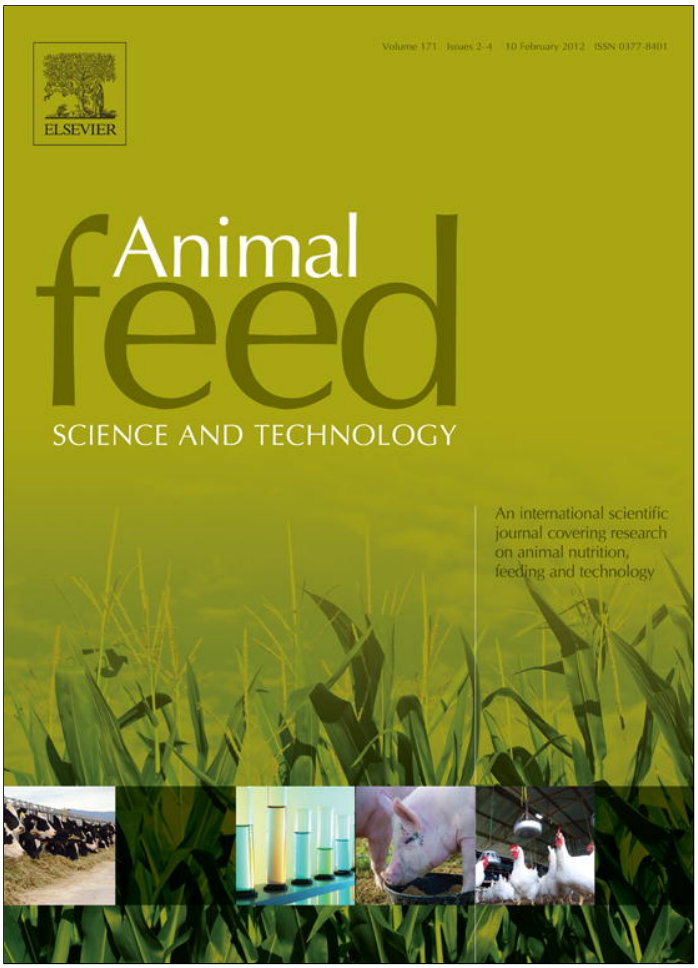

Animal Feed Science and Technology 171 (2012) 128–135

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed *Atriplex halimus* foliages

A.Z.M. Salem[a,e,*], A.A. Hassan[b], M.S. Khalil[b], H.M. Gado[c], H. Alsersy[e], J. Simbaya[d]

[a] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, Mexico*
[b] *Animal Production Research Institute, Ministry of Agriculture, Dokki, Gizza, Egypt*
[c] *Department of Animal Productions, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*
[d] *Department of Animal Science, School of Agricultural Sciences, University of Zambia, P.O. Box 32379, Lusaka, Zambia*
[e] *Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt*

ARTICLE INFO

Article history:
Received 27 May 2011
Received in revised form 28 October 2011
Accepted 28 October 2011

Keywords:
Atriplex halimus
N utilization
Secondary compounds
Exogenous enzymes

ABSTRACT

This study was conducted to assess effects of sun-drying and/or addition of an exogenous enzyme (ENZ) preparation on intake, digestibility of nutrients and recovery values of secondary metabolites (SM) in the gastrointestinal tract of sheep fed *Atriplex halimus* (AH) foliages. A randomized block design for 28 d was used for four experimental treatments based on either fresh (AH-F) or sun-dried (AH-S) *A. halimus* foliages in the absence (−ENZ) or presence (+ENZ) of 10 g/sheep/d of the exogenous of ZADO® enzyme preparation. Three adult sheep weighing 51 ± 2.7 kg were fed for each experimental treatment. The ENZ was added daily with a small amount of concentrate to help balance the dietary metabolizable energy concentration. Nutrient intake and digestibility, N balance and recovery of SM (*i.e.*, total phenolics (TP), saponins (SP), alkaloids (AK), aqueous fraction (AF)) in the gastrointestinal tract were determined. Levels of most nutrients did not differ between AH-F and AH-S foliages, but the AH-S contained less than half of the SM in AH-F. Drying of *A. halimus* and ENZ addition increased (P=0.001) intake as well as OM and NDFom digestibility (P=0.02). Feed intake and digestibility were higher (P=0.01) in AH-S with ENZ addition. Intake of N by sheep fed the treatment diets depended on DM intake as the dietary concentration of N in the diets was similar. Thus AH-S sheep supplemented with ENZ had higher (P=0.001) N intake. Digestibility of N was similar to DM and OM digestibility, and was higher (P=0.03) in AH-S sheep supplemented with ENZ. Drying and ENZ addition to the diet increased (P=0.004) recovery of all SM. The fate of these compounds in the rumen needs to be evaluated considering that SM have been implicated in fiber and protein degradation in the rumen. The study showed that there are beneficial impacts of sun-drying and/or dietary exogenous enzyme addition for sheep fed *A. halimus*.

