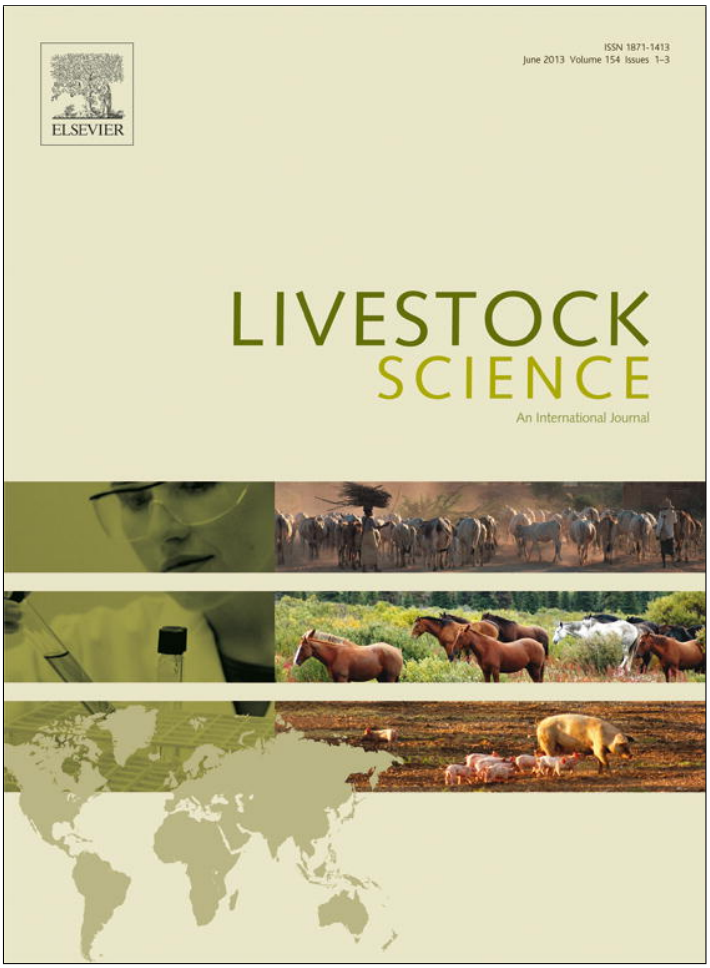

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# Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers

A.Z.M. Salem [a,d,*], H.M. Gado [b], D. Colombatto [c], M.M.Y. Elghandour [a]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México
[b] Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[c] Facultad de Agronomía, Universidad de Buenos Aires, Argentina
[d] Faculty of Agriculture (El-Shatby), Alexandria University, Egypt

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ABSTRACT

Forty crossbred steers (Baladi × Friesian, average BW 153 ± 5.14 kg) were used to evaluate the effects of exogenous enzyme (ENZ) addition on nutrient intake, digestion, ruminal fermentation and feed conversion in beef steers. Steers were randomly assigned to two groups of 20 animals and fed individually a total mixed ration (TMR) without (CTRL) or with addition of 40 g/hd/d of an enzyme mixture (ZADO®). The ENZ mixture was added for 220 days and *in vivo* apparent digestibility was measured on days 210–220. Enzyme addition did not affect ($P=0.1$) DM intake, whereas it increased ($P<0.05$) total tract apparent digestibility of all nutrients. The magnitude of improvement in digestibility varied among nutrients, with the highest improvement occurring in digestibility of NDF and ADF (21.8% and 26.7%, respectively). Addition of ENZ also increased ($P<0.05$) concentrations of rumen ammonia N and total short chain fatty acids (SCFA) before and 3 h post-feeding. Allantoin concentration total purine derivates were increased ($P=0.04$) with enzyme addition while uric acid was not affected ($P=0.05$). Live-weight gain was also higher ($P<0.01$) in steers supplemented with ENZ. In conclusion, adding the exogenous enzyme product increased live-weight gain by 16% due to increased nutrient digestibility.



## 1. Introduction

Recent research has demonstrated that feeding cattle on diets supplemented with fiber-degrading enzymes can improve feed utilization and animal performance by enhancing fiber degradation (Arriola et al., 2011; Holtshausen et al., 2011).

