion of poor-quality forage than other ruminants living (Robinson *et al.* 2006).

In some areas, many camel herders forced to settle in the nearby cities because of long term impact of natural disasters has aggravated. Moreover, to keep pace with the alarming nutritional crisis and make the economical ration for sustainable production of the camel, diets for camels by conventional and non-conventional feed resources were formulated.

Many microorganisms are in camel which by producing effective enzyme breakdown and digest plants. One of the most abundant organisms of camel is *Bacteroides fragilis* that digests plant materials and has been improved animal digestion.

Thus, an important contribution to high abundance and diversity of carbohydrate utilization found in the camel metagenome may be a result of a high level of complex polysaccharides in the camel diet. This may be because of rumen pH, together with microbial population, substrates, temperature, and cations and soluble carbohydrates that governing bacterial attachment.

It is concluded that the cellulolytic activity of camelids microbes in degradation of low-digestible wheat straw was 20% higher than the other ruminants and these differences in digestive ability appear after 24 h in forestomach. Camelids have sufficient enzymatic activity to hydrolyze the cell wall carbohydrates and fermentation of hydrolyzed oligosaccharides (Kayouli *et al.* 1991).

There is rare information available on the foregut microbial community of the camels (Ghali *et al.* 2011). Moreover, many researches showed when the low quality roughages were fed, the microbial efficiency (Lemosquet *et al.* 1996) and the nutrient digestibility were significantly higher in llamas than sheep because of cellulolytic activity in the forestomache of camelids was higher (Dulphy *et al.* 1997).

But very little information is available on the bacterial population of alpaca's rumen (Pei *et al.* 2010). Generally, researchers showed a greater ability for digestion of fibrous forage in the camels than other ruminants. This can be attributed to efficient nitrogen recycling in camel due to the lower renal excretion (San Martin, 1991) and a higher rumen retention time for the solid phase (Dulphy *et al.* 1994) which facilitates cell walls degradation via a greater exposure time to microorganisms. Another factor is pH of ruminal contents, which is closer to neutrality favoring as cellulolytic digestion.

Due to the lack of sufficient information about the one-humped camel rumen microbial activity and especially in terms of change in diet systems, this experiment carried out to investigate of fiber digestibility, fermentation pattern and enzyme activity by rumen cellulolytic bacteria of dromedary camels fed C_3 cultivable and C_4 pasture forage.

MATERIALS AND METHODS

Diet and animal

This trial was performed during the spring season in Khuzestan province-Iran. Four fistulated dromedary camels about 2 years old and 150-200 kg weight of the Arabian breed were selected. The animals were kept in individual stalls equipped with feeders and water pails. Camels were fed for 35 days in 2 periods as cross over design with two types of diets (4 camels per each) that one of them contained alfalfa hay and wheat straw (C_3 forage or cultivable diet) and the other contained *Atriplex L.*, *Suaeda F.* and *Seidlitzia R.* (C_4 forage or pasture diet) (Table 1).

Isolation procedure

Isolation of cellulolytic microorganisms was done from the rumen of Iranian (one-humped) fistulated camels (4 camels for each diet in 2 periods). The camels were fed for 35 days at a level of 40 g/kg metabolic weight for adaptation. Rumen fluid was taken about 2 h after morning feeding. About 3 to 4 samples of rumen fluid of each animal that fed with cultivable and pasture forage were taken and mixed. Rumen contents were strained by two layers of cheesecloth into pre-warmed thermo flasks.

The cellulolytic bacteria isolation was carried out by using specific media of rumen cellulolytic bacteria and during the isolation, the anaerobic conditions were maintained (Makkar and McSweeney, 2005). The media contained: 15 mL mineral solution I (KH_2PO_4 3.0 g; $(NH_4)_2SO_4$ 6.0 g; NaCl 6.0 g; $MgSO_4$ 0.6 g and $CaCl_2 \cdot 2H_2O$ 0.795 g/L), 15 mL mineral solution II (K_2HPO_4 3 g/L), 0.5 g yeast extract,

0.5 g trypticase, 0.1 mL resazurine (0.1%), 0.2 g microcrystaline cellulose, 0.1 g cellubiose, 0.8 g sodium carbonate, 30 mL clarified rumen fluid to support volatile fatty acids (VFA) mix, 0.5 g L- cysteine HCl-H_2O, 0.05 g benomyle and metalaxyle [200 U/mL] (anti fungi) and distilled water added to final volume of 100 mL. This trial contains two factors 1: camel's diets and 2: media substrate. Diets were containing cultivable and pasture forage (Table 1). Also the substrate involves 1: wheat straw and 2: Atriplex. Therefore, treatments included:

1) Rumen fluid of camels fed cultivable forage × straw substrate
2) Rumen fluid of camels fed cultivable forage × Atriplex substrate
3) Rumen fluid of camels fed pasture forage × straw substrate
4) Rumen fluid of camels fed pasture forage × Atriplex substrate

An attempt was made to investigate the effects of the source of rumen fluid, media substrate and interaction effects among them. Particularly, due to adaptation of rumen fluid microbes with their substrate, the best comparison about plant's type was done for the first and fourth treatments.

***In vitro* degradation by cellulolytic bacteria**

A 100 mL calibrated glass vial was used in triplicate and 1 g substrate (Atriplex or wheat straw) was added to each vial. The reducing solution, bacteria inoculant (were prepared as described before) and specific media of rumen cellulolytic bacteria was added to vials. While rumen fluid was being added to the vials, the headspace of each vial was gassed with CO_2.

The vials fitted with crimp sealed caps to ensure that no gas could escape, and then it was placed in an incubator 37 ˚C for 96 h.

For low degradable feeds, it is important or essential that the incubation time be long enough (e.g. 72 h or even up to 96 h). During sampling, three vials (for each substrate) were removed from the incubator after 24, 48 and 96 h incubation (3 repeats for each time and substrate). The media pH was recorded and the content of each vial was used for determination of ammonia N concentration by using phenol-hypochlorite as the main reagent. Then the content of each vial was filtered and oven-dried at 105 ˚C for dry matter determination and was analyzed for nautral detergent fiber and organic matter and digestibilities by rumen cellulolytic bacteria measured (Mohammadabadi and Chaji, 2010).

