doi: 10.17221/8133-CJAS

Feed intake, nutrient digestibility, nitrogen utilization, and ruminal fermentation activities in sheep fed *Atriplex halimus* ensiled with three developed enzyme cocktails

A.Z.M. Salem[1], H. Alsersy[2], L.M. Camacho[3], M.M. El-Adawy[2], M.M.Y. Elghandour[1], A.E. Kholif[4], N. Rivero[5], M.U. Alonso[1], A. Zaragoza[5]

[1]Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico, Mexico
[2]Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[3]Faculty of Veterinary and Animal Science, Autonomous University of Guerrero, Altamirano, Mexico
[4]Dairy Science Department, National Research Centre, Giza, Egypt
[5]Autonomous University of Hidalgo State, Institute of Agricultural Sciences, Academic Area of Veterinary Medicine and Animal Science, Tulancingo, Mexico

ABSTRACT: The effects of feeding *Atriplex halimus* treated with three developed enzyme cocktails to Barki sheep on feed intake, nutrient digestibility, N utilization, and ruminal fermentation were assessed. *A. halimus* was ensiled with two developed enzyme cocktails of ZAD1® (Z1) and/or ZAD2® (Z2) as liquid enzyme preparations (2 l/t) with 5% molasses and ensiled for 30 days. Three Barki rams (45 ± 3.2 kg) were used per treatment in five consecutive digestibility trials, while three ewes fitted with a permanent rumen fistula were used as source of inoculum for *in vitro* rumen fermentation trials. Barley grain (300 g/animal/day) was fed as energy supplement during the experimental trial for all diets. Five diets were composed as follows: *A. halimus* (leaves and stems) (D1); untreated *A. halimus* plus 4 g/animal/day ZADO® (Z) (enzyme preparation in powder form) (D2); *A. halimus* ensiled with Z1 and barley plus 4 g/animal/day Z (D3); *A. halimus* ensiled with Z2 and barley (D3) plus 4 g/animal/day Z (D4); *A. halimus* ensiled with a combination of Z1 and Z2 (1:1) and barley plus 4 g/head/day Z (D5). For all trials, *ad libitum A. halimus* was offered twice a day at 9:00 and 16:00 h while barley grain was given once a day at 10:00 h. Both D1 and D2 diets increased (P <0.001) dry matter intake of *A. halimus* and total dry matter intake. Addition of 4 g/day of Z to Z1 and/or Z2 ensiled diets improved (P < 0.0001) organic matter, crude protein, crude fibre, and neutral detergent fibre digestibilities. Diets D1 and D2 increased (P < 0.001) N intake, whereas the direct addition of Z to D3, D4, and D5 decreased (P < 0.001) N balance and N balance/N absorption ratio. Sheep fed on Z in addition to Z2 ensiled *A. halimus* showed higher improvements for total volatile fatty acids (P < 0.001), ammonia N (P = 0.007), and microbial protein production (P = 0.003). It can be concluded that feeding sheep on *A. halimus* ensiled with Z1 and Z2 with direct feeding of Z enzyme preparation improved intake, digestibility, nitrogen balance and utilization, as well as rumen fermentation.

Keywords: direct-fed enzyme; exogenous enzymes; nutritive value; performance; saltbushes

INTRODUCTION

There is a wide range of browse species capable of growing under saline soil and water conditions. Many of these plants represent a feed resource for livestock during the dry season (Salem et al. 2012a). In general, they have a low content of crude protein (CP) with high fibre and ash content during summer (Papanastasis et al. 2008). However, saltbushes contain high levels of CP (Norman et

doi: 10.17221/8133-CJAS

al. 2004; Al-Owaimer et al. 2011). Much of the nitrogen is associated with non-protein compounds such as nitrates and proline. The CP and digestibility of neutral detergent fibre (NDF) and acid detergent fibre (ADF) vary, but it is possible that salinity levels may not affect these parameters (Masters et al. 2007).

Saltbushes (*Atriplex* spp.) represent an important group of browses. They produce considerable amounts of biomass that can be used when herbaceous forage is scarce. *A. halimus* (AH) is found in semi-arid environments in the Mediterranean basin, where Egypt is located, and is valued as livestock forage when herbage availability is low (Le Houerou 1993). However, AH is not highly used because of its deficiency in available carbohydrate and high fibre content and, therefore, barley grain has been suggested as energy source to stimulate the usage of saltbush. Feeding a high-energy supplement to saltbush pastures can increase its nutritive value and stimulate ruminal microbes to produce microbial protein (MP) and enhance carbohydrate digestion and detoxification of secondary compounds (Norman et al. 2008). The AH contains up to 10% sodium chloride and secondary plant metabolites (Makkar 2003; Salem et al. 2006) which considerably affects its palatability and nutritive value (Abd El-Rahman et al. 2006; Salem et al. 2006). The processing of AH (succulent and foliage parts) by ensiling enhances its acceptability in sheep and goats providing sufficient total digestible nutrients (TDN) and digestible CP (DCP) (Abd El-Rahman et al. 2006; Alsersy et al. 2015).

