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Influence of Exogenous Enzymes on *In Vitro* Ruminal Degradation of Ensiled Rice Straw with DDGS#

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ABSTRACT

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The objective of this study was to determine the effect of exogenous enzymes (ENZ) on nutrient profile and ruminal degradability of rice straw (RS), distillers dried grains with solubles (DDGS) and their mixture (RS with 10% DDGS). Ten samples of each fibrous feed were mixed with ZAD® (mixture of cellulases, xylanaxes, proteases and alpha amylase). ENZ was added at 0, 1 and 3 L to one ton of the fibrous feeds and the mixture was ensiled for 30 days. Feed samples were incubated for 72 h in rumen liquor of sheep to determine the degradability of DM, NDF and ADF. Pretreatment of feeds and their mixture (RS and DDGS) with ENZ at 3 L were increased ($P<0.01$) the degradation of NDF and ADF. Degradation fractions (a, b, (a+b) and c) of feeds were improved ($P<0.01$) at 3 L of ENZ, except the *c* of NDF and ADF of RS which were not affected by ENZ treatment. The results suggested a strong potential in improving digestion of RS and DDGS as well as their mixture with the pretreatment with ENZ. The dose of 3 L/ton of fibrous product improved the DM, NDF and ADF degradability.

Key words: DDGS, Degradability, Exogenous enzymes, Fiber fractions, Rice straw.

INTRODUCTION

Fibre degradation in the rumen is not fully efficient because the fibre fraction recovered from faeces is fermentable (Krause *et al.,* 2003). In recent years, abundant researches focused on addition of exogenous enzyme on fibre digestibility of *in vitro* rumen fermentation (Hristov *et al.,* 2008; Salem *et al.,* 2012), however, as opinions vary, no defined conclusion can be drawn about the effect of exogenous enzymes products on fibre digestibility. The enzymes preparation ZAD® is biotechnical product made from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes. Gado *et al*. (2011) reported that ZAD® improved nutrients digestibility, live body weight gain and feed conversion of wheat straw in sheep. The

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ZAD® is mixture of enzymes from anaerobic bacteria that had a beneficial effect on digestibility of low quality roughages. DDGS are highly valued as an animal feed for its nutrient content (Kingsly *et al.,* 2010). High protein and high energy content make distillers DDGS a unique ingredient for ruminant diets, but variation in composition reduces nutritional quality and market value (Belyea *et al.,* 2010). The objectives of the present study aimed at investigation of the effect of an anaerobic enzyme (ENZ) on nutrient profile and ruminal degradability of rice straw, DDGS and their mixture (RS with 10% DDGS).

MATERIALS AND METHODS

Ten samples of one kg for each of fibrous feeds sample (RS, DDGS) or their mixture (RS with 10% DDGS), on a DM basis, were collected from different sites of each fibrous material bulk and mixed with an enzyme product ZAD® (ENZ, according to the manufacturer; Bactizad Inc., Cairo, Egypt). The ZAD® is a product in a powder form manufactured by the Academy of Scientific Research and Technology, Egypt, and contains enzymes of cellulases (7.1 unit g^{-1}), xylanases (2.3 unit g^{-1}), α-amylase (61.5 unit g^{-1}) and protease (29.2 unit g^{-1}) obtained through an anaerobic fermentation process of anaerobic bacteria. Enzymes activities of ZAD® were determined according to the methods mentioned in Salem *et al.* (2013). Chemical composition of fibrous feeds are presented in Table 1.

Table 1. Chemical composition (%±SD) of rice straw, DDGS and their mixture [*n=10*]

	Rice straw (RS)	DDGS	RS with 10% DDGS
DM	90.0±2.31	90.0±1.23	96.1±2.11
CP	3.8±0.98	26.8±1.22	6.0±0.82
NDF	65.5±2.13	43.6±1.34	50.1±2.40
ADF	48.0±1.37	10.7±0.43	46.7±1.56

Samples of each fibrous material (*i.e.,* RS, DDGS and RS with DDGS) were chopped at 5 cm and mixed with ENZ at three levels (0, 1 or 3 L/ton of fibrous material) and moistened to a relative humidity of approximately 50%. Sugarcane molasses was added at 10 kg/ton of DM fibrous material. Enzymes of ZAD® was sprayed inside the mixture in a unify way to all of the contents without changing the humidity contents between treatments. After the mixture, the whole contents were transferred to a baling machine to press the whole contents together that followed by a plastic raving machine to isolate the whole contents from air (anaerobic condition) and ensiled for 30 days. Samples of ensiled RS and DDGS or their mixture were ground through a 1 mm screen (Wiley mill, Arthur H. Co., Philadelphia, PA, USA) for chemical analysis (Table 1). Samples were analysed for DM (934.01), ash (942.05), N (954.01) and EE (920.39) as per AOAC (1997). The NDF (Van Soest *et al.*, 1991), ADF and lignin (AOAC 1997; 973.18) were analysed using an ANKOM 200 Fibre Analyzer Unit (ANKOM Technology Corporation, Macedon, NY, USA). NDF was assayed with heat-stable alpha-amylase and with sodium sulfite in the NDF. Both NDF and ADF were expressed without residual ash.

