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ORIGINAL ARTICLE

Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep

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ABSTRACT

The aim of this study was to assess the effects of feeding *Atriplex halimus* (AH) silage treated with two developed enzyme cocktails to sheep on feed intake, nutrient digestibility and ruminal fermentation. The AH silage was treated without or with 2 L of ZAD1® or ZAD2®/1000 kg with 5% molasses and ensiled for 30 days. Barley grain (300 g/head/day) was fed as an energy supplement once daily at 10.00 hours and AH silage with or without enzyme treatment was offered *ad libitum* to animals twice daily at 09.00 and 16.00 hours. Sheep were fed on four experimental forage diets comprised of AH silage and barley (D1), AH silage treated with ZAD1® and barley (D2), AH silage treated with ZAD2® and barley (D3) and AH silage treated with a combination of ZAD1® and ZAD2® (1:1) and barley (D4). Ensiling AH with enzymes reduced its contents of neutral detergent fiber and acid detergent fiber. The dry matter intake of AH of D2, D3 and D4 decreased ($P < 0.001$) as compared to D1. However, enzyme-treated diets had greater total digestible nutrients intake ($P < 0.001$) as compared to D1. The nutrients digestibility for D2, D3 and D4 were higher than those for D1 ($P < 0.001$), and were higher for D3 as compared to both D2 and D4. Sheep fed on D3 had highest ($P < 0.001$) ruminal total volatile fatty acids concentration, ammonia nitrogen concentration and microbial protein yield. It could be concluded that AH silage treated with ZAD1® or ZAD2® improved digestibility and rumen fermentation in sheep.

Key words: *Atriplex halimus, exogenous enzymes, feed intake, nutrient digestibility, ruminal fermentation.*

INTRODUCTION

The main constraint to develop animal production in developing countries is feed shortage, both in terms of quality and quantity. Halophytic and saltbush forage shrubs are used for ruminant feed across a range of saline and arid production environments (Le Houerou 1992). Saltbushes contain high levels of crude protein (CP) (Norman *et al.* 2004; Al-Owaimer *et al.* 2011), however, much of the nitrogen (N) is associated with non-protein compounds such as nitrates and proline. These non-protein compounds may be converted into microbial protein in the rumen or converted to ammonia depending on the availability of metabolizable energy (Pearce *et al.* 2010). In Egypt, tree and shrub forages play an important role in ruminant feeding in arid and semi-arid regions of the northern region of Egypt where halophyte shrubs of the genus *Atriplex* tend to predominate in these areas (Salem *et al.* 2012). However, *Atriplex halimus* is not highly utilized because of its deficiency of available carbohydrate and high fiber content, and therefore, barley grain as an energy source has been suggested to

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stimulate the utilization of saltbush. Feeding a high-energy supplement such as barley grains can improve the feeding value of saltbush pastures and providing energy to ruminal microbes, stimulating carbohydrate digestion and detoxifying secondary metabolites (Mayberry *et al.* 2008; Norman *et al.* 2008). *Atriplex halimus* has high ash and crude fiber, and low crude fat contents. However, it contains up to 10% sodium chloride and secondary plant metabolites, including tannins, flavonoids, saponins, alkaloids and resins (Makkar 2003; Salem *et al.* 2006) which considerably affect its palatability and nutritive value (Abd El-Rahman *et al.* 2006). The processing of *A. halimus* by ensiling enhanced its acceptability in sheep and goats providing sufficient metabolizable energy and digestible CP to animals (Abd El-Rahman *et al.* 2006).