The use of exogenous fibrolytic enzymes to enhance quality and digestibility of fibrous forage is on the verge of delivering practical benefits to ruminant production systems (Togtokhbayar et al. 2015). It is well known that addition of exogenous enzymes to animal diets can improve feed utilization and animal performance by improving fibre degradation *in vitro* (Giraldo et al. 2008; Elghandour et al. 2013; Salem et al. 2015), *in situ* (Chung et al. 2012), and *in vivo* (Gado et al. 2009, 2011; Salem et al. 2013; Valdes et al. 2015), increasing feed intake and digestion rate (Salem et al. 2012a, b).

Proposed modes of action of direct-fed enzymes include solubilization of dietary fibre before ingestion, provision of readily fermentable substrate for ruminal microorganisms, and/or enhancement of microbial enzyme activity in the rumen (McAllister et al. 2001). A variety of factors, such as the specific activity of the enzymes, their mode and way of application, as well as the type of animal and its diet, may affect enzyme efficacy. Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Morgavi et al. 2000; Nsereko et al. 2000) to increase rate of degradation of fibre in the rumen (Giraldo et al. 2008; Gado et al. 2013), as well as rumen MP synthesis (Yang et al. 1999; Nsereko et al. 2002; Gado et al. 2013) and forestomach digestibility.

ZAD1® (Z1), ZAD2® (Z2), and ZADO® (Z) are three enzyme cocktails obtained from anaerobic bacteria to increase the level of cellulolytic enzymes in ruminal anaerobic bacteria, which specifically transform polysaccharides into monosaccharides (Gado et al. 2009, 2011), enhancing poor quality roughages. Gado et al. (2009) and Khattab et al. (2011) reported that Z1, Z2, and Z improved ruminal fermentation, feed intake, N balance, nutrients digestibility, and milk production as well as live weight gain and feed conversion of low quality roughages in sheep and goats. Activity of Z enzyme preparation starts immediately after feeding it to the animals.

The objective of the present work was to investigate the effect of adding Z powdered enzyme cocktail to untreated Z1 and/or Z2 (liquid form) ensiled AH on animal performance in terms of feed intake, nutrient digestibility, N metabolism, rumen fermentation, and rumen MP synthesis.

MATERIAL AND METHODS

A. halimus preparation. Leaves and stems of fresh Mediterranean saltbush (AH) were collected from the north-western desertic region, air dried, and chopped to 3 to 5 cm lengths and stored in a dry environment. Secondary metabolite concentrations in fresh (g/kg) and dried AH were: 13.3 and 7.2 total phenolics, 5.5 and 2.2 total tannin, and 7.3 and 2.6 alkaloids, respectively.

Animals and treatments. Three Barki rams (45 ± 3.2 kg) per treatment were housed in individual cages and used in five consecutive trails for nutrient feed intake and digestibility determination, while three ewes (40 ± 2.6 kg) per treatment fitted with a permanent rumen fistula, as the inoculum source for *in vitro* rumen fermentation trials.

Barki sheep were fed on a basal diet formulated to meet their maintenance requirements (rams for

doi: 10.17221/8133-CJAS

nutrient feed intake and digestibility, and ewes for rumen fermentation experiments) (NRC 1985) composed of barley grain (300 g/animal/day) plus either treated or untreated AH *ad libitum*.

The AH was moistened until an average dry matter (DM) content of 40% was reached, and was added or not with Z1 and/or Z2 (2 l/1000 kg of AH, on fresh matter basis) and molasses (5% of AH, on fresh matter basis), and then sealed with polyethylene sheet to be ensiled for 4 weeks of each treatment. Each diet was prepared a month before feeding it to animals. Barley grain (300 g/animal/day) was fed as energy supplement during the experimental period for all diets.

Five experimental diets comprised of: AH (leaves and stems) (D1); untreated AH plus 4 g/animal/day ZADO® (Z enzyme) (D2); AH silage treated with Z1 and barley plus 4 g/animal/day Z enzyme (D3); AH silage treated with Z2 and barley (D3) plus 4 g/animal/day Z enzyme (D4); AH silage treated with a combination of Z1 and Z2 (1 : 1) and barley plus 4 g/head/day Z enzyme (D5) were proven. For all treatments, *ad libitum* AH was offered to animals twice a day at 9:00 and 16:00 h while barley grain was given once a day at 10:00 h. Feed intake was measured and expressed both as air dried feed and on DM basis. The D1 treatment is the same control diet of Alsersy et al. (2015).

Three recently developed enzyme cocktails Z1, Z2, and Z were used in this study. Both Z1 and Z2 (liquid form) are live anaerobic bacteria with their enzymes, while Z (enzyme preparation in powder form) is a multi-mix containing cellulases, xylanases, protease and α-amylase, added to the related anaerobic bacteria which produce them, and coated with starch and glycol (Table 1).