The *in vitro* degradation of samples was carried out according to the method of Tilley and Terry (1963) in ruminal fluid collected from three sheep of 45 kg live weight and feed on total mixed ration of concentrate and roughages (1.1). Sheep were fitted with permanent rumen fistula. Ruminal fluid was collected before the morning feeding and filtrated through four layers of cheesecloth and filled with O_2-free CO_2 headspace. Samples (1 g) were weighed in polypropylene tubes with runner stopper. Forty ml of saliva were added to samples with and 10 ml of filtrated ruminal liqueur. Tubes were then incubated in water bath at 39°C for 2, 4, 6, 8, 12, 24, 48, 72 h. after incubation, tubs were filtrated and undigested residuals were recovered and dried at 65°C for 24 h and weighed. Subsample of the undigested residual was used for NDF and ADF contents determination. Incubations were done in different runs and days.

It used five tubs for each treatment during the *in vitro* incubation. Kinetics of *in vitro* degradation was measured by fitting the data in the Gompertz model (Susmel *et al.,* 1999) as follows:

$$dis(t) = (a+b) * \exp[(-c)\exp(-Dt)]$$

Where, dis(t) is the degradation of sample (g/kg) at time 't'; 'a' is the rapid soluble fraction (g/kg) at t=time (h); 'b' is the insoluble, but potentially degradable fraction (g/kg); 'c' is the degradation rate of a+b; 'D' is a parameter to measure the degradation. According to Gompertz model, the fractional rate of degradation varies as a function of time, and the average value (*i.e.*, a constant comparable to the exponential rate of degradation).

Data of DM, NDF and ADF degradability at each incubation time were fitted in the "NLIN" procedure of SAS (1999) for calculation the degradation fraction of a, b and c. Data of *in vitro* degradation wasanalysed as complete randomized design using the GLM procedure of SAS (1999) considering the individual samples within each fibrous material as experimental unit. Linear and quadratic orthogonal contrasts within each fibrous species were analysed.

RESULTS

The RS showed the highest ($P<0.05$) NDF and ADF contents compared to the DDGS while the CP content was the highest in DDGS. Application of ENZ at 3 L increased the quadratically ($P<0.01$) the degradation of NDF and ADF of RS, DDGS and their mixture, and was followed by treatment of 1 L of ENZ versus control treatment (Table 2). Increasing the level of ENZ from 0-3 L before ensiling RS, DDGS and their mixture improved the quadratically ($P<0.01$) degradation fractions (a, b, (a+b) and c) of nutrients but had no effect on the c of NDF and ADF of RS. In general, the ENZ increased the potentially degradable fractions of NDF and ADF of DDGS by 11%, the potentially degradation fraction of NDF and ADF of RS plus DDGS by 6 and 8%, respectively and the degradation rate of NDF and ADF of RS by 24% (Table 3).

Table 2. Effect of level (0, 1 and 3 L ton^{-1} of the fibrous feed) of ENZ on fiber fraction (%) of ensiled RS, DDGS and their mixture for sheep

		ENZ (L ton^{-1})			SEM	Contrast†	
		0	1	3		L	Q
RS	NDF	65.5	60.1	50.2	4.45	0.009	0.024
	ADF	48.0	41.2	32.6	3.26	0.007	0.035
	ADL	9.0	8.7	8.6	0.78	0.181	0.062
DDGS	NDF	43.7	39.8	31.2	5.30	0.009	0.034
	ADF	18.7	13.4	10.1	2.89	0.008	0.016
	ADL	3.0	2.8	2.7	0.65	0.151	0.205
RS with 10% DDGS	NDF	63.6	58.4	49.3	4.82	0.008	0.016
	ADF	46.2	40.0	30.1	4.53	0.006	0.034
	ADL	8.0	7.9	7.8	0.34	0.191	0.042

†Probability of a linear (L) or quadratic (Q) effect of ENZ level.