Gas production by cellulolytic bacteria

In vitro gas production was determined as described by Blümmel *et al.* (1997). About 500 mg of samples was weighed accurately into 100 mL glass vials, then bacteria buffered medium and reducing solution were added to each vial. Vials fitted with plungers and placed in a pre-warmed incubator at 39 ˚C. Gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h using a digital pressure gauge (Model SDPG0015PG5, SenSym ICT, Honeywell Inc., Morris NJ) fitted with a 21 mm gauge needle. 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 (Ayaşan *et al.* 2018). *In vitro* gas production values (mL/g organic matter (OM)) were fitted to the following non-linear model (Orskov and McDonald, 1979).

$$Y= b(1-e^{-ct})$$

Where:
Y: gas volume at time t (mL).
b: potential of gas production (mL/g OM).
t: time (h).
c: gas production rate (mL/h).

After 96 h of incubation, fermentation was stopped and the contents of the vials mixed with 20 mL neutral detergent fiber solution, boiled for 1 hour, filtered, dried and ashed. Then gas production parameters (partitioning factor, microbial biomass and actual digested organic matter) estimated as fallow (Blümmel *et al.* 1999):

Partitioning factor (mg/mL)= actual digested organic matter (mg) / Gas production (mL)

Microbial biomass (mg)= actual digested organic matter (mg) - (gas production×2.2)

Microbial biomass efficiency (%)= (microbial biomass (mg)/actual digested organic matter (mg)) × 100

Enzyme assay

The enzyme activity of cellulolytic bacteria was determined by the dinitro salicylic acid (DNS) method (Colombatto and Beauchemin, 2003), which is based on the measurement of reducing sugars content released during the enzyme reaction with a defined substrate. In this assay, sampling contained two vials (for each substrate) that were removed from incubator after 24, 48 and 96 hours' incubation. For endoglucanase assay, 1% (wt/vol) medium viscosity carboxy methyl cellulose (CMC; Sigma Chemicals Co., St. L-

ouis, MO, USA) was used as the substrate and exoglucanase activity was determined using 1% (wt/vol) solution of microcrystalline cellulose (Avicel, Sigmacell 50; Sigma Chemical Co.) as the substrate.

For this assay, 0.1 M citrate-phosphate buffer (pH 6.0), substrate and distilled water were mixed and incubated for 10 min at 39 ˚C in water bath for equilibration. Tubes contained only substrate (no enzyme) and only enzyme (no substrate) also was included to correct for background of reducing sugars in the enzyme samples. Reaction was terminated by adding 3.0 mL of DNS reagent. Then the tubes were placed in a boiling bath 300 s and cooled in water. The absorbance was read at 540 nm by using a spectrophotometer. The amount of reducing sugars released was determined using a standard curve made with glucose. Then the units of activity were expressed as μmol of glucose equivalents / min.mL of undiluted enzyme product.

Statistical analysis

Data were analyzed with ANOVA using the general linear model procedure of SAS (2005) with the main effects of rumen fluid source, substrate and their interaction effects. Means were separated using LSD test and were considered to be significant different at $P < 0.05$. The Statistical model was:

$$Y_{ijk}= \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

Where:

Y_{ijk}:observed value.

μ: general mean.

α_i: main effects of the source of camel rumen content.

β_j: main effects of substrate.

$\alpha\beta_{ij}$: interaction effects.

e_{ijk}: experimental error.

RESULTS AND DISCUSSION

The estimated gas production parameters are presented in Table 2.

The results of this experiment revealed that gas production potential (b) and gas production rate (c) were not influenced by the type of rumen fluid. Whereas the total produced gas in inoculated media by rumen fluid of camels fed C_4 forage was significantly higher after 96 hour incubation ($P\leq0.01$).

The type of inoculated substrate to the media affected the gas production kinetics as well as the total amount of the produced gas, hence the gas production potential (b) with Atriplex substrate was increased but at the end of 96 hours of incubation, the gas production rate (c) and the volume of produced gas were higher in wheat straw substrate treatments ($P\leq0.01$).

Haddi *et al.* (2009) found that gas production kinetics (b and c) by dromedary's rumen content were 207 mL/g OM and 2.1/h for *Atriplex halimus* and 284 mL/g OM and 1.5/h for commercial hay, respectively. The results of interaction effects between rumen fluid and substrate demonstrated that b was the highest in treatments 2 and 4 and lowest for treatments 1 and 3 after 96 h incubation ($P\leq0.01$). Overhand, c in treatment 1 was the highest and was lowest for treatments 2 and 4. The produced gas in treatments 1 and 4 are not significantly higher after 96 h; however, 1 is equal to 3 and in treatment 2 was the lower amount.

The amount of gas production during the incubation of isolates with rumen liquor is closely related to the degradability and volatile fatty acids reaction with bicarbonate buffer to release CO_2 (Ayaşan *et al.* 2018). The results indicated that the main effect as well as the interaction effects of the experimental conditions had no effect on PF, microbial biomass, and the microbial biomass efficiency (Table 3), but the actual digested organic matter was influenced by the interaction effects of rumen fluid and substrate, therefore treatments 1 and 4 had the organic matter digestion higher than treatments 2 and 3 ($P\leq0.01$). Treatment 2, which was inoculated by the rumen fluid of C_3 cultivable diet and Atriplex substrate, had the lowest organic matter digestibility. This procedure was predictable because the rumen fluid microbes had the lowest adaptation with their substrate in treatments 2 and 3.