Abbreviations: ADFom, acid detergent fiber; AH-F, fresh *A. halimus*; AH-S, sun-dried *A. halimus*; AF, aqueous fraction; AK, alkaloids; CP, crude protein; EE, ether extract; ENZ, exogenous enzymes; ME, metabolizable energy; NDFom, neutral detergent fiber; OM, organic matter; SM, secondary metabolites; SP, saponins; TP, total phenolics.

* Corresponding author at: Departamento de Nutrición Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, Mexico. Tel.: +52 1 722 296 55 42; fax: +52 1 722 180 61 94.
E-mail address: asalem70@yahoo.com (A.Z.M. Salem).

0377-8401/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.anifeedsci.2011.10.011

1. Introduction

Tree and shrub forages play an important role in ruminant feeding in arid and semi-arid regions of the northern region of Egypt (Salem et al., 2006, 2007, 2010). In Africa alone, about 52% of cattle, 57% of sheep, 65% of goats and virtually all camels are in the arid and semi-arid regions where fodder trees and shrubs predominate as the main feed resource (Von Kaufmann, 1986). In North Africa, shrub lands cover about 940,000 km^2 of which 65,000, 350,000 and 525,000 km^2 are located in semiarid, arid and desert regions, respectively (Le Houérou, 1989).

Halophyte shrubs of the genus Atriplex tend to predominate in these areas, mostly because of their resistance to drought which may be coupled with low or high ambient temperatures (Uchiyama, 1987). The leaves have a high content of sodium chloride and secondary metabolites (SM: Valderrábano et al., 1996), including tannins, flavonoids, saponins, alkaloids and resins (Makkar, 2003; Salem et al., 2006). The high content of sodium chloride and SM in *Atriplex halimus* tends to reduce fodder palatability and feed intake (Valderrábano et al., 1996) of sheep and goats. In addition to high levels of SM, the nutrient composition of Atriplex species is not well-balanced due to low metabolizable energy (ME) concentrations. The protein fraction in *A. halimus* leaves also contains high levels of non-protein N (Le Houérou, 1992).

Several methods have been devised to alleviate deleterious effects of SM found in tree and shrub fodder foliages. These include treatment with alkalis such as urea, ammonia, calcium hydroxide, sodium hydroxide and potassium hydroxide (Vitti et al., 2005), metal ions and oxidizing agents such as potassium dichromate and potassium permanganate (Makkar, 2003). Despite being effective in overcoming acute toxic effects of tannins (Mueller-Harvey, 2006), chemical methods are expensive, unavailable in rural areas and require expertise for successful application. The chemicals are also corrosive and, if not properly managed, could be poisonous to humans, animals and the environment (Vitti et al., 2005).

Oven, freeze and sun air-drying techniques have also been used to lessen adverse effects of phenols in browse legumes (Stewart et al., 2000; Vitti et al., 2005). However oven and freeze drying methods require expertise, sophisticated equipment and electricity (Ahn et al., 1997; Stewart et al., 2000), which are often not available in rural communities in northern Africa. Though less effective compared to other methods, sun-drying is a simple, inexpensive and eco-friendly technique which uses readily and abundantly available resources (Dzowela et al., 1995). Sun-drying could, therefore, be a more acceptable and feasible alternative for resource-poor beef producers. Dried tree legume leaf-meal diets have improved performance of animals, which was attributed to improved utilization of endogenous N in the rumen and changes in the solubility of protein, and increased rumen escape dietary protein, as well as the amount and profile of amino acid absorbed post-ruminally from leaf meal (Rubanza et al., 2007).

Addition of exogenous enzymes is also a method to improve the nutritive value of tree leaves. Some research has demonstrated that supplementing diets of dairy cows and feedlot cattle with fiber degrading enzymes could improve feed utilization and animal performance by enhancing fiber degradation *in vitro* (Gado et al., 2007), *in situ* (Tricarico et al., 2005; Krueger et al., 2008), and *in vivo* (Gado and Salem, 2008; Gado et al., 2009, 2011; Salem et al., 2011).

Proposed modes of action of direct fed enzymes include solubilization of dietary fiber 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 level of application, as well as the type of animal and its diet, may affect enzyme efficacy. Direct fed enzymes could 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 fiber in the rumen (Tricarico et al., 2005; Giraldo et al., 2008), rumen microbial protein synthesis (Nsereko et al., 2002; Gado et al., 2009) and forestomach digestibility.

The aim of this study was to investigate impacts of sun-drying and/or addition of an exogenous enzyme preparation on nutrient intake and digestibility, as well as N utilization and intestinal recovery of secondary metabolites of *A. halimus* fed to sheep.

2. Materials and methods

2.1. Collection and preparation of A. halimus foliages

Fresh consumable parts of desert shrubs *A. halimus* (AH) consisting of leaves and small twigs were randomly harvested from young and adult wild growing plants by hand plucking. The harvesting was every 3 d during the experimental period and harvested materials were either fed fresh (AH-F) or after drying in the sun on a cement concrete floor for 3–5 d. After drying, leaves and twigs were put into bags and stored in a shed until used as hay (AH-S). Part of the harvested materials were thoroughly mixed and sampled for chemical analysis. Before being subjected to chemical analysis, the samples were dried at 40 °C for 72 h, after which they were ground to pass a 1 mm sieve and stored in a dry environment.

