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Rumen degradation and nutritive utilization of wheat straw, corn stalks and sugarcane bagasse ensiled with multienzymes

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ABSTRACT

The aim of this study was to determine the effect of anaerobic ensiling of raw agricultural wastes with a fibrolytic enzyme cocktail (EZ) as a cleaner and sustainable biological product for animal feed. Ten 1-kg samples of wheat straw, corn stalks and sugarcane bagasse were chopped at 5 cm length and mixed with EZ at three levels of 0, 1 or 3 L enzyme/ton of feed, moistened to a relative humidity of approximately 50% and ensiled in plastic bales for 30 days. Additionally, fibrous samples were incubated for 72 h with rumen liquor to determine the digestion of dry matter (DM), neutral detergent fibre and acid detergent fibre. Increasing enzyme level lowered ether extract and nitrogen-free extract contents of fibrous feeds and increased the biodegradation of acid detergent lignin of wheat straw. Anaerobic ensiled corn stalks and sugarcane bagasse with EZ improved the biodegradation of DM and fibre fractions. It could be concluded that ensiling fibres of the three wastes with EZ improved and enhanced their ruminal digestion with the biodegradation rate at 3 L/ton and subsequently produced a cleaner product for animal feed from agriculture wastes.

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Digestion; raw agricultural wastes; enzymes

1. Introduction

Agricultural wastes such as wheat straw, corn stalks and sugarcane bagasse are carbohydrate-rich materials with a large potential as a source of dietary energy for ruminants. However, such feeds have poor nutritional value with low nitrogen and high fibre content (Tang et al. 2013; Ghorbani et al. 2014; Kholif et al. 2014). Although ruminant production systems depend mainly on forages as the main nutritional components, the digestion of these fibrous forages in the rumen is limited by their high content of fibre and inefficient fibre degradation (Krause et al. 2003; Khattab et al. 2013), thus limiting their use as the sole feed for actively growing or high-performing ruminants (Dean et al. 2013). The high fibre content also prevents the access of ruminal hydrolytic enzymes to cellulose and hemicellulose (Chesson 1984).

The use of exogenous fibrolytic enzymes is one of the applications of biotechnology for ruminant nutrition to improve the digestibility of structural carbohydrates in fibrous feeds (Khattab et al. 2011; Díaz et al. 2013). The application of exogenous fibrolytic enzymes has been shown to improve nutrient utilization and fibre digestibility *in vitro* (Gado et al. 2013; Ghorbani et al. 2014; Togtokhbayar et al. 2015), *in vivo* (Salem et al. 2013; Valdes et al. 2015) and *in situ* (Chung et al. 2012). The enzyme preparation is a biotechnical product made from anaerobic bacteria which convert polysaccharides to monosaccharides by specific enzymes, with many beneficial effects on nutrient digestibility of fibrous feeds (Khattab et al. 2011; Gado et al. 2013). Gado et al. (2011) and Khattab et al. (2011) reported that the enzyme cocktail (EZ) improved nutrients digestibility, body weight gain, milk production and composition and feed conversion of fibrous feeds in lambs and *in vitro*, respectively. Gado et al. (2013) reported that there was a strong potential for EZ to improve dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) digestion of rice straw at 3 L/ton of fibrous product. Therefore, the objective of the current study was to determine the effect of anaerobic ensiling of wheat straw, corn stalks and sugarcane bagasse with a fibrolytic EZ on nutrient profile and ruminal digestion as a cleaner and sustainable biological product for animal feed.

2. Materials and methods

2.1. Experimental design

Three raw agricultural wastes with low nutritive values, that is, wheat straw, corn stalks and sugarcane bagasse, were evaluated for their chemical composition and nutrient digestion before and after treatment with the EZ. The wheat straw, corn stalks and sugarcane bagasse were collected from different sites, and each feed was bulked and mixed with the enzyme product.

The EZ contains 7.1 unit/g cellulases, 2.3 unit/g xylanases, 61.5 unit/g α-amylase and 29.2 unit/g protease obtained through an anaerobic fermentation process. These enzymes'

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activities were determined according to the methods of Valdes et al. (2015).