In recent years, some species of the *Atriplex* genus, in particular *Atriplex halimus,* has been cultivated as forage because of its tolerance to severe conditions of drought, and it can also grow in very alkaline and saline soils (Aharonson *et al.* 1969). *Atriplex halimus* has been studied in relation to its adaptability, productive potential, palatability and nutritive value (Norman *et al.* 2008; Otal *et al.* 2010). It is well known that addition of exogenous enzymes to animal diets can improve feeding value and animal performance by improving fiber degradation and increasing feed intake and digestion (Khattab *et al.* 2011; Salem *et al.* 2013) as indicated by *in vitro* studies (Giraldo *et al.* 2008; Elghandour *et al.* 2013), *in situ* studies (Miller *et al.* 2008a; Chung *et al.* 2012) and *in vivo* studies (Gado *et al.* 2009; Holtshausen *et al.* 2011; Salem *et al.* 2013). Moreover, enzymes addition at forage ensiling increased both feed intake and digestion rate (Miller *et al.* 2008b; Salem *et al.* 2012). A two recently developed enzyme cocktails ZAD1® and ZAD2® are biotechnical products made from anaerobic bacteria from natural sources to elevate the level of cellulolytic enzymes from anaerobic bacteria, which convert polysaccharides into monosaccharide by specific enzymes (Gado *et al.* 2009, 2011) and their products have beneficial effects on nutritive value of poor quality roughages (Gado 1997). ZAD1® differs from ZAD2® in its different enzyme concentrations.

The objective of the present work was to evaluate the effect of treating *A. halimus* with different enzymes of ZAD1® or ZAD2® on sheep feed intake, nutrient digestibility, N utilization, rumen fermentation and rumen microbial protein synthesis in sheep.

MATERIALS AND METHODS

A. halimus preparation

Leaves and stems of fresh Mediterranean saltbush (*A. halimus*) were collected from the north-western desert region in Egypt, dried and chopped to 3-5 cm in lengths and stored in a dry environment. The secondary metabolite concentrations of fresh (g/kg) and dried *A. halimus* (g/kg dry matter (DM)), respectively, were 13.33 and 7.21 for total phenolics, 5.45 and 2.23 for total tannin and 7.27 and 2.62 for alkaloids.

Animals and treatments

Three Barki rams (45 ± 3.2 kg) were used in four consecutive trails for *ad libitum* feed intake and nutrients digestibility determination; and three ewes (40 ± 2.6 kg) fitted with permanent rumen fistulas were used for rumen fermentation trials as the source of inoculum for *in vitro* rumen fermentation trials.

All animals were housed in individual cages. The sheep were fed with basal diet comprised of *A. halimus* silage treated with or without enzymes, *ad libitum*, and barley grain (300 g/day). The diet was formulated to meet maintenance requirements (NRC 1994).

A. halimus was moistened into DM content in an average of 40% without or with addition of ZAD1® or ZAD2® (2 L/1000 kg of *A. halimus*, in fresh matter basis) and molasses (5 % *A. halimus*, in fresh matter basis), and then sealed with a polyethylene sheet to be ensiled for 4 weeks in each treatment. Every diet was prepared a month before feeding to animals. Barley grain (300 g/head/day) was fed as an energy supplement during the experimental period for all diets. Four experimental diets comprised of: AH silage (leaves and stems; D1); AH silage treated with ZAD1® and barley (D2), AH silage treated with ZAD2® and barley (D3) and AH silage treated with a combination of ZAD1® and ZAD2® (1:1) and barley (D4). For all treatments, *A. halimus* was offered to animals *ad libitum* twice daily at 09.00 and 16.00 hours, while barley grain was given once daily at 10.00 hours.

The two recently developed enzyme cocktails ZAD1® and ZAD2® (patent No: 22155) were obtained from the laboratory of the Rumen Ecology Center, Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, according to the procedure of (Gado 1997). Both ZAD1® and ZAD2® are live anaerobic bacteria with their enzymes (Table 1).

