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Effect of Exogenous Enzymes on the Growth Performance and Digestibility of Growing Buffalo Calves

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ABSTRACT

This study was carried out to investigate the effect of an enzymatic mixture with cellulase, xylanase, alpha-amylase and polyglacturenase activities on the performance of buffalo male calves. Sixteen buffalo male calves were distributed randomly on four treatments with four animals each. The feeding trial lasted for 180 days. Four metabolism trials were carried out at the end of the experimental period. The four experimental rations were as follows. R_1: 2% of body weight fed concentrate feed mixture + *Pennisetum glaucum* (pearl millet) *ad-lib* (control ration). R_2: 2% of body weight fed concentrate feed mixture supplemented with SBP at 0.2% (W/W) + *Pennisetum glaucum ad-lib*. R_3: 2% of body weigh fed concentrate feed mixture supplemented with sugar beet pulp supplemented with experimental enzymes (SBP) at 0.4% (W/W) + *Pennisetum glaucum ad-lib*. R_4: 2% of body weight fed concentrate feed mixture supplemented with SBP at 0.6% (W/W) + *Pennisetum glaucum ad- lib*. The results indicated that: Feed intake was not affected ($P < 0.05$) by enzyme supplementation among experimental groups. Supplementation with exoenzymes caused a significant ($P < 0.05$) increase in both of average daily gain and total body weight gain. The highest values of total body weight gain (kg) and daily body weight gain (g) were recorded with group R_4 compared to other groups. Supplementation with exoenzymes showed significant ($P < 0.05$) increase in feed conversion as (Kg DM/kg gain) and (Kg TDN/kg gain). The highest value of (kg DM/kg gain) was recorded with R_4 group. However, the highest value of (Kg TDN/Kg gain), were recorded with R_2 and R_3 groups. Supplementation with exoenzymes increased ($P < 0.05$) digestibility coefficients of DM, OM and CF for R_4 group compared to other groups. However, digestibility of CP, EE and NFE were significantly higher ($P < 0.05$) for all exoenzyme groups compared to the control group. Total digestible nutrients (TDN) was significantly ($P < 0.05$) higher for all exoenzymes groups compared to the control group, the highest value of TDN was recorded with R_4 group but the differences between R_2 and R_3 groups were not significant. All of the blood serum parameters were within the range of the normal values for healthy buffalo calves. Results indicated that, exogenous enzymes treatment were good methods for enhancing the growth performance and digestibility of ration containing pearl millet without any hazard on buffalo health.

Key Words: Enzymes; Buffalo; Growth performance; Nutrients digestibility; Blood serum

INTRODUCTION

Forages have always provided the base upon, which ruminant nutrition is built. It is evident that the ruminant animals consume grasses, leaves and stems rich in cellulose, hemicellulose and legnin. These animals do not produce the enzymes responsible for degradation of lignocelluloses but are dependent on associated microbial populations. Exogenous polysaccharidases may survive for a considerable period of time in the small intestine and they probably maintain activity against target substrates in this environment (Morgavi *et al*., 2001).

Interest in applying fibrolytic enzymes to ruminant diet has increased recently due to enzyme-mediated increases in feed digestion *in vitro* (Lewis *et al*., 1996; Bowman *et al*., 2002; Kung *et al*., 2002) and diet use *in vivo* (Schingoethe *et al*., 1999; Yang *et al*., 1999). However, in certain studies (Bowman *et al*., 2002; Vicini *et al*., 2003) exogenous enzyme supplementation did not consistently improve animal performance. Where improved performance was observed, the mechanism was not always confirmed by improved digestion (Mandebvu *et al*., 1999).

These inconsistencies were due to various factors such as enzyme type, concentration and activity, application method, substrate to which enzyme is added and animal differences (Bowman *et al*., 2002). Additional factors that may be implicated include prevailing temperature and pH, presence of cofactors and inhibitors, and enzyme and substrate concentration.

Nevertheless, feed enzymes have been used to improve the use of a wide range of diets containing legumes, grasses, haylage, straw and other feedstuffs (Beauchemin *et al*., 2003). The mode of action of these enzymes in ruminants is not fully understood. They can enhance feed colonization by increasing the numbers of ruminal fibrolytic microbes (Morgavi *et al*., 2000; Nsereko *et al*., 2000) and thus can increase the rate of degradation in the rumen (Yang *et al*., 1999).

Enzymes can also partially solubilize NDF, ADF, and release reducing sugars in the process. Colombatto *et al*.

(2003) observed that fibrolytic enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and postincubation effects. Most of the studies on fibrolytic enzyme treatment of ruminant feed have been done using temperature feedstuffs. Little is known about their effectiveness on tropical or subtropical forages, which tend to be poorly digested. Exogenous fibrolytic enzymes might enhance attachment and/or improve access to the cell wall matrix by ruminal microorganisms and by doing so, accelerate the rate of digestion (Nsereko *et al*., 2000).

