Effect of ZADO®, as enzymes from anaerobic bacterium, on extent of ruminal fermentation, nutrient digestibilties and average daily gain in steers.

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Abstract

A series of experiments were undertaken to determine effects of a mixture of exogenous enzymes (ZADO®) from anaerobic bacteria on nutrient intake, digestion, ruminal fermentation and feed conversion in beef steers fed supplemented concentrate feed mixture (CFM) with or without ZADO®. Forty cross bred steers (Baladi x Friesian, average initial weight 153 ± 5.14 kg) were randomly assigned in two groups of twenty animals each, and fed ration without (CTRL) or with addition of 40 g/h/d of ZADO® enzyme powder. The ZADO® supplementation occurred for 219 days with digestibility measurements on 16 weeks after commencement. The ZADO® treatment resulted in increased daily voluntary DM intakes ($P<0.01$), addition of enzymes increased ($P<0.05$) rumen microbial N synthesis. Digestibility of all nutrients was higher ($P<0.05$) in the total tract of supplemented steers. Although the magnitude of the improvement varied among nutrients, with highest improvement in NDF and ADF increased (41.7 – 50.8% and 32.2 – 40.8% respectively) than the other nutrients. Supplementation of enzymes also increased ($P<0.05$) rumen ammonia N and total volatile catty acids (TVFA's) concentrations before and 3 h post-feeding. Daily gain was higher (1.25 – 1.45 kg, respectively; $P<0.01$) for steers fed the ZADO® supplemented diet, due to positive effects on nutrient intake and digestibility, extent of ruminal fermentation and microbial protein synthesis.

Introduction

The shortage of animal feeds in Egypt is considered as the main problem in animal production and consequently meat production in Egypt have not yat reached an acceptable level of self sufficiency because of a limited livestock population in addition to the low productivity oh native breeds (Baraghit et al., 1999).

Recent research has demonstrated that supplementing diet of feed at cattle with fiber degrading enzymes can improve feed utilization and animal performance by enhancing fiber degradation (Gado et al., 2007;Salem et al., 2007; Gado and Salem , 2008).

Proposed modes of action of direct-fed enzymes include solubilization of dietary fiber before ingestion, provision of readily fermentable substrate for ruminal microorganisms and for enhancement of microbial enzyme activity in the rumen (McAllister et al., 2001) . A variety of factors, such as the specific activity of the enzymes, their mode and level of application, as well as. the type of animal and its diet, may affect enzyme efficacy. Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Morgavi et al., 2000; Nsereko et al., 2000) to increase rate of degradation of fiber in the rumen (Tricarico et al., 2005 ; Giraldo et al., 2008) rumen microbial protein synthesis (Yang et al., 1999 ; Nsereko et al., 2002) and fore stomach digestibility. Positive effect of adding exogenous enzymes to ruminant diets have been reported for feedlote cattle, fibrolytic enzyme have improved live weight gain by as much as 35% and feed conversion ratio by up to 10% (Beauchemin et al., 1995).

A commercial exogenous enzyme mixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility, as well as live weight gain and feed conversion of wheat straw in sheep and goats (Salem et al., 2007 ; Gado. and Salem, 2008) our objective was to evaluate the effects of exogenous enzyme (ZADO®) supplemented to a concentrate feed mixture (CFM) of steers on feed intake and digestibility, ruminal fermentation and microbial protein synthesis and average daily gain (ADG).

Materials and methods

A commercial bacterial culture called ZADO® was used; (patent. No. : 22155) it is biotechnical product made from natural sources of anaerobic ruminal bacteria (including 7.1 unit/g of cellulose, 2.3 unit/g of xylanase, 61.5 unit/g of α – amylase and 29.2 unit/g of proteases) to elevate level of cellulose enzyme from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes. It is produced in monosaccharide by specific enzymes it is produced in the Molecular Biology Lab, Animal Production Department, Ain-Shams University according to the procedure of Gado (1997).

The study was conducted at a private beef farm at Sharkia, Egypt and the chemical and statistical analysis was completed at the Molecular Laboratory of the Department of Animal Production of

Ain Shams University in Cairo as well as the laboratory of Animal Nutrition of the Faculty of Agriculture of Alexandria University.