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

Ingredients	C_3 cultivable forage	C_4 pasture forage
Atriplex L.	0	80
Suaeda F.	0	10
Seidlitzia R.	0	10
Alfalfa hay	40	0
Wheat straw	60	0
Chemical composition		
Dry matter (DM)	89.3	83.0
Crude protein (CP)	7.20	7.07
Natural detergent fiber (NDF)	68.1	61.8
Acid detergent fiber (ADF)	43.45	38.73
Ash	8.2	18.8
Organic matter (OM)	91.8	81.2

Table 2 Gas production kinetics of straw and atriplex by rumen bacteria of camels fed with experimental diets

Effects	Gas production kinetics[1]		
	b (mL for 1 g)	c (/h)	Total gas of 96 h (mL)
Source of rumen content			
Rumen fluid of fed C_3 cultivable forage	134	0.012	53[b]
Rumen fluid of fed C_4 pasture forage	151	0.010	62[a]
SEM	12.5	0.0006	0.9
Significant	NS	NS	**
Substrate			
Wheat straw	76[b]	0.020[a]	61[a]
Atriplex	209[a]	0.003[b]	54[b]
SEM	12.5	0.0006	0.9
Significant	**	**	**
Interaction effects[2]			
Treatment 1	75[b]	0.021[a]	62[ab]
Treatment 2	192[a]	0.003[c]	44[c]
Treatment 3	77[b]	0.018[b]	60[b]
Treatment 4	226[a]	0.003[c]	64[a]
SEM	17.6	0.0008	1.2
Significant	**	**	**

[1] c: gas production rate (%) and b: potential gas production (mL); (n=3).
[2] Treatment 1: rumen fluid of camels that fed C_3 cultivable forage × straw as substrate; Treatment 2: rumen fluid of camels that fed C_3 cultivable forage × atriplex as substrate; Treatment 3: rumen fluid of camels that fed C_4 pasture forage × straw as substrate and Treatment 4: rumen fluid of camels that fed C_4 pasture forage × atriplex as substrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01).
NS: non significant.
SEM: standard error of the means.

Table 3 Gas production parameters of straw and atriplex by rumen bacteria of camels fed with experimental diets

Effects	PF (mg/mL)	Microbial biomass (mg)	Efficiency of microbial biomass (%)	Actual digested organic matter (mg)
Source of rumen content				
Rumen fluid of fed C_3 cultivable forage	5	85	58.8	143
Rumen fluid of fed C_4 pasture forage	5	86	55.5	154
SEM	0.3	5.7	2.04	4.9
Significant	NS	NS	NS	NS
Substrate				
Wheat straw	4.9	81	54.5	147
Atriplex	5.6	90	59.8	150
SEM	0.3	5.7	2.04	4.9
Significant	NS	NS	NS	NS
Interaction effects[1]				
Treatment 1	5	88.1	56	156[ab]
Treatment 2	5	80.9	61	130[c]
Treatment 3	4	72.9	53	138[bc]
Treatment 4	5	99.3	59	169[a]
SEM	0.4	8.02	2.9	6.9
Significant	NS	NS	NS	**

[1] Treatment 1: rumen fluid of camels that fed C_3 cultivable forage × straw as substrate; Treatment 2: rumen fluid of camels that fed C_3 cultivable forage × atriplex as substrate; Treatment 3: rumen fluid of camels that fed C_4 pasture forage × straw as substrate and Treatment 4: rumen fluid of camels that fed C_4 pasture forage × atriplex as substrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01).
NS: non significant.
SEM: standard error of the means.

The results of pH and ammonia N are showed in Table 4. The results showed that pH was significantly higher in treatments inoculated by rumen fluid of camels fed with C_4 pasture forages at 24, 48, and 96 h incubation. But the type of substrate had no significant effect on pH at 24 h incubation whereas as significantly higher pH was recorded at 48 and 96 h in inoculated treatments with Atriplex substrate.

However, investigation of the interaction effect shows that pH was not influenced by the experimental conditions in any of the incubation times. The ammonia-N was influenced by the type of rumen fluid only after 96 hours and rumen fluid was higher in treatments inoculated by cultivable forage (P≤0.05). The amount of ammonia-N was variable at 24, 48, and 96 h times among the treatments, as the

ammonia-N for wheat straw treatments was higher at 24 h but this procedure became inverse at 48 and 96 h and it was increased in inoculated groups with Atriplex substrate (P≤0.01). The results of interaction effects indicated that the ammonia-N was influenced by treatments at 24 and 48 h (P≤0.05), that was the highest for treatment 1 and the lowest for treatment 2 after 24 h incubation. After 48 h, treatment 2 and 4 had the highest ammonia-N and treatments 1 and 3 had the lowest amount. The amount of ammonia-N was not influenced by treatments at 96 h incubation.

This result suggests that tropical forage have the lower nitrogen availability because C_4 pasture forages protein in highly vascularized bundle sheath cells, which is deterrent to insectivorous and bacterial degradation.

Table 5 provides the results of dry matter, organic matter, and natural detergent fiber digestibility at different times of incubation. Digestibility of dry matter (P≤0.01) and organic matter (P≤0.05) in rumen fluid of C_4 pasture forage were significantly higher at 24 h incubation, however; natural detergent fiber digestibility was not affected by the type of rumen fluid at 24 h. Moreover, dry matter (P≤0.05) and natural detergent fiber (P≤0.01) digestibility at 48 h were higher in rumen fluid of C_4 pasture forages but the organic matter digestibility was not changed by the rumen fluid type.

Digestibility of dry matter and organic matter at 96 h incubation had not any significant differences but natural detergent fiber digestibility in treatments inoculated with pasture diets was highest (P≤0.05). Only the substrate type affected the dry matter digestibility at 24 h so that in inoculated treatments with wheat straw substrate, it was higher than Atriplex group (P≤0.05).

Ehleringer and Monson (1993) have reported that protein concentration in the protected bundle sheath cells of C_4 pasture plants should even reduce their dry matter and nitrogen digestibility further.

It was observed that all of the parameters were affected by the experimental conditions (P≤0.01). Dry matter disappearance of treatment 2 was the lowest but treatments 1, 3, and 4 had not any differences after 24 h incubation. The organic matter and natural detergent fiber digestion in treatments 1 and 4 were the highest and likewise treatment 2 had the lowest digestion. At 48 h incubation, the procedure was slightly changed so that the digestion of dry matter, organic matter, and natural detergent fiber in treatment 4 had more increment in comparison with the other treatments and similarly treatment 2 had the lowest digestion. Similar to the 48 h, treatment 4 had the highest digestion at 96 h. It can be certainly demonstrated that one of the reasons for digestibility reduction in treatment 2 is depended to the presence of tannin in Atriplex.