Due to the importance of forage in ruminant diets, the possibility of improving its nutritive value by adding exogenous enzymes should be explored. The objective of this study was to evaluate the effects of three doses of a mixture of enzymes on their changes of growth performance and nutrient digestibilities of calves feed treated concentrates and *Pennisetum glaucum.*

MATERIALS AND METHODS

The field study was carried out at Nubaria Experimental Station at El-Hussein Village of El-Bostan. Province-Nubaria area is a new reclaimed land in the western desert of Egypt. Chemical analysis, were undertaken at National Research Center Laboratories, Dokki, Giza.

Biologically Treated Sugar Beet Pulp

Microorganisms. Trichodermareesei F- 418 was obtained from Microbial Chemistry Department, National Research Center; Dokki, Cairo, Egypt. The organisms were maintained on PDA medium. Sugar beet pulp (SBP) the secondary by-product of sugar industry from sugar beet was obtained from El-Fayoum sugar Factory-El-Fayoum, Egypt.

Preparation of fungal inoculum. The fungal inoculum was prepared in 250 mL capacity conical flasks containing 50 mL of a medium of (g/L) petome, 5.0 yeast extract, 3.0, malt extract, 3.0 and sucrose, 10.0. The flasks were seteilized by autoclaving at 121°C for 15 min. The cooled sterilized flasks were inoculated by a loop of 3 days old fungal cultures. The inoculated flasks were incubated in a rotary shaker (GFL) 150 rpm at 30°C for 48 h. The fungal mycelial were used to inoculated the experimental flasks at 10% (V/W).

Experimental flasks. Five hundred capacity conical flasks containing 25 g SBP moituring at solid: liquid ratio 1:2 with salt medium of (g/L) urea 5; ammonium sulphate, 75, KH_2PO_4, 5; magnesium sulphate 0.125 in 0.05 m citrate buffer pH 5.2. The flasks were autoclaved at 121°C for 30 min. The cooled sterilized flasks were inoculated with above inoculum, then incubated under static condition 30°C $\pm$ C for 72 h. The fermented substrate was used inoculum for the following containers.

Scaling up methodology of fungal biomass. The treatment was scaled up in 20 L capacity flasks each containing 400 g SBP moistened with above basal liquid medium at solid liquid ration 1:2 (The moistend SBP was sterilized in heating bags at autoclaving for 121°C for 30 min). The containers were sterilized by ethanol. The flasks were inoculated by the above growing fungal spores at 10% (W/W). The inoculated flasks were incubated at room temperature (28 - 34°C) for 5 days.

Harvesting. At the end of incubation period the fermented SBP was dried in conditional air flow at 20°C tell constant weight. The dried products, was analyzed for cellulase, xylanase, amylase and polyglacturinase enzymes.

Enzymes assays. Cellulase as carboxymethyl cellulase (CMCase) was assayed according to Mandls *et al*. (1974), xylanase according to the method described by Bailey (1985). Alpha amylase was determined according to the methods of Degtyarev, *et al.* (1989) and polyglactuernase was determined according to the method of Hancook *et al*. (1964).

Enzyme	IU/g fermented SBP
Cellulase	114.2
Xylanase	662.1
Alpha-amylase	1065.1
Polyglacturenase (pectinase)	216.2

Growth trial. Sixteen buffalo male calves aged 11 months old weighing in average 185.4 kg were divided in to four similar groups (4 calves in each group) and housed in semi-opened pens where they were individually fed. The experimental calves were fed on the tested feed mixtures at 2% level of their body weight, while the forage *Pennisetum glaucum* (*Pearl millet*) offered *ad-lib*. Tested rations were offered twice daily in two equal portions at 8.00 a.m. and 2.00 p.m. The fermented sugar beet pulp (SBP) was supplemented at 0.2, 0.4 and 0.6% (W/W) to concentrate feed mixture (CFM) and mixed well just before feeding for R_2, R_3 and R_4 treatments, respectively. The chemical composition of un-treated and biologically treated concentrate feed mixture is given in Table I.

The growth trial extended for 6 successive months. The offered and refused feeds were daily recorded. The experimental calves were individually weighed bi-weekly, and offered feeds were weekly adjusted according to changes of body weight. Mineral blocks and fresh water were available freely at all time throughout the experimental period.

Digestibility trials. Four metabolism trials were carried out at the end of the experimental period. Three animals were randomly chosen for each experimental ration to evaluate the effect of treatments on nutrient digestibilities. Animals were fed the treated concentrate feed mixture at 2% level of their body weight and *Pennisetum glaucum* was fed *ad lib*. The collection period was 7 days. Drinking water was freely available. Chemical composition of feeds and feces were determined according to A.O.A.C. (1999). Digestibility trials were carried out using acid insoluble ash (AIA) technique of Van Keulen and Young (1977).

Blood samples were taken bi-weekly at 8 a.m. before feeding from jugular vein of experimental calves using

heparinized tube and blood serum was separated by centrifugation at 3500 rpm, then stored at -20°C till analysis. Serum was analysed for total protein (as described by (Armstrong & Carr, 1964), albumin (Doumas *et al*., 1971), urea (Patton & Crouch, 1977), transaminases GOT and GPT activities (Reitman & Frankel, 1957), and creatinine (measured by spectrophotometer using kits delivered from sentinel, CH, Milano, Italy).