2.1. Animals and Feeding

Forty cross bred steers (Baladi x Friesian, average initial weight 153±5.14kg) were randomly assigned in two groups of twenty animals each, and fed a ration without (CTRL) or with addition of 40 g/h/d of ZADO ®enzyme powder. Feed was offered twice daily at 8.00 and 14.00 hr. fresh water was available at all times. Feed and orts samples were collected twice weekly to calculate dry matter (DM) intake. The TMR was balanced for minerals and vitamins and formulated to meet the nutrient requirements of steers according to NRC (1996) recommendation (Table, 1) the daily amount of enzymes was mixed individually for each steer with the TMR fed.

2.2. Measurements of Digestibility,

Rumen fermentation feed and orts samples were collected twice weekly to calculate dry matter (DM) intake. Apparent digestibility was determined by adding chromic oxide (55.1 mg of Cr/kg of DM) to the diets and sampling feces from the rectum of each steer at five equally spaced times per day on week 16th of the study. Fecal samples, for determination of digestibility, were composite by steer, dried at 55ºC, ground to pass a”1″mm screen and retained for chemical analysis.

On the 16th week of the study, samples of rumen fluid were withdrawn from each steer by stomach tube before the morning feeding (i.e., Oh) and 3h after feeding on one day. Samples (50 ml/cow) were immediately filtered and stored for total volatile fatty acids (TVFA ` s) and ammonia-N analysis. To determine microbial N synthesis, urine from each steer was collected for a period of 24h and diluted to a fixed volume with water, and one sub-sample was stored at -20ºC for later analysis of purine derivatives.

Table 1

Ingredient (g/ kg of DM) and chemical composition % of the concentrate mixture and rihan straw as a two component of the TMR fed to the steers.

Ingredient composition	**Treatments**	
	CTRL	ZADO®
Corn	110	110
Agwa (Palm date)	85	85
Biscuits	265	265
Sugar cane molasses	100	100
Sesamine cake	190	190
Soya bean meal	20	20
Beans	60	60
Rihan straw	147	142
Salt	10	10
Limestone	10	10
Mineral and vitamine mix*	3	3
Zado	0	5
Chemical composition (%)		
Dry matter	66	
Crude protein	13.6	
Ash	7	
Calcium	0.92	
Phosphorous	0.57	
NDF	29	
ADF	38.3	

*Mineral and vitamin mixture: ca, 190 g/d; p, 115 g/d; Mg, 63 g/d; cl, 167 g/d; K, 380 g/d; Na, 70 g/d; S, 53 g/d; Co, 3.3 mg/d; Cu 97 mg/d; Fe, 360 mg/d; Mn,900 mg/d; Se, 2 mg/d; Zn, 810 mg/d; Vit. A 940 (1000 IU); Vit, D, 165 (1000 IUD) Vit, E, 374 (1000 IULD).

2.3. Sample Analysis

Amounts of TMR offered and refused were recorded daily by steer. Feed refusals from individual steers were collected, mixed within treatment and analyzed for DM, and the DM content of feed ingredients was determined weekly to adjust dietary formulations (if necessary) to account for small changes in ingredient DM contents. To determine DM, feed samples were dried at 60 ◦C for 48 h in a forced air oven.

Dried samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) using a 1mm screen. Analytical DM content of the samples (feed, orts, feces) was determined by drying at 135 ◦C for 3 h, and organic matter (OM) was determined as the weight loss on ashingf at 550 ◦C. Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) were determined using the procedures of Van Soest et al. (1991). Sodium sulfite was not used in the procedure for aNDFom determination, but pre-treatment with heat stable amylase (type XI-A from Bacillus

subtilis; Sigma, Sharkia, Egypt) was included. Both aNDFom and ADFom are expressed without residual ash.

Chromium, as a marker to calculate nutrient digestibility, was determined in fecal samples by atomic absorption spectrophotometry according to AOAC (2000; ID 952.02). Collected feed, refusals and fecal samples were analyzed for N, aNDFom and ADFom. A Kjeldahl method (AOAC, 1990; ID 954.01) was used to determine N.