Tannins in rumen environment can affect the microbial digestion of organic matter through making complexes with a lot of nutrients such as carbohydrates and proteins (Frutos *et al.* 2004). This procedure was less effective with regard to treatment 4 because the rumen fluid microorganisms of this treatment were already adapted to Atriplex.

The result was showed the effect of treatments on cellulose activity of media culture (Table 6) can be reduced that at the first 24 hours of incubation, the activity of none of the 2 types of evaluated enzymes was influenced by the type of rumen fluid. The activity of endoglucanase (P≤0.05) and exoglucanase (P≤0.01) in inoculated treatments with C_4 pasture forage rumen fluid at 48 and 96 h of incubation were significantly higher. With regard to the effect of media substrate, it is understood that the endoglucanase (P≤0.01) and exoglucanase (P≤0.05) activity in the treatments being fed with wheat straw at 24 h were higher.

The procedure was continued for 48 and 96 h and the endoglucanase and exoglucanase activity in the treatments being fed with wheat straw were higher (P≤0.01). The results of interaction effects show that the activity of endoglucanase and exoglucanase after 24 h was not influenced by the treatments, however; after 48 h, treatments 1 and 3 had the greatest enzyme activity (P≤0.01), and treatment 2 had the lowest.

Treatment 4 did not have any difference with treatment 1 and 3 but treatment 2 had the lowest enzyme activity (P≤0.01). Also, the results of *in vitro* disappearance showed the same results as those of the enzymatic analysis of the treatments.

Cellulolytic bacteria growth depended to the optimum temperature and pH and if they are not optimum, gas production will decrease (Adesogan, 2002). The produced gas in this experiment reveals that the process of gas production in media with Atriplex substrate has been delayed, so that it is followed by decline in the measured parameters for Atriplex, especially at the beginning hours of incubation. It can be suggested that Atriplex species contains secondary metabolites such condensed tannins which may restrict digestibility and gas production.

Hassan (2009) concluded that Atriplex contains the high salt that can limit intake and digestion by ruminants. Also, energy value of Atriplex is low and non-protein nitrogen is around 65% of nitrogen (Ben Salem *et al.* 2005).

Benhammou *et al.* (2009) showed that saponins content of *Atriplex h.* was 0.33% and saponins are the antiprotozoal agent. Also phenolic components present in plants such Atriplex may disrupt protozoal membranes and enzymes, and decrease nutrients and ions essential for protozoa metabolism (Patra and Saxena, 2011). However, it is reported defaunation reduces the methane production in the rumen by 20-30% (Santra *et al.* 1994).

Table 4 The pH and ammonia-N of straw and atriplex incubated by rumen bacteria of camels fed experimental diets

Rumen parameters	Time					
	24 h		48 h		96 h	
	pH	Ammonia-N	pH	Ammonia-N	pH	Ammonia-N
Source of rumen content						
Rumen fluid of fed C_3 cultivable forage	6.6^{b}	6	6.5^{b}	7	6.4^{b}	7^{a}
Rumen fluid of fed C_4 pasture forage	6.9^{a}	6	6.8^{a}	7	6.7^{a}	6^{b}
SEM	0.08	0.2	0.01	0.2	0.04	0.2
Significant	*	NS	**	NS	**	*
Substrate						
Wheat straw	6.7	7^{a}	6.6^{b}	5^{b}	6.4^{b}	5^{b}
Atriplex	6.7	5^{b}	6.7^{a}	9^{a}	6.6^{a}	7^{a}
SEM	0.08	0.2	0.01	0.2	0.04	0.2
Significant	NS	**	**	**	*	**
Interaction effects[1]						
Treatment 1	6.5	7^{a}	6.5	5^{b}	6.3	6
Treatment 2	6.6	5^{c}	6.6	$9.^{a}$	6.5	8
Treatment 3	6.9	6^{b}	6.7	5^{b}	6.6	5
Treatment 4	6.9	5^{bc}	6.7	10^{a}	6.8	7
SEM	0.11	0.4	0.02	0.3	0.06	0.3
Significant	NS	*	NS	*	NS	NS

[1] Treatment 1: rumen fluid of camels that fed C_3 cultivable forage × straw as substrate; Treatment 2: rumen fluid of camels that fed C_3 cultivable forage × atriplex as substrate; Treatment 3: rumen fluid of camels that fed C_4 pasture forage × straw as substrate and Treatment 4: rumen fluid of camels that fed C_4 pasture forage × atriplex as substrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01) and * (P<0.05).
NS: non significant.
SEM: standard error of the means.

Table 5 Disappearance of dry matter (DM), organic matter (OM) and natural detergent fiber (NDF) of straw and atriplex incubated by rumen bacteria of camels fed experimental diets

Disappearance (g/kg DM)	Time								
	24 h			48 h			96 h		
	DM	OM	NDF	DM	OM	NDF	DM	OM	NDF
Source of rumen content									
Rumen fluid of fed C_3 cultivable forage	266^{b}	407^{b}	416	466^{b}	645	576^{b}	620	830	756^{b}
Rumen fluid of fed C_4 pasture forage	318^{a}	441^{a}	434	497^{a}	667	652^{a}	656	824	795^{a}
SEM	7.2	9.7	6.0	7.0	12.2	8.5	14.4	8.9	8.4
Significant	**	*	NS	*	NS	**	NS	NS	*
Substrate									
Wheat straw	312^{a}	434	431	474	645	609	637	814	769
Atriplex	271^{b}	414	420	489	667	619	638	840	780
SEM	7.2	9.7	6.0	7.0	12.2	8.5	14.4	8.9	8.4
Significant	**	NS	NS	NS	NS	NS	NS	NS	NS
Interaction effects[1]									
Treatment 1	313^{a}	459^{a}	445^{a}	487^{b}	681^{a}	609^{b}	663^{ab}	837^{a}	782^{b}
Treatment 2	218^{b}	355^{c}	387^{c}	444^{c}	609^{b}	543^{c}	577^{c}	824^{ab}	730^{c}
Treatment 3	311^{a}	409^{b}	416^{b}	460^{bc}	609^{b}	609^{b}	611^{bc}	791^{b}	758^{bc}
Treatment 4	325^{a}	473^{a}	452^{a}	534^{a}	725^{a}	695^{a}	700^{a}	856^{a}	831^{a}
SEM	10.2	13.7	8.6	10.0	17.3	12.0	20.3	12.7	11.8
Significant	**	**	**	**	**	**	**	*	**