Metabolism study

Each metabolism trial was conducted for 3 weeks as a preliminary period and 1 week for sample collection. All animals were kept individually in stainless metabolic cages at room temperature and had free access to water. Beneath each cage was a stainless steel screen having 4 mm mesh to retain feces but free passage of urine, which was collected through a

Table 1 Composition of exogenous enzymes† of ZAD1® and ZAD2®

Samples	Xylanase‡	a-amylase§	Cellulase¶	Protease††
ZAD1®	2.32 U/mL	61.5 U/mL	7.05 U/mL	29.2 U/mL
ZAD2®	6.93 U/ mL	69.4 U/mL	8.16 U/mL	12.3 U/mL

†ZAD1,2®: moisture contents of 40%. ‡One unit (U) is defined as the amount (g or mL) of enzyme needed to release 1 μmol xylose per minute from 5 mg/mL xylan solution (pH 5.5 and 37°C). §One unit (U) is defined as enzyme needed to produce 1 mg glucose from starch in 1 h by 1 mL ZAD[1,2]® (pH 4.6 and 40°C). ¶One unit (U) is defined as enzyme activity required to release 1 μmol reducing sugar from 4 mg/mL Na carboxymethyl cellulose in 1 min (pH 5.5 and 37°C). ††One unit (U) is defined as enzyme needed to produce 1 μmol amino acids from protein in 1 min (pH 5.5 and 37°C).

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funnel. Feces and urine were collected once daily before the morning feeding and stored at −10°C for later analysis.

The samples of feces were mixed within 7 day's collections, composited and stored for later routine analyses. Fecal samples were dried at 60°C for 72 h and then ground through a 1 mm screen using a Wiley mill grinder (Arthur H. Thomas, Philadelphia, PA, USA).

Conventional analysis of feed and fecal samples was carried out according to AOAC (1997) for DM, crude ash, N, crude fiber (CF) and ether extract (EE). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Van Soest *et al*. (1991) using ANKOM200 Fibre Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of an alpha amylase but with sodium sulfite. Both NDF and ADF are expressed without residual ash. Urinary N was analyzed according to AOAC (1997).

The value of total digestible nutrients (TDN) intake was calculated according to NRC (2001) using values of digestible nitrogen free extract (NFE), CP, EE and CF.

Ruminal fermentation activities

In vitro zero order technique (Carroll & Hungate 1954) was applied for measuring rates of ammonia (NH_3-N) and volatile fatty acids production. Rumen contents were collected from three rumen fistulated Barki ewes fed on each experimental diet consecutively at 08.00 hours before feeding and at 1, 3 and 6 h after feeding.

The rumen samples were mixed, homogenized and filtered through a sieve with a pore size of 1 mm under continuous CO_2 flushing, closed with a tightly fitting rubber with an outlet Bunsen valve and incubated at 39°C in a thermostatically controlled water bath. Each sample was composed of two-thirds fibrous material and one-third liquid (El-Shazly & Hungate 1965). At zero time incubation, two sub-samples were transported, poured into another jar containing formalin (1 mL/100 g rumen contents) and swirled vigorously to stop metabolic activity.

After 1 h incubation, rumen samples treated with formalin were used to estimate NH_3-N and total volatile fatty acids (tVFA) concentration. Rumen samples taken at zero and at 1 h fermentation were strained through four layers of cheesecloth. Fifty milliliters of rumen liquor were deproteinized using 0.03 mol/mL of sulphuric acid (50 mL) and the volume was diluted with water to 500 mL in a volumetric flask and filtered. The supernatant was used for determination of NH_3-N concentration using (MgO) distillation method (Al-Rabbat *et al*. 1971) and tVFA were estimated using steam distillation as described by Warner (1964).

The microbial protein (MP) synthesized in the rumen fed the four experimental diets were calculated by the following equation (Borhami *et al*. 1992):

$$\text{MP (g/day)} = \text{tVFA production (mol/day)} \times 2 \times 13.48 \times 10.5 \times 6.25/100$$

tVFA production = tVFA concentration (mol/100 mL; obtained from the in vitro zero order technique) × 10 × total rumen digesta (L).