Ten 1-kg samples of each of the fibrous feeds were chopped at 5 cm length and mixed with EZ in plastic bales at three levels of 0, 1 or 3 L enzyme/ton of feed and moistened to a relative humidity of approximately 50%. Before ensiling, 10 kg of molasses was added per ton of DM fibrous feed and thoroughly mixed. The EZ was then sprayed on the mixtures without changing the humidity content and the mixtures remixed. A baling machine was used to press the whole content together, and then a plastic raving machine was used to isolate the whole content from air, and the content was allowed to ferment anaerobically for 30 days.

2.2. DM, NDF and ADF biodegradation

The technique of Tilley and Terry (1963) was used to determine the *in vitro* biodegradation of the fibrous feeds. Ruminal contents, rich in anaerobic microflora, were collected from three fistulated sheep of 45 ± 2 kg live weight which were fed a total mixed ration of commercial concentrate and roughages (1:1) according to National Research Council (NRC) (1994) before morning feeding. Fresh water was available to sheep at all times during the rumen inoculum collection phase. Rumen contents, from each sheep, were filtered through four layers of cheesecloth into a flask with O_2-free CO_2 headspace. Five feed samples (1 g) were weighed into polypropylene tubes with a rubber stopper and 10 mL of particle-free ruminal fluid was added followed by 40 mL of artificial saliva in 1:4 (v/v) proportion with no trypticase added according to Goering and Van Soest (1970). The tubes were then incubated in a water bath at 39°C for 2, 4, 6, 8, 12, 24, 48 and 72 h of incubation. At the end of the incubation, the tubes were filtered and undigested residuals were recovered and dried at 65°C for 24 h and then weighed. Subsample of the undigested residual was used for NDF and ADF contents determination. Incubations were done in different runs and days. Data were fitted using the Gompertz model (Susmel et al. 1999) for the determination of *in vitro* degradation kinetics as follows:

$$\mathrm{dis}(t) = (a + b) * \exp[(-c)\exp(-\mathrm{D}t)],$$

where dis(t) is the biodegradation of the 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 biodegradation. The Gompertz model shows that the fractional rate of degradation varies as a function of time, and average value (i.e. a constant comparable to the exponential rate of degradation).

2.3. Chemical analysis

Samples of ensiled fibrous feeds were ground through a 1-mm screen (Wiley mill, Arthur H. Co., Philadelphia, PA, USA) for DM (#934.01), ash (#942.05), crude fibres (CF) (#962.09) and ether extract (EE) (#920.39) according to Association Of Analytical Communities (AOAC) (1997). NDF (Van Soest et al. 1991), ADF and lignin (AOAC 1997; #973.18) were analysed using an ANKOM 200 Fibre Analyser Unit (ANKOM Technology Corporation, Macedon, NY, USA). NDF was assayed with heat-stable alpha-amylase and with sodium sulphite in the neutral detergent solution. Both NDF and ADF are expressed without residual ash.

2.4. Calculations and statistical analysis

Data on DM, NDF and ADF digestion at each incubation time were fitted in the 'nonlinear' procedure of SAS (1999; Version 8, Cary, NC, USA) for calculating the biodegradation fraction of a, b and c. Data on *in vitro* biodegradation were analysed as complete randomized design using the generalized linear model procedure of SAS (1999) with the individual samples within each fibrous feed as an experimental unit. Linear and quadratic orthogonal contrasts within each fibrous species were fitted.

3. Results

3.1. Effect of EZ on nutrient content

Interactions ($P < .05$) were observed between feed type and enzyme level for all nutrient contents (Table 1). For all forages, increasing the level of enzyme lowered EE (linear effect, $P < .0001$; quadratic effect, $P < .05$) and nitrogen-free extract (NFE) (linear and quadratic effects, $P < .001$) contents. For wheat straw, increasing the level of EZ linearly increased CF ($P = .003$) and acid detergent lignin (ADL) ($P = .0129$) biodegradation without affecting ($P > .05$) DM, organic matter (OM), NDF and ADF contents. The EZ increased the biodegradation of corn stalks and sugarcane bagasse CF (linear and quadratic effects, $P < .0001$), NDF (linear effect, $P < .001$), ADF (linear and quadratic effects, $P < .0001$) and ADL (linear and quadratic effects, $P < .0001$) compared to that of the untreated ones (Table 1).