2.2. Animals, management and feeding

Twelve adult male sheep of 51 ± 2.7 kg live weight, were used (3/treatment) to evaluate the effect of processing (fresh (AH-F) or sun-dried (AH-S) of *A. halimus* (*i.e.,* AH) foliages) or addition of exogenous of ZADO® enzyme preparation (*i.e.,* ENZ) on the nutrient intake, digestibility, N utilization and losses of secondary metabolites (*i.e.,* SM). Treatments based on either AH-F or AH-S desert shrub forages of AH in absence (−ENZ) or presence (+ENZ) of 10 g/sheep/d of

the enzyme preparation ZADO® which is a patented product manufactured by the Academy of Scientific Research and Technology in Cairo, Egypt containing a mixture of anaerobic bacteria and their enzymes of cellulases (7.1 unit/g), xylanases (2.3 unit/g), α-amylase (61.5 unit/g), protease (29.2 unit/g) in a powder form obtained through an anaerobic fermentation process. Daily ENZ feeding amounts were added to a small amount of a concentrate. All sheep of each treatment were fed an amount of concentrate to meet 70% of their ME requirements for maintenance (NRC, 1985). It contained whole cotton seed meal (300 g/kg), ground yellow corn grain (355 g/kg), wheat bran (300 g/kg), limestone (30 g/kg), salt (10 g/kg) and 5 g/kg of trace mineral and vitamin premix. Expressed on a DM basis, the premix contained vitamin A, 2,000,000 IU; vitamin D3, 150,000 IU; vitamin K, 0.33 mg; vitamin B1, 0.33 g; vitamin B2, 1.0 g; vitamin B6, 0.33 g; vitamin B12, 1.7 mg; pantothenic acid, 3.33 g; biotin, 33.0 mg; folic acid, 0.83 g; choline chloride, 200 mg; Zn, 11.7 g; Mn, 5.0 g; Fe, 12.5 g; Mg, 66.7 mg; Se, 16.6 mg; Co, 1.33 mg; Cu, 0.5 g; I, 16.6 mg and antioxidant, 10.0 g).

Sheep were fed experimental treatments and the concentrate separately. The concentrate was fed daily at 9:00 h, which was 1 h prior to the treated foliages (*i.e.,* at 10:00 h). Foliages were offered *ad libitum*, as well as a free access to clean drinking water to all sheep during the experiment.

Sheep were housed in individual pens and allowed to adapt to dietary treatments for 15 d. After every feeding, refusals were removed and weighed to calculate intake. The concentrate portion offered prior to the foliage was consumed by all sheep within 60 min of offer on all occasions. Thus, any orts were assumed to be AH foliage.

2.3. Metabolism experiment

After the 15 d of adaptation of sheep to the experimental treatments in individual pens, they were housed in metabolism cages for a 10 d digestibility period. Sheep were acclimatized for 3 d following and then by 10 d for fecal collection of total consumed feeds (*i.e.,* concentrate, foliages), orts, feces and urine to determine apparent nutrient digestibility and N utilization. During the collection period (*i.e.,* 10 d), samples of foliage orts, concentrate and feces were collected and dried at 105 °C as well as a daily representative sample of urine kept at −20 °C until N analysis. Individual samples of foliage refusals, concentrate and feces were collected and dried at 35 °C to determine the SM recovery from the gastrointestinal tract of sheep.

2.4. Chemical composition analysis

All collected samples including AH foliages, orts and feces were dried at 105 °C for 24 h in a forced air oven to determine DM. Ash was determined by igniting samples in a muffle furnace at 550 °C for 4 h. The crude protein (CP) and ether extract (EE) fractions were determined using a Kjeldahl (AOAC, 1990; ID 954.01) and Soxhlet (AOAC, 1990; ID 920.39) method, respectively. Neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and lignin(sa) were determined by methods of Van Soest et al. (1991). NDFom was assayed without use of an alpha amylase but with sodium sulfite. Both NDFom and ADFom are expressed without residual ash.

2.5. Secondary metabolites assay

To determine the content and composition of secondary metabolites (*i.e.,* SM), ~200 mg of ground samples of AH-F, AH-S and the concentrate, as well as feces were extracted. Ten ml of extract was fractionated by funnel separation with a double volume of ethyl acetate (99.7/100, Sigma®–Aldrich) to determine total phenolics (TP) by drying and quantifying the TP layer in the funnel. After TP separation, a double volume of n-butanol (99.9/100, Sigma®–Aldrich), was added to fractionate the saponins (SP) (Makkar et al., 1998). For the alkaloid (AK) extract, dried samples were first extracted with ethanol and then dissolved in dilute hydrochloric acid. This solution was filtered and extracted with petroleum ether to remove fat (Arambewela and Ranatunge, 1991). The remaining solution was considered to be the aqueous fraction (AF) which contained other SM including lectins, polypeptides and starch (Cowan, 1999).

