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SHORT NOTE [NOTA CORTA]

EFFECTS OF EXOGENOUS ENZYMES ON NUTRIENTS DIGESTIBILITY AND GROWTH PERFORMANCE IN SHEEP AND GOATS

[EFECTO DE LAS ENZIMAS EXOGENAS SOBRE LA DIGESTIBILIDAD DE NUTRIENTES Y EL COMPORTAMIENTO PRODUCTIVO EN OVINOS Y CAPRINOS]

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SUMMARY

Six crossbred sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) were used in 2 × 2 factorial design to evaluate the effect of exogenous enzymes of ZADO® (*i.e.,* ENZ) and on nutrients digestibility and growth performance. Animals were fed on wheat straw *ad libitum* and restricted amount of commercial concentrate with (+ENZ) or without (-ENZ) 10 g/animal/day of ZADO® to cover 120% of their maintenance requirements. Nutrients digestibilities were increased ($P<0.01$) in +ENZ groups and it was better in goats than sheep. Goats had the lowest total dry matter intake (DMI) that associated with an increased in digestibility and average daily gain (ADG) more than sheep. Feed efficiency, metabolizable protein intake and net energy for growth occurred sheep and goats diets of +ENZ and the highest ($P<0.01$) values were observed in goats received +ENZ diet. These results suggested a good performance of goats than sheep received ENZ under the critical conditions of shortage feedstuffs in Egypt.

Key words: enzymes; sheep; goats; digestibility; average daily gain.

RESUMEN

Seis corderos encastados (32±0.603 kg de peso vivo (PV)) y 6 cabras Baladi (18±0.703 kg de PV) fueron usados en un experimento factorial 2x2 para evaluar el efecto de las enzimas exógenas de ZADO® (ENZ), sobre la digestibilidad de nutrientes y el comportamiento productivo. Los animales se alimentaron con paja de trigo *ad libitum* y cantidades restringidas de un concentrado comercial con (+ENZ) y sin (-ENZ) 10 g/animal/día de ZADO® para cubrir el 120% de las necesidades de mantenimiento. La digestibilidad de los nutrientes se incrementó ($P<0.01$) en los grupos de +ENZ y la respuesta fue mejor en las cabras que en los borregos. Las cabras presentaron el más bajo consumo de materia seca (CMS) que estuvo asociado con un incremento en la digestibilidad y en la ganancia diaria de peso (GDP), comparadas con los borregos. La eficiencia alimenticia, el consumo de proteína metabolizable y de energía neta para el crecimiento, presentaron los valores más altos ($P<0.01$) en las cabras recibiendo las dietas de +ENZ. Estos resultados sugieren un buen comportamiento de las cabras recibiendo enzimas (+ENZ) comparadas con los borregos, bajo las condiciones críticas por los cortos periodos de disponibilidad de alimento en Egipto.

Palabras clave: Enzimas; borregos; cabras; digestibilidad; ganancia diaria de peso.

INTRODUCTION

Twenty-two million tons of plant by-products or crop residues are produced annually in Egypt (United States Department of Agriculture, 2002). These by-products are generate plentiful, inexpensive and available year round, but are not efficiently utilized. Indeed more than eight million tons, which could potentially be used as animal feed, is wasted or burnt. Some of these residues, especially wheat and rice straws, are used as fuels, or as bulky residues on walkways. Use of these by-products in animal feeds could decrease the pollution of atmosphere. Only about four million tons of cereal straws, mainly wheat straw, are available at present as feeds for livestock (Hathout, 1984). These lignocellulotic by-products are widely spread. Such residues usually contain high amounts of fibrous and low protein content. They are characterized by a poor nutritive value that affects negatively the feed intake and digestibility. Offering the crop residues in large amount may allow animals, particularly sheep and goats.

