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Short communication

Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs

H.M. Gado[a], A.Z.M. Salem[b,d,*], N.E. Odongo[c], B.E. Borhami[b]

[a] Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[b] Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Egypt
[c] Animal Production and Health Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
[d] Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, km 67.5 Carr. Toluca-Tejupilco, Estado de México, 51300, Mexico

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ABSTRACT

Twenty-four Ossimi male lambs were used to evaluate effects of inclusion of ensiled orange pulp (EOP) in lamb diets either with or without addition of exogenous enzymes (ENZ) of ZADO® on digestion and growth performance. Lambs (21.1 ± 1.01 kg body weight (BW)) were assigned to one of three groups of 8 animals/group in a randomized complete block design being: Control (basal diet with 0 g/kg EOP), EOP (Control with 150 g/kg EOP) or EOP + ENZ (EOP with 5 g/kg of ZADO®) in a 90-day experiment. Ensiling the orange pulp increased the crude protein, ether extract and metabolizable energy of the silage by 29, 46 and 8%, respectively, and reduced the secondary metabolites, such as total phenolics, saponins and alkaloids. Silage lactic acid and ethanol were increased by 35% and 54%, respectively for EOP and EOP with ENZ, but all silage quality parameters were in the normal range. Concentration of NH_3–N before feeding was decreased ($P < 0.05$) by 11 and 13% in EOP and EOP + ENZ, respectively, whereas at 3 and 6 h after feeding ruminal VFA concentration was increased ($P < 0.05$) by 23 and 9% respectively, only in EOP + ENZ lambs. NDFom intake was increased ($P = 0.036$) by 52 and 59%, whereas the ADFom increased ($P = 0.032$) by 8 and 11% in EOP and EOP + ENZ lambs, respectively. Nutrients digestion were higher ($P < 0.05$) in EOP + ENZ than EOP lambs. Digestible DM was increased by 18%, whereas the fiber fractions (NDFom and ADFom) were increased by 93 and 47% with similar EOP + ENZ. DM intake among groups, whereas feed efficiency was higher ($P = 0.042$) by 19 and 31% in EOP and EOP + ENZ lambs compared to control diet. Live weight gain increased ($P = 0.038$) by 92% in EOP + ENZ lambs whereas it increased by 54% in EOP lambs. Addition of EOP to the diet improved feed efficiency and live weight gain suggesting a good quality feed, which could probably be used to replace a part of the concentrate in ruminant diets.

1. Introduction

Citrus pulp is an industrial by-product, used mainly in ruminant diets (Gohl, 1981), and could be fed fresh, ensiled or dried. Fresh citrus pulp, due to its high moisture content, cannot be stored for long and so ensiling is a practical option

Abbreviations: ADFom, acid detergent fiber; BW, body weight; CP, crude protein; DM, dry matter; ENZ, exogenous enzymes of ZADO®; EOP, ensiled orange pulp; NDFom, neutral detergent fiber; OM, organic matter.

* Corresponding author at: Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México, km 67.5 Carr. Toluca-Tejupilco, Estado de México 51300, Mexico. Tel.: +52 1 7162665171; fax: +52 1 7162665171.
E-mail address: asalem70@yahoo.com (A.Z.M. Salem).

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Table 1
Ingredient and chemical composition of the lamb diets.

	Diets[a]		
	Control	EOP	EOP + ENZ
Ingredients (g/kg)			
Yellow corn grain	300	230	230
Molasses, liquid	5.8	0	0
ZADO®	0	0	5
Cottonseed cake	165	90	90
Soybean meal	120	120	120
Berseem (*Trifolium alexandrinum*) hay	150	150	150
Ensiled orange pulp	0	150	150
Rice straw	231	231	230
Salt	6	6	5
Limestone	20.2	21	18
Mineral/vitamin premix[b]	2	2	2
Chemical composition (g/kg DM)			
Organic matter	914	918	919
Crude protein	125	127	126
Ether extract	26	36	35
Neutral detergent fiber (om)	296	310	307
Acid detergent fiber (om)	201	221	223
Lignin (sa)	101	116	112
Metabolizable energy[c]	2.49	2.49	2.64