Table 1. Composition of exogenous enzymes[1] of Z1, Z2, and Z

	Xylanase[2]	α-Amylase[3]	Cellulase[4]	Protease[5]
Z1 (U/ml)	2.32	61.50	7.05	29.20
Z2 (U/ml)	6.93	69.40	8.16	12.30
Z (U/g)	0.058	3.39	0.892	1.56

Z1 = ZAD1®, Z2 = ZAD2®, Z = ZADO®

[1]Z1 and Z2 moisture contents = 40%; Z is a powdered multi-mix which contains cellulases, xylanases, protease, and α-amylase

[2]1 U = amount (g or ml) of enzyme needed to release 1 μmol xylose/min from 5 mg/ml xylan solution (pH 5.5 and 37°C)

[3]1 U = amount of enzyme needed to produce 1 mg glucose from starch in 1 h by 1 ml Z1 and Z2 or 1 g Z (pH 4.6 and 40°C)

[4]1 U = amount of 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)

[5]1 U = amount of enzyme needed to produce 1 μmol amino acids from protein in 1 min (pH 5.5 and 37°C)

Nutrient digestibility. Each metabolism trial was conducted for three weeks as preliminary period and one week for sample collection. All animals were individually kept in stainless steel metabolic cages at room temperature with *ad libitum* access to water. Beneath each cage a 4 mm stainless steel mesh was placed to retain faeces with free passage for urine, which was collected through a funnel. Faeces and urine were collected once a day before the morning feeding and stored at –10°C for later analysis.

Seven-day collections of faecal samples were mixed and stored for later routine analysis. Faecal samples were dried out at 60°C for 72 h and ground through a 1 mm screen using a Wiley mill grinder (Arthur H. Thomas, Philadelphia, USA), using a 20 g faecal sample from each animal for analysis. Digestibility of DM was determined and expressed on DM basis.

Conventional analyses of feed (Table 2) and faecal samples were carried out according to AOAC (1997) for DM (method No. 934.01), ash (method No. 942.05), N (method No. 954.01), and ether extract (EE) (method No. 920.39). The NDF (Van Soest et al. 1991), ADF, and lignin (AOAC 1997; method No. 973.18) were analyzed using an ANKOM^{200} Fibre Analyser unit (ANKOM Technology Corporation, Macedon, USA). NDF was assayed without the use of alpha amylase but with sodium sulfite. Both NDF and ADF are expressed without residual ash.

The values of TDN intake as a percentage from total DM intake were calculated according to NRC (2001) by using total digestible nitrogen free extract (NFE) (tdNFE), CP (tdCP), EE (tdEE), crude fibre (CF) (tdCF) values according to the following equation:

TDN (%) = tdNFE + tdCP + (tdEE – 2.25) + tdCF.

Ruminal fermentation activities. *In vitro* zero order technique (Carroll and Hungate 1954) was applied for measuring rates of ammonia (NH_3-N) and total volatile fatty acids production (tVFA). Rumen contents were collected from three rumen fistulated Barki ewes fed on each experimental diet consecutively at 8.00 h before feeding, and 1, 3, and 6 h after feeding.

doi: 10.17221/8133-CJAS

Table 2. Chemical composition of ingredients used in experimental diets (g/kg DM) (after Alsersy et al. 2015)

	Barley	AH	AH ensiled with		
			Z1	Z2	Z1 + Z2
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 fibre	86.3	248.2	226.5	222.5	224.9
Neutral detergent fibre	189.0	551.8	403.0	406.0	401.2
Acid detergent fibre	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

AH = *Atriplex halimus,* DM = dry matter, Z1 = ZAD1®, Z2 = ZAD2®

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 thermostatically controlled water bath. Each sample was composed to comprise two thirds of fibrous material and one third of liquid (El-Shazly and Hungate 1965). At zero time incubation, two subsamples were transported, poured into another jar containing formalin (1 ml/100 g rumen contents), and vigorously swirled to stop metabolic activity.

After one-hour incubation, rumen samples treated with formalin were used to estimate NH_3-N and tVFA concentration. Rumen samples taken at zero and at one-hour fermentation were strained through 4 layers of cheesecloth. Fifty ml of rumen liquor were deproteinized using 0.03 mol/ml of sulphuric acid (50 ml) and the volume 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).

MP synthesized in the rumen and fed in the four experimental diets was calculated as follows (Borhami et al. 1992):

MP (g/day) = tVFA production (mol/day) × 2 × 13.48 × 10.5 × 6.25/100

tVFA production = tVFA concentration (mol/100 ml; obtained from the *in vitro* zero order technique) × 10 × total rumen digesta (l)

(when multiplicated by 10, the rate of VFA production/1 h/l is obtained, while multiplicated by 24 the rate of VFA production/24 h/l is obtained).

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

Statistical analysis. Data with those of Alsersy et al. (2015) were statistically analyzed according to a randomized design using the PROC MIXED procedure of SAS (Statistical Analysis System, Version 9.0, 2002) according to the following statistical model:

$$Y_{ij} = \mu + D_i + e_{ij}$$

where:

Y_{ij} = observation on individual j

μ = overall mean

D_i = fixed effect of the i^{th} diet

e_{ij} = random error assumed to be normally distributed with mean = 0 and variance = δ^2

Tukey's test was used for the multiple comparisons among mean values for different treatments.