It is well established that the nutritive value could be improved substantially by different methods of chemical, physical and for microbiological treatments. Microbial treatment probably conducted by administration of microbial cells, microbial extracts or microbial enzymes such as cellulases. However, microbial treatments improve the nutritive value of these lignocellulosic by -products, as well as palatability, feed intake, and animal performance (Colombatto *et al.*, 2007; Di Francia *et al.*, 2007; Gado *et al.*, 2009 and 2011). A wide range of fibrolytic enzymes are currently commercially available for this purpose to be used with ruminants (Beauchemin *et al.*, 2000; Colombatto *et al.*, 2007; Gado *et al.*, 2009 and 2011). Furthermore, exogenous enzymes work in synergy with rumen microbial enzymes, which increases their hydrolytic potential within the rumen (Eun *et al.*, 2007; Gado *et al.*, 2009 and 2011). To date, it has not been possible to predict the potential of exogenous feed enzymes to increase cell wall degradation in the rumen based on their biochemical characterization alone (Colombatto et al., 2003). Nsereko et al. (2000) reported that enzyme products containing xylanases and esterases had stimulatory effects on fiber degradation of alfalfa hay *in vitro*, whereas Colombatto et al. (2003) indicated that some xylanases and proteases improved *in vitro* degradation of alfalfa hay.

The objective of the current study was to evaluate the effect of exogenous enzymes of a commercial product of enzymes on growth performance and digestibility in sheep and goats fed on wheat straw *ad libitum* as well as a restricted amount of commercial concentrate to cover 120% of their maintenance requirements.

MATERIALS AND METHODS

In vitro study

Treatment of what straw with ZADO®

Two hundred grams of chopped what straw (1-3 cm) were treated with ZADO® enzymes (*i.e.*, ENZ) with the proportion of one liters of ENZ per ton) in a plastic bag (60 × 60 cm) according to the procedures of Gado (1997). 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), alpha amylase (61.5 unit/g), protease (29.2 unit/g) in a powder form obtained through an anaerobic fermentation process (Gado, 1997). Enzymes solution was mixed with water at the rate of 2:1 of the amount of what straw, 4% w/w molasses, 3% urea, 0.3% w/w formic acid and 0.3% w/w acetic acid, before applied on the straw. The bag was sealed and kept under the room temperature for 30 days before used in the *in vitro* study.

In vitro digestibility

The two stage technique of Tilley and Terry (1963) was used to study the effect of the biological treatment of the tested roughages on *in vitro* dry matter digestibility. Rumen liquor was obtained from two fistulated crossbred rams kept on high quality hay. The fluid was strained through four layers of cheese cloth. The McDougal buffer was prepared, then it was added mixed with rumen liquor at the ratio of (2 buffer: 1 rumen liquor).This mixture was saturated with CO_2 and warmed at 39°C in a water bath. A volume of 250 ml of the mixture was added to each polyethylene tube containing 0.5g air dried sample (in triplicate), then saturated with CO_2 and tightly closed with rubber stopper fitted with outlet valve and incubated at 39°C in a water bath for 48 hours. Tubes were well shaken twice daily during incubation period. At the end of the incubation period, 6 ml of HCl (20%) was added gradually to each tube followed by 2 ml of 5% pepsin. Tubes were closed and incubated in the water bath at 39°C for another 24 hours. Samples were filtered using a dry weighed Whatman filter paper (No. 54) and the residue was washed with boiled water; the filter paper and residue was dried at 100°C overnight and weighed after cooling in desiccators.

In vivo study

Animals, management and growth performance trial

Six crossbred sheep (32.00±0.603 kg body weight (BW)) and 6 Baladi goats (18.00±0.703 kg BW) were used to evaluate effects of ENZ on feed intake, apparent digestibility and animal performance. The growth study was lasted for 65 days followed by one week for a digestibility trail. Sheep and goats were randomly divided into two groups of three to create two experimental groups per each species. Animals were fed on wheat straw *ad libitum* and restricted amount of commercial concentrate (Table 1) were offered with or without 10 g/animal/day of ENZ to cover 120% of their maintenance requirements. Animals were individually fed and had free access to fresh water. Changes in body weight were recorded each 15 days during the experimental period. Wheat straw offered and orts were measured daily during the experimental periods.