Multiplication by 10 obtains the rate of VFA production/h/L, while multiplication by 24 obtains the rate of VFA production/24 h (i.e. day)/L.

It was assumed that 1 mol tVFA yields 2 moles ATP (Walker 1965), 1 mole ATP produces 13.48 g microbial cells (DM basis) (Borhami *et al*. 1979) and N concentration of microbial cells is 10.5% (Hungate 1965). Rumen digesta weight (kg) was estimated using the colorimetric method of chromium ethylenediaminetetraacetate (Cr-EDTA) according to the method of El-Shazly *et al*. (1976).

Statistical analysis

Data were statistically analyzed according to a randomized block design using the PROC MIXED procedure of SAS (2002) according to the following statistical model:

$$Y_{ijk} = \mu + D_i + T_j + (D * T)_{ij} + e_{ijk}$$

where:

Y_{ijk} = observation on individual k, μ = overall mean, D_i = fixed effect of the ith diet, T_j = fixed effect of the jth time, $(D*T)_{ij}$ = interaction between diet and time, e_{ijk} = random error = $\hat{o}^2$. Tukey's test was used for the multiple comparisons among mean values for different treatments.

RESULTS

Treating of *A. halimus* with enzymes reduced its contents of CF, NDF and ADF without affecting CP and EE contents. The combination of ZAD1® + ZAD2® and ZAD2® were more effective in lowering CF, NDF and ADF than ZAD1® (Table 2).

Addition of *A. halimus* silage treated with enzymes (D2, D3 and D4) improved the intake as air dried or TDN (g/day) compared to D1 (*A. halimus* silage). However, when compared based on DM, D1 had the highest intake, although enzyme-treated groups (D2, D3 and D4) decreased ($P < 0.001$) it (Table 3). D2 and D3 had almost the same total DM intake; however, D2, D3 and D4 almost had the same TDN/total DM intake compared to D1 which had the lowest ($P < 0.001$) percent. All treated diets improved ($P = 0.032$) the intake of air dried *A. halimus* silage. All

Table 2 Chemical composition of ingredients used in the experimental diets (g/kg dry matter)

	Barley	*Atriplex halimus* silage	*A. halimus* silage treated with ZAD1®	*A. halimus* silage treated with ZAD2®	*A. halimus* silage treated with ZAD1® + ZAD2®
Organic matter	960.0	766.0	760.0	761.0	759.0
Crude protein	118.9	117.4	115.2	114.4	113.7
Crude fiber	86.3	248.2	226.5	222.5	224.9
Neutral detergent fiber	189.0	551.8	403.0	406.0	401.2
Acid detergent fiber	72.0	339.6	253.0	258.0	250.2
Ether extract	29.3	19.2	19.3	19.2	18.8
Nitrogen free extract	725.3	380.7	399.2	404.4	401.8

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Table 3 Feed intake, nutrient digestibility and nitrogen utilization of sheep fed† *Atriplex halimus* silage treated with enzymes and barley

