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Utilization of Cellulolytic Enzymes to Improve the Nutritive Value of Date Kernels and the Investigation of the Impact of Adding these Enzymes to Lactating Goat's Diets on Rumen Fermentation and Nutrients Digestibility

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ABSTRACT

Two experiments were carried out to evaluate the effects of cellulases supplementation on *in vitro* degradation of date kernels (the first trial) and *in vivo* (rumen fermentation and nutrients digestibility) by lactating Zaraibi goats (the second trial). In the *in vitro* experiment, dry matter and organic matter disappearance (IVDMD and IVOMD) were determined for date kernels supplemented separately with (Asperozym) and commercial cellulolytic enzyme source (Veta-Zyme Plus®) at 3 levels (15, 30 and 45 U kg^{-1} DM) compared with the control. The highest values ($p<0.05$) of IVDMD and IVOMD were observed with Asperozym supplementation level at 45 U kg^{-1} DM compared to control. While, Veta - Zyme Plus® gave the highest ($p<0.05$) IVDMD and IVOMD values at 15 U kg^{-1} DM compared to control. In the *in vivo* experiment, nine lactating Zaraibi goats after 7 days of parturition were divided into three groups, three animals each, using 3×3 Latin square designs. The first group was fed 37.5% Concentrate Feed Mixture (CFM), 12.5% date kernel and 50% berseem hay (control diet). The second group was fed control diet supplemented with Veta-Zyme Plus® at level 15 U kg^{-1} DM (T_1). The third group was fed control diet supplemented with Asperozyme at level 45 U kg^{-1} DM (T_2). The results indicated that Asperozym and Veta-Zyme Plus® supplementation significantly ($p<0.05$) increased nutrients digestibility, nutritive values, ruminal Total Volatile Fatty Acids (TVFA's) and ruminal ammonia nitrogen (NH_3-N) for treated groups compared with the control group.

Key words: Cellulases, date kernels, lactating zaraibi goats, digestibility, rumen

INTRODUCTION

Date kernels like other agricultural by-products or agro-industrial by-products are characterized by high levels of lignocellulose content. The problems of feeding lignocellulosic materials to farm animals are in general, low protein content, high crude fiber, low digestibility coefficients , low palatability and containing some anti-nutritional factors such as tannins and alkaloids (Kholif *et al.,* 2005). Therefore, to increase digestibility of these lignocellulosic materials, it is important to destroy the linkage between cellulose, hemicellulose and lignin or destroy the compact nature of the tissue. There have been attempts to increase the nutritive values of the by-products by mechanical, chemical or biological treatments (McHan, 1986a; Iyo and Antai, 1988; Hunt *et al*., 1992; Singh *et al.,* 1993).

Biological treatments of some agricultural by-products become essential in order to degrade lingo-cellulosics into lignin, cellulose and hemicellulose and improve crude protein content. It is well known that biological treatments could be conducted by administration of the microbial cells, microbial extracts or microbial enzymes such as cellulase enzyme (McHan, 1986b; Morrison, 1988) Cellulases are among the industrially important hydrolytic enzymes and are of great significance in present day biotechnology. These enzymes produced by numerous microorganisms such as *Aspergillus*, *cladosporium*, *Fusarium*, *Geotrichum*, *Myrothecium*, *Paecilomyces*, *Penicillium* and *Trichoderma* species (Haight, 2005; Azzaz, 2009). Cellulases are a group of fibrolytic enzymes which cooperatively hydrolyze plant cell wall fibers into glucose, cellobioses or oligosaccharides (Murad and Azzaz, 2010; Chinedu *et al*., 2010).

Cellulase as one of exogenous fibrolytic enzymes was used to improve the digestibility and nutritive value of poor quality roughages. Increasing digestibility of the diet by using exogenous feed enzymes will lead to the beneficial effects on animal performance, so such treatments are likely to be greatest for ruminants in negative energy balance, such as animals in early lactation (Rode *et al.,* 1999). Two experiments were carried out to evaluate the effects of cellulases supplementation on *in vitro* degradation of date kernels (the first trial) and *in vivo* (rumen fermentation and nutrients digestibility) by lactating Zaraibi goats (the second trial).

MATERIAL AND METHODS

This study was carried out at Agricultural Experimental Station, Sheep and Goat Research Unit, Faculty of Agriculture, Cairo University, Giza, Egypt. In cooperation with Dairy Science Department, National Research Center (NRC), Dokki, Giza, Egypt.