Table 1. Chemical composition (g/kg DM) of sheep and goats diet (wheat straw and commercial concentrate)

	Wheat straw	Commercial concentrate
Organic matter	856	926
Crude protein	57	176
Ether extract	13	46
Neutral detergent fiber	720	250
Acid detergent fiber	520	120
Acid detergent lignin	92	65

Metabolism trial

At the end of the growth trial of the 65 day experiment, all lambs of each treatment were used in a digestibility trial. Feed offer and feed refusals and total feces output were daily recorded for one week to determine the effect of ENZ and animal species on the apparent digestibility. Representative samples of straw, concentrate, refusals and faeces were collected daily and dried at 55ºC to determine daily intake of DM for each animal. Other sub-samples of each material were bulked, mixed, and ground through a 1mm sieve for further laboratory analysis.

Analytical methods

Ground samples of feeds, refusals and feces were analyzed for dry matter (DM) by drying samples at 105ºC for 24 h in a forced air oven. Ash was determined after igniting samples in a muffle furnace at 550ºC for 4 h. Crude protein (CP) was determined by a Kjeldahl method (AOAC, 1990; ID 954.01). Ether extract (EE) was determined by Soxhlet method (AOAC, 1990; ID 920.39). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin were determined. NDF was assayed without the use of an alpha amylase but with use of sodium sulfite. Both NDF and ADF are expressed without residual ash.

Calculations

Metabolizable energy intake (kJ/kg $BW^{0.75}$) was estimated according to Luo et al. (2004) as:

Metabolizable energy intake = 533 + (43.2 × ADG (g/kg $BW^{0.75}$))

where: ADG is the average daily gain (g/kg $BW^{0.75}$) of sheep and goats during the 65 days of the growth performance experiment.

Net energy required for growth (Mcal/kg $BW^{0.75}$/d) was estimated according to NRC, (1985) as:

Net energy required for growth = 644 – 2.61 × MBW

where: MBW is the metabolic body weight (MBW = kg $BW^{0.75}$)

Statistical analysis

A 2 × 2 factorial experimental design (2 animal species (sheep and goats) ×2 treatments (with or without ENZ)) were used to analyze the data of body weight changes, feed intake, feed efficiency, Metabolizable energy intake, and net energy required for gain during the growth trial. Also the nutrient components of total feed intake, straw consumption and digestibility during the digestibility trial. The 'PROC GLM' of SAS (1999), with methods of Steel and Torrie (1980), was used to determine differences due to animal species and ENZ. In the case of significant interactions (*i.e.*, P<0.05), Duncan's multiple-range test (Duncan, 1955) was used to separate means within animal species.

RESULTS

Addition of enzymes (*i.e.*, ENZ) improved (P<0.05) *in vitro* dry matter digestibility during the first 4 h and from 24 to 48 h after incubations, while decried at 6h (Table 2).

Total dry matter intake (*i.e.*, DMI) was increased from 76 to 84 g/kg $BW^{0.75}$ in goats, but it was not affected in sheep. Digestibility of DM was increased (P<0.01) by 5 and 11%, in sheep and goats respectively by addition of ENZ, while NDF digestibility was increased (P<0.01) by 7 and 9%, respectively. OM and CP digestibility were increased (P<0.01) also in both animal species with ENZ diets. However, the nutritive value was improved in both species and it appears to be higher (P<0.05) in goats than in sheep (Table 3).

In the growth performance trial, DMI was increased (P<0.05) by addition the daily addition of ENZ to sheep and goats diets during the 65 days of the experimental. Total DMI was increased (P<0.01) in sheep than goats, while average daily gain (ADG) was increased (P<0.01) goats than sheep with the addition of ENZ (2.2 and 6.3 g/kg $BW^{0.75}$, respectively). Feed efficiency was increased in goats than sheep with ENZ addition. An increased (P<0.01) in metabolizable protein intake and net energy for growth occurred by ENZ addition sheep and goats diets and the highest (P<0.01) values were observed in goats received ENZ diet (Table 4).