Rumen liquor (RL) samples were collected by intubation under vacuum before and 3 h after the morning feeding on the day following the digestibility study to provide an indication of the influence of ZADO® treatment on rumen fermentation characteristics. Steers were fed sequentially to maintain the 3 h period between feeding and sampling. The RL sample was strained through nylon stocking and pH measured immediately with a digital pH meter. At each sampling time, duplicate RL samples were collected for NH3-N determination using steam distillation, and a single sample collected for total volatile fatty acid (TVFA's) determination using methods as outlined by Lopez et al. (2003).

Concentrations of ruminal NH3-N were determined using the colorimetric method described by Rhine et al. (1998). A standard curve was made to determine whether a linear relationship existed between varying concentrations of ammonium sulfate standard solution and intensity of color produced by Nesslerization. Ten test tubes containing 0.10–1.0 ml of standard solution were prepared. To each test tube, 0.04 ml of Nessler's reagent was added and volume was made up to 5ml with distilled water. The intensity of color thus developed was measured at a wavelength of 420 nm on Spectronic 21 within 5–10 min after setting it at 0 absorbance with the blank.

Sub-samples of urine were analyzed for allantoin by high-performance liquid chromatography with pre-column derivation according to Chen et al. (1993) and for uric and hypoxanthine plus xanthine according to Chen et al. (1990). In the latter method, hypoxanthine and xanthine were determined collectively as uric acid after treatment with xanthine oxidase. Urine samples were diluted with distilled water before the assays, by 40 times for allantoin and 10 times for uric and hypoxanthine plus xanthine. The N content of urine was determined by the method of Davidson et al. (1970). All daily urine samples were analyzed individually. Rumen microbial N was calculated depending on the total purine derivatives (i.e., allantoin and uric acid measured) according to Chen et al. (1990).

2.4. Statistical Analysis

Collected date on nutrient intake and digestibility ruminal fermentation and average daily gain parameters in the two steers groups (i.e., CTRL and ZADO enzymes) were analyzed as factorial design using the general linear models procedure of SAS (2001), with methods of Steel and Torrie (1980) to determine differences due to enzymes addition. The significance level of the test was $P<0.05$.

Results

Ingredients digestibility of DM, OM, CP, NDF and ADF was higher ($P<0.05$) for steers fed enzyme supplemented diet (Table 2).

Enzyme supplemented steers had higher TVFAs and ammonia – N concentration (Table 3) pre-feeding (113 versus 100 mmo 1/1; 67 versus 55 mg/1 respectively) and α 3 h post-feeding (120 versus 110 mm01/1; 65 versus 54 mg/1, vespectively). Microbial N synthesis was higher in enzyme supplemented steers (200 versus 170 g/d; $P<0.01$).

Output of daily gain and feed per gain was higher ($P<0.01$) for steers fed the enzyme supplemented diet (Table 4).

Table 2

Dry matter intake and nutrients digestibility of the TMR fed to steers supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture.

Item	CTRL	ZADO®	P value
DM intake, kg/ day	7.3[b]	7.8 [a]	0.01
Digestibility %			
DM	61.7 [b]	69.1 [a]	0.05
OM	67.4 [b]	75.3 [a]	0.01
CP	83.5 [b]	87.4 [a]	0.05
aNDFom	41.7 [b]	50.8 [a]	0.01
ADFom	32.2 [b]	40.8 [a]	0.01

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. [a]TMR: total mixed ration without (CTRL) or with (ZADO ®) the commercial exogenous enzyme mixture.

Table 3

Ruminal pH, TVFA's, ammonia N concentrations (after 0 and 3h of feeding); microbial nitrogen synthesis of the TMR[a] fed to steers supplemented with (ZADO® or without (CTRL) the exogenous enzymes mixture.