[1] Treatment 1: rumen fluid of camels that fed C_3 cultivable forage × straw as substrate; Treatment 2: rumen fluid of camels that fed C_3 cultivable forage × atriplex as substrate; Treatment 3: rumen fluid of camels that fed C_4 pasture forage × straw as substrate and Treatment 4: rumen fluid of camels that fed C_4 pasture forage × atriplex as substrate.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01) and * (P<0.05).
NS: non significant.
SEM: standard error of the means.

Furthermore, the results of this experiment showed that in treatments 2 and 3, rumen fluid microorganisms have not adapted with substrate so that the gas production decreased. With respect to this issue, the researchers demonstrated that the adaptation of rumen's microorganisms exposed to the feed type had impacted the gas production (Macome, 2017). Our observation about extent of gas production potential (b) was partly in line with Niekerk *et al.* (2006) experiment that deduced the potential of gas production for Atriplex species ranged between 125 to 164 mL/g dry matter. These values are very low when compared with values of 289.5 to 334 mL/g recorded for four browse leaves (Kamalak *et al.* 2004). Although in experiment of Niekerk *et al.* (2006), the degree of the total gas production at 72 h ranged between 74 to 96 mL/g dry matter, whereas it was observed in this experiment that it was between 64 to 44 mL for Atriplex and 62 to 59 mL for wheat straw.

The highly digestible feed will produce more gas than the less digestible one (Ouda *et al.* 2005; Macome, 2017). In our experiment the gas production rate for media with Atriplex substrate was lower than that for media with wheat straw substrate (0.003/h for Atriplex versus 0.02/h for wheat straw). Niekerk *et al.* (2006) reported that the average degradation rate of 0.053/h for Atriplex species that is lower than the average values of 0.09 /h reported by Kaitho *et al.* (1998) for *Atriplex halimus*. Moreover, El-Hassan *et al.* (2000) reported that the value of degradation rate of dry matter (/h) was 0.032 to 0.110 for some African foliage species. In general, the ranges of gas production rate (c) in this study were lower than values reported by Ammar *et al.* (2005) (0.04/h) for shrub species in the Mediterranean and by those reported by Apori *et al.* (1998) for Ghanaian browse species (0.0361 to 0.0654/h). The researchers reported that feed with higher PF had more degraded matter and led to the lower microbial mass and the methane output. However, the feeds with similar digestibility, the one that produces the lower gas production, have higher nutritive value because most of its degraded fraction is likely to be incorporated into the microbial biomass or directly be absorbed by the animal (Elghandour *et al.* 2015). Generally, in treatments that contain Atriplex, the pH was higher and it seems that this result is due to the reduction of fermentation and volatile fatty acids production. Ferme *et al.* (2004) also explained that the inhibition of ammonia producer bacteria (*Prevotella ruminantium* and *Prevotella bryantii)* resulted in the reduction of NH_3-N concentration. In addition, protozoa play a important role in regulating bacterial N turnover in the rumen and supply the soluble protein for microbial growth. Protozoa are not able to use NH_3-N, so a fraction of previously engulfed insoluble protein is later returned to the rumen fluid in the form of soluble protein (Dijkstra, 1994), that this is one of the main reasons for decreasing NH_3-N concentration in the rumen by defaunation. However, it is reported that the amount of recycled nitrogen in camelids is higher (San Martin, 1991) and the ammonia concentration in the camelids forestomach is lower, that could be the result of the lower protozoa concentration as discussed by Jouany (1991). Mehrez *et al.* (2001) found that 20.61 mg NH_3-N/100 mL rumen liquid, would satisfy microbial needs for N and hence maximize the fermentation rate of the in the rumen. Hassan (2009) also indicated microbial protein synthesis in the rumen requires an adequate supply of nitrogen to support maximal efficiency. On the other hand, it was reported that reduction of protein degradability in the rumen is due to tannin's binding to feed protein and also reduction of proteolytic bacteria growth leads to ammonia N reduction (Min *et al.* 2005). The results of this study showed the reduction of degradability of dry matter, organic matter, and natural detergent fiber in treatments with Atriplex substrate at the first hours of incubation, but the fermentation process was developed with more speed and the primary delay was compensated at the last hours of incubation. But this recompense seems to be not efficient in *in vivo* condition because the feed stays in the rumen for a shorter time and the opportunity for degradation and digestion is less.

The obtained results may be due to the presence of salt as the major component in Atriplex species (Salem *et al.* 2012) that leds to the increment of animal water intake and reduction of the rumen turnover time that have consequential effects on rumen physiology and metabolism (Warner and Casson 1992; Ahmed *et al.* 2015). Baan *et al.* (2004) in an experiment done with the aim of determining the digestibility of *Atriplex nummularia*, found that organic matter digestibility of Atriplex was 39.5% by using Telly and Terry test and was 30.8% by gas production technique. Shawket (1999) indicated that the digestibility coefficients of dry matter, organic matter, crude protein, crude fiber and nitrogen free extract for diets containing Atriplex were lower than those of the diet containing Atriplex and concluded that the utilization of Atriplex was enhanced by the energy supplementation.

Ahmed *et al.* (2001) showed that the total digestible nutrient value was lower as it was 7.15% for ration containing Atriplex in comparison to diet containing berseem hay (73.19% *vs.* 67.95%). Lee *et al.* (2000) observed that *in vitro* degradation of orchard grass cell walls by rumen bacterial of Jersey cow was about 46% after 96 h incubation whereas the present study's data were around 72 to 83% after 96 h incubation. Fayed *et al.* (2010) reported apparent digestibility of organic matter, crude fiber, nitrogen free extract, acid detergent fiber and acid detergent lignin of fresh alfalfa were higher than diet containing Atriplex in sheep.