However, there is still controversy regarding the mode of action and effectiveness of these products in ruminants. Proposed modes of action of direct-fed enzymes include hydrolysis of dietary fiber before ingestion, provision of readily fermentable substrates for ruminal microorganisms and synergistic enhancement of microbial enzyme activity in the rumen. 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 (Beauchemin et al., 2004; McAllister and Cheng, 1996). Direct-fed enzymes have been shown to enhance microbial colonization of feeds by increasing numbers of ruminal fibrolytic microbes (Morgavi et al., 2004; Nsereko et al., 2002) and to increase the rate of ruminal fiber degradation (Giraldo et al., 2008; Tricarico et al., 2005), ruminal microbial protein synthesis (Nsereko et al., 2002; Yang et al., 1999) and total tract digestibility (Gado et al., 2011, 2009). Production benefits of adding exogenous fibrolytic enzymes to ruminant diets have included

* Corresponding author at: Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, México.
Tel.: +52 1722 296 55 42; fax: +52 1722 180 61 94.
*E-mail addresses:* asalem70@yahoo.com, asalem70@hotmail.com (A.Z.M. Salem).

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increased live weight gain by as much as 35% and increased feed conversion ratio by up to 10% in steers (Beauchemin et al., 1995) and 92% in lambs (Gado et al., 2011). In contrast, other studies have shown no results when enzymes have been added to ruminants, as reviewed by Beauchemin et al. (2004).

Recently, some promising results were reported when a specific mixture of enzyme activities (ZADO®, Cairo, Egypt) was added to lambs (Gado et al., 2011) and dairy cows (Gado et al., 2009), but less published research is available regarding its effects on beef cattle performance. The aim of the present work was to evaluate the effects of an exogenous enzyme mixture (ZADO®) added to a total mixed ration fed to beef steers on feed intake, digestibility, rumen fermentation. Concentrations of allantoin and uric acid were estimated in the urine as predictors of microbial protein synthesis and live-weight gain was also determined.

## 2. Materials and methods

The study was conducted at a private beef farm at Sharkia, Egypt, while the chemical and statistical analyses were completed at the Molecular Laboratory of the Department of Animal Production of Ain Shams University in Cairo (Egypt) as well as in the laboratory of Animal Nutrition of the Faculty of Agriculture of Alexandria University (Egypt).

### 2.1. Enzyme product and activity determination

The commercial enzyme product, ZADO® (Patent no.: 22155, Cairo, Egypt) is produced from *Ruminococcus flavefaciens* by the Academy of Scientific Research and Technology in Egypt. Prior to this work, the enzyme mixture was assayed for several enzymatic activities, and it was found to contain 7.1 U/g of endoglucanase, 2.3 U/g of xylanase, 61.5 U/g of α-amylase and 29.2 U/g of protease activity.

Briefly, endoglucanase activity was assayed by liberating glucose from carboxymethyl cellulose, which was determined calorimetrically using alkaline copper reagent, as described by Robyt and Whelan (1972). One unit of endoglucanase catalyzes the liberation of 1 mmol of glucose per minute from sodium carboxymethyl cellulose at 40 °C and pH 4.5. Furthermore, α-amylase was assayed by its ability to produce reducing groups from starch, which were measured by the reduction of 3,5-dinitrosalicylic acid (Bernfeld, 1955). One unit of α-amylase catalyzes the liberation of 1 mmol of reducing groups per minute from soluble starch at 25 °C and pH 6.0, calculated as maltose equivalents. Protease activity was determined by the hydrolysis of dimethyl casein (DMC) and the liberated amino acids were determined using 2,4,6-trinitrobenzene sulfonic acid (Lin et al., 1969). One DMC-U catalyzes the cleavage of 1 mmol of peptide bond per minute from DMC at 25 °C and pH 7.0, expressed in terms of newly formed terminal amino groups. Xylanase catalyzes the hydrolysis of xylan from oat spelts, and the reducing groups liberated were determined using alkaline copper reagent (Robyt and Whelan, 1972). One unit catalyzes the liberation of 1 mmol of reducing groups per hour from xylan at 37 °C and pH 5.5, expressed as xylose equivalents.

### 2.2. Animals and feeding

Forty crossbred steers (Baladi × Friesian, average BW 153 ± 5.14 kg) were randomly assigned on the same day to two experimental groups of 20 animals housed in individual pens and fed a ration without (CTRL group) or with addition of 40 g/hd/d of ZADO® enzyme powder (ENZ group) for 220 days. The application rate was chosen based on previous research using this same enzyme mixture (Gado et al., 2011, 2009).

Steers were fed individually *ad libitum* a total mixed ration (TMR) that was formulated to meet the nutrient requirements of steers according to National Research Council (NRC) (1996) recommendations (Table 1) with the target to reach 450 kg BW in 220 days of experimental period. The daily amount of enzymes was mixed individually for each steer with the TMR fed at 0800 h. Feed was offered twice daily at 0800 and 1400 h, and fresh water was available at all times.