3.2. Effect of EZ on in vitro biodegradation of DM

Interactions between forage type and enzyme levels were observed for all tested DM digestion parameters (a, b, $a + b$, c; $P < .0001$), and ADF and NDF digestion parameters (b, c; $P < .05$) (Table 2). For DM digestion, ensiling wheat straw, corn stalks and sugarcane bagasse with EZ at 1 and 3 L/ton linearly ($P < .001$) lowered the values of the soluble fraction, the potentially degradable fraction, total degradation and degradation rates compared with untreated forages (Table 2). Moreover, EZ treatments linearly ($P < .001$) increased the potentially degradable fraction and the rates of NDF biodegradation of the three tested forages. The same effects were observed for ADF digestion parameters. Enzyme treatment linearly ($P < .001$) increased the potentially degradable fraction of ADF in addition to linearly ($P < .001$) increasing the rates of ADF biodegradation of the three forages (Table 2).

4. Discussion

Enzymatic treatment improved the anaerobic bio-utilization of nutrient content of all tested fibrous feeds. The improvements were in the form of increased crude protein and lowered fibre

Table 1. Effect of ensiling wheat straw, corn stalks and sugarcane bagasse with a fibrolytic EZ for 30 days on nutrient content (%).

Feed	Enzyme level	DM	OM	EE	NFE	CF	NDF	ADF	ADL
Wheat straw	0	85.53	80.00	1.50	32.33	42.67	64.07	45.70	11.33
	1	85.74	78.18	1.75	25.27	40.03	62.73	44.51	10.78
	3	86.41	80.87	1.88	27.32	39.45	62.90	44.53	10.68
	SEM	10.07	9.27	0.18	3.31	4.72	7.29	5.20	1.32
	Linear	.5592	.53	<.0001	<.0001	.003	.3013	.1625	.0129
	Quadratic	.8541	.0939	.0402	<.0001	.124	.4314	.3785	.2006
Corn stalks	0	87.90	90.27	2.02	48.87	35.06	61.63	43.16	9.79
	1	87.21	77.30	2.14	36.56	26.13	57.16	39.16	7.88
	3	87.40	75.65	3.05	33.64	24.00	56.10	38.21	7.44
	SEM	9.01	8.36	0.26	4.14	2.97	6.00	4.14	0.87
	Linear	.7101	<.0001	<.0001	<.0001	<.0001	.0006	.0001	<.0001
	Quadratic	.7028	.0015	<.0001	<.0001	<.0001	.0591	.0238	.0005
Sugarcane bagasse	0	92.94	98.49	1.72	44.55	50.55	71.64	51.49	13.21
	1	88.65	90.93	3.40	45.07	35.28	64.01	44.66	9.96
	3	86.53	88.75	3.82	39.13	31.60	62.17	43.02	9.17
	SEM	9.20	9.54	0.33	4.43	4.11	6.79	4.79	1.12
	Linear	.0026	.0004	<.0001	.0001	<.0001	<.0001	<.0001	<.0001
	Quadratic	.3719	.0607	<.0001	.001	<.0001	.0132	.0045	.0001
Pooled SEM		5.03	4.81	0.14	2.10	2.12	3.57	2.51	0.59
P value									
Feed		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Enzyme level:									
Linear		.0027	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		.5175	<.0001	<.0001	<.0001	<.0001	<.0001	.0004	<.0001
Feed × enzyme level		.0132	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Abbreviations: DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract; CF, crude fibres; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; SEM, standard error of the mean.

and fibre fraction content. The same trends were obtained by Khattab et al. (2011) with the same EZ. Alsersy et al. (2015) stated that treating forages anaerobically with exogenous fibrolytic enzymes is accompanied with biodegradation of various fibre fractions. Lowered fibre and fibre fractions may be due to the reduction of structural polysaccharide fractions (Facchini et al. 2011). Gado et al. (2009) showed that ensiling rice straw, bagasse and corn stalks anaerobically with EZ lowered the CF in the range of 30.3–36.6%.