Table 6 Enzyme activity of incubated medium by rumen fluid of camels fed experimental diets (µmol glucose/min.mL)

Type of enzyme	Time					
	24 h		48 h		96 h	
	Endo G.	Exo G.	Endo G.	Exo G.	Endo G.	Exo G.
Source of rumen content						
Rumen fluid of fed C_3 cultivable forage	7	4	8^b	5.1^b	10^b	7^b
Rumen fluid of fed C_4 pasture forage	7	4	9^a	5.5^a	11^a	8^a
SEM	0.3	0.3	0.1	0.07	0.2	0.1
Significant	NS	NS	*	**	*	**
Substrate						
Wheat straw	8^a	5^a	10^a	5.8^a	11^a	8^a
Atriplex	6^b	4^b	8^b	4.8^b	10^b	7^b
SEM	0.3	0.3	0.1	0.07	0.2	0.1
Significant	**	*	**	**	**	**
Interaction effects						
Treatment 1	9	5	10^a	6^a	12^a	8^a
Treatment 2	6	3	8^c	4^c	9^b	7^b
Treatment 3	8	4	10^a	6^a	11^a	9^a
Treatment 4	6	4	9^b	5^b	11^a	8^a
SEM	1.8	0.8	0.2	0.1	0.2	0.2
Significant	NS	NS	**	**	**	**

[1] Treatment 1: rumen fluid of camels that fed C_3 cultivable forage × straw as substrate; Treatment 2: rumen fluid of camels that fed C_3 cultivable forage × atriplex as substrate; Treatment 3: rumen fluid of camels that fed C_4 pasture forage × straw as substrate and Treatment 4: rumen fluid of camels that fed C_4 pasture forage × atriplex as substrate.
Endo G: endoglucanase and Exo G: exoglucanase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01) and * (P<0.05).
NS: non significant.
SEM: standard error of the means.

These results may be due to higher salt content of Atriplex which leading to shortening the rumen turnover time that influences digestion and metabolism. Such results might also be attributed to the secondary metabolites such as oxalates, tannins, and saponins in Atriplex which might decrease the rumen microbial activity (Shawket *et al.* 2009; Abu Zanat and Tabbaa, 2005). Saponins are the antiprotozoal agents (Patra and Saxena, 2011), and reduction of fiber digestibility in defaunated ruminants may be due to the elimination of large entodiniomorphid ciliates which have better cellulolytic activity for ruminal fiber degradation and stabilization of the rumen environment favoring the development of cellulolytic microbes and a stimulatory effect of ciliate protozoa over rumen bacteria (Hegarty *et al.* 1991). With regard to these issues, it can be deduced from the obtained results that protozoa decline caused to the reduction of fermentation in treatments with Atriplex substrate in primal time of incubation. Maybe microorganism's adaptation to anti nutritional materials of Atriplex in treatment 4 caused to the observed lower disorder at last. It is reported that rumen microbial population might adapt to the plant and degrade antiprotozoal components such as saponins (Teferedegne *et al.* 2000).

On base of the current result, the activity of rumen bacterial enzymes with C_4 pasture forage was significantly higher that prove the result of Dadvar *et al.* (2016).

Although the activity of carboxymethyl cellulase from endoglucanase gene could be detected as a zone of clearing in a carboxy methyl cellulose plate, this activity was very low in culture media.

Instability of the enzyme altered processing by the host strain, and a deletion in the promoter region of endoglucanase accounted for the enzyme's poor activity (Whitehead and Flint, 1995). In the experiment of Lee *et al.* (2000), carboxy methyl cellulase activity of bacterial fraction was approximately 20 after 24 h and 37 after 96 h incubation whereas the data of the present study were around 5.9-8.7 for 24 h and 8.9-11.5 for 96 h incubation. Kayouli *et al.* (1991) observed that dromedaries were able to digest low-quality roughages more efficiently than sheep that these differences in digestive ability appear after 24 h in forestomach.

The more recent studies show that one extracellular protein released by cellulolytic bacteria inhibit fungal activity and ability of fungi for cellulose hydrolyzes (Bernalier *et al.* 1993). Also Stern *et al.* (1997) suggested that the biological validity of the results could be limiting as a result of incomplete enzymatic activity.

The enzyme concentration must be sufficient to saturate the substrate; if enzyme concentration is limiting, accumulation at the end of products during incubation can lead to the inhibition of enzyme activity.

CONCLUSION

All observations in this experiment revealed that Atriplex forage caused to a delay in starting digestion and fermentation process by rumen cellulolytic bacteria of camels, especially at the beginning hours of incubation, then process was developed with more speed and primary delay was compensated. It is also recommended to use adopted rumen fluid with the substrate *in vitro* research, particularly in the cases that investigate halophyte forages. Also according to the current result, feeding of C_4 pasture forage to camels improved the fiber degradability and enzyme activity of rumen bacteria in compared with C_3 cultivable forage. Since little researches has done about the camel's rumen microbes, it needs to be studied more.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Ramin Agriculture and Natural Resources University of Khuzestan for their laboratory and financial support.

REFERENCES

Abu-Zanat M.M.W. and Tabbaa M.J. (2005). Effect of feeding Atriplex brows to lactating ewes on milk yield and growth rate of their lambs. *Small Rumin. Res*. **64,** 152-161.

Adesogan A.T. (2002). What are feeds worth? A critical evaluation of selected nutritive value methods. Pp. 33-37 in Proc. 13th Ann. Florida Rumin. Nutr. Symp., Gainesville, Florida.

Ahmed M.H., Khatab I.M., Borhami E.B. and Fahmy W.G. (2001). Effect of energy source supplementation of the utilization of some desert forage by growing lambs. *Int. J. Adv. Agric.* **6,** 255-277.

Ahmed M.H., Salem A.Z.M., Zeweil H.S., Sun X.Z., Kholif A.E., Elghandour M.M.Y. and Bahar M.S.I. (2015). Growth performance and carcass characteristics of lambs fed halophytes as a partial or whole replacement of berseem hay. *Small Rumin. Res*. **128,** 1-9.