### 2.3. Measurements of digestibility and ruminal fermentation

Feed and ort samples were collected daily, and composited to one weekly sample to determine dry matter (DM) intake. Apparent digestibility was determined for 10 days (d 210–220) by adding chromic oxide (55.1 mg of Cr/

**Table 1**
Ingredient and chemical composition (g/kg of DM) of the TMR[a] fed to the steers.

| Ingredient composition | g/kg |
|---|---|
| Yellow corn grain, ground | 110 |
| Agwa (Palm date ) | 85 |
| Biscuits | 265 |
| Molasses, liquid | 100 |
| Sesamine cake | 190 |
| Soya bean meal | 20 |
| Beans | 60 |
| Rihan straw | 147 |
| Salt | 10 |
| Limestone | 10 |
| Mineral and vitamin mix[b] | 3 |
| *Chemical composition* | |
| Dry matter | 660 |
| Crude protein | 136 |
| Ash | 70 |
| Neutral detergent fiber | 383 |
| Acid detergent fiber | 290 |
| Calcium | 9.2 |
| Phosphorous | 5.7 |

[a] TMR: total mixed ration.
[b] Mineral and vitamin mixture per kg: Ca, 190 g; P, 115 g; Mg, 63 g; Cl, 167 g; K, 380 g; Na, 70 g; S, 53 g; Co, 3.3 mg; Cu, 197 mg; Fe, 360 mg; Mn, 900 mg; Se, 2 mg; Zn 810 mg; Vit. A 9401000 IU); Vit. D 165 (1000 IU); and Vit. E 374 (1000 IU).

kg of DM) to the diets and sampling feces from the rectum of each steer at five equally spaced times at 10, 12, 14, 16 and 18 h post first feeding per day. Fecal samples were composited by steer, dried at 55 °C, ground to pass a 1-mm screen and stored for further chemical analysis.

On the day 202 of the study, samples of ruminal fluid were withdrawn from each steer using a stomach tube before the morning feeding (*i.e.*, 0 h) and 3 h after feeding on one day (d 202 of the experiment). Samples (50 mL/steer) were immediately filtered using four layers of cheesecloth. Strained rumen liquor was stored in glass bottles (45-mL) with a few drops of toluene and paraffin oil to cover the surface and stored at −18 °C for total short chain fatty acids (SCFA) and ammonia-N analyses. To determine the purine derivatives (allantoin and uric acide) as a prediction of microbial N synthesis, urine was collected daily from each steer during the 10 days of feces collection (days 210–220) for a period of 24 h and diluted to a fixed volume with water, and one sub-sample was stored at −20 °C for analysis of purine derivatives (Perry et al., 1966).

### 2.4. Chemical analysis

Feed samples and refusals were dried at 60 °C for 48 h (Association of Official Analytical Chemists (2000): ID 934.01) in a forced air oven before analysis. Dried samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). Dry matter contents of feed, orts and feces were determined by drying at 105 °C for 4 h (Association of Official Analytical Chemists (2000): ID 934.01).

Neutral detergent fiber (NDF—Van Soest et al., 1991) and acid detergent fiber (ADF—AOAC, 2000: ID: 973.18) contents were determined using an $ANKOM_{200}$ Fiber Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA).

Chromium oxide, used as a marker to calculate nutrient digestibility, was determined in fecal samples by atomic absorption spectrophotometry (Z-2000 Series Polarized Zeeman Atomic Absorption spectrophotometer, PerkinElmer Inc., Shelton, CT) according to AOAC (2000: ID 952.02). Collected feed, refusals and fecal samples were also analyzed for crude protein (Association of Official Analytical Chemists (2000): ID 954.01).

Ruminal fluid samples were strained through cheesecloth, and pH was measured immediately with a digital pH meter. At each sampling time, duplicate ruminal samples were collected for $NH_3$-N determination using steam distillation (Rhine et al., 1998) and a sample collected for total SCFA determination as outlined by Lopez et al. (2003).

Sub-samples of urine were analyzed for allantoin by high-performance liquid chromatography, according to Chen et al. (1993) and for uric and hypoxanthine plus xanthine, according to Chen et al. (1990). The N content of urine was determined by the method of Davidson et al. (1970). Rumen microbial N was calculated depending on the total purine derivatives (*i.e.*, allantoin and uric acid measured), according to Chen et al. (1990).