Item	CTRL	ZADO®	P value
Before- feeding (0h)			
pH	6.8 [a]	6.4 [b]	0.03
TVFA's, mmol/1	100[b]	113[a]	0.01
Ammonia N, mg/1	55 [b]	67[a]	0.01
Post-feeding (3h)			
pH	6.1 [a]	5.9	N.S.
TVFA's, mmol/1	110 [b]	120[a]	0.05
Ammonia N, mg/1	54 [b]	65[a]	0.05
Microbial N, g/d	170 [b]	200[a]	0.01

[a]TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture.

Table 4

Daily gain, and feed conversion (feed per gain) of the TMR[a] fed to steers supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture.

Item	CTRL	ZADO®	P value
NO. of animal	20	20	
NO. of days	219	219	
Initial weight, kg	156	151	N.S.
Final weight, kg	430 [b]	470[a]	0.05
Gain, kg	274 [b]	319[a]	0.01
Daily gain, kg	1.25 [b]	1.45[a]	0.01
DM intake, kg/day	7.3 [b]	7.8[a]	0.01
Feed per gain	5.8	5.3	0.01

[a]TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture.

Discussion

4.1. Nutrient intake and digestibility

Increased DM intake by addition of enzymes may be partly due to increased digestibility, which is consistent with previous results with the same enzyme mixture (Soliman, 2006; Gado et al., 2007; Salem et al., 2007; El-Adawy et al., 2008; Gado and Salem, 2008).

In the current study, DM intake and digestibility were improved by about 6.8 and 12% respectively also, OM and CP digestibility were improved by about 11.7 and 4.7% respectively with enzyme addition. Other reports have also shown in DM, particularly fiber, digestibility with fibrolytic enzyme addition (Gado and Salem, 2008; Hristov et al., 2008). Bowman et al. (2002), for example, reported a 25% increase in total tract aNDFom digestibility with a fibrolytic enzyme product, but it appeared that most of the impact was post-ruminal. Our results are consistent with Beauchemin et al .(2003b), Miller et al. (2008), Mohamed et al. (2009) and Gado et al.(2009), who reported that the average increase in DM intake due to ZADO® supplementation was 2.1 kg/d in dairy cows.

Generally, digestion of aNDFom varies due to the chemical composition of the diet (Varga et al.,1998), the size of the indigestible aNDFom fraction, the digestion rate of potentially digestible aNDFom and rumen outflow rate (Firkins et al., 1998), as well as use of feed additives. Exogenous fibrolytic enzymes would be expected to increase fiber digestion by increasing the rate of ruminal digestion of the potentially digestible aNDFom fraction (Yang et al., 1999), but increases in fiber digestion may also be, in part, due to reduced digesta viscosity (Hristov et al., 2000), alterations in ruminal fermentation (Nsereko et al., 2002) and/or enhanced attachment and colonization to the plant cell wall by ruminal microorganisms (Nsereko et al., 2000; Wang et al., 2001) and/or by synergism with enzymes in rumen fluid (Morgavi et al., 2000). However, increased fiber digestion is unlikely the result of supplemental enzyme activity alone because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin et al., 2001). Morgavi et al. (2000) demonstrated synergism between exogenous enzymes and ruminal enzymes such that the net combined hydrolytic effect in the rumen was much greater than that estimated from individual enzyme activities. Wang et al. (2001) reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more total polysaccharidase activity to digest feedstuffs.

Previous studies in our laboratory (i.e., Gado et al., 2007) showed that this ZADO enzyme product improved in vitro DM and aNDFom digestibility of wheat straw in the first 24 h of incubation, which is consistent with Feng et al. (1996), Miller et al. (2008) and Wang et al. (2004), who suggested that exogenous fibrolytic enzymes increase rate of DM digestion. Our exogenous enzyme, rich in xylanolytic, cellulase, α-amylase and protease activity, had positive effects on digestion of aNDFom in TMR, consistent with Krause et al. (1998), who suggested that enzymes can improve nutrient degradation in high concentrate diets. Perhaps the net effects of fibrolytic enzyme mixtures are not limited to the dietary component to which the enzymes are

applied, which may explain why fibrolytic enzymes can be effective in improving digestibility of the non-fiber carbohydrates in addition to increasing digestibility of fiber when enzymes are added to the concentrate portion of a diet, or to high-concentrate diets (Beauchemin et al., 2003a).