Items	*A. halimus* silage and 300 g barley (D1)	ZAD1® and 300 g barley (D2)	*A. halimus* silage treated with enzymes of		SEM	*P*-value
			ZAD2® and 300 g barley (D3)	ZAD1® + ZAD2® and 300 g barley (D4)		
A. halimus intake, g/day						
Air dried	571.8[c]	1106.6[a]	1091.5[ab]	978.3[b]	19.16	0.032
Dry matter (DM)	510.7[a]	390.8[b]	390.1[b]	346.1[c]	16.72	< .0001
Total DM intake	776.7[a]	656.8[b]	656.1[b]	612.1[b]	17.73	< .0001
Total TDN intake, g/day	407.0[a]	374.8[b]	383.2[ab]	350.6[b]	15.45	< .0001
TDN/Total DM intake, %	52.4[b]	57.1[a]	58.4[a]	57.3[a]	2.23	< .0001
Digestibility,%						
Dry matter	57.9[b]	62.1[a]	63.8[a]	61.8[a]	2.88	< .0001
Organic matter	60.8[b]	65.2[a]	66.8[a]	65.2[a]	2.60	< .0001
Crude protein	43.5[c]	53.2[b]	56.6[a]	55.7[ab]	3.65	< .0001
Crude fiber	39.8[c]	49.5[b]	52.7[a]	50.7[ab]	2.40	< .0001
Neutral detergent fiber	55.8[c]	60.2[b]	62.2[ab]	65.0[a]	2.03	< .0001
Ether extract	64.6[c]	74.8[ab]	76.1[a]	72.3[b]	3.80	< .0001
Nitrogen free extract	72.8	72.5	73.0	71.3	3.70	0.0016
Nitrogen utilization, g/day						
Nitrogen intake	14.7[a]	12.26[b]	12.2[b]	11.35[c]	1.02	< .0001
Nitrogen retention	0.81[c]	2.08[b]	2.65[a]	2.34[ab]	0.06	< .0001
Nitrogen retention /Nitrogen absorption	12.7[c]	31.96[b]	40.2[a]	37.04[a]	3.12	< .0001

†All diets supplemented with 300 g air-dried barley per head per day or 266 g DM/head/day. [a,b,c]Means in the same row with different superscripts are significantly different ($P < 0.05$).

enzyme-treated diets (D2, D3 and D4) improved ($P < 0.001$) OM, CP, CF, NDF and EE digestibilities compared with D1. No differences ($P = 0.0016$) were observed for NFE digestibility between different diets. The D3 tended to show higher nutrient digestibility than D2 and D4. Feeding *A. halimus* silage treated with different enzymes (D2, D3 and D4) to sheep lowered ($P < 0.001$) nitrogen retention (NR) and nitrogen retention /nitrogen absorption (NA) ratio (NR/NA) compared to untreated *A. halimus* silage (D1) which increased ($P < 0.001$) nitrogen intake (NI) compared to other diets (Table 3).

No differences ($P > 0.05$) were observed for ruminal pH among the dietary treatments. Sheep fed on ZAD2® treated *A. halimus* silage (D3) showed numerically higher values for all measured rumen fermentation parameters. Animals fed on ZAD2® + ZAD2® treated *A. halimus* silage (D4) had higher rumen fermentation parameters than ZAD1® treated or untreated *A. halimus* silage. The concentration of ruminal tVFA, NH_3-N, and microbial protein production was increased with advancing of time to reach the maximum value around 3 h after feeding and then later decreased (Table 4).

DISCUSSION

Composition of enzymes in treated or untreated *A. halimus* silage

Treating forages like *A. halimus* with exogenous fibrolytic enzymes is accompanied, in most cases, with degradation of various fiber fractions. In our study, ZAD1®, ZAD2® and their combination reduced CF content by 8.7, 10.4 and 9.4%; NDF by 27, 26.4 and 27.3%; ADF by 25.5, 24 and 26.3%, respectively. This may be due to reduction of structural polysaccharide fractions (Facchini *et al.* 2011). The difference in response between ZAD1® and ZAD2® or their combination depends on their different enzyme contents (Table 1). Gado *et al.* (2009) showed that ensiling rice straw, bagasse and corn stalks with ZAD® decreased the CF in a range of 30.3% to 36.6%. The present study showed that enzyme-treated or untreated *A. halimus* silage can be used as a basal diet for ruminant production in arid and semi-arid regions (Haddi *et al.* 2009; Otal *et al.* 2010).