### 2.5. Statistical analysis

Data on nutrient intake, digestibility, ruminal fermentation parameters and growth performance in the two treatments were analyzed as a completely randomized design using the general linear models procedures of SAS (2001) with the model

$$Y_{ikl} = \mu + T_i + A_k + E_{ikl}$$

where $Y$ expressed every observation of the $k$th animal in the $i$th treatment, $\mu$ expressed the general mean, $T$ expressed the treatment effect, $A$ expressed the animal effect and $E$ expressed the experimental error. Unless stated otherwise, significance was declared when $P < 0.05$, and trends were discussed when $P < 0.10$.

## 3. Results

Intake of DM was not affected ($P=0.11$) by addition of ENZ, whereas digestibility of DM, OM, CP, NDF and ADF were higher ($P < 0.05$) in enzyme-supplemented steers (Table 2). Enzyme-supplemented steers had lower ruminal pH before feeding ($P=0.03$), while there was only a trend towards lower pH 3 h post feeding ($P=0.06$). Enzyme addition increased ($P < 0.05$) SCFA and ammonia N concentrations (Table 3) pre-feeding and 3 h post-feeding. Allantoin concentration increased ($P=0.04$) with enzyme addition while uric acid was not affected ($P=0.05$) in steers. However, total purine derivates were higher ($P=0.04$) in enzyme-supplemented steers. Live-weight gain was higher ($P=0.01$) by 16% and feed conversion was improved ($P=0.04$) by 9% in steers fed the enzyme-supplemented diet (Table 4).

## 4. Discussion

### 4.1. Nutrient intake and digestibility

Nutrient digestibility was improved by about 12%, while DM intake was not affected with the addition of ENZ. Organic matter and CP digestibility were improved by about 11.7% and 4.7%, respectively due to ENZ supplementation. Previous reports using the same enzyme product have also shown that digestibility of DM, and particularly fiber, were increased (Gado et al., 2011).

**Table 2**
Dry matter intake and total tract nutrient digestibility of the TMR[a] supplemented with (ENZ) or without (CTRL) the exogenous enzyme mixture in steers.

| | CTRL | ENZ | SEM | *P* |
|---|---|---|---|---|
| Dry matter intake (kg/d) | 7.3 | 7.8 | 0.3 | 0.11 |
| Digestibility (g/kg DM) | | | | |
| Dry matter | 617 | 691 | 12.2 | 0.04 |
| Organic matter | 674 | 753 | 151 | 0.01 |
| Crude protein | 835 | 874 | 7.6 | 0.04 |
| Neutral detergent fiber | 417 | 508 | 12.3 | 0.01 |
| Acid detergent fiber | 322 | 408 | 15.1 | 0.01 |

[a] TMR: total mixed ration supplemented with (ENZ, 40g/hd/d) or without (CTRL) the exogenous enzymes mixture.

Digestion of NDF varies due to the chemical composition of the diet, the size of the indigestible NDF fraction, the degradation rate of potentially digestible NDF and rumen outflow rate (Firkins et al., 1998). Exogenous fibrolytic ENZ would be expected to increase fiber digestion by increasing the potentially digestible NDF fraction (Yang et al., 1999). However, increases in fiber digestion may also be, in part, due to reduced digesta viscosity (Hristov et al., 2000), altered ruminal fermentation (Nsereko et al., 2002), enhanced attachment and colonization to the plant cell wall by rumen microorganisms (Nsereko et al., 2002; Wang et al., 2001) and/or by synergism with ENZ in rumen fluid (Morgavi et al., 2004). Morgavi et al. (2004) demonstrated synergism between exogenous and ruminal ENZ such that the net combined hydrolytic effect in the rumen was much greater than that estimated from individual enzyme activities. Colombatto et al. (2003) and Wang et al. (2001) reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system. 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.

### 4.2. Rumen fermentation

Previous studies have shown that enzyme addition before feeding, or incubation with rumen fluid, enhanced the beneficial effects of ENZ on rumen fermentation (Gado et al., 2011; Giraldo et al., 2008). As pointed out by Colombatto et al. (2003), some have suggested that these phenomena could be due to creation of a stable enzyme–feed complex (Kung et al., 2000). Others have indicated the possibility of alteration in the fiber structure, which would stimulate microbial colonization (Arriola et al., 2011; Gado et al., 2009). Wang et al. (2001) suggested that changes in rumen fermentation patterns might reflect a shift in the species profile of colonizing bacteria in response to enzyme treatment of feed with ENZ. The average concentration of SCFA was higher in ENZ *vs.* CTRL before and post feeding. The increase in SCFA concentration with the ENZ supplementation is consistent with the increase in nutrient digestibility due to ENZ treatment in this study, and with the trend towards lower ruminal pH post feeding.