Collecting date kernel: Date kernels powdered were obtained from Siwa Oasis, Marsa Matrouh, Egypt.

Enzyme sources

Veta-Zyme Plus®: A commercial enzymes source produced by Vetagri® Consulting Inc, Canada. Each 1 g of this enzyme contains 400 unit of cellulase, 550 unit of amylase, 2000 unit of protease, *Lactobacillus acidophiles* 200 million Colony Forming Unit (CFU) and carrier (calcium carbonate up to 1 g).

Asperozym: Laboratory produced cellulase from *Aspergillus niger*. Each 1 g contains 133 unit of cellulase.

***In vitro* study:** Thirty five incubation flasks (250 mL volume) were used to determine the *in vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) for date kernels. Samples of 1 g of date kernels powder were accurately weighed into each flask. These flasks were separately supplemented with solution of Asperozym and Veta-Zyme Plus®. (5 flasks per each enzyme level) at different levels (0, 15, 30 and 45 U kg^{-1} DM). The *in-vitro* technique was carried out according to Fondevila and Perez-Espes (2008). The procedures were using flasks filled with 140 mL of incubation solution prepared under a CO_2 atmosphere, including a buffer solution, macro-mineral and trace mineral solution, a reduction solution and rumen inoculum. Rumen liquor was obtained from rams fed berseem hay ration using stomach tube. Whole rumen contents was obtained before

morning feeding, squeezed through four layers of gauze and liquor was collected in a pre-warmed thermos flask. Flasks were sealed and maintained at 39°C in a shaking water bath (20 oscillations/ min) for 48 h.

Feeding and management: Nine Zaraibi lactating goats (about 3 years old and weighing on average 30 kg) after 7 days of parturition were randomly assigned into three groups of three animals each using 3×3 Latin square design. The experimental periods were 12 weeks (84 days) and consisted of three equal periods (28 day each). The goats were fed on ration consisted of 50% concentrate and 50% roughage adlibtum. The concentrate feed mixture consisted of 33.33% yellow corn, 13.33% soybean meal, 20% wheat bran, 26.67% cotton seed meal, 4% minerals-vitamins premix and 2.67% molasses. The first group was fed on 37.5% Concentrate Feed Mixture (CFM), 12.5% date kernels and 50% berseem hay (Control diet). The experimental enzymes were supplemented at the recommended rate from the *in vitro* experiment. Accordingly, the second group was fed the control diet supplemented with Veta-Zyme Plus® at 15 U kg^{-1} DM. (T_1), while the third group was fed the control diet supplemented with Asperozym at 45 U kg^{-1} DM (T_2). The concentrate feed mixture, date kernels and berseem hay were divided into two portion then twice a day at 8.00 am and 4.00 pm. The enzymes were mixed well with the date kernels and introduced once a day to each group of animal. Fresh water was available at all times. The chemical composition of feed ingredients used in feeding experiment (DM basis) (Table 1).

Digestibility: A grab sample method was applied at which Acid Insoluble Ash (AIA) was used as an internal marker according to Gallup *et al*. (1945) and Forbes and Garrigus (1948) for determining the nutrients digestibility. Fecal grab samples were collected at 12 pm, for three successive days at the end of the experiment (25-27th day) from each animal. Feed consumption and residues were recorded daily.

The digestion coefficient of a certain nutrient was calculated according to the following formula:

$$\text{Digestion coefficient} = 100 - \left[\frac{\text{Indicator in feed}(\%)}{\text{Indicator in feces}(\%)} \times \frac{\text{Nutrient in feces}(\%)}{\text{Nutrient in feed}(\%)} \times 100 \right]$$

Table 1: Chemical composition of feed ingredients used in feeding experiment (DM basis)

Items	CFM	Berseem hay	Date kernels
DM	92.50	93.60	89.10
Chemical composition (%)			
OM	89.70	86.70	97.16
CP	16.49	17.47	4.60
EE	3.32	1.50	6.76
CF	7.24	19.41	13.22
NFE	62.65	48.32	72.58
Ash	10.30	13.30	2.84
Cell wall constituents (%)			
NDF	24.70	43.76	52.11
ADF	13.77	35.96	46.04
ADL	4.65	10.34	11.63
Hemicellulose	10.93	7.80	6.07
Cellulose	9.12	25.62	34.41

Hemicellulose: NDF-ADF, Cellulose: ADF-ADL, CFM: Concentrate feed mixture, DM: Dry matter, OM: Organic matter, CP: crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, NDF, Nitrogen detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin

Feeds and feces analysis: Chemical analysis of feedstuffs and feces samples were carried out to determine the percentage of Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash content according to the methods of AOAC (1995). The Nitrogen Free Extract (NFE) was calculated by difference. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined in feeds and feces according to Goering and van Soest (1970).