[a] Containing/kg of diet: control (0 g/kg EOP); EOP (150 g/kg EOP); and EOP + ENZ (150 g/kg EOP with 5 g/kg ZADO®).
[b] Mineral/vitamin premix(per kg): Ca, 190 g; P, 115 g; Mg, 63 g; Cl, 167 g; K, 380 g; Na, 70 g; S, 53 g; Co, 3.3 mg; Cu, 197 mg; Fe, 360 mg; Mn, 900 mg; Se, 2 mg; Zn 810 mg; Vit. A 940 1000 IU; Vit. D 165 1000 IU; Vit. E 374 1000 IU.
[c] MJ/kg DM calculated according to Nsahlai et al. (2004).

to facilitate year-round feeding, particularly in the dry season when feeds are often scarce. These by-products (*i.e.*, citrus pulps) are widely available in Mediterranean countries, especially Egypt, and it is inexpensive relative to its nutritive value (Guessous et al., 1989; Gado et al., 2009). It contains a relatively high amount of pectin and soluble carbohydrates therefore dried citrus pulp has successfully been used to replace cereals in ruminant diets (Fegeros et al., 1995; Piquer et al., 2009). Use of citrus pulp has been associated with beneficial effects on ruminal fermentation, fiber digestion and microbial protein synthesis (Miron et al., 2001; Gado et al., 2009). Beneficial effects have also been reported in dairy cattle (Gado et al., 2009) and in small ruminants (Fegeros et al., 1995; Aregheore, 2000). Supplementation of low quality feeds with citrus pulps is therefore a strategy to improve voluntary feed intake and digestibility, that has been attributed to increased rate of forage digestion and passage (Scerra et al., 2001).

Research in the area of exogenous enzyme supplements for ruminants has focused on fibrolytic enzyme preparations and their effects on fiber digestion. In addition increased ruminal fiber digestion often explains improvements in ruminant productivity resulting from dietary supplementation with fiber degrading enzymes (Gado et al., 2009; Arriola et al., 2011; Holtshausen et al., 2011). Such enzymes have been shown to improve growth of steers fed some dry forages (Beauchemin et al., 1995), and milk production of dairy cows (Gado et al., 2009; Arriola et al., 2011; Holtshausen et al., 2011). However, information is scarce on effects of addition of exogenous enzymes during ensilage of citrus pulp on performance of ruminants. The objective of this experiment was to evaluate the effect of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs.

2. Materials and methods

2.1. Animals, management and feeding

Twenty-four Ossimi male lambs of 20.9–21.4 kg body weight (BW) were used in a 90-day experiment and were randomly assigned to three treatment groups (8 animals of each) in a complete block design. Treatments (Table 1) were – Control group 0 g/kg EOP; EOP 150 g/kg EOP and EOP + ENZ was (150 g/kg EOP plus 5 g/kg ZADO®). For the EOP, it was ensiled fresh orange pulp with chopped 200 g/kg of chopped rice straw (*i.e.*, 3–5 cm) in a ratio 80:20 (DM) to limit ensiling losses due to the high moisture content of the orange pulp. After mixing, all the contents were transferred to a baling machine and pressed together into a plastic raving machine to remove air before ensiling for 30 days. The same process was used for the EOP + ENZ, except for the extraction of some EOP fluid to mix and dissolve the ZADO. ZADO® is a patented product manufactured by the Academy of Scientific Research and Technology, Egypt, and contains a mix 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. Ingredients and chemical composition of the diets are presented in Table 1. The concentrates were mixed and given separately from the silage to lambs. Lambs were fed the silage offered *ad libitum*. Rations were fed twice daily in equal portions at 8:00 and 16:00 h. Daily feed intake was calculated after weighing residues during the experimental period. Fresh water was always available and the sheep were weighed monthly before the morning feeding.

2.2. Metabolism study

During the last day of the experiment, four lambs from each experimental group were acclimatized to metabolism cages for 3 days and total feces collected at 8:00 h for seven consecutive days from each animal. A solution of 100 ml/l concentrated H_2SO_4 and 100 ml/l formalin was added to representative samples prior to drying in an oven at 60 °C for 24 h. Dried feces samples from each sheep was mixed and stored at −18 °C for later chemical analysis.