RESULTS

Intake and digestibility. Sheep fed the diets containing untreated AH (D1 and D2) showed higher ($P < 0.001$) DM intake of AH and total DM intake compared to those fed AH ensiled with different enzymes preparations (D3, D4, and D5). However, all diets containing AH ensiled with Z1 and/or Z2 enzyme preparations (D3, D4, and D5) improved ($P = 0.032$) the intake of air dried

doi: 10.17221/8133-CJAS

Table 3. Feed intake, nutrients digestibility, and N utilization of sheep fed[1]AH treated with enzyme and barley

	D1	D2	D3	D4	D5	SEM	*P*-value
AH intake (g/h/day)							
Air-dried	571.8^{d}	643.3^{c}	1093.0^{a}	1029.3^{ab}	1023.0^{b}	19.16	0.032
Dry matter	510.7^{a}	574.8^{a}	386.1^{b}	367.9^{b}	362.0^{b}	16.72	< 0.0001
Total DM intake	776.7^{a}	798.5^{a}	652.1^{b}	633.9^{b}	628.0^{b}	16.73	< 0.0001
Total TDN intake (g/day)	407.0^{a}	547.7	578.0	591.1	578.5	15.45	< 0.0001
Digestibility (mg/g DM)							
Dry matter	578.8^{c}	614.6^{b}	630.8^{ab}	644.0^{a}	629.8^{ab}	18.75	< 0.0001
Organic matter	607.9^{c}	638.8^{b}	660.7^{ab}	674.2^{a}	660.4^{ab}	16.00	< 0.0001
Crude protein	435.0^{d}	508.8^{c}	548.5^{b}	585.0^{a}	565.7^{ab}	36.50	< 0.0001
Crude fibre	397.5^{c}	474.8^{b}	509.9^{b}	543.3^{a}	522.9^{ab}	24.00	< 0.0001
Neutral detergent fibre	558.0^{c}	596.0^{b}	653.2^{a}	661.2^{a}	674.1^{a}	20.34	< 0.0001
Ether extract	645.5^{d}	683.4^{a}	750.9^{b}	751.0^{b}	726.1^{c}	38.00	< 0.0001
Nitrogen free extract	728.4	734.1	729.3	731.3	720.3	17.00	0.0016
N utilization (g/h/day)							
N intake	14.7^{a}	15.9^{a}	12.2^{b}	11.8^{c}	11.6^{c}	1.02	< 0.0001
N balance	0.81^{c}	1.67^{b}	2.58^{a}	2.99^{a}	2.55^{a}	0.06	< 0.0001
N balance/N absorption	12.7^{d}	20.69^{c}	38.62^{b}	43.23^{a}	43.23^{a}	0.12	< 0.0001

Z1 = ZAD1®, Z2 = ZAD2®, Z = ZADO®, AH = *Atriplex halimus*, DM = dry matter, TDN = total digestible nutrients, D1 = untreated AH, D2 = untreated AH supplemented with Z enzyme at 4 g/animal/day, D3 = Z1 ensiled AH supplemented with Z enzyme at 4 g/animal/day, D4 = Z2 ensiled AH supplemented with Z enzyme at 4 g/animal/day, D5 = Z1 + Z2 ensiled AH supplemented with Z enzyme at 4 g/animal/day

[1]all diets supplemented with 300 g air-dried barley/animal/day or 266 g DM/animal/day

[a–d]means in the same row with different superscripts are significantly different ($P < 0.05$)

AH. Direct feeding of Z enzyme preparation to sheep fed untreated AH showed the highest ($P < 0.001$) values of both DM intake of AH and total DM intake compared to other diets. Addition of Z to untreated AH (D2) improved ($P < 0.001$) all nutrient digestibility vs D1. Moreover, addition of Z to Z1 and/or Z2 ensiled diets improved ($P < 0.0001$) organic matter (OM), CP, CF, and NDF digestibilities. Addition of Z to Z2 ensiled AH (D4) was the best diet. Diets containing untreated AH (D1 and D2) showed increased ($P < 0.001$) N intake (NI) compared to those containing Z1 and/or Z2 ensiled AH. However, D2 had numerically higher ($P > 0.05$) NI than D1. On the contrary, direct addition of Z to AH ensiled with different enzyme preparations (i.e. D3, D4, and D5) to sheep increased ($P < 0.001$) N balance and N balance/N absorption ratio (Table 3).

Ruminal fermentation and microbial protein synthesis. Sheep fed on Z in addition to Z2 ensiled AH (D4) showed higher improvements for all measured rumen fermentation parameters (tVFA, $P < 0.001$; NH_3-N, $P = 0.007$; MP production, $P = 0.003$) followed by those of D5 fed Z1 + Z2 ensiled AH, when compared to those fed Z1 ensiled AH. Moreover, direct feeding of Z to sheep fed untreated AH (D2) numerically improved these fermentation parameters compared to those fed untreated AH without addition of Z (D1). No differences ($P > 0.05$) were observed for ruminal pH with different treatments. The production of tVFA, NH_3-N, and MP was increased as time advanced reaching a peak 3 h after feeding and then gradually decreased (Table 4).

