IN VITRO CAECAL GAS PRODUCTION AND DRY MATTER DEGRADABILITY OF SOME BROWSE LEAVES IN PRESENCE OF ENZYMES FROM ANAEROBIC BACTERIUM IN NZW RABBITS

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ABSTRACT

This work was conducted to assess the nutritive value of four browse leaves (*Acacia saligna*, *Atriplex halimus*, *Lucena leucocephala,* and *Eucalyptus camaldulensis*) grown in the northern region of Egypt using the ceacum content obtained directly after slaughtering of 12 New Zealand White rabbits. ZADO®, enzymic preparation containing cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium (patent No. 22155 of Egypt) was added at 0, 2 or 5 mg/ml of buffered caecal content. *In vitro* caecal gas production after 24 h of incubations ($IVGP_{24}$) and dry matter degradability (IVDMD) were determined. Crude protein content of browse leaves ranged from 105 g/kg DM (*A. halimus*) to 221 g/kg DM (*L. leucocephala*). The highest ether extract (68 g/kg) was in *L. leucocephala*, while this species had the lowest neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin content. *E. camaldulensis* had the highest NDF, ADF and lignin. Total extractable phenolics (TEP), saponins (SAP) and alkaloids (ALK) contents were low in *L. leucocephala* and high in *A. halimus* and *E. camaldulensis*. The $IVGP_{24}$, and IVDMD varied ($P<0.001$) among leaves, with the highest ($P<0.001$) value in *A. halimus*, intermediate in *A. saligna* and *E. camaldulensis,* and the lowest ($P<0.001$) in *L. leucocephala*. When comparing all leaves species, addition of ZADO® improved ($P<0.05$) caecal $IVGP_{24}$, and DMD in all leaves without a significant difference ($P>0.05$) between the two ZADO® levels (2 or 5 mg ZADO®/ml). ZADO® increased the rate of gas production during the first 6 h of incubations. Our results suggest a positive effect of ZADO® addition on browse leaves degradation in rabbits. ZADO® could have a positive influence on the caecal microbial activity and nutrient digestion, as well as the degradation of secondary compounds of the browse leaves.

Key words: Gas production, Dry matter degradability, ZADO®, Ceacal activity, Probiotics, Rabbits.

INTRODUCTION

Tree and shrub leaves are important sources of forage for small ruminants (Salem *et al*., 2006) in the semi-arid conditions of northern Egypt, but the use of locally available foliage in the nutrition of the rabbit is a subject relatively unexplored in tropical countries. This is in spite of the fact that tropical foliages are locally available and at low cost, which is in contrast with the scarcity and often high cost of commercial feeds. However, most tropical browse species contain substantial amounts of phenolic compounds, mainly tannins (Makkar *et al*., 1995; Salem, 2005), as well as other secondary compounds such are phenolics, saponins and alkaloids, lectins, polypeptides, and essential oils (Salem *et al*., 2006, 2007), which can reduce their nutritional value, as most of tannins bind to feed proteins thereby making them unavailable to caecal and ruminal microorganisms. Use of tree and shrub leaves by herbivores may be restricted by negative effects of secondary compounds on digestion (Provenza, 1995; Salem, 2005; Salem *et al*., 2006, 2007). ZADO®, as a product containing cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium, showed a positive effect on ruminant performance and nutrient utilization of low quality forages *in vivo* (Gado, 1997) and *in vitro* (Gado *et al*., 2007). Probiotics are food ingredients which are supposed to stimulate the activity and potentially alter the composition of the gut flora by providing energy to selected species of the microbial community (Guerra *et al*., 2007). Studies on the effect of probiotics for improving the nutritive utilization of tree and shrubs leaves in rabbits are limited. Therefore, the objective of the current experiment was to evaluate the utilization of four browse leaves using the ceacal contents of rabbits in the presence of ZADO® which is a mixture of cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium.

MATERIALS AND METHODS

Samples of leaves of the browse species *Acacia saligna*, *Atriplex halimus*, *Leucaena leucocephala*, and *Eucalyptus camaldulensis* were randomly and manually harvested from different parts of both young and mature leaves. Leaf samples were dried at 40°C for 72 h in a forced air oven in triplicate to constant weight, ground in a hammer mill to pass 1 mm sieve, and stored in plastic bags for subsequent determination of chemical components, secondary compounds and *in vitro* fermentation using the caecal contents from NZW rabbits. Methods of chemical analyses, i.e. ash, N, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin, as well as the secondary compounds, i.e. total extractable phenolics (TEP), saponins (SAP), alkaloids (ALK), and aqueous fraction (AF) were as previously described (Salem *et al*., 2006).