2.6. Statistical analysis

Data on nutrient intake, digestibility and N utilization, as well as SM recovery in the gastrointestinal tract were analyzed according to a randomized block design using the MIXED procedure of SAS (2001), where sheep were the experimental unit as:

$$Y_{ikl} = \mu + T_i + A_k + E_{ikl}$$

where Y expressed every observation of the kth animal in the ith treatment, T (1–4) expressed the treatment effect, A (1–3) expressed the sheep effect and E expressed the experimental error.

Table 1
Nutrient and secondary metabolite levels (g/kg DM) of *A. halimus* foliages and the concentrate mixture.

	A. halimus		Concentrate mixture
	Fresh	Sun-dried	
Chemical composition			
Organic matter	798	785	956
Crude protein	105	101	136
Ether extract	14.4	13.7	28.0
Neutral detergent fiber (om)	544	552	201
Acid detergent fiber (om)	339	340	113
Lignin(sa)	101	105	85
Secondary compounds			
Total phenolics	112.9	54.1	2.1
Saponins	123.8	45.7	5.3
Alkaloids	2.3	1.6	ND[a]
Aqueous fraction	47.5	13.0	1.2

[a] Not detected.

3. Results

The levels of most nutrients, including organic matter (OM), CP, EE and dietary fiber components did not change between fresh (AH-F) and sun-dried (AH-S) foliages. This was expected since drying of forages does not usually result in changes in the level of nutrients. As expected, the concentrate had high concentrations of all nutrients but was lower in fiber components (Table 1). The AH-S contained less than half of all the SM in AH-F, which may be an indication of destruction of SM during drying, or the SM could have been rendered insoluble during drying and thus less prone to extraction. However the concentration of SM in the concentrate was minimal.

Drying foliages of *A. halimus* (*i.e.,* AH) and ENZ addition increased (P=0.01) intake of all nutrients, while the digestibility was increased only in OM (P=0.04) and NDFom (P=0.02). Feed intake and digestibility of nutrients were improved (P=0.01) in AH-S *versus* AH-F sheep with ENZ addition (Table 2).

Overall intake of N by sheep depended on DM intake as the dietary concentration of N among treatments was similar (Table 3). Thus, AH-S sheep supplemented with ENZ had higher (P=0.001) N intake per DM consumption. The N digestibility was similar to DM and OM digestibility and higher (P=0.03) in AH-S sheep supplemented with ENZ. Drying of AH forages also improved digestibility of the same nutrients and this is an indication of improvements in utilization of N in tree fodder forages as a result of post-harvest processing and application of the exogenous enzyme preparation.

The SM were either destroyed or absorbed from the gastrointestinal tract of sheep as very small amounts were excreted in feces (Table 4). Drying and ENZ addition to the diet increased (P=0.004) all SM (*i.e.,* TP, SP, AK, AF) recoveries. It may be that most SM were degraded in the gastrointestinal tract, as only small amounts of the SM were in feces.

Table 2
Feed intake (g/d) and digestibility coefficients (g digested/g ingested) in sheep fed fresh (AH-F) or sun-dried (AH-S) *A. halimus* foliages in the absence (−ENZ) or presence (+ENZ) of the exogenous enzymes preparation.

Processing (Pr)	AH-F		AH-S		SEM	P		
Enzymes (ENZ)	−ENZ	+ENZ	−ENZ	+ENZ		Pr	ENZ	Pr×ENZ
Dry matter (DM) intake								
Atriplex (g/d)	396^{b}	376^{b}	372^{b}	745^{a}	35.3	0.01	0.01	0.01
(g/kg$^{0.75}$)	20.8^{b}	19.6^{b}	19.4^{b}	38.9^{a}	2.15	0.01	0.01	0.01
Concentrate[1]	381^{b}	416^{ab}	471^{a}	434^{ab}	16.8	0.01	0.95	0.06
Total (g/d)	777^{b}	791^{b}	842^{b}	1178^{a}	41.5	0.01	0.01	0.01
(g/kg$^{0.75}$)	40.7^{b}	41.3^{b}	44.1^{b}	61.5^{a}	2.85	0.01	0.01	0.02
DM digestibility	0.619^{d}	0.670^{b}	0.641^{c}	0.724^{a}	0.0410	<0.01	<0.01	0.01
Organic matter								
Intake	680^{b}	697^{b}	742^{b}	999^{a}	34.6	0.01	0.01	0.01
Digestibility	0.618^{d}	0.677^{b}	0.648^{c}	0.723^{a}	0.0321	<0.01	0.01	0.04
Crude protein								
Intake	93^{b}	96^{b}	102^{b}	135^{a}	4,7	0.01	0.01	0.01
Digestibility	0.535	0.603	0.574	0.648	0.0682	0.01	<0.01	0.72
Ether extract								
Intake	16^{b}	17^{b}	18^{b}	22^{a}	0.8	0.01	0.01	0.05
Digestibility	0.669	0.719	0.684	0.728	0.0784	0.17	0.01	0.71
Neutral detergent fiber (om)								
Intake	295^{b}	291^{b}	299^{b}	498^{a}	20.3	0.01	0.01	0.01
Digestibility	0.496^{b}	0.555^{a}	0.558^{a}	0.571^{a}	0.0833	0.01	0.01	0.02

Means in the same row with different superscript letters differ (P<0.05).