DISCUSSION

Composition of A. halimus. Ensiling AH with exogenous fibrolytic enzymes is accompanied, in most cases, by degradation of various fibre fractions. In the current study, Z1, Z2 and their combination decreased 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

doi: 10.17221/8133-CJAS

Table 4. Ruminal pH, total volatile fatty acids (tVFA), ammonia-N (NH_3-N), and microbial protein production at different times of incubation of sheep fed[1]AH ensiled with enzyme and barley

Time after feeding (h)	D1	D2	D3	D4	D5	SEM	*P*-value
pH							
0	6.91	6.87	6.75	6.78	6.78	0.06	
1	6.63	6.58	6.61	6.61	6.64	0.06	
3	6.32	6.31	6.30	6.27	6.31	0.05	
6	6.76	6.68	6.68	6.63	6.68	0.06	
Mean	6.60	6.61	6.59	6.57	6.60	0.04	0.8138
tVFA (mmol/100 ml rumen liquor)							
0	6.53	6.77	8.01	8.26	8.17	0.44	
1	7.77	8.03	8.85	9.39	9.27	0.48	
3	9.65	10.22	11.75	12.69	12.46	0.44	
6	7.66	7.93	8.60	9.02	9.01	0.49	
Mean	7.90^{c}	8.24^{b}	9.30^{b}	9.84^{a}	9.73^{a}	0.41	< 0.0001
NH_3-N (mg N/100 ml rumen liquor)							
0	8.96	10.30	12.18	12.53	12.27	0.36	
1	10.02	10.95	13.55	14.80	13.77	0.39	
3	11.93	12.76	15.63	17.00	15.82	0.38	
6	9.66	10.53	13.29	14.45	13.50	0.39	
Mean	10.14^{c}	11.14^{b}	13.66^{ab}	14.70^{a}	13.84^{ab}	0.31	0.0071
Microbial protein production (g/day)							
0	15.52	19.12	21.12	28.52	21.89	3.48	
1	19.65	23.33	26.54	39.98	30.19	3.41	
3	32.44	39.86	43.86	62.35	46.57	3.62	
6	15.64	20.61	23.62	32.76	23.84	3.61	
Mean	20.81^{c}	25.73^{bc}	28.79^{b}	40.90^{a}	30.62^{b}	3.42	0.0003

Z1 = ZAD1®, Z2 = ZAD2®, Z = ZADO®, AH = *Atriplex halimus*, D1 = untreated AH, D2 = untreated AH supplemented with Z enzyme at 4 g/animal/day, D3 = Z1 ensiled AH supplemented with Z enzyme at 4 g/animal/day, D4 = Z2 ensiled AH supplemented with Z enzyme at 4 g/animal/day, D5 = Z1 + Z2 ensiled AH supplemented with Z enzyme at 4 g/animal/day

[1]all diets supplemented with 300 g air-dried barley/animal/day or 266 g dry matter/animal/day

[a–d]means in the same row with different superscripts are significantly different ($P < 0.05$)

of structural polysaccharide fractions (Facchini et al. 2011). The different response between Z1 and Z2 or their combination depends on the content of different enzymes (Table 1). The current study showed that enzyme treated or untreated AH silage can be used as a basal diet for ruminant production in arid and semiarid regions (Alsersy et al. 2015).

Feed intake and digestibility. Treating AH with Z1 and/or Z2 decreased total DM intake and increased air dried intake from AH silage. This may be due to the moisture concentration (up to 60%) of silage which may increase the satiety of sheep and decrease feed intake (Alsersy et al. 2015). Increased air dried intake from enzymes treated AH silage may be due to increased palatability of the diet due to sugars released by pre-ingestive fibre 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; Alsersy et al. 2015). Addition of Z enzyme preparation improved feed intake when it was added alone to untreated diet. Khattab et al. (2011) found that diets ensiled with Z1 or direct addition of Z did not affect feed intake in goats. Salem et al. (2011) reported that feed intake of animals was improved when fed AH added with Z .

doi: 10.17221/8133-CJAS

Nutrients digestibility and N utilization. The AH ensiled with Z1, Z2 and addition of Z to barley showed the highest values for all nutrients digestibility with the exception of nitrogen free extract. These results are similar to other reports that showed an increase in nutrients digestibility with the same exogenous fibrolytic enzymes preparations of Z1 and Z2 or Z (Khattab et al. 2011; Salem et al. 2012a, b). The AH ensiled with Z2 showed the highest values for all nutrients digestibility with the exception of NFE. The improved digestibilities with the addition of Z after Z2 than Z1 and/or Z2 preparations may be related to their enzymes contents. Z2 contains more xylanase, cellulose, and α-amylase than Z1 preparation which encourages pre-ingestive fibre hydrolysis. Addition of both enzyme preparations was less effective than that of Z2, maybe because the increasing enzyme concentrations prevented binding of enzyme to substrate receptors, which reduced proportional attachment by ruminal microorganisms to fibre (Treacher and Hunt 1996). Direct addition of Z improved ruminal fermentation kinetics which positively improved rumen fermentation resulting in higher digestibilities (Valdes et al. 2015). These results are similar to other reports that showed an increase in nutrients digestibility with the same exogenous fibrolytic enzymes preparations of Z1, Z2, and Z (Khattab et al. 2011; Kholif et al. 2012). Exogenous fibrolytic enzymes are supposed to increase fibre digestion by many mechanisms: by increasing the rate of ruminal digestion of the potentially digestible fibre (Yang et al. 1999), reducing digesta viscosity (Hristov et al. 2000), and alterations in ruminal fermentation (Nsereko et al. 2002). Exogenous fibrolytic enzymes also enhance attachment and colonization to the plant cell wall by ruminal microorganisms (Wang et al. 2001; Chung et al. 2012) and/or by synergism between exogenous enzymes and enzymes in rumen fluid (Eun et al. 2006). Eun et al. (2006) demonstrated synergism between exogenous enzymes and ruminal enzymes in such a way that the combined net hydrolytic effect in the rumen was much greater than that estimated from individual enzyme activities. Moreover, Wang et al. (2001) reported that diet supplementation with enzymes increased the numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more total polysaccharides activity to digest different feeds and degrade secondary metabolites. This possibility, however, was not supported by Nsereko et al. (2002) and 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 MP available to animal metabolism, and this may increase fibre digestibility and metabolizable energy level of diets.