Feed intake, nutrient digestibility and N utilization

Feeding *A. halimus* silage treated with enzymes (i.e. D2, D3 and D4) to sheep increased its air dried intake. This may be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However, post-ingestive enzyme effects, such as increased digestion rate or extent of digestion (Krueger *et al.* 2008) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Adesogan 2005). In contrast, comparing feed intake on a DM basis, feeding *A. halimus* silage treated with ZAD1® or ZAD2® caused a decreased total DM intake. This may be due to the moisture concentration

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Table 4 Ruminal pH, total volatile fatty acids (tVFA), NH_3-N and microbial protein production at different times of incubation of sheep fed† *Atriplex halimus* silage treated with enzymes and barley

Time after feeding (h)	*A. halimus* silage and 300 g barley (D1)	*A. halimus* silage treated with enzymes of			SEM	*P*-value
		ZAD1® and 300 g barley (D2)	ZAD2® and 300 g barley (D3)	ZAD1® + ZAD2® and 300 g barley (D4)		
pH						
0	6.91	6.79	6.79	6.83	0.06	
1	6.63	6.56	6.61	6.67	0.06	
3	6.32	6.28	6.28	6.31	0.05	
6	6.76	6.68	6.65	6.7	0.06	
Mean	6.60	6.58	6.58	6.63	0.04	0.8138
tVFA, mmol/100 mL rumen liquor						
0	6.53	7.79	8.15	8.03	0.44	
1	7.77	8.82	9.18	8.95	0.48	
3	9.65	11.56	12.41	12.1	0.44	
6	7.66	8.4	8.8	8.66	0.49	
Mean	7.90[c]	9.14[b]	9.64[a]	9.44[ab]	0.41	< .0001
NH_3-N, mg N/100 mL rumen liquor						
0	8.96	12.08	12.51	12.16	0.36	
1	10.02	13.44	14.64	13.66	0.39	
3	11.93	15.36	16.86	15.69	0.38	
6	9.66	13.18	14.18	13.34	0.39	
Mean	10.14[c]	13.52[b]	14.55[a]	13.71[ab]	0.31	0.0071
Microbial protein production (g/day)						
0	15.52	19.86	28.09	20.92	3.48	
1	19.65	24.67	38.88	28.31	3.41	
3	32.44	41.64	57.97	43.79	3.62	
6	15.64	21.92	31.25	23.58	3.61	
Mean	20.81[c]	27.02[b]	39.05 [a]	29.15[b]	3.42	0.0003

†All diets supplemented with 300 g air-dried barley per head per day or 266 g DM/head/day. [a,b,c]Means in the same row with different superscripts are significantly different ($P < 0.05$).

of silage (up to 60% moisture) which may increase the satiety of sheep, resulting a decreased feed intake (Forbes 2007). However, Khattab *et al.* (2011) found that diets treated with the same enzyme preparations did not affect feed intake. Intake of TDN of sheep fed enzyme-treated *A. halimus* silage (i.e. D2, D3 and D4) was lower than for those fed *A. halimus* silage untreated with enzymes (i.e. D1). However, when the TDN intake was compared as a percent from total DM intake, the results may be completely reversed. At the same time, comparing obtained TDN intake (%) for enzyme-untreated or treated *A. halimus* silage showed that these values were higher than the tabular value from NRC (1994) for daily maintenance requirements from TDN intake (%) which is about 54.5% TDN at 45 kg of body weight. This may give an indication for sufficient energy allowances for the animals in different treatments.