There were interactions between enzyme levels and fibrous feed type during the anaerobic ensiling, indicating that the effectiveness of each EZ level varied with the different forages. These interactions were expected because enzyme–feed specificity is often considered as an important determinant of enzyme

Table 2. Effect of ensiling wheat straw, corn stalks and sugarcane bagasse with a fibrolytic EZ for 30 days on *in vitro* digestion of dry matter and fibre fractions.

		DM[d] biodegradability[e]				NDF[d] biodegradability[e]		ADF[d] biodegradability[e]	
Feed	Enzyme level	*a*	*b*	*a* + *b*	*c*	*b*	*c*	*b*	*c*
Wheat straw	0	17.90	37.17	57.87	2.67	39.93	1.97	41.80	2.77
	1	23.25	42.53	66.77	3.58	44.32	2.38	45.91	3.28
	3	28.12	46.41	78.80	4.87	46.90	2.78	49.29	3.78
	SEM	2.83	5.13	8.32	0.47	5.33	0.31	5.60	0.39
	Linear	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Quadratic	.5144	.2816	.1769	.0169	.2173	.835	.6153	.9209
Corn stalks	0	12.71	37.53	47.83	2.38	43.28	2.23	40.50	2.57
	1	13.48	39.79	50.70	2.52	45.88	2.36	42.94	2.73
	3	14.29	42.18	53.75	2.67	48.63	2.50	45.51	2.89
	SEM	1.40	4.10	5.23	0.25	4.73	0.26	4.43	0.29
	Linear	.0002	.0002	.0002	.0002	.0002	.0003	.0002	.0002
	Quadratic	.8959	.8962	.8968	.8761	.899	.9593	.8992	.964
Sugarcane bagasse	0	11.49	35.85	44.86	2.28	41.69	2.28	40.90	2.67
	1	12.06	37.64	47.11	2.39	43.78	2.39	42.95	2.81
	3	12.67	39.52	49.46	2.51	45.97	2.51	45.10	2.95
	SEM	.1.24	3.88	4.86	0.26	4.51	0.26	4.43	0.29
	Linear	.0005	.0005	.0005	.0006	.0005	.0006	.0005	.0005
	Quadratic	.9162	.9304	.9293	.9593	.9308	.9593	.9272	.964
Pooled SEM[f]		1.03	2.31	3.32	0.18	2.54	0.14	2.52	0.17
P value									
Feed		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Enzyme level:									
Linear		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic		.6477	<.0001	.3294	.0172	.4996	.9566	.8474	.9818
Feed × enzyme level		<.0001	<.0001	<.0001	<.0001	.0114	<.0001	.0029	<.0001

[d]DM, dry matter; NDF, neutral detergent fibre; ADF, Acid detergent fibre.
[e]*a*, soluble fraction; *b*, potentially degradable fraction; *a* + *b*, total degradation; *c*, degradation rate.
[f]SEM, standard error of the mean.

action (Beauchemin et al. 2004). Díaz et al. (2013) reported significant interactions between enzymes and forage type. However, Arriola and Adesogan (2013) observed a lack of substrate × EZ interaction between most feed nutrients and enzymes.

Moreover, enzymatic treatments improved the DM biodegradations, increasing both the potentially degradable fraction and the degradation rate of NDF and ADF of the three fibrous feeds. In addition, significant increases were observed for NDF and ADF degradabilities. These results suggest the catalytic effect of the enzymes on the fibrous feeds, which indicated that the fermentation efficiency of fibrous feeds might be improved when enzymes were used as a pretreatment in ruminant feedstuffs (Tang et al. 2013).

The mode of action by which enzymes can improve biodegradation is still subject to speculation (Togtokhbayar et al. 2015). Arriola and Adesogan (2013) showed that anaerobic pretreatment using an exogenous fibrolytic enzyme caused a preingestive enzyme–feed interaction which may enable partial hydrolysis and bio-utilization of NDF and ADF. Anaerobic hydrolysis of NDF and ADF using ruminal contents rich in anaerobic microflora can release reducing sugars (Krueger & Adesogan 2008), modify plant cell wall structure and thereby increase fibre anaerobic digestion (Feng et al. 1996). Moreover, pretreatment with an enzyme enhances the binding of the enzyme to the feed and increases the resistance of the enzyme to ruminal proteolysis (Fontes et al. 1995).