Although enzyme preparations (Z1, Z2, and Z) decreased ($P < 0.001$) NI per DM consumption, with improving ($P < 0.001$) CP digestibility, N balance was higher in diets with directly added Z1, Z2, and Z. This indicates N utilization improvements in tree fodder forages as a result of application of the exogenous fibrolytic enzyme preparations (Salem et al. 2012a).

Ruminal fermentation activities. Treatments of Z1 and Z2 or addition of Z enzyme preparations to diets improved ($P < 0.001$) the production of tVFA and NH_3-N, and slightly ($P > 0.05$) affected pH values. The increase in ruminal tVFA and NH_3-N suggested that treatments of Z1, Z2 or addition of Z preparations treated AH were more efficient and yielded more tVFA and NH_3-N than the control. tVFA results suggested that treatment of Z2 with addition of Z improved the anaerobic ruminal fermentation of AH 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 D4. Increased NH_3-N concentration in animals fed Z2 ensiled AH with addition of Z supported its capability to enhance rumen protein degradation, probably because it contained protease enzymes (Gado et al. 2009; Khattab et al. 2011). It may also be due to the synergistic effect of the exogenous enzyme and the endogenous enzyme, especially the proteolytic enzyme, or the enhanced activity of microorganisms.

Rumen MP of sheep was significantly improved by feeding AH ensiled with Z2 and/or Z1 with the addition of Z preparations. It is well known that MP synthesis is a good indicator of beneficial effect of feed utilization. The MP has the most significant impact on both quantity and quality of metabolizable protein absorbed from the small intestine.

Feeding the enzyme preparation may have stimulated and/or increased total viable rumen bacterial

doi: 10.17221/8133-CJAS

numbers because rumen microbial N synthesis was increased, which may be due, at least in part, to increased fibre digestion and an improved capacity of rumen bacteria to digest feed. This possibility, however, is not supported by Nsereko et al. (2002) and Krueger et al. (2008). The use of Z1, Z2 and/or Z preparation in the present study indicated that enzyme supplementation increased the quantity of MP available to animal metabolism, which might be more efficient for enhancing fibre digestibility and consequently providing more nutrients for ruminal microorganisms beneficial for microbial growth.

CONCLUSION

Our results suggested that treating *A. halimus* with Z1, Z2 and addition of Z enzyme preparation, separately or in combination, may improve nutrient digestibility, nitrogen balance and utilization, rumen fermentation (tVFA and NH_3-N), as well as MP production.

REFERENCES

Abd El-Rahman H.H., Mohamed M.I., Gehad A.E.A., Awadallah I.M. (2006): Ameliorating the anti-nutritional factors effect in *Atriplex halimus* on sheep and goats by ensiling or polyethylene glycol supplementation. International Journal of Agriculture and Biology, 8, 766–769.

Adesogan A.T. (2005): Improving forage quality and animal performance with fibrolytic enzymes. In: Proc. Florida Ruminant Nutrition Symposium, Gainesville, USA, 91–109.

Al-Owaimer A.N., El-Waziry A.M., Koohmaraie M., Zahran S.M. (2011): The use of ground date pits and *Atriplex halimus* as alternative feeds for sheep. Australian Journal of Basic and Applied Sciences, 5, 1154–1161.

Al-Rabbat M.F., Baldwin R.L., Weir W.C. (1971): *In vitro* ^{15}Nitrogen-tracer technique for some kinetic measures of ruminal ammonia. Journal of Dairy Science, 54, 1150–1161.

Alsersy A., Salem A.Z.M., Borhami B.E., Olivares J., Gado H.M., Mariezcurrena M.D., Yacuot M.H., Kholif A.E., El-Adawy M., Hernandez S.R. (2015): Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. Animal Science Journal, 86, 51–58.

AOAC (1997): Official Methods of Analysis. 16th Ed. Arlington, USA.

Borhami B.E., El-Shazly K., Abou-Akkada A.R., Naga M.A., Abaza A.M. (1979): Nitrogen (N^{15}) utilization and microbial protein synthesis in the rumen of cattle. Journal of Animal Science, 49, 1306–1311.