Table 2. *In vitro* DM digestibility of wheat straw in absence (-ENZ) or presence (+ENZ) of enzymes during different time of incubation. (*n* = 3)

	In vitro digestibility, g/kg DM				
	2 h	4 h	6 h	24 h	48 h
-ENZ	87.7	154.0	284.7	369.0	687.7
+ENZ	98.7	181.0	273.0	395.0	815.7
SEM	5.50	13.50	5.83	13.00	64.00

DISCUSSION

Nutrients digestibility

It was speculated that the addition of ZADO® (*i.e.*, ENZ) increased (P<0.05) total feed intake in sheep and goats (Tables 3 and 4). Some results reported an effect of providing exogenous fibrolytic enzymes on feed intake of dairy cows or beef steers (Schingoethe *et al.*, 1999; Gado *et al.*, 2009). DM digestibility was improved by addition of ENZ by 4 and 11%, in sheep and goats, respectively. Improvement of nutrient digestibility by addition of ENZ probably due to the beneficial effects of ENZ on fiber hydrolysis and rumen fermentation activity (Gado *et al.*, 2006, 2009 and 2011) and this results were confirmed *in vitro* (Table 2). Beauchemin *et al.* (2003) reported that supplementing the diet with *E. faecium* increased (P<0.05) the proportion of propionate and, consequently, decreased (P<0.10) the proportion of butyrate in ruminal fluid compared with the control regarding to its roles in stimulating the presence of lactic acid-utilizing bacteria, which produce propionate. Similarly, Kim *et al.* (2000) reported increased propionate concentrations in steers fed *L. plantarum,* another lactic acid-producing bacterium. NDF digestibility was increased (P<0.01) in sheep and goats treated groups by about 7 and 9%, respectively (Table 2). This effect may be due to the ability of ENZ to lose lignocellulotic bonds in fiber (Bassuny *et al.*, 2003).

Improve the NDF digestibility by addition of ENZ may be also due to increase their degradability in the rumen which could reduce the physical fill in the rumen over time thereby allowing higher voluntary feed intake (Dado and Allen, 1995). Improved the nutrient digestibility in animals could be also refer to the improvement in ruminal fermentation activities, by addition of ENZ to diet (Gado *et al.*, 2006, 2009 and 2011). ENZ may improve the stimulation of ruminal microorganism's activity by reducing the NH_3-N concentration in the rumen liquor throughout incorporation of NH_3-N into microbial protein (Gado *et al.*, 2006). This effect attributed to an increase in microbial colonization of feed particles and speculated that exogenous enzymes may act similarly to primary bacterial colonization Yang *et al.* (1999), and increase the dietary energy utilization and rumen fermentation. Colombatto *et al.* (2007) reported an improvement *in vitro* ruminal fermentation activity and cell wall digestibility of alfalfa stems by addition of exogenous fibrolytic enzyme in cow. Zadrazil (1977) reported an increase of 12 % in the *in vitro* digestibility of wheat straw incubated with *Pleurotus florida,* and Ibrahim and Pearce (1980) observed an increase in digestibility of barley straw by 10 units, with *Peniophora gigantea.*

Growth performance

Improvement of the average daily gain (*i.e.,* ADG, Table 4) by addition of ENZ probably due to the improvement in nutrient digestibility of diets (Table 3) which reflect on improving the feed efficiency (Table 4). Supplementing diets with enzymes has been shown to improve feed efficiency and daily gain of feedlot cattle (Swinney-Floyd *et al.*, 1999; Galyean *et al.*, 2000). Ware *et al.* (1988) Improving the nutrient digestibility and animal's performance may be due to the positive effect of ENZ on ruminal fermentation activity. Gado *et al.* (2006) reported an improvement in ruminal fermentation activity by treating of rice straw with ENZ.