Table 3
Nitrogen utilization in sheep fed fresh (AH-F) or sun-dried (AH-S) *A. halimus* foliages in the absence (−ENZ) or presence (+ENZ) of the exogenous enzymes preparation.

Processing (Pr)	AH-F		AH-S		SEM	P		
Enzymes (ENZ)	−ENZ	+ENZ	−ENZ	+ENZ		Pr	ENZ	Pr×ENZ
Nitrogen intake (g/d)								
Atriplex	6.7^{b}	6.3^{b}	6.0^{b}	12.1^{a}	0.59	0.003	0.001	0.001
Concentrate	8.3^{b}	9.1^{ab}	10.3^{a}	9.4^{ab}	0.37	0.012	0.954	0.064
Total	14.9^{b}	15.5^{b}	16.3^{b}	21.6^{a}	0.75	0.010	0.004	0.012
NB (g/d)	1.3	4.2	2.8	6.1	0.19	<0.01	<0.01	0.370
ND (g digested/d)	8.0^{b}	9.3^{b}	9.3^{b}	14.0^{a}	0.40	<0.01	<0.01	0.003
NB/N intake	0.09^{c}	0.27^{a}	0.17^{b}	0.28^{a}	0.011	0.003	<0.01	0.010
NB/ND	0.16^{c}	0.45^{a}	0.30^{b}	0.43^{a}	0.021	0.022	<0.01	0.008

Means in the same row with different superscript letters differ (P<0.05).
NB, N balance; ND, N digestibility.

Table 4
Secondary metabolites intake, excretion in feces (g/d) and values of recovery in the whole gastrointestinal tract of sheep fed fresh (AH-F) or sun-dried (AH-S) *A. halimus* foliages in the absence (−ENZ) or presence (+ENZ) of the exogenous enzymes preparation.

Processing (Pr)	AH-F		AH-S		SEM	P		
Enzymes (ENZ)	−ENZ	+ENZ	−ENZ	+ENZ		Pr	ENZ	Pr×ENZ
Total phenolics								
Intake	45.5^{a}	43.3^{a}	21.1^{b}	41.2^{a}	3.90	0.009	0.051	0.021
In feces	6.87	1.79	0.34	0.26	2.034	0.083	0.241	0.253
Recovery	0.848^{b}	0.958^{b}	0.981^{a}	0.993^{a}	0.0213	0.003	0.001	0.004
Saponins								
Intake	51.0^{a}	48.7^{ab}	19.5^{c}	36.3^{b}	4.27	0.001	0.127	0.054
In feces	1.56	0.18	0.42	0.14	0.783	0.475	0.318	0.450
Recovery	0.971^{b}	0.996^{a}	0.974^{b}	0.997^{a}	0.0474	0.002	0.126	0.093
Alkaloids								
Intake	0.91^{ab}	0.86^{b}	0.58^{c}	1.16^{a}	0.078	0.822	0.009	0.004
In feces	0.01^{b}	0.00^{b}	0.00^{b}	0.02^{a}	0.001	0.001	0.001	0.001
Recovery	0.989^{b}	1.000^{a}	1.000^{a}	1.000^{a}	0.0080	0.887	0.009	0.005
Aqueous fraction								
Intake	192.8	183.6	53.9	101.7	16.32	0.001	0.270	0.119
In feces	3.28	2.07	3.99	3.14	1.128	0.455	0.390	0.877
Recovery	0.983^{a}	0.989^{a}	0.926^{b}	0.970^{b}	0.0170	0.001	0.265	0.033

Means in the same row with different superscript letters differ (P<0.05).

4. Discussion

4.1. Effect of sun drying

Improving the feed intake, digestibility and N utilization with sun-drying of these foliages may be due to improving foliage palatability, reducing SM concentrations and increasing SM recovery in the digestive tract of sheep. It is possible that sun-drying of AH could polymerize SM, especially phenolics, which would then have a lower inhibitory effect on ruminal bacteria. However, in the process of polymerization SM may be complexed and become less available to negatively affect ruminal microorganisms. Sun-drying has been reported to reduce condensed tannins by 15–30% relative to fresh foliage (Ahn et al., 1997) and, in our study, the TP, SP, AK and AF were reduced by 52%, 63%, 30% and 73%, respectively.

Depending on prevailing conditions, drying for a long period at temperatures above 40 °C can cause losses in water soluble carbohydrates due to respiration and decomposition, which in turn reduce digestibility (Ahn et al., 1997). Our reduction of SM levels might be due to the former possibility due to the reducing sugars which would invariably be present in leaf materials and the protection has little to do with secondary plant metabolites. However, that tannins may be implicated was shown by Ahn et al. (1997), as the extractable tannin content of Gliricidia leaves was zero after drying but, when dried leaf meal was fed to sheep, straw intake increased and feed and N digestibility together with N balance also increased. Dalzell (1996) has reported that drying reduces extractable condensed tannins to 25% of those obtained from freeze dried samples. If the concept that tannin–protein complexes were too strongly cross-linked in dried materials to provide a digestible rumen escape protein to the intestines is correct, then a mild Browning reaction in drying catalyzed by sugars in the leaf could make more protein available to the animal post-ruminally.