In vitro caecal gas production (IVGP) was determined according to Menke *et al*. (1979). Approximately 500 mg of dry matter (DM) of each sample were placed in 60 ml syringes with 0, 2, or 5 mg/ml of caecal buffered liquor of ZADO®, containing cellulase (7.1 U/g), xylanase (2.3 U/g), α-amylase (61.5 U/g) and proteases (29.2 U/g). ZADO® is the patent No. 22155 of Egypt (Molecular Biology Laboratory of the Ain Shams University in Cairo), prepared according to the procedure of Gado (1997). The piston of each syringe was closed with a hard rubber to prevent inflow of water into the syringe during incubation. The experiments were carried out using 12 NZW rabbits. Rabbits were about 14 weeks of age and 2235 g of LW. Rabbits were fed a concentrate mixture (Table 1). Caecal contents were collected directly after slaughtering of rabbits in three runs (four rabbits for each). Caecal contents were combined, homogenized and kept at 39°C in a water bath, flushed with CO_2 before use, and diluted (1:4 w/v) with the culture medium of Makkar et al. (1995) containing bicarbonate buffer, macro-mineral, micro-mineral, resazurine and reducing solutions. Caecal buffered liquor (30 ml) was pipetted into each syringe and syringes were immediately placed into a water bath at 39°C. Gas volumes were recorded at 2, 4, 6, 10, 12, and 24 h of the incubation. Blank syringes were incubated in each run. At the end of the incubation (24 h), content of each syringe was centrifuged at 15,000 *g* for 20 min at 4°C. The residual moisture was removed by drying the tubes overnight at 60°C, and then tubes were weighed and dry matter degradability (IVDMD) calculated from differences between initial and residue weights, minus blank tubes. The study was completed at the laboratory of Animal Production, Alexandria University.

Rate of gas production (RGP) and gas yields (GY) were calculated as follow: RGP_4 h (ml/g DM/h) = (volume of gas produced at 6 h − volume of gas produced at 2 h)/4 × sample weight (g). Gas yields (GY_{24} h) were calculated as the volume of gas produced after 24 h (ml gas/g DM) of incubation divided by the amount of substrate apparently degraded. Gas production readings data (ml/0.5g DM) were fitted using the NLIN option of SAS (1999) to the model of France et al. (2000) as: $G = b \times (1 - e^{-k(t-L)})$, where: 'G' was the volume of gas production at time 't'; 'b' was the asymptotic gas production (ml/0.5 g DM); 'k' was the rate of gas production (per h) from the slowly fermentable feed fraction 'b', and 'L' was the discrete lag time prior to gas being produced. Data were analyzed as a 4 x 3 factorial experiment (4 browse leaves species × 3 treatments: 0, 2 and 5mg of ZADO®/ml) using the 'GLM' option of SAS (1999) with methods of Steel and Torrie (1980), to determine differences due to tree species and ZADO®. In the case of significant differences, Duncan (1995) multiple-range test was used to separate means within species.

RESULTS AND DISCUSSION

Crude protein content of the browse leaves ranged from 105 to 221 g/kg DM. *L. leucocephala* had the lowest NDF, ADF and lignin contents, *E. camaldulensis* had the highest, and *A. saligna* and *A. halimus* the intermediate. Secondary compounds, i.e. total extractable phenolics (TEP), saponins (SAP) and alkaloids (ALK)) were at low concentration in *L. leucocephala* (36, 3 and 0.2 g/kg DM, respectively) and at high concentration in *A. halimus* (113, 124 and 2.3 g/kg DM, respectively) as well as in *E. camaldulensis* (102, 15 and 5 g/kg DM, respectively). Aqueous fraction (AF) of lectins, polypeptides and starch, was higher in *A. halimus* than in the other browse species. Tannins (TEP) concentrations were higher than 50 g/kg of DM in all leaves except those of *L. leucocephala* (Table 1). However, high levels of secondary compounds may reduce the feed intake, impair the nutrient digestibility or even be toxic in rabbits (Sreekanth *et al*., 2006). Some variation among different browse leaves could be due to genotypic characteristics in relation to the type of secondary compounds (Salem *et al*., 2006). In Figure 1 and Table 2, caecal fermentation parameters (i.e. $IVGP_{24}$ and its parameters B, k, L; and IVDMD) demonstrate differences in nutritional value of the four shrub species that are closely related to their chemical composition (Salem, 2005). The high $IVGP_{24}$ and IVDMD in *A. halimus* suggest a higher extent of fermentation in the first 24 h of fermentation in comparison with the other leaves, especially with *L. leucocephala*. Differences in $IVGP_{24}$ and IVDMD among the leaves could be due to the different content and nature, of their fibre (Rubanza *et al*., 2003) and secondary compounds (Salem *et al*., 2007). Indeed, the higher fibre level, as well as high (P<0.05) levels of secondary compounds in *L. leucocephala* (Salem *et al*., 2006), are almost certainly responsible for reduced gas production in comparison with the other foliages.