A. halimus silage treated with ZAD2® showed numerically highest values for nutrient digestibility with the exception of NDF and NFE. The relatively higher digestibilities with ZAD2® than ZAD1® + ZAD2® may be related to its enzyme contents. ZAD2® contains higher xylanase, cellulase and a-amylase than ZAD1® which encourages pre-ingestive fiber hydrolysis. Addition of both enzymes together was less effective than ZAD2®. This is related with higher enzyme concentrations when both enzymes were added; resulting in preventing binding of enzymes to substrate receptors, which reduced proportional attachment by ruminal microorganisms to fiber (Treacher & Hunt 1996). These results are similar to other reports that had shown an increase in nutrient digestibility with the same exogenous fibrolytic enzymes of ZAD1® and ZAD2® (Khattab *et al.* 2011; Kholif *et al.* 2012). Exogenous fibrolytic enzymes would increase fiber digestion by many mechanisms, increasing the rate of ruminal digestion of the potentially digestible fiber (Yang *et al.* 1999), reducing digesta viscosity (Hristov *et al.* 2000) and alterations in ruminal fermentation (Nsereko *et al.* 2002). It also enhances attachment and colonization to the plant cell wall by ruminal microorganisms (Wang *et al.* 2001; Chung *et al.* 2012) or by synergism between exogenous enzymes and enzymes in rumen fluid (Eun *et al.* 2006). They demonstrated a synergism between exogenous enzymes and ruminal enzymes such that the net combined hydrolytic effect in the rumen was much greater than that estimated from individual enzyme activities. Moreover, Wang *et al.* (2001) reported that supplementation diets with enzymes increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with

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rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more polysaccharidase activity to digest different feeds and degrade secondary metabolites. Although this possibility may not be supported by Nsereko *et al.* (2002) or Krueger *et al.* (2008), who showed that while cellobiose and glucose utilizing bacteria were stimulated, effects on the fibrolytic population were negligible. Our results indicate that enzyme supplementation could increase the quantity of microbial protein available to animal metabolism, and this may increase fiber digestibility and the metabolizable energy density of diets.

Although addition of enzyme-treated *A. halimus* silage had decreased ($P < 0.001$) N intake per DM consumption, with improving ($P < 0.001$) CP digestibility especially with ZAD2® and ZAD1® + ZAD2®, nitrogen retention was higher than the other diets. This is an indication of improvements in utilization of N in tree fodder forages as a result of application of the exogenous fibrolytic enzymes being contained in ZAD2®.

Ruminal fermentation and microbial protein synthesis

Addition of ZAD2® and ZAD1® + ZAD2® treated *A. halimus* silage improved ($P < 0.001$) the production of tVFA and NH_3-N. The increase in ruminal tVFA and NH_3-N suggested that ZAD2® and ZAD1® + ZAD2® treated *A. halimus* silage was more efficient and yielded more tVFA and NH_3-N than control. Results of tVFA suggested that feeding of ZAD2® treated *A. halimus* silage improved anaerobic ruminal fermentation which stimulated it to yield more VFA. This improved yield of VFA may be due to the increases of OM digestibility in treated diets, especially D3. Increased NH_3-N concentration in animals fed ZAD2® and ZAD1® + ZAD2® (i.e. D3 and D4) diets supports its capability to enhance rumen protein degradation, probably because of its protease enzyme contents (Gado *et al.* 2009; Khattab *et al.* 2011). It may be also due to the fact that exogenous enzymes had a synergism with endogenous enzymes, especially the proteolytic enzyme, or enhanced the activity of microorganisms.

Rumen microbial protein of sheep was improved by feeding *A. halimus* silage treated with ZAD2® or ZAD1® + ZAD2®. It is well known that microbial protein synthesis is a good indicator of beneficial effect of feed utilization. Microbial protein has the most significant impact on both quantity and quality of metabolizable protein absorbed from the small intestine. These results suggest that *A. halimus* silage treated with ZAD2® or ZAD1® + ZAD2® may be more efficient for enhancing fiber digestibility, resulting in provision of more nutrients for ruminal microorganisms which are beneficial for the growth of microbes.

Conclusions

Feeding *A. halimus* silage treated with ZAD1® and ZAD2®, separately or in combination, to sheep improved digestibility coefficients, nitrogen retention and utilization and rumen fermentation (tVFA and NH_3-N), as well as microbial protein production. The enzyme cocktail of ZAD2® was more effective than ZAD1® followed by ZAD1® + ZAD2® combination.

However, there is a need for further studies on the effect of these exogenous enzymes on ruminal microbial populations, animal performance properties and the mechanisms of actions between ZAD1® and ZAD2® with endogenous enzymes and microbes.

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