Sun-drying has been shown to improve palatability in some browse species (Ahn et al., 1997). In practice, it has been recommended to reduce astringency of Acacia species, thereby increasing feed intake by ruminants (Maasdorp et al., 1999). Intake of *Acacia karroo* was, however, shown to increase over time as the animals adapted to the diet (Mapiye et al., 2009). It is, therefore, important to prolong feed adaptation to 8 weeks or mix *A. karroo* leaf-meal with locally available palatable

feed resources such as natural pasture hay before feeding. Feeding browse grass mixtures dilute or reduce toxic effects of tannins and minimize the problem of palatability (Dube and Ndlovu, 1995). Recent studies have confirmed that feeding a combination of sun air-dry *A. karroo* leaf-meal and natural pasture hay improves beef production (Mapiye et al., 2009, 2010).

Sun-drying improves degradability and digestibility of leguminous tree leaves (Lowry et al., 1996). In addition, drying might remove some SM (Norton, 1994), including condensed tannins. Heat or drying appear to produce rumen escape proteins from the soluble proteins of tree foliages, but the mechanisms by which this occurs is not clear and may not necessarily involve reactions with tannins (FAO, 1997), which was also observed in our study. This is the type of response which might be related to a change in the solubility of the protein thereby increasing the rumen escape protein content of the diet. Nolan and Leng (1972) showed that about 60% of the protein in sun-dry lucerne apparently escaped rumen fermentation when the legume was fed as the sole diet to sheep. Tropical legumes are generally richer in tannins than temperate legumes and therefore should function better as sources of rumen escape protein.

4.2. Effect of exogenous enzymes

As demonstrated in our study, use of exogenous microbial enzymes has potential to improve utilization of tree fodder forages by ruminants (McSweeney et al., 2001; Makkar, 2003; Salem et al., 2011). Feeding enzymes is often accompanied by increased feed intake, which may partly 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 and/or extent of digestion (Gado and Salem, 2008; Krueger et al., 2008; Gado et al., 2009, 2011; Salem et al., 2011) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Adesogan, 2005). Increased nutrient digestibility in our study may be a reason for improved feed intake by ENZ addition, which is consistent with previous results with the same mixture (Gado et al., 2007, 2009, 2011; El-Adawy et al., 2008; Gado and Salem, 2008; Salem et al., 2011).

In this study, DM intake and digestibility improved about 46% and 11% respectively with ENZ addition. Other reports have also shown increases in DM, particularly fiber digestibility with fibrolytic ENZ addition (Gado and Salem, 2008; Hristov et al., 2008; Gado et al., 2009, 2011; Salem et al., 2011). Addition of the same ENZ during ensiling also improved digestion of some nutrients (*i.e.,* DM, OM, CP, NDFom, ADFom) compared to a control in dairy cows (Gado et al., 2011). It is well known that digestion of NDFom varies with the chemical composition of the diet (Rodríguez-Prado et al., 2004; Arriola et al., 2011), the size of the indigestible NDFom fraction, the digestion rate of potentially digestible NDFom and the rumen outflow rate (Firkins et al., 1998; Arriola et al., 2011). Fibrolytic ENZ could increase rate of ruminal digestion of the potentially digestible NDFom fraction (Arriola et al., 2011) resulting in increased nutrient digestibility (Arriola et al., 2011; Gado et al., 2009, 2011; Salem et al., 2011). Fibrolytic ENZ supplementation of ruminant diets could also partly reduce digesta viscosity (Hristov et al., 2000) to alter ruminal fermentation (Arriola et al., 2011; Gado et al., 2009, 2011) and/or enhance attachment and colonization to the plant cell wall by ruminal microorganisms (Wang et al., 2001).

Feeding the enzyme preparation may have stimulated and/or increased total viable rumen bacterial numbers because rumen microbial N synthesis was increased, which may be due (at least in part), to increased fiber digestion and an improved capacity of rumen bacteria to digest feed and degrade SM. Although this possibility may not be supported by Nsereko et al. (2002) and Krueger et al. (2007), 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 ME density of diet.

However increased protein degradation and utilization with addition of ENZ may also reflect the more neutral rumen pH with enzyme addition, thereby increasing ruminal bacterial colonization of feed particles (Morgavi et al., 2000; Nsereko et al., 2000). However, Colombatto et al. (2007), working with an enzyme product rich in xylanolytic activity, concluded that exogenous enzymes had higher activity close to pH neutrality and the hypothesis that exogenous enzymes have an effect on digestion when pH values were not optimal for fiber degradation is not supported. Research in the area of exogenous ENZ supplements for ruminants has focused on fibrolytic ENZ preparations and their effects on fiber digestion. However increased ruminal fiber digestion often explains improvements in ruminant productivity resulting from dietary supplementation with fiber degrading enzymes (Arriola et al., 2011; Gado et al., 2009; Holtshausen et al., 2011).