Increased ammonia N concentration in steers fed the ENZ supplemented diet supports its capability to enhance rumen protein degradation, probably because it contained protease enzymes, or because there was a shift in microbial populations. Colombatto et al. (2007), working with an ENZ product rich in xylanase activity, concluded that the effect of exogenous ENZ was more pronounced at pH values closer to neutrality, implicating that the hypothesis that exogenous ENZ have an effect on digestion when pH values are not optimal for fiber degradation is not valid.

Feeding the ENZ preparation may have stimulated and/or increased total viable rumen bacterial numbers, which is supported by the increase of total purine derivates as an indicator of rumen microbial protein synthesis. Thus, the net result was an increase in fiber digestion and the improvement in the capacity of rumen bacteria to digest feed. Our results indicate that ENZ supplementation increased the quantity of microbial protein available to animal metabolism. Increasing fiber digestibility increased the net energy density of the ENZ diet. This may create conditions in which supplementary fibrolytic exogenous ENZ will have beneficial effects (Beauchemin et al., 2004).

**Table 3**
Ruminal pH, concentrations of short chain fatty acids (SCFA) and ammonia N (after 0 h and 3 h of feeding); allantoin, uric acid and total purine derivatives in steers fed a TMR[a] supplemented with (ENZ) or without (CTRL) the exogenous enzyme mixture.

| | CTRL | ENZ | SEM | *P* |
|---|---|---|---|---|
| Before feeding (0h) | | | | |
| pH | 6.8 | 6.4 | 0.11 | 0.03 |
| SCFA (mmol/L) | 100 | 113 | 2.1 | 0.01 |
| Ammonia N (mg/L) | 55 | 67 | 4.8 | 0.01 |
| Post-feeding (3 h) | | | | |
| pH | 6.1 | 5.9 | 1.83 | 0.06 |
| SCFA (mmol/L) | 110 | 120 | 6.8 | 0.05 |
| Ammonia N (mg/L) | 54 | 65 | 8.9 | 0.05 |
| Allantoin (mmol/d) | 200 | 210 | 2.34 | 0.04 |
| Uric acid | 18 | 20 | 1.17 | 0.05 |
| Total purine derivatives | 218 | 230 | 3.16 | 0.04 |

[a] TMR: total mixed ration supplemented with (ENZ, 40 g/h/d) or without (CTRL) the exogenous enzymes mixture.

### 4.3. Animal performance

A major finding of our study was that live-weight gain and feed conversion were improved in ENZ supplemented steers by 16% and 9%, respectively. The higher digestibility and ruminal fermentation activities due to feeding ENZ probably resulted in the observed increased live-weight gain. Other studies on ENZ supplementation to steer diets

**Table 4**
Live weight and feed efficiency of steers fed a TMR[a] supplemented with (ENZ) or without (CTRL) the commercial exogenous enzyme mixture.

| | CTRL | ENZ | SEM | *P* |
|---|---|---|---|---|
| No. of steers | 20 | 20 | | |
| Initial live-weight (kg) | 156 | 151 | 4.6 | 0.12 |
| Final live-weight (kg) | 430 | 470 | 11.3 | 0.05 |
| Live-weight gain (kg/d) | 1.25 | 1.45 | 0.22 | 0.01 |
| Feed efficiency (kg DM/kg live-weight gain) | 5.8 | 5.3 | 0.21 | 0.04 |

[a] TMR: total mixed ration supplemented with (ENZ, 40 g/h/d) or without (CTRL) the exogenous enzymes mixture.

also have shown increased gain (Krueger et al., 2008; ZoBell et al., 2000), probably due to increased digestibility and energy availability for growth and production (Gado et al., 2011; Tricarico et al., 2005).

Gado et al. (2011) found that treatment of ensiled orange pulp with the same product used in the present study increased nutrient digestibility, ruminal fermentation and live weight gain by lambs. The ENZ improved the lamb's average daily gain by 92% and digestible DM by 18%. Titi and Lubbadeh (2004) noted that exogenous fibrolytic ENZ addition improved feed conversion of fattening Awassi sheep.

## 5. Conclusions

In conclusion, addition of the exogenous enzyme product to steers fed a TMR increased live-weight gain by 16% due to enhanced ruminal fermentation and digestion as well as increased total purine derivatives.

## Conflict of interest statement

The authors declare that no conflict of interest, financial or other, exists.

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