Table 1: Nutrient and secondary compound levels (g/kg DM) of browse leaves and also the commercial concentrate fed to NZW rabbit during the experiment

	A. saligna	*A. halimus*	*L. leucocephala*	*E. camaldulensis*	Concentrate[a]
Chemical composition[b]					
OM	902	798	901	945	903
CP	149	105	221	154	207
EE	39	14	68	41	62
CF	239	324	203	98	97
NDF	389	544	372	615	350
ADF	302	339	208	542	148
lignin	134	101	121	192	30
Secondary compounds[c]					
TEP	61	112.9	35.74	102.31	ND[d]
SAP	23.5	123.8	3.14	14.58	ND
ALK	3.19	2.3	0.2	5.02	ND
AF	68	47.5	7.28	2.4	ND

[a]Commercial concentrate used in NZW rabbit feeding during the *in vitro* experiment; [b]OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; lignin, acid detergent lignin; [c]TEP, total extractable phenolic compounds; SAP, saponins; ALK, alkaloids; AF, aqueous fraction (lectins, polypeptides, starch; Cowan, 1999); [d]below 0.01 g/kg DM

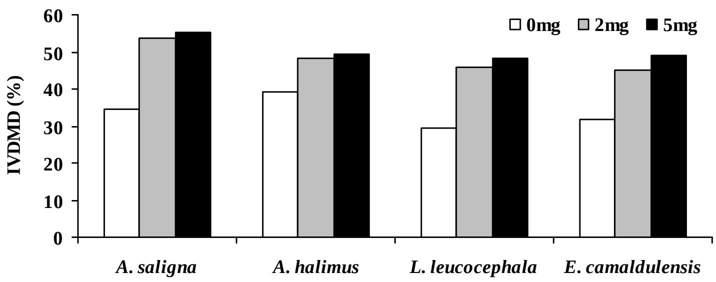

Figure 1: *In vitro* caecal dry matter degradability (IVDMD) of the four shrub species in the presence of 0, 2, or 5 mg of ZADO®[1] /ml of caecal buffered liquor after 24h of incubation in NZW rabbit. [1]ZADO® is a mixture of cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium

Increased $IVGP_{24}$ and IVDMD in *E. camaldulensis* than in *L. leucocephala* may be due to its lower condensed tannins concentration in leaves. However, differences in degradability among browses could be also due to the different extent of lignification of NDF (Van Soest, 1994). Salem (2005) reported a similar relationship between $IVGP_{24}$ and NDF using the rumen fluid of sheep, cattle and buffalo. Low nutritive value of *L. leucocephala* and *E. camaldulensis* could be due to its NDF binding with polyphenolics (Ndlovu and Nherera, 1997). Probiotics are live bacteria or enzymes mixtures, used as feed supplements (Yoon and Stern, 1995). The original concept of feeding live microbes to livestock was based primarily on potentially beneficial effects, including improved establishment of beneficial gut microflora (Fuller, 1999). Addition of low levels of the enzymes mixture from an anaerobic bacterium (ZADO®) improved (P<0.001) parameters of the caecal microbial activity ($IVGP_{24}$ and IVDMD) in all cultures supplied with browse leaves. The improvement varied between the browse leaves and was more pronounced at higher addition of ZADO®. Presumably, this could be due to the beneficial effect of ZADO® on fibre hydrolysis (Gado *et al*., 2007; Juskiewicz *et al*., 2007). Addition of the exogenous fibrolytic enzyme mixture such ZADO® improved *in vitro* rumen fermentation activity and cell wall digestibility of alfalfa stems (Colombatto *et al*., 2007). However, the mode of action of live microbials in the rumen and the caecum is not completely understood. The presence of lactate-producing bacteria is thought to help the caecal and ruminal microflora to adapt to the presence of lactic acid (Beauchemin *et al*., 2003; Guerra *et al*., 2007; Marinho *et al*., 2007). Beauchemin *et al*. (2003) reported that the supplementation of the diet with *E. faecium* increased (P<0.05) the proportion of propionate and decreased (P<0.10) proportion of butyrate in ruminal fluid compared with the control. This may be explained by stimulating of lactic acid-utilizing bacteria, which produce propionate.