Ammar H., Lopez S. and Gonzalez J.S. (2005). Assessment of the digestibility of Mediterranean shrubs by *in vitro* techniques. *Anim. Feed Sci. Technol*. **119,** 323-331.

Apori S.O., Castro F.B., Shand W.J. and Orskov E.R. (1998). Chemical composition, in sacco degradation and *in vitro* gas production of some Ghanaian browse plants. *Anim. Feed Sci. Technol*. **76,** 129-137.

Ayaşan T., Ülger I., Kaliber M., Ergül Ş., İnci H., Mart D. and Türkeri M. (2018). Comparison of *in vitro* gas production, nutritive value, metabolizable energy and organic matter digestibility of some chickpea varieties. *Iranian J. Appl. Anim. Sci.* **8,** 131-136.

Baan A.V.D., Niekerk W.A.V., Rethman N.F.G. and Coertze RJ. (2004). The determination of digestibility of *Atriplex nummularia*. De Kock (Oldman's Saltbush) using different *in vitro* techniques. *South Africa J. Anim. Sci*. **34,** 95-97.

Benhammou N., Bekkara F.A. and Panovska T.K. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *C. R. Chim.* **12(12),** 1259-1266.

Ben Salem H., Nefzaoui A., Makkar H.P.S., Hochlef H., Ben Salem I. and Ben Salem L. (2005). Effect of early experience and adaptation period on voluntary intake, digestion and growth in Barbarine lambs given tannin – containing (*Acacia cyanophylla*) or tannin – free (oaten hay) diets. *Anim. Feed Sci. Technol.* **122,** 59-77.

Bernalier A., Fonty G., Bonnemoy F. and Gouet P. (1993). Inhibition of the cellulolytic activity of Neocallimastix frontalis by *Ruminococcus flavefaciens*. *J. Gen. Microbiol*. **139,** 873-880.

Blummel M., Makkar H.P.S. and Becker K. (1997). *In vitro* gas production: A technique revisited. *J. Anim. Physiol. Anim. Nutr*. **77,** 24-34.

Blummel M., Mgomezulu R., Chen X.B., Makkar H.P.S., Becker K. and Orskov E.R. (1999). The modification of in *in vitro* gas production test to detect roughage related differences in *in vivo* microbial protein synthesis as estimated by the excretion of purine derivates. *J. Agric. Sci.* **133,** 335-340.

Colombatto D. and Beauchemin KA. (2003). A proposed methodology to standardize the determination of enzymic activities present in enzyme additives used in ruminant diets. *Canadian J. Anim. Sci.* **83,** 559-568.

Dadvar P., Mohammadabadi T., Sari M. and Fayazi J. (2016). Ruminal microbial fermentation of dromedary camel in a dual flow continuous culture system using cultivable and pasture forages. *J. Livest. Sci. Technol.* **4(1),** 17-24.

Dereje M. and Uden P. (2005). The browsing dromedary camel: I. Behaviour, plant preference and quality of forage selected. *Anim. Feed Sci. Technol.* **121,** 297-308.

Dijkstra J. (1994). Production and absorption of volatile fatty acids in the rumen. *Livest. Prod. Sci.* **39,** 61-69.

Dulphy J.P., Dardillat C., Jailler M. and Joveny J.P. (1994). Comparison of the intake and digestibility of different diets in llamas and sheep: a preliminary study. *Ann. Zootech*. **43,** 379-387.

Dulphy J.P., Dardillat C., Jailler M. and Ballet J.M. (1997). Comparative study of forestomach digestion in llamas and sheep. *Reprod. Nutr. Dev.* **37,** 709-725.

Ehleringer J.R. and Monson R.K. (1993). Evolutionary and ecological aspects of photosynthetic pathway variation. *Ann. Rev. Ecol. Evol. Syst*. **24,** 411-439.

Elghandour M.M.Y., Kholif A.E., Bastida A.Z., Martinez D.L.P. and Salem A.Z.M. (2015). *In vitro* gas production of five diets of different concentrate and maize silage ratios influenced by increasing levels of chemically characterized extract of *Salix babylonica*. *Turkish J. Vet. Anim. Sci*. **39,** 186-194.

El Hassan S.M., Kassi A.L., Newbold C.J. and Wallace R.J. (2000). Chemical composition and degradation characteristics of foliage of some African multipurpose trees. *Anim. Feed Sci. Technol*. **86,** 27-37.

Fayed A.M., El-Essawy A.M., Eid E.Y., Helal H.G., Abdou A.R. and El-Shaer H.M. (2010). Utilization of alfalfa and Atriplex for feeding sheep under saline conditions of South Sinai, Egypt. *Am. J. Sci.* **6(12),** 1447-1461.

Ferme D., Banjac M., Calsamiglia S., Busquet M., Kamel C. and Avgustin G. (2004). The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. *Folia Microbiol*. **49,** 151-155.

Frutos P., Hervás G., Giráldez F.J. and Mantecón A.R. (2004). Review tannins and ruminant nutrition. *Spanish J. Agric. Res*. **2(2),** 191-202.

Haddi M.L., Arab H., Yacoub F., Hornick J.L., Rollin F. and Mehennaoui S. (2009). Seasonal changes in chemical composition and *in vitro* gas production of six plants from Eastern Algerian arid regions. *Livest. Res. Rural. Dev*. **21(4),** 47-52.

Hassan A.A. (2009). Effect of some Enrichment and Nawaz biologicaltreatments on a melioration utilization of *Atriplex-nummularia* fed by sheep. *Egyptian J. Nutr. Feeds.* **12(3),** 553-566.

Hegarty R.S., Nolan J.V. and Leng R.A. (1991). Sulphur availability and microbial fermentation in fauna free rumen. *Arch. Anim. Nutr*. **41,** 725-736.

Jouany J.P. (1991). Defaunation of the rumen. Pp. 239-261 in Rumen Microbial Metabolism and Ruminant Digestion. J.P. Jouany, Ed. INRA Editions, Science Update. Versailles, France.