Improved ADG in sheep and goats could be also explained by an increase in the available nutrients to animals for deposition and growth (Lewis *et al.*, 1999). ENZ addition increased the ADG by 51 and 69% in sheep and goats, respectively. These results are in agreement with Beauchemin *et al.* (1995) who reported significant improved growth and body weight gain by adding an enzyme product containing xylanase and cellulase activities to alfalfa hay of growing beef cattle by up to 30% or high concentrate ration (Beauchemin *et al.*, 1999). Meanwhile, McAllister *et al.* (1999) observed that average daily gain was related to the enzyme concentration. Dong *et al.* (1995) reported that direct fed cellulases had the ability to improve the growth of Holstein calves when administrated orally. However, improving the growth performance of sheep and goats with addition of ENZ suggested to have a marked effect on increasing the total microbial population in the rumen (Yang *et al.*, 1999), and increased microbial protein synthesis (Gado *et al.*, 2009). Yang *et al.* (1999) and Beauchemin *et al.* (2000) reported that the increase in nutrient digestibility (Table 3) is due to the increase or stimulation in the total microbial population. However, Beauchemin *et al*. (1995) and Lewis *et al*. (1999) indicated that the improved performance might be due to increased digestibility which yields more energy and/or nutrient availability to rumen microbes because of enzyme feeding. Beauchemin *et al.* (1997) and Yang *et al.* (1999) concluded that fibrolytic enzymes can be used to improve the digestibility of treated diets and provide more nutrients for production. Fibrolytic enzymes contain a wide variety of polysaccharidase enzymes that solubilize fiber and provide some essential nutrients or growth factors to rumen microorganisms. Di Francia *et al*. (2007) who observed an improvement in average daily gain and digestive efficiency of fibre with supplementation of *A. oryzae* fermentation extract and *S. cerevisiae* culture on the calf starter.

Feeding efficiency expressed as kg feed/kg body weight was significantly improved (P<0.05) for ENZ addition groups compared to control (Table 4). However, this response was mainly due to the effect of cellulase feeding on feed intake and digestibility. However, feed efficiency (P<0.05) was improved by 35% with addition of ENZ, and this was associated with increased of 60% in ADG. Beauchemin *et al.* (2000) reported an 11% improvement in feed conversion ratio resulting from a 5% decrease in feed intake from enzyme treated ration associated with a 6% increase in body weight gain. En earlier study of the same author of Beauchemin *et al*. (1995) reported a 10% improvement in the feed conversion ratio of steers fed dry forages resulting from improved digestibility of enzyme-supplemented ration.

Effect of animal species

Sheep and goats increased the amount of straw intake when the amount offered was increased to allow animals to refuse more than the rate of 10 to 20% of amount offered, normally adopted in *ad libitum* feeding trials. Our research has aimed to define how much straw needs to be consumed in goats and sheep, to enable them to select the more nutritious components of straw, after eating the restricted amount of concentrate in absence of presence of ENZ. Differences in gut morphology, rumen size, and physiology, such as rumen motility (Tisserand *et al.*, 1991), offer an explanation as to differences between the two species. Attempts have been made to explain the observed differences in diet selection, on grounds of nutrient requirements and physiology, which postulate that goats differ from sheep in energy, nitrogen, dietary fibre requirements, digestive capacity and the rate of passage of undigested residues (Brown and Johnson, 1985; Lu, 1988).

Table 3. Feed intake and digestibility in sheep and goats fed on diet without (-ENZ) or with (+ENZ) addition of enzymes. (n = 3)