Table 2: *In vitro* caecal gas production $IVGP_{24}$ (gas production after 24 h of incubation, in ml/500mg DM)), gas production rate (ml/g DM at 2, 4, 5 and 6 h), gas yield (GY_{24h}, ml/g DM disappeared), and gas production parameters of leaves of four shrub species in the presence of 0, 2, or 5 mg of ZADO®[1] per ml of caecal buffered liquor after 24h of incubation in NZW rabbits

Species (S)	*A. saligna*			*A. halimus*			*L. leucocephala*			*E. camaldulensis*			SEM	Probability		
ZADO® (Z)	0mg	2mg	5mg	0mg	2mg	5mg	0mg	2mg	5mg	0mg	2mg	5mg		S	Z	S×Z
$IVGP_{24}$	33.9^{b}	75.7^{a}	79.9^{a*}	45.3^{b}	73.9^{a}	75.5^{a*}	30.5^{b}	69.6^{a}	78.2^{a**}	36.7^{b}	57.8^{a}	73.4^{a*}	2.86	0.08	<0.01	0.31
Rate of gas production																
RGP_{2h}	10.4	15.1	12.1	8.2	13.3	12.2	10.2	13.9	12.4	9.1	12.7	11.2	3.79	0.87	0.16	0.89
RGP_{4h}	8.8	10.2	14.1	6.6	13.4	13.0	5.7	14.1	15.0	5.6^{b}	14.1^{a}	15.6^{a*}	1.59	0.94	0.01	0.55
RGP_{5h}	8.7	12.5	13.7	7.8^{b}	14.2^{a}	13.2^{a*}	5.0^{b}	14.3^{a}	14.0^{a*}	6.2	6.3	14.0	1.55	0.26	0.01	0.23
RGP_{6h}	8.1	12.2	12.6	7.2^{b}	14.2^{a}	12.4^{a*}	4.3^{b}	15.2^{a}	11.9^{a*}	5.6	6.7	13.2	1.43	0.27	0.01	0.13
GY_{24h}	137^{b}	170^{a}	173^{a*}	151^{b}	173^{a}	174^{a*}	117^{b}	160^{a}	166^{a***}	134^{b}	158^{a}	168^{a**}	37.1	0.01	<0.01	0.14
Gas production parameters[2]																
B	58.7^{b}	107.8^{a}	122.2^{a**}	83.8^{b}	123.4^{a}	116.6^{ab*}	57.1^{b}	$124..2^{a}$	118.6^{a*}	55.7^{c}	92.3^{b}	121.9^{a**}	6.91	0.13	<0.01	0.21
k	0.19	0.21	0.20	0.14	0.20	0.22	0.23	0.24	0.22	0.24	0.24	0.22	0.126	0.40	0.46	0.40
L	1.9	2.3	1.9	1.0^{b}	2.0^{a}	2.4^{a*}	0.7	2.2	1.9	0.9^{b}	2.1^{a}	2.3^{a*}	0.32	0.61	0.04	0.55

[1] ZADO® is a mixture of cellulases, xylanases, α-amylase and proteases from an anaerobic bacterium. [2]'B' is the asymptotic gas production (ml/0.5g DM); 'k' is the rate of gas production (/h); 'L' is the initial delay before gas production begins (h). a, b, and c are superscripts following means within a row and foliage species which indicate differences at *P<0.05, **P<0.01, ***P<0.001

CONCLUSIONS

In Egypt, which is a semi-arid country, the incorporation of shrubs and tree leaves into rabbit diet is restricted. Probiotics could improve the utilization of nutrients of the browse leaves *in vitro* as shown in increasing the caecal gas production and the degradability of dry matter. *In vivo* experiments are required to complete this work using a large scale of animals. Our findings suggest a positive effect of ZADO® addition to browse leaves in rabbits. ZADO® could have a positive effect on the activity of caecal microorganisms in nutrient digestion and degrading secondary compounds of browse leaves.

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