2.3. Ruminal activity trial

Rumen contents were sampled from four animals in each group by stomach tube from all lambs on the last 2 days of the experiment. Samples were collected before the morning feeding (*i.e.*, $t = 0$), 3 and 6 h after the morning feeding. Rumen contents were filtered through four layers of cheesecloth and used immediately for the measurement of pH. Strained rumen liquor was stored in glass bottles (45 ml) with a few drops of toluene and paraffin oil to cover the surface and stored at −18 °C.

2.4. Analytical methods

Chemical component analysis of feed and faecal samples included dry matter (DM), crude protein (CP), ether extract (EE) and ash content according to (AOAC, 1990; ID: 07.007, 920.39, 923.03 and 954.01, respectively). Neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and lignin (sa) were also determined. NDFom was assayed without the use of α-amylase but with sodium sulfite (Van Soest et al., 1991). Both NDFom and ADFom are expressed without residual ash. Secondary metabolites (*i.e.*, total extractable phenolics, saponins, alkaloids and aqueous fraction) in EOP samples, were assayed as described by Salem et al. (2006). Fresh silage was extracted and used for pH, lactic acid, total and individual VFA (acetic, propionic and butyric acids), ethanol (Mo and Tjørnholm, 1978) and NH_3–N determination (Krizsan and Randby, 2007). The concentration of NH_3–N in rumen fluid was determined by a Kjeldahl distillation method according to AOAC (1995; ID: 954.01) and ruminal total volatile fatty acids (VFA) were determined by steam distillation as described by Warner (1964).

2.5. Statistical analysis

Data were analyzed using SAS (1999) software and Tukey's test for mean separation. Rumen liquor data was analyzed as repeated measures using the model:

$$Y_{ijk} = \mu + T_i + A_j(T_i) + S_k + (T \times S)_{ik} + E_{ijk}$$

where, Y expressed every observation of the jth animal in the kth sampling time given ith treatment, T (1–3) expressed the treatments effect, $A(T)$ expressed the animal within treatments, S (1–2) expressed the sampling time effect, $T \times S$ expressed the interaction between the treatments and sampling times effect and E expressed the experimental error.

Data on nutrients intake, digestibility coefficients, growth performance and feed efficiency were analyzed in completely randomized design using the model:

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

where, Y expressed every observation of the kth animal in the ith treatment, T (1–3) expressed the treatment effect, A (1–84) expressed the animals effect and E expressed the experimental error.

3. Results

Crude proteins (CP), ether extract (EE) and metabolizable energy were increased following ensiling of orange pulp by 29, 46 and 8%, respectively. All secondary metabolites in the orange pulp reduced following addition of enzymes (*i.e.*, ENZ) and during ensiling (Table 2). Silage quality parameters (*i.e.*, pH, lactic acid, butyric acid, ethanol and NH_3–N) were all in the normal range and trace amount of butyric and propionic acids were detected. Silage lactic acid and ethanol were increased by 35% and 54%, respectively with the ensiled of ENZ with EOP, whereas the NH_3–N was reduced by 9% (Table 2).

NH_3–N concentration before feeding was decreased ($P < 0.05$) by 11 and 13% in EOP and EOP + ENZ, respectively, whereas at 3 and 6 h after feeding ruminal VFA concentration was increased ($P < 0.05$) by 23 and 9% respectively, only in EOP + ENZ lambs (Table 3). The intake of nutrients was not changed with the EOP or EOP + ENZ lambs, except the fiber fractions (*i.e.*, NDFom and ADFom). NDFom intake was increased ($P = 0.036$) by 52 and 59%, whereas the ADFom increased ($P = 0.032$) by 8 and 11% in EOP and EOP + ENZ lambs, respectively. Digestible fraction of all nutrients, except EE, were increased ($P < 0.05$) in EOP and EOP + ENZ lambs. Digestible DM was increased by 18%, whereas the fiber fractions (NDFom and ADFom) were

Table 2
Primary and secondary metabolites, as well as silage quality of the ensiled orange pulp (EOP), the presence (EOP + ENZ) or absence (EOP) of an exogenous enzymes mixture[a].