Statistical analysis: Data obtained from this study was statistically analyzed by SAS (1998) according to general linear model procedures outlined by Snedecor and Cochran (1982). These procedures were:

Latin square design: Latin square design for nutrients digestibilities using the general linear model procedure:

$$Y_{ijk} = \mu + R_i + C_j + T_k + E_{ijk}$$

where, Y_{ijk} is the parameter under analysis of the ijk trait, μ is the overall mean, R_i is the effect due to the lactation period on the parameter under analysis, C_j is the effect due to the animals on the parameter under analysis, T_k is the effect due to treatment on the parameter under analysis and E_{ijk} is the experimental error for ijk on the observation, assumed to be randomly distributed (0‘ σ^2).

Repeated measures for rumen liquid parameters:

$$Y_{ijk} = \mu + R_i + T_j + (RT)_{ij} + B_k + (TB)_{jk} + E_{ijk}$$

where, R_i is replicate, T_j is treatment, $(RT)_{ij}$ is interaction, B_k is sampling time, $(TB)_{jk}$ is interaction (TB) and E_{ijk} is experimental error, assumed to be randomly distributed (0‘ σ^2).

The Duncan's multiple range tests (Duncan, 1955) were used to test the significance among means for data of cellulase production trials, *in vitro* and *in vivo* experiments, milk yield, milk composition, nutrients digestibilities, rumen parameters and blood parameters.

RESULTS AND DISCUSSION

***In vitro* study:** Table 2 showed that the highest values ($p<0.05$) of IVDMD and IVOMD were observed with Asperozym supplementation level at 45 U kg^{-1} DM compared to control. While, Veta - Zyme Plus gave the highest ($p<0.05$) IVDMD and IVOMD values at 15 U kg^{-1} DM compared to control. This result may be related to some different biochemical properties of the experimental enzymes such as source organism, molecular size, etc. (Vahjen and Simon, 1999).

Digestibility and nutritive value: Data of Table 3 clearly show that both of diets supplemented with Veta-Zyme PlusÒ (T_1) and Asperozym (T_2) significantly ($p<0.05$) improved all nutrients digestibility, fiber fraction digestibility and nutritive values compared with the control diet. Also, digestibility of goats fed Asperozym (T_2) diet showed significantly ($p<0.05$) improvement compared with those fed Veta-Zyme Plus® (T_1) diet.

Table 2: Cellulases enzymes effect on (*in vitro*) dry matter and organic matter disappearance of date Kernels

Enzymes source	Enzyme levels (U kg^{-1})	IVDMD (%)	Enzyme efficiency[1] (%)	IVOMD (%)	Enzyme efficiency[2] (%)
Control	0	15.93^{c}	0.00	19. 05^{c}	0.00
Asperozym	15	16.72^{c}	4.96	19.48^{c}	2.26
	30	19.44^{c}	22.03	22.91^{c}	20.26
	45	30.30^{ab}	90.21	34.92^{ab}	83.31
Veta-Zyme Plus®	15	32.89^{a}	106.47	37.78^{a}	98.32
	30	24.03^{bc}	50.85	26.89^{bc}	41.15
	45	22.78^{bc}	43.00	26.07^{bc}	36.85

[1]Enzyme efficiency (%) (DM) = IVDMD% (sample)-IVDMD (%) (control)/IVDMD (%) (control)*100, [2]Enzyme efficiency (%) (OM) = IVOMD (%) (sample)-IVOMD (%) (control)/IVOMD (%) (control)*100 [a,b,c]means designated with the same letter in the same column are not significantly different at 0.05 level of probability, IVOMD: *In vitro* organic matter disappearance, IVDMD: *In vitro* dry matter disappearance

Table 3: Cellulase effects on digestion coefficients and nutritive values of experimental diets fed to lactating goats