5. Conclusions

Sun-drying of *A. halimus* fodder foliages reduced concentrations of secondary metabolites, but had little effect on the primary nutrient composition. There was an increase in intake and digestibility of DM, OM and NDFom, as well as N utilization, by feeding sheep dried Atriplex foliages supplemented with ENZ. Most secondary metabolites were metabolized in the gastrointestinal tract with only a low amounts being secreted in the feces. There is a need for further studies to determine the functions of secondary metabolites in metabolism. The fate of metabolized compounds and their effect on animal performance also needs to be established.

Acknowledgment

The authors gratefully acknowledge Dr. Peter H. Robinson (Department of Animal Science, University of California, Davis, CA, USA) for his assistance and advices during revision of the manuscript.

References

Adesogan, A.T., 2005. Improving forage quality and animal performance with fibrolytic enzymes. In: Florida Rum. Nutr. Symp, pp. 91–109.

Ahn, J.H., Elliott, R., Norton, B.W., 1997. Oven drying improves the nutritional value of *Calliandra calothyrsus* and *Gliricidia sepium* as supplements for sheep given low-quality straw. J. Sci. Food Agric. 75, 503–510.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Arambewela, L.S.R., Ranatunge, T., 1991. Indole alkaloids from *Tabernaemontana divaricata*. Phytochemistry 30, 1740–1741.

Arriola, K.G., Kim, S.C., Staples, C.R., Adesogan, A.T., 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J. Dairy Sci. 94, 832–841.

Colombatto, D., Mould, F.L., Bhat, M.K., Owen, E., 2007. Influence of exogenous fibrolytic enzyme level and incubation pH on the *in vitro* ruminal fermentation of alfalfa stems. Anim. Feed Sci. Technol. 137, 150–162.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–582.

Dalzell, S., 1996. Sampling of condensed tannin analysis of Leucaena foliage. Leucnet News 2, 5.

Dube, J.S., Ndlovu, L.R., 1995. Feed intake, chemical composition of faeces and nitrogen retention on goats consuming single browse species or browse mixtures. Zim. J. Agric. Res. 33, 133–141.

Dzowela, B.H., Hove, L., Mafongoya, P.L., 1995. Effect of drying method on chemical composition and *in vitro* digestibility of multi-purpose tree and shrub fodders. Trop. Grasslands 29, 263–269.

El-Adawy, M.M., Salem, A.Z.M., Borhami, B.E., Gado, H.M., Khalil, M.S., Abo-Zeid, A., 2008. *In vitro* cecal gas production and dry matter degradability of some browse leaves in presence of enzymes from anaerobic bacterium in NZW rabbit. In: The 9th WRSA World Rabbit Congress, Verona, Italy, June 10–13, pp. 643–647.

FAO, 1997. Bypass proteins from tree foliages. In: Leng, R.A. (Ed.), Tree Foliage in Ruminant Nutrition. , pp. 72–86.

Firkins, J.L., Allen, M.S., Oldick, B.S., 1998. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. J. Dairy Sci. 81, 3350–3369.

Gado, H.M., Salem, A.Z.M., 2008. Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed of orange pulp silage in total mixed ration. In: 59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania, August 24–27, p. 228 (Abstract).

Gado, H.M., Metwally, H.M., Soliman, H., Basiony, A.Z.L., El Galil, E.R., 2007. Enzymatic treatments of bagasse by different sources of cellulase enzymes. In: The 11th Conf. Animal Nutr., Al-Aqsor-Aswan, Egypt on 2 November, 13–18, vol. 10, p. 607.

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. Anim. Feed Sci. Technol. 154, 36–46.

Gado, H.M., Salem, A.Z.M., Odongo, N.E., Borhami, B.E., 2011. Effect of exogenous enzymes ensiled with orange pulp on digestion, blood metabolites and growth performance in lambs. Anim. Feed Sci. Technol. 165, 131–136.

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. Anim. Feed Sci. Technol. 141, 306–325.

Holtshausen, L., Chung, Y.-H., Gerardo-Cuervo, H., Oba, M., Beauchemin, K.A., 2011. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. J. Dairy Sci. 94, 899–907.

Hristov, A.N., McAllister, T.A., Cheng, K.-J., 2000. Intraruminal supplementation with increasing ruminal levels of polysaccharide degrading enzymes: effect on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci. 78, 477–487.

Hristov, A.N., Basel, C.E., Melgar, A., Foley, A.E., Ropp, J.K., Hunt, C.W., Tricarico, J.M., 2008. Effect of exogenous polysaccharide degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. Anim. Feed Sci. Technol. 145, 182–193.

Krueger, N.A., Adesogan, A.T., Staples, C.R., Littell, R.C., Dean, D.B., Krueger, W.K., 2007. The potential to increase the digestibility of tropical grasses with a fungal ferulic acid esterase enzyme preparation. Anim. Feed Sci. Technol. 145, 95–108.

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. J. Anim. Sci. 86, 882–889.

Le Houérou, H.N., 1992. The role of saltbushes (Atriplex spp) in arid land rehabilitation in the Mediterranean Basin: a review. Agroforestry Systems 18, 107–148.

Le Houérou, H.N., 1989. The biology and utilization of shrubs. In: McKell, C.M. (Ed.), The Shrublands of Africa. Academic Press, pp. 119–143.