	EOP	EOP + ENZ
Primary compounds (g/kg DM)		
Organic matter	920	920
Crude protein	73	94
Ether extract	46	67
Metabolizable energy[b]	2.39	2.58
Secondary metabolites (g/kg DM)		
Crude extract	299	184
Total phenolics	167	99
Saponins	59	31
Alkaloids	2.7	1.8
Aqueous fraction	55	197
Silage quality		
pH	3.85	3.78
Lactic acid (g/kg DM)	42.4	57.1
VFA (meq/100 g silage)	4.63	4.11
Acetic acid (g/kg DM)	11.6	15.3
Propionic acid (g/kg DM)	0.50	0.70
Butyric acid (g/kg DM)	0.30	0.20
Ethanol (g/kg DM)	13.0	20.0
Ammonia–N (mg/g of CP)	58	53

[a] Containing/kg of diet: EOP (150 g/kg EOP without addition of ZADO®) and EOP + ENZ (150 g/kg EOP with 5 g/kg ZADO®).
[b] MJ/kg DM calculated according to Nsahlai et al. (2004).

Table 3
Ruminal volatile fatty acids (VFA, meq/dl) and ammonia N (NH_3–N, mg/dl) concentrations of lambs fed a control diet, control diet with the addition of ensiled orange pulp (EOP) or EOP and enzymes (EOP + ENZ).

	Diets[a]			SEM	P value
	Control	EOP	EOP + ENZ		
Total VFA					
0 h	7.65	8.39	9.14	1.323	0.35
3 h	8.89b	9.11b	10.93a	0.424	0.042
6 h	8.54b	7.78b	9.32a	0.560	0.036
NH_3–N					
0 h	17.51a	15.73b	15.22b	0.791	0.035
3 h	20.56	22.14	23.61	0.974	0.28
6 h	18.9	17.94	18.73	0.871	0.33

a,b Means in the same row with different letters differ significantly ($P < 0.05$).
[a] Containing per kg of diet: control (0 g/kg EOP), EOP (150 g/kg EOP) and EOP + ENZ (150 g/kg EOP with 5 g/kg ZADO®).

increased by 93 and 47% with the ensiled of EOP with ENZ (Table 4). DM intake was similar among groups, whereas feed efficiency was higher ($P = 0.042$) by 19 and 31% in EOP and EOP + ENZ lambs compared to control diet. Live-weight gain increased ($P = 0.038$) by 92% in EOP + ENZ lambs whereas it increased by 54% in EOP lambs (Table 5).

4. Discussion

4.1. Ruminal fermentation

Ruminal fermentation improvement in lambs fed EOP was perhaps due to the beneficial effects of orange pulp on ruminal microbial activity. Higher increase in VFA concentration with ENZ addition to EOP diet (Table 3) may be due to the beneficial effects of enzymes on fiber hydrolysis and rumen fermentation activity (Gado et al., 2009; Arriola et al., 2011) or due to lower secondary metabolite concentrations in diets (Table 2). However, orange pulp has a high concentration of pectin, which could lead to a fast rate of degradation in rumen, releasing energy for a rapid microbial growth and creating better rumen conditions for fiber fermentation and its high level of potential degradable dry matter (Silva et al., 1997; Arriola et al., 2011). All treatments resulted in a higher rumen pH after feeding although the relative magnitude of the increase was the same across treatments. Moreover, average pH values were relatively high (6–7.2) and well in excess of the suggested values for maintenance of optimal ruminal microbial synthesis and fiber degradation (Russell et al., 1992) suggesting that citrus pulp may not depress ruminal pH as much as starchy concentrates.

Table 4
Intake (g/d) and digestible nutrients of lambs fed a control diet, control diet with the addition of ensiled orange pulp (EOP) or EOP and enzymes (EOP + ENZ).