	Experimental diets			
Items	Control	T_1	T_2	±SE
Nutrient digestibilities (%)				
DM	58.53^{c}	64.99^{b}	71.12^{a}	1.83
OM	60.02^{c}	65.77^{b}	72.29^{a}	1.78
CP	58.69^{c}	66.17^{b}	71.34^{a}	1.88
CF	52.14^{c}	63.00^{b}	68.89^{a}	2.48
EE	62.05^{c}	70.54^{b}	73.63^{a}	1.81
NFE	62.88^{c}	67.13^{b}	73.32^{a}	1.58
NDF	50.01^{c}	57.74^{b}	64.37^{a}	2.18
ADF	53.80^{c}	62.49^{b}	68.17^{a}	2.11
ADL	49.64^{c}	57.45^{b}	61.73^{a}	1.80
Nutritive values (%)				
TDN	56.05^{c}	61.65^{b}	66.96^{a}	1.45
DCP	9.09^{c}	10.02^{b}	11.06^{a}	0.22

[a,b,c]Means designated with the same letter in the same row are not significantly different at 0.05 level of probability. SE: standard error. T_1: Veta - Zyme Plus®, T_2: Asperozym, CP: crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, NDF: Nitrogen detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, DM: Dry matter, OM: Organic matter

Goats fed T_1 and T_2 diets showed significant ($p<0.05$) improvement in TDN and DCP compared to those fed the control diet. Goats fed Asperozym (T_2) diet showed significant ($p<0.05$) improvement in TDN and DCP compared to those fed Veta-Zyme Plus® (T_1) diet. Responses variation to fibrolytic enzymes supplementation could be attributed to the retention time of different types of fiber in the rumen; length of time that fiber is exposed to the fibrolytic enzymes process, rate of particle size reduction, particle density and rate of digestion (Nsereko *et al.,* 2000a, b). Enzymes supplementation were affected also by diet composition, type of enzyme used, level of enzyme provided, enzyme stability and method of application (Rode *et al*., 2000).

Rumen parameters: Data of Table 4 clearly show that ruminal pH showed lower ($p<0.05$) values by goats fed on T_2 and T_1 diets than goats fed on control diet. These results may be due to the intensive fermentation process of both nonstructural and structural carbohydrates and the production of volatile fatty acids. Such results are supported by the finding of Khattab *et al*. (1996) and Azzaz (2009) who observed that fibrolytic enzymes treatment significantly decreased ruminal pH. Ruminal Total Volatile Fatty Acids (TVFA's) concentration showed higher ($p<0.05$) values by goats fed T_2 and T_1 diets than those fed control diet. The pattern of TVFA's values reflects the pattern of fermentation activity in the rumen (Shafie and Ashour, 1997). Lewis *et al.* (1996) and Azzaz (2009) observed that fibrolytic enzymes treatment significantly decreased ruminal pH and increased TVFA's concentration in the rumen. Ruminal ammonia nitrogen (NH_3-N) showed significant increase ($p<0.05$) by goats fed T_2 and T_1 diets compared with goats fed control diet. The increase of ammonia nitrogen concentration with the fibrolytic enzymes treatments may be due

Table 4: Rumen parameters of lactating goats fed the different experimental diets

	Experimental diets			Overall mean of sampling time (h)		
Items	Control	T_1	T_2	0	3	6
pH	6.86[a]	6.71[b]	6.60[b]	6.92[a]	6.55[c]	6.70[b]
±SE	0.03	0.05	0.07	0.045	0.046	0.047
TVFA's (meq dL^{-1})	9.42[b]	11.02[a]	11.68[a]	8.25[c]	13.18[a]	10.70[b]
±SE	0.46	0.53	0.51	0.22	0.52	0.27
NH3-N (mg dL^{-1})	18.04[b]	20.86[a]	21.25[a]	15.54[c]	24.37[a]	20.24[b]
±SE	1.07	0.92	1.38	0.79	1.06	0.93

[a,b,c]Means designated with the same letter in the same row are not significantly different at 0.05 level of probability. SE: Standard error. T_1: Veta-Zyme Plus®, T_2: Asperozym, TVFA's: Total volatile fatty acids

to higher CP digestibility (Table 3) and higher fermentation rate in fibrolytic enzymes treated diets. EL-Ashry *et al*. (1997) and Khorshed (2000) observed that ruminal ammonia-N increased in rumen of sheep and goats when fed on rations treated with biological treatments.

CONCLUSION

Using Asperozym and Veta-Zyme Plus®, *in vitro* studies were very efficient for improving IVDMD and IVOMD of date kernels. Data of the *in vivo* studies showed that both of diets supplemented with Asperozym and Veta-Zyme Plus® increased ($p<0.05$) all nutrients digestibility, nutritive values, ruminal Total Volatile Fatty Acids (TVFA's) and ruminal ammonia nitrogen (NH_3-N) by lactating Zaraibi goats compared with the control diet.

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