Kaitho R., Nsahlai I., Williams B., Umunna N., Tamminga S. and Van Bruchem J. (1998). Relationships between preference, rumen degradability, gas production and chemical composition of browses. *Agroforest. Syst*. **39,** 129-144.

Kamalak A., Canbolat O. and Gurbuz Y. (2004). Comparison between *in situ* dry matter degradation and *in vitro* gas production of tannin containing leaves from four tree species. *South Africa J. Anim. Sci.* **34(4),** 233-240.

Kayouli C., Jouany J.P. and Ben Amor J. (1991). Comparison of microbial activity in the forestomachs of the dromedary and the sheep measured *in vitro* and in sacco on Mediterranean roughages. *Anim. Feed Sci. Technol.* **33,** 237-245.

Lee S.S., Ha J.K. and Cheng K.J. (2000). Relative contributions of bacteria, protozoa, and fungi to *in vitro* degradation of orchard grass cell walls and their interactions. *Appl. Environ. Microbiol*. **66,** 3807-3813.

Lemosquet S., Dardillat C., Jailler M. and Dulphy J.P. (1996). Voluntary intake and gastric digestion of two hays by llamas and sheep: influence of concentrate supplementation. *J. Agric. Sci*. **127,** 539-548.

Macome F.M. (2017). *In vitro* prediction of methane production by lactating dairy cows. Ph D. Thesis. Utrecht Univ., the Netherlands.

Makkar H.P.S. and McSweeney C.S. (2005). Methods in Gut Microbial Ecology for Ruminants. International Atomic Energy Agency. Springer-Verlag Berlin Heidelberg Publisher, Germany.

Mehrez A.Z., Soliman E.M., El-Ayek M.Y., El-Ayouty E.A. and El-Kholany M.E. (2001). Influence of roughage to concentrate ratio and type of roughage on digestibility, some rumen parameters and fiber fractions degradability of treated rations with ruminants. *Egyptian J. Nutr. Feeds.* **4,** 193-209.

Min B.R., Attwood G.T., McNabbb W.C., Molanb A.L. and Barry T.N. (2005). The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Anim. Feed Sci. Technol*. **121,** 45-58.

Mohammadabadi T. and Chaji M. (2010). Effect of exogenous enzyme on *in vitro* fermentation of sesame straw by rumen bacteria culture. *J. Appl. Anim. Res*. **39,** 161-163.

Niekerk W.A.V, Hassen A., Vermaak P.J., Rethman N.F.G. and Coertze R.J. (2006). Ruminal degradation and *in vitro* gas production characteristics of foliage from Atriplex species and Cassia sturtii. *South Africa J. Anim. Sci*. **36,** 54-58.

Orskov E.R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurement weighted according to rat of passage. *J. Agric. Sci.* **92,** 499-503.

Ouda J.O., Njehia G.K., Moss A.R., Omed H.M. and Nsahlai I.V. (2005). The nutritive value of forage sorghum genotypes developed for the dry tropical highlands of Kenya as feed source for ruminants. *South Africa J. Anim. Sci.* **35,** 55-60.

Patra A.K. and Saxena J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric*. **91,** 24-37.

Pei C.X., Liu Q., Dong C.S., Li H.Q., Jiang J.B. and Gao W.J. (2010). Diversity and abundance of the bacterial 16S rRNA gene sequences in forestomach of alpacas (Lama pacos) and sheep (Ovis aries). *Anaerobe.* **16,** 426-432.

Robinson T.F., Sponheimer M., Roeder B.L., Passey B., Cerling T.E., Dearing MD. and Ehleringer J.R. (2006). Digestibility and nitrogen retention in llamas and goats fed alfalfa, C_3 grass, and C_4 grass hays. *Small Rumin. Res.* **64,** 162-168.

Salem A.Z.M., Hassan A.A., Khalil M.S., Gado H.M., Alsersy H. and Simbaya J. (2012). Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed *Atriplex halimus* foliages. *Anim. Feed Sci. Technol*. **171,** 128-135.

San Martin F. (1991). Nutrition y alimentacion. Pp. 72-99 in Advances and Perspectives in the Knowledge of South American Camelids. S. Fernandez-Baca, Eds. FAO. Oficina Regional Para America Latina el Caribe. Santiago, Chile.

Santra A., Kamra D.N., Pathak N.N. and Khan M.Y. (1994). Effect of protozoa on the loss of energy in Murrah buffalo (*Bubalua bubalis*) calves. *Buff. J.* **3,** 249-253.

SAS Institute. (2005). SAS®/STAT Software, Release 6.08. SAS Institute, Inc., Cary, NC. USA.

Shawket S.M. (1999). Effect of energy level supplementation on the utilization of some pastures plants by goats. *J. Agric. Sci.* **24,** 4565-4573.

Shawket S.M., Safinaz M. and Ahmed M.H. (2009). Effect of prolonged feeding Atriplex (saltbush) to camels on digestibility, nutritive value and nitrogen utilization. *Egyptian J. Nutr. Feeds*. **12(3),** 205-214.

Stern M.D., Bach A. and Calsamiglia S. (1997). Alternative techniques for measuring nutrient digestion in ruminants. *J. Anim. Sci.* **75,** 2256-2276.

Teferedegne B., McIntosh F., Osuji P.O., Odenyo A., Wallace R.J. and Newbold C.J. (2000). Influence of foliage from different accessions of the sub-tropical leguminous tree, *Sesbaniasesban*, on ruminal protozoa in Ethiopia and Scottish sheep. *Anim. Feed Sci. Technol.* **78,** 11-20.

Warner B.E. and Casson T. (1992). Performance of sheep grazing salt tolerant forages on revegetated salt land. *Proc. Australian Soc. Anim. Prod.* **19,** 237-241.

Whitehead T.R. and Flint H.J. (1995). Heterologous expression of an endoglucanase gene (end A) from the ruminal anaerobe *Ruminococcus flavefaciens* 17 in *Streptococcus bovis* and *Streptococcus sanguis*. *FEMS Microbiol. Lett.* **126,** 165-170.

Yagil R. (1994). The Camel in Today's World. Gifrid, Bonn, Germany.