Borhami B.E., Fahmy W.G., El-Shazly K. (1992): Rumen environment microbial protein synthesis and nitrogen balance in sheep. In: Proc. Internat. Conference Manipulation of Rumen Microorganisms, Alexandria, Egypt, 85–97.

Carroll E.J., Hungate R.E. (1954): The magnitude of microbial fermentation in the bovine rumen. Journal of Applied Microbiology, 2, 205–214.

Chung Y.H., Zhou M., Holtshausen L., Alexander T.W., McAllister T.A., Guan L.L., Oba M., Beauchemin K.A. (2012): A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. Journal of Dairy Science, 95, 1419–1427.

Elghandour M.M.Y., Salem A.Z.M., Ronquillo M., Borquez J.L., Gado H.M., Odongo N.E., Penuelas C.G. (2013): Effect of exogenous enzymes on *in vitro* gas production kinetics and ruminal fermentation of four fibrous feeds. Animal Feed Science and Technology, 179, 46–53.

El-Shazly K., Hungate R.E. (1965): Fermentation capacity as a measure of net growth of rumen microorganisms. Appllied Microbiology, 13, 62–69.

El-Shazly K., Ahmed E.I.A., Naga M.A., Borhami B.E.A. (1976): A colorimetric technique using chromium-ethylene diamine tetra acetate for measuring rumen volume. Journal of Agricultural Science, 87, 369–373.

Eun J.S., Beauchemin K.A., Hong S.H., Bauer M.W. (2006): Exogenous enzymes added to untreated or ammoniated rice straw: effects on *in vitro* fermentation characteristics and degradability. Animal Feed Science and Technology, 131, 87–102.

Facchini F.D., Vici A.C., Benassi V.M., Freitas L.A., Reis R.A., Jorge J.A., Terenzi H.F., Mde L. (2011): Optimization of fibrolytic enzyme production by *Aspergillus japonicus* C03 with potential application in ruminant feed and their effects on tropical forages hydrolysis. Bioprocess and Biosystems, 34, 1027–1038.

Gado H.M., Salem A.Z.M., Robinson P.H., Hassan M. (2009): Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Animal Feed Science and Technology, 154, 36–46.

Gado H.M., Salem A.Z.M., Odongo N.E., Borhami B.E. (2011): Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Animal Feed Science and Technology, 165, 131–136.

Gado H.M., Salem A.Z.M., Camacho L.M., Elghandour M.M.Y., Salazar M.C. (2013): Influence of exogenous enzyme on *in vitro* ruminal degradation of ensiled rice straw with DDGS. Animal Nutrition and Feed Technology, 13, 569–574.

doi: 10.17221/8133-CJAS

Giraldo L.A., Tejido M.L., Ranilla M.J., Carro M.D. (2008): Effects of exogenous fibrolytic enzymes on *in vitro* ruminal fermentation of substrates with different forage : concentrate ratios. Animal Feed Science and Technology, 141, 306–325.

Hristov A.N., McAllister T.A., Cheng K.J. (2000): Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: effects on nutrient digestion in cattle fed a barley grain diet. Journal of Animal Science, 78, 477–487.

Hungate R.E. (1965): The Rumen and Its Microbes. Academic Press, New York, USA.

Khattab H.M., Gado H.M., Kholif A.E., Mansour A.M., Kholif A.M. (2011): The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. International Journal of Dairy Science, 6, 267–277.

Kholif S.M., Gado H.M., Morsy T.A., El-Bordeny N., Abedo A.A. (2012): Influence of exogenous enzymes on nutrient digestibility, blood composition, milk production and its composition as well as milk fatty acids profile in dairy buffaloes. Egyptian Journal of Nutrition and Feeds, 15, 13–22.

Krueger N.A., Adesogan A.T., Staples C.R., Krueger W.K., Kim S.C., Littell R.C., Sollenberger L.E. (2008): Effect of method of applying fibrolytic enzymes or ammonia to Bermuda grass hay on feed intake, digestion, and growth of beef steers. Journal of Animal Science, 86, 882–889.

Le Houerou H.N. (1993): Environmental aspects of fodder trees and shrubs plantation in the Mediterranean Basin. In: Papanastasis V. (ed.): Agriculture A Grimed Research Program: Fodder Trees and Shrubs in the Mediterranean Production Systems: Objectives and Expected Results of the EC Research Contract. Commission of the European Communities, Brussels, Belgium, 11–34.

Makkar H.P.S. (2003): Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of tannin rich feeds. Small Ruminant Research, 49, 241–256.

Masters D.G., Benes S.E., Norman H.C. (2007): Biosaline agriculture for forage and livestock production. Agriculture Ecosystems and Environment, 119, 234–248.

McAllister T.A., Hristov A.N., Beauchemin K.A., Rode L.M., Cheng K.-J. (2001): Enzymes in ruminant diets. In: Bedford M., Partridge G. (eds): Enzymes in Farm Animal Nutrition. CABI Publishing, Wallingford, UK, 273–298.