4.2. Ruminal fermentation and microbial protein synthesis

Previous studies have shown that treatment of diets with enzymes before feeding, or incubation with ruminal fluid, enhanced beneficial effects of enzymes on ruminal fermentation (Wang et al., 2001; Giraldo et al., 2004; Gado and Salem, 2008). As pointed out by Colombatto et al. (2003), some have suggested that this could be due to creation of a stable enzyme–feed complex (Kung et al., 2000), but others have indicated the possibility of alteration in the fiber structure, which would stimulate microbial colonization (Nsereko et al., 2000; Giraldo et al., 2008; Ranilla et al., 2008). Wang et al. (2001) suggested that changes in rumen fermentation patterns may reflect a shift in the species profile of colonizing bacteria in response to enzyme treatment of feed with exogenous enzymes.

The average concentration of VFA was higher in ZADO® than CTRL before and post feeding. In general, total volatile fatty acids in the rumen of steers increased after feeding to reach

its peak at 3hr post- feeding in all treatments. It is clear the VFA followed the opposite trend of the pH values this results agree with Baraghit et al. (2009). the resulted increase in TVFA's concentration with the inzymes supplement is correlated with the increase in nutrients digestibilties seen in this study. This results agreement with Omar et al .(2009), who concluded that supplementation of exogenous enzymes to steers rations as fibrolytic enzymes. Improved digestibilities and rumen TVFA's concentrations.

Increased ammonia N concentration in cows fed the enzyme supplemented diet supports its

capability to enhance rumen protein degradation, probably because it contained protease enzymes.

However increased protein degradation may also reflect the more neutral rumen pH with enzyme

addition, thereby increasing ruminal bacterial colonization of feed particles (Yang et al., 1999; Morgavi et al., 2000; Nsereko et al., 2000). However, Colombatto et al. (2007),working with an enzyme product rich in xylanolytic activity, concluded that exogenous enzymes had higher activity close to pH neutrality and that the hypothesis that exogenous enzymes have an effect on digestion when pH values were not optimal for fiber degradation is not supported.

Feeding the enzyme preparation may have stimulated and/or increased total viable rumen bacterial numbers because rumen microbial N synthesis was increased which may be due, at

least in part, to increased fiber digestion and an improved capacity of rumen bacteria to digest feed. Although this possibility may not be supported by Nsereko et al. (2002) and Krueger et al. (2007) who showed that while cellobiose and glucose utilizing bacteria were stimulated, effects on the fibrolytic population were negligible. Our results indicate that enzyme supplementation increased the quantity of microbial protein available to animal metabolism, and that increased fiber digestibility increased the net energy density of the ZADO enzyme diet. This may create conditions in which upplementary fibrolytic exogenous enzyme (EE) will have beneficial effects (Beauchemin et al., 2001). These results of exogenous enzyme might be related to the more utilization of the dietary energy and positive fermentation in the rumen.

4.3 Daily gain and feed conversion:

Major finding of our study was that daily gain and feed conversion was higher in enzyme supplemented steers (1.25 versus 1.45 kg/d and 5.3 versus 5.8 feed/gain) the enzyme diet was a higher average daily gain and DM intake. The large intense in DM intake, digestibity and ruminal fermentation activities suggest that increased gain due to feeding enzymes.

Studies on enzyme supplementation to steers diets have shown increased gain (Beauchemin et al., 1995; Lewis et al., 1996, Krause et al., 1998, ZoBell et al., 2000, Krueger et al., 2008), probably due to increased digestibility and energy available for growth and production (Yang et al., 1999; ZoBell et al., 2000, Tricarico et al., 2005).

Titi (2004) noted that exogenous fibrolytic enzyme resulted in improved feed conversion of fattened Awassi sheep. The same author also, indicated that fobrolytic enzymes could enhance the growth of fattened lambs and improve their conversion rations mainly through improving digestibility.

Conclusions

The exogenous enzyme product (ZADO®), sourced from anaerobic bacterium and added to the TMR of steers increased daily gain due to enhanced nutrient intake, and nutrient digestibility, as well as increased rumen microbial protein synthesis.

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