Lowry, J.B., McSweeney, C.S., Palmer, B., 1996. Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. Aust. J. Agric. Res. 47, 829–842.

Maasdorp, B.V., Muchenje, V., Titterton, M., 1999. Palatability and effect on dairy cow milk yield of dried fodder from the forage trees *Acacia boliviana*, *Calliandra calothyrsus* and *Leucaena leucocephala*. Anim. Feed Sci. Technol. 77, 49–59.

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 Rum. Res. 49, 241–256.

Makkar, H.P.S., Sen, S., Blummel, M., Becker, K., 1998. Effects of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Acacia auriculo-formis* on rumen fermentation. J. Agric. Food Chem. 46, 4324–4328.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Marufu, M.C., Muchenje, V., 2009. Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with *Acacia karroo* leaf-meal. Lives. Sci. 126, 206–214.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E., Muchenje, V., 2010. Meat quality attributes of Nguni steers supplemented with *Acacia karroo* leaf-meal. Meat Sci. 8 (4), 621–627.

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, Oxon, UK, pp. 273–298.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci. Technol. 82, 227–241.

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. Rumen Function, Chicago, IL, USA, p. 33.

Mueller-Harvey, I., 2006. Review: unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.

Nolan, J.V., Leng, R.A., 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 27, 177–194.

Norton, B.W., 1994. Anti-nutritive and toxic factors in forage tree legumes. In: Gutteridge, R.C., Shelton, H.M. (Eds.), Forgage Tree Legumes in Tropical Agriculture. CAB International, Wallingford, Oxford, pp. 202–215.

NRC, 1985. Nutrient Requirements of Domestic animals. National Research Council, Washington, DC, 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*. Anim. Feed Sci. Technol. 88, 153–170.

Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Yang, W.Z., Wang, Y., 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Can. J. Microbiol. 48, 14–20.

Rodríguez-Prado, M., Calsamiglia, S., Ferret, A., 2004. Effects of fiber content and particle size of forage on the flow of microbial amino acids from continuous culture fermenters. J. Dairy Sci. 87, 1413–1424.

Rubanza, C.D.K., Shem, M.N., Bakengesa, S.S., Ichinohe, T., Fujihara, T., 2007. Effects of *Acacia nilotica*, *A. polyacantha* and *Leucaena leucocephala* leaf meal supplementation on performance of Small East African goats fed native pasture hay basal forages. Small Rum. Res. 70, 165–173.

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. Anim. Feed Sci. Technol. 127, 251–267.

Salem, A.Z.M., Robinson, P.H., El-Adawy, M.M., Hassan, A.A., 2007. *In vitro* fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. Anim. Feed Sci. Technol. 138, 318–330.

Salem, A.Z.M., Robinson, P.H., Lopez, S., Gohar, Y.M., Rojo, R., Tinoco, J.L., 2010. Sensitivity of sheep intestinal lactic acid bacteria to secondary compounds extracted from *Acacia saligna* leaves. Anim. Feed Sci. Technol. 61, 85–93.

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. Trop. Subtrop. Agroecosyst. 14, 867–874.

SAS, 2001. Users Guide: Statistics (Release 8.01). SAS Inst. Inc., Cary, NC, USA.

Stewart, J.L., Mould, F., Mueller-Harvey, I., 2000. The effect of drying treatment on the fodder quality and tannin content of two provenances of *Calliandra calothyrsus* Meissner. J. Sci. Food Agric. 80, 1461–1468.

Tricarico, J.M., Johnston, J.D., Dawson, K.A., Hanson, K.C., McLeod, K.R., Harmon, D.L., 2005. The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. Anim. Sci. 81, 365–374.

Uchiyama, Y., 1987. Salt tolerance of *Atriplex nummularia*. Technical Bulletin of the Tropical Agricultural Research Center No. 22. Tropical Agricultural Research Center, Yatabe, Tsukuba, Ibaraki, Japan, p. 69.

Valderrábano, J., Muñoz, F., Delgado, I., 1996. Browsing ability and utilization by sheep and goats of *Atriplex halimus* L. shrubs. Small Rum. Res. 19, 131–136.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Vitti, D.M.S.S., Nozella, E.F., Abdalla, A.L., Bueno, I.C.S., Silva Filho, J.C., Costa, C., Bueno, M.S., Longo, C., Vieira, M.E.Q., Cabral Filho, S.L.S., Godoy, P.B., Mueller-Harvey, I., 2005. The effect of drying and urea treatment on nutritional and anti-nutritional components of browses collected during wet and dry seasons. Anim. Feed Sci. Technol. 122, 123–133.

Von Kaufmann, R., 1986. An introduction to the sub-humid zone of West Africa and the ILCA subhumid zone programme. In: Livestock Systems Research in Nigeria's Sub-humid Zone. Proceedings of the Second ILCA/NAPRI Symposium held in Kaduna, Nigeria, 29 October–2 November 1984. ILCA, Addis Ababa, Ethiopia.

Wang, Y., McAllister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D., Yang, W., 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rusitec). Br. J. Nutr. 85, 325–332.