Morgavi D.P., Beauchemin K.A., Nsereko V.L., Rode L.M., McAllister M., Wang Y. (2000): A trichoderma feed enzyme preparation enhances adhesion of *Fibrobacter succinogenes* to complex substrates but not to pure cellulose. In: 25th Conf. on Rumen Function, Chicago, USA, 33.

Norman H.C., Dynes R.A., Rintoul A.J., Wilmot M.G., Masters D.G. (2004): Sheep production from saline land – productivity from old man and river Saltbush and the value of grain and straw supplements. In: Australian Society of Animal Production (ed.): Animal Production in Australia. Proc. 25th Biennial Conf. of the Australian Society of Animal Production, Melbourne, Australia, 289.

Norman H.C., Masters D.G., Wilmot M.G., Rintoul A.J. (2008): Effect of supplementation with grain, hay or straw on the performance of weaner Merino sheep grazing old man (*Atriplex nummularia*) or river (*Atriplex amnicola*) saltbush. Grass and Forage Science, 63, 179–192.

NRC (1985): Nutrient Requirements of Sheep. 6th Ed. The National Academies Press, Washington, USA.

NRC (2001): Nutrient Requirements of Dairy Cattle. 7th Ed. The National Academies Press, Washington, USA.

Nsereko V.L., Morgavi D.P., Rode L.M., Beauchemin K.A., McAllister T.A. (2000): Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms *in vitro*. Animal Feed Science and Technology, 88, 153–170.

Nsereko V.L., Beauchemin K.A., Morgavi D.P., Rode L.M., Furtado A.F. (2002): Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Canadian Journal of Microbiology, 48, 14–20.

Papanastasis V.P., Yiakoulaki M.D., Decandia M., Dini-Papanastasi O. (2008): Integrating woody species into livestock feeding in the Mediterranean areas of Europe. Animal Feed Science and Technology, 140, 1–17.

Salem A.Z.M., Salem M.Z.M., El-Adawy M.M., Robinson P.H. (2006): Nutritive evaluations of some browse tree foliages during the dry season: secondary compounds, feed intake and *in vivo* digestibility in sheep and goats. Animal Feed Science and Technology, 127, 251–267.

Salem A.Z.M., El-Adawy M.M., Gado H.M., Camacho L.M., Ronquillo M., Alsersy H., Borhami B. (2011): Effects of exogenous enzymes on nutrients digestibility and growth performance in sheep and goats. Tropical and Subtropical Agroecosystems, 14, 867–874.

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

Salem A.Z.M., Szumacher-Strabel M., Lopez S., Khalil M.S., Mendoza G.D., Ammar H. (2012b): *In situ* degradability of soyabean meal treated with *Acacia saligna* and *Atriplex halimus* extracts in sheep. Journal of Animal and Feed Sciences, 21, 447–457.

doi: 10.17221/8133-CJAS

Salem A.Z.M., Gado H.M., Colombatto D., Eghandour M.M.Y. (2013): Effect of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livestock Science, 154, 69–73.

Salem A.Z.M., Buendia-Rodriguez G., Elghandour M.M.Y., Mariezcurrena Berasain M.A., Pena Jimenez F.J., Pliego A.B., Chagoyan J.C.V., Cerrillo M.A., Rodriguez M.A. (2015): Effects of cellulase and xylanase enzymes mixed with increasing doses of *Salix babylonica* extract on *in vitro* rumen gas production kinetics of a mixture of corn silage with concentrate. Journal of Integrative Agriculture, 14, 131–139.

Togtokhbayar N., Salem A.Z.M., Jigjidpurev S., Shinekhuu J., Urantulkhuur D., Nergui D., Odongo N.E., Kholif A.E. (2015): Effect of xylanase on rumen *in vitro* gas production and degradability of wheat straw. Animal Science Journal, doi: 10.1111/asj.12364.

Treacher R.J., Hunt C.W. (1996): Recent developments in feed enzymes for ruminant rations. In: Proc. Pacific Northwest Animal Nutrition Conference, Seattle, USA, 37–54.

Valdes K.I., Salem A.Z.M., Lopez S., Alonso M.U., Rivero N., Elghandour M.M.Y., Dominguez I.A., Ronquillo M.G., Kholif A.E. (2015): Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. The Journal of Agricultural Science, doi: 10.1017/S0021859614000975.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Walker D.J. (1965): Energy metabolism and rumen microorganisms. In: Physiology of Digestion in the Ruminants. Butterworth Inc., Washington, USA.

Wang Y., McAllister T.A., Rode L.M., Beauchemin K.A., Morgavi D.P., Nsereko V.L., Iwaasa A.D., YangW. (2001): Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen simulation technique (Rusitec). British Journal of Nutrition, 85, 325–332.

Warner A.C. (1964): Production of volatile fatty acids in the rumen: methods of measurement. Nutrition Abstracts and Reviews, 34, 339–352.

Yang W.Z., Beauchemin K.A., Rode L.M. (1999): Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. Journal of Dairy Science, 82, 391–403.

Received: 2013–09–09
Accepted after corrections: 2014–09–02

Corresponding Author

Dr. Abdelfattah Zeidan Mohamed Salem, Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico, Toluca P.O. 50000, Mexico
Phone: +52 17 222 965 542, e-mail: asalem70@yahoo.com