Another probable mechanism was postulated by Wang et al. (2002) and Togtokhbayar et al. (2015) who stated that exogenous fibrolytic enzymes have the ability to increase the initial rate of DM anaerobic digestion. Increased anaerobic bacterial colonization of feed particles (Giraldo et al. 2008) is another mechanism. Exogenous enzymes can stimulate increases in the total number of viable anaerobic bacteria, causing increased fibre digestion and improving the ability of rumen anaerobic bacteria to ingest and biodegrade feed (Valdes et al. 2015). It was suggested that the improved biodegradation of feeds with EZ could be related to the enhanced ruminal anaerobic enzymes' activities as a result of increment of soluble carbohydrates released from undigested feed particles (Arriola & Adesogan 2013). Increase in soluble carbohydrates released from cell walls could stimulate the production of anaerobic bacterial glycocalyx, and increase the attraction of bacteria to the site of digestion and enhance the adhesion of bacteria to undigested feed particles (Wang et al. 2002). Sutton et al. (2002) showed that the released soluble carbohydrates provide additional energy for microbial growth and shorten the lag time for microbial colonization in the rumen (Gado et al. 2013). Anaerobic pretreatment of the fibrous feeds with EZ for 30 days encourages the biodegradation of fibre and fibre fractions, leading to the release of the soluble fraction (Giraldo et al. 2008; Gado et al. 2013) as a result of NDF and ADF anaerobic biosolubilization.

Data on DM, NDF and ADF biodegradability showed that EZ treatments at levels 3 and 1 L/ton of fibrous feeds increased and enhanced the biodegradable fraction of these forages. This is in agreement with data obtained from rice straw (Gado et al. 2013), alfalfa (Eun & Beauchemin 2007), corn silage (Chen et al. 2013), and maize stover, wheat straw and rice straw (Tang et al. 2013). Gado et al. (2013) noted that the treatment of rice straw with the same EZ cocktail increased NDF and ADF biodegradation with improved degradation fractions (*a*, *b*, *a* + *b* and *c*). In addition, pretreatment of wheat straw with EZ increased both the potentially degradable fraction and the degradation rate of NDF and ADF (Togtokhbayar et al. 2015). Tang et al. (2013) suggested that the increased degradable fraction was due to the hydrolysis of macromolecules to simpler and degradable ones as enzymes were supplemented in forages. Other reasons may be related to the activity of anaerobic endoglucanase, which hydrolyses cellulose chains at random, and exoglucanase, which hydrolyses cellulose chains from the nonreducing end in the enzyme system (Bhat & Hazlewood 2001).

The effectiveness of exogenous fibrolytic enzymes depends on many factors including enzyme type and level in addition to the type of the diet (Díaz et al. 2013). Therefore, it is imperative to test an enzyme on different forages at different levels to identify the optimum enzyme level for a particular forage. In the current study, the high level of the enzyme (3 L/ton) was more effective than the low level (1 L/ton). This may be related to the higher enzymatic activity as the level of enzyme increased (Gado et al. 2013). In a study of Gado et al. (2013) with the same EZ, they found that addition at 3 L/ton of rice straw was more effective than addition of 1 L/ton.

5. Conclusion

Agriculture wastes such as straw are carbohydrate-rich materials with a large potential as a source of dietary energy for ruminants. However, in most developing countries, they are burnt in the field causing environmental problems. The aim of this study was to determine the effect of anaerobic ensiling of raw agricultural wastes with a fibrolytic EZ as a cleaner and sustainable biological product for animal feed. The use of EZ enhanced the anaerobic bio-utilization of nutrient contents and the biodegradation of fibrous feeds used in the current study. The level of 3 L/ton of fibrous feed improved the DM, NDF and ADF biodegradation than 1 L/ton.

Disclosure statement

No potential conflict of interest was reported by the authors.

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