	Diets[a]			SEM	*P* value
	Control	EOP	EOP + ENZ		
DM	780	790	820	5.7	0.28
Digestible DM	538c	580b	636a	4.3	0.039
OM	700	710	730	3.8	0.19
Digestible OM	520b	549b	580a	3.1	0.040
CP	94.5	96	100	2.4	0.26
Digestible CP	79b	82b	87a	1.9	0.047
EE	2.0	2.2	2.7	0.9	0.30
Digestible EE	1.6	1.9	2.1	0.5	0.24
NDFom	207c	315b	330a	9.6	0.036
Digestible NDFom	102c	156b	197a	7.2	0.038
ADFom	165c	179b	183a	6.8	0.032
Digestible ADFom	68c	79b	100a	4.5	0.047

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber; ADFom, acid detergent fiber.
a,b,c Means in the same row with different letters differ significantly ($P < 0.05$).
[a] Containing per kg of diet: control (0 g/kg EOP), EOP (150 g/kg EOP) and EOP + ENZ (150 g/kg EOP with 5 g/kg ZADO®).

Table 5
Live weight of lambs fed a control diet, control diet with the addition of ensiled orange pulp (EOP) or EOP and enzymes (EOP + ENZ).

	Diets[a]			SEM	*P* value
	Control	EOP	EOP + ENZ		
No. of lambs	8	8	8	–	–
Initial live-weight (kg)	21.4	20.9	21.2	0.25	0.31
Final live-weight (kg)	32b	38ab	42a	5.1	0.033
Live-weight gain (kg/d)	0.13b	0.20ab	0.25a	0.062	0.038
Feed efficiency (kg DM/kg) live-weight gain	6.2c	4.0b	3.2a	0.07	0.042

a,b,c Means in the same row with different letters differ significantly ($P < 0.05$).
[a] Containing per kg of diet: control (0 g/kg EOP), EOP (150 g/kg EOP) and EOP + ENZ (150 g/kg EOP with 5 g/kg ZADO®).

4.2. Nutrients digestion

Improvement of the digestion of nutrients (*i.e.*, DM and fiber fraction of NDFom and ADFom – Table 4) was probably due to the beneficial effects of EOP on rumen microflora (Gado et al., 2009). Increased DM digestion with inclusion of EOP in our experiment can be attributed to both high content of pectins as the major component and highly digestible NDFom (Aregheore, 2000). Because the fiber in citrus pulp is highly fermentable (Aregheore, 2000), this might lead to increased NDFom digestion with EOP supplementation. Addition of ENZ during ensiling process also improved the digestion of some nutrients (*i.e.*, DM, OM, CP, NDFom and ADFom) compared to control group. 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 flow rate (Firkins et al., 1998; Arriola et al., 2011). Fibrolytic ENZ could increase the rate of ruminal digestion of the potentially digestible NDFom fraction (Arriola et al., 2011), resulting in increased nutrient digestibility (Gado et al., 2009; Arriola et al., 2011). Fibrolytic ENZ supplementation of ruminant diets could also partly reduced digesta viscosity (Hristov et al., 2000) and alterate ruminal fermentation (Gado et al., 2009; Arriola et al., 2011) and/or enhancing attachment and colonization to the plant cell wall by ruminal microorganisms (Wang et al., 2001), by synergism with enzymes or stimulate the rumen microbial numbers.

4.3. Live weight performance

Improvement in the average live weight gain of lambs fed orange pulp (Table 5) is probably due to increased ruminal fermentation activities and nutrients digestion (Table 4). Addition of ENZ to EOP also improved the average live weight that probably due to the increased nutrients digestion of the diet, which is attributable to improved feed efficiency and/or increased availability of nutrients to the animals (Lewis et al., 1999). Beauchemin et al. (1995) indicated that the improved live weight with ENZ might be due to increased digestibility, which yields more energy and/or nutrient availability to rumen microbes. Probiotic are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion, and increased flow of microbial protein from the rumen (Salem et al., 2004), which may be beneficial for live weight of lambs. However, ENZ has been shown to have marked positive effects on increasing the total microbial population in the rumen and increased microbial protein synthesis (Gado et al., 2009) in sheep.

5. Conclusions

Addition of EOP to the diet improved feed efficiency and live weight gain suggesting a good quality feed, which could probably be used to replace a part of the concentrate in ruminant diets. Moreover, a higher improvement of the nutritive value when EOP was ensiled with enzymes compare to the other diets had beneficial results of improving the digestion of nutrients, ruminal fermentation and live weight of lambs. Ensiled of EOP with enzymes improved the lamb's average daily gain by 92% and digestible DM by 18%.

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