Table 3. Effect of ENZ levels on *in vitro* degradation† of DM and fibre fractions of RS, DDGS and their mixture in sheep

		ENZ, L ton^{-1}			SEM‡	Contrast§	
		0	1	3		L	Q
RS	DM						
	a (%)	11.0	17.3	22.6	4.66	0.006	0.024
	b (%)	33.4	39.2	44.7	3.12	0.009	0.035
	a+b (%)	44.4	56.5	67.3	2.88	0.006	0.041
	c (%/h)	2.3	3.4	4.6	0.68	0.008	0.021
	NDF						
	b (%)	41.5	44.6	48.6	1.45	0.009	0.016
	c (%/h)	2.1	2.4	2.7	0.42	0.240	0.340
	ADF						
	b (%)	38.2	41.1	44.7	1.44	0.008	0.019
	c (%/h)	2.4	2.6	2.8	0.61	0.190	0.048
DDGS	DM						
	a (%)	28.7	29.4	31.4	1.44	0.005	0.036
	b (%)	46.1	49.8	53.4	2.62	0.009	0.042
	a+b (%)	74.8	79.2	84.8	1.89	0.007	0.026
	c (%/h)	3.8	4.0	4.9	0.79	0.007	0.042
	NDF						
	b (%)	45.4	47.4	51.5	1.93	0.008	0.035
	c (%/h)	3.2	3.5	3.9	0.53	0.008	0.012
	ADF						
	b (%)	49.9	50.6	55.6	1.99	0.009	0.043
	c (%/h)	3.4	3.5	3.9	0.29	0.006	0.051
RS with 10% DDGS	DM						
	a (%)	15.6	26.4	33.8	3.73	0.009	0.043
	b (%)	34.2	46.3	55.1	2.47	0.008	0.031
	a+b (%)	49.8	72.7	88.9	4.83	0.008	0.026
	c (%/h)	3.2	4.3	5.4	0.29	0.006	0.04
	NDF						
	b (%)	42.5	48.6	53.6	3.81	0.007	0.042
	c (%/h)	2.8	3.1	3.6	0.33	0.006	0.043
	ADF						
	b (%)	41.8	42.2	45.8	1.69	0.008	0.036
	c (%/h)	2.6	3.1	3.4	0.29	0.006	0.046

†a, soluble fraction; b, potentially degradable fraction; a+b, total degradation; c, degradation rate.

‡SEM, standard error of the mean.

§Probability of a linear (L) or quadratic (Q) effect of ENZ level.

DISCUSSION

Enzyme level and fiber degradability

The DM degradation of RS was improved by enzymes; however, ENZ increased both potentially degradable fraction and degradation rate of NDF and ADF. The positive results of enzymes on *in vitro* degradation of rice straw fibre are consistent with those reported by Yang *et al*. (1999). However, the mode of action by which ENZ improves degradation has not been elucidated, although it has been suggested that it could be related to the fact that ENZ may enhance rumen enzyme activity (Hristov *et al.,* 2008) due to increments of soluble carbohydrates released from undigested feed particles, which provides additional energy for microbial growth and shortening the lag time for microbial colonization (Sutton *et al.,* 2002).

Additionally, it could contribute to the increase of the soluble fraction that would be expected if feeds are directly treated with enzymes, because this pretreatment has been shown to start fiber degradation and to reduce the NDF content of different feeds (Giraldo *et al.,* 2008). The release of sugars from feeds arises at least partially from the solubilization of NDF and ADF (Hristov *et al.,* 2008). This is consistent with increased soluble fraction and rate of *in situ* digestion (Hristov *et al.,* 2008). The DDGS pretreatment with ENZ could assist in elevating NDF and ADF degradability as it appeared in the obtained results.

Enzymes and type of fiber

Our results showed that the ENZ were effective on RS and DDGS and their mixture plus ENZ would be expected to increase fiber digestion by increasing the rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al.,* 1999). The large effects of the ENZ preparation on RS could be due to their enzymatic activities and levels. The ENZ in our study at 3 L, have improved the degradation of RS followed by DDGS. The mixture of RS and DDGS showed a positive associative effect on the increase of soluble part of their fiber. Salem *et al*. (2012) reported that sun-drying of roughage and addition of ENZ had a beneficial impact on fibre digestibility. Different type of ENZ affected the highest specific growth rates of bacteria or yeast (Lamsal *et al.,* 2012).

The ruminal insoluble potentially degradable fraction (b) of grass hay DM and its fractional rate of degradation (c) were increased ($P<0.05$) by ENZ treatment. Supplementation with ENZ also increased ($P<0.01$) effective and potential degradability of grass hay DM and NDF (Giraldo *et al.,* 2008). This result was consistent with nutrients digestion were higher ($P<0.05$) in ensiled orange pulp with ENZ and digestible DM was increased by 18%, whereas the fiber fractions (NDF and ADF) were increased by 93 and 47% with similar ensiled orange pulp with ENZ (Gado *et al.,* 2011).

CONCLUSIONS

The use of exogenous enzymes showed strong potential in improving the degradation of fibrous materials such as RS and DDGS. A dose of 3 L of ENZ/ton of fibrous feed product improved the DM, NDF and ADF degradation of RS, DDGS and their mixture. The increase in soluble fraction, potentially degradable fraction, total degradation and degradation rate were significantly higher at 3 L of ENZ treatment than 1 L with respect to the source of fiber.

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