	Sheep		Goats		SEM
	-ENZ	+ENZ	-ENZ	+ENZ	
Dry matter intake:					
Straw (g/d)	295 [a]	283 [a]	155 [b]	151 [b]	9.15
(g /kg $BW^{0.75}$)	22.0	20.9	16.9	18.1	0.74
Concentrate[1]	547	547	547	547	-
Total DM intake (g/d)	842 [a]	830 [a]	702 [b]	697 [b]	9.22
(g /kg $BW^{0.75}$)	62.4 [c]	61.3 [c]	76.2 [b]	83.6 [a]	5.54
Digestibility, g/kg	545 [b]	589 [b]	604 [a]	709 [a]	36.53
Organic matter:					
Intake, g/d	759 [a]	748 [a]	639 [b]	635 [b]	7.83
Digestibility, g/kg	567 [b]	609 [b]	663 [a]	727 [a]	85.85
Crude Protein:					
Intake, g/d	113 [a]	112 [a]	105 [b]	105 [b]	0.52
Digestibility, g/kg	599 [c]	613 [c]	702 [b]	749 [a]	9.53
Ether Extract:					
Intake, g/d	29 [a]	29 [a]	27 [b]	27 [b]	0.11
Digestibility, g/kg	778 [b]	724 [c]	859 [a]	859 [a]	10.34
Neutral detergent fiber (g/d):					
Intake, g/d	349 [a]	340 [b]	249 [c]	245 [c]	5.4
Digestibility, g/kg	475 [d]	548 [b]	525 [c]	612 [a]	12.51

[1] Concentrate intake (g DM/animal/d) was not statistically analyzed as it was offered at a flat rate.
[a,b,c] Means in the same row with different letters differ (P < 0.05).

Table 4. Performance of sheep and goats fed on diet without (-ENZ) or with (+ENZ) addition of enzymes. (n = 3)

	Sheep		Goats		SEM
	-ENZ	+ENZ	-ENZ	+ENZ	
Feed consumption					
Wheat straw (g/d)	195^{a}	197^{a}	80^{b}	79^{b}	1.4
(g /kg BW$^{0.75}$)	14.5	14.5	8.6	9.4	0.32
Concentrate[1](g/d)	547	547	547	547	-
Total DM intake (g/d)	742^{a}	744^{a}	626^{b}	625^{b}	1.1
(g /kg BW$^{0.75}$)	55.2	54.9	67.9	75.1	2.00
Body weight changes (kg)					
Initial	32.0^{a}	32.3^{a}	19.3^{b}	17.0^{b}	0.85
Final	36.7^{a}	39.0^{a}	23.7^{b}	24.3^{b}	0.91
Average daily gain (g/d)	71.8^{c}	102.6^{b}	66.7^{c}	112.8^{a}	5.13
(g /g /kg BW$^{0.75}$)	5.4^{c}	7.6^{b}	7.2^{b}	13.5^{a}	0.52
Feed efficiency, kg diet/kg gain	10.4^{a}	7.3^{b}	9.5^{a}	5.6^{c}	0.57
Metabolizable energy intake[2]	764^{c}	860^{b}	846^{b}	1117^{a}	22.5
Net energy for growth[3]	630^{a}	624^{b}	625^{b}	609^{c}	1.36

[1] Concentrate intake (g DM/animal/d) was not statistically analyzed as it was fed at a flat rate.
[a,b] Means in the same row with different letters differ ($P < 0.05$).
[2] kJ/kg BW$^{0.75}$ and were calculated according to equations of Luo et al. (2004).
[3] (Mcal/kg BW$^{0.75}$)/d and was calculated according to NRC, (1985).

Sheep and goats in our study were fed less than 60% of their DM required for the growth (NRC, 1985) to give the animal chance to increase the amount ingested from the wheat straw. Goats had the lowest (P<0.05) total DM intake (Table 4) and associated with an improvement in nutrient digestibility and ADG more than sheep (625 and 743g/animal/d, for goats and sheep, respectively). Both goats and sheep increased their total intake with increasing amounts of straw offered, under addition of ENZ in concentrate, and this was reflected in increasing rates of live- weight gain. Growth rates of goats were higher than for sheep; although intakes were higher in sheep than goats. The species differences were probably also a reflection due to the higher mature weight of the sheep genotype used than goats, and may be reflect also the higher efficiency of goats than sheep in feed utilization.

CONCLUSIONS

Addition of enzymes of ZADO® enzymes improved nutrients digestibility in sheep and goats. The improvement was better in goats than in sheep. Goats had the lowest total dry matter intake that associated with an improvement in nutrient digestibility and average daily gain more than sheep. These results suggested a good performance of goats than sheep under the critical conditions of shortage feedstuffs during the dry season in Egypt.

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