Data were statistically analyzed using the general linear model program of SAS (1996). The significance of the differences among treatments were tested by Duncan's Multiple Range test (1955).

RESULTS AND DISCUSSION

1. Digestion coefficients and nutritive values. Data presented in Table II showed that the concentrate and roughage intake and consequently total dry matter intake were significantly higher ($P < 0.05$) with all enzymatic treatments than that of control rations. While, total dry matter intake as (g/kg Bw) and total DM intake as ($g/w^{0.75}$) were nearly the same for the different experimental rations. There were no effects of exoenzymes on DM intake throughout the experimental period. These results are agreement with previous studies, which found no effect of adding different fibrolytic enzymes on feed intake of lactating cows (Rode *et al*., 1999; Lewis *et al*., 1999; Zheng *et al*., 2000), lactating sheep (Flores, 2004) and lactating goats (Titi & Lubbadeh, 2004; Gonzijlez, 2004). In contrast; others have reported increased feed intake of dairy cows and feedlot cattle supplemented with cellulase enzymes (Lewis *et al*., 1999; McAllister *et al*., 1999).

The digestion coefficient of DM, OM and CF were significantly higher ($P < 0.05$) in R_4 ration than that of the other experimental rations. In addition EE, CP and NFE digestibility and TDN were significantly higher ($P < 0.05$) in R_4, R_2 and R_3 diets than R_1 diet, but the differences between R_2 and R_3 rations were not significant.

The digestion coefficient of DM, OM and CF were significantly higher ($P < 0.05$) in R_4 ration that of different rations, suggesting that level lower than 0.6% of SBP mixed with enzymes supplemented with concentrate feed mixture diets did not increase the beneficial effects of the enzymatic preparation used. On the same trend several authors (Titi & Tabbaa, 2004; Wang *et al*., 2004; Dean *et al*., 2005; Eun & Beauchemin, 2005; Yu *et al*., 2005; Mohamed *et al*., 2005), showed an increase in DM, OM, N, ADF and hemicellulose digestibility regardless of level of forage in animal diets, when exogenous enzymes as cellulase and xylanase were supplemented in animal diets.

Supplementation with exoenzymes increased ($P < 0.05$) digestibility of CP, EE and NFE for all exoenzymes groups compared to the control one. Rojo-Rubio *et al*. (2001) found that addition of thermostable amylase of *B. licheniformis* increased the *in vitro* starch digestion of sorghum and maize; therefore, this enzyme could improve *in vivo* starch digestion and feed efficiency of grain based diets with low and intermediate rates of starch fermentation. However, Mora-Jaimes *et al*. (2002) suggest that alpha-amylase from *B. licheniformis* and glucoamylase from *A. niger* could be used as additives to improve ruminal digestibility of sorghum grain starch. Moreover, Gutierrez *et al*. (2005) reported that both amylolytic thermostable enzymes have the potential to become feed additives to improve ruminal digestibility of corn and sorghum, and are stable at low humidity conditions which may facilitate incorporation with grain during feed processing.

It is noteworthy that the improving digestibility of nutrients lead to improvement in nutritive value of diet. So the supplementation with exoenzymes increased the nutritive value of the different exoenzymes supplementation. The best TDN and DCP were observed with R_4 group.

Dean *et al*. (2005) found that the nutritive value and fermentation of Bermuda-grass silage can be improved by treating it with fibrolytc enzymes compared with control silages.

Table I. Chemical composition (%) of feedstuffs and rations on DM basis

Item		DM	OM	CP	CF	EE	NFE	Ash
Concentrate feed mixture (CFM)*		90.01	92.77	14.62	15.38	2.88	59.89	7.23
Penniselum glaucum		84.30	86.65	8.94	33.84	0.97	42.90	13.35
Calcuated composition of tested rations								
Control	R_1	35.14	90.74	12.73	21.46	2.25	54.30	9.26
	R_2	35.45	90.75	12.74	21.35	2.23	54.43	9.25
	R_3	34.66	90.70	12.70	21.60	2.23	54.17	9.30
	R_4	35.00	90.73	12.72	21.49	2.24	54.28	9.27

The (CFM)* contained: 42% yellow corn, 25% undecorticated cotton seed meal, 16% wheat bran, 14% berseem hay, 2% limestone and 1% common salt.

Table II. Digestion coefficients and nutritive values of calves fed experimental rations

Item	R_1	R_2	R_3	R_4	± SE
No of animals	3	3	3	3	-
Mean body Wt.Kg	342.67	374.33	367.67	371.67	±41.454
Mean DMI (DM bais), kg/h/d					
Concentrate feed mixture	6.973^{b}	7.566^{a}	7.433^{a}	7.500^{a}	±822.815
Roughage	3.413^{b}	3.640^{a}	3.783^{a}	3.733^{a}	±374.315
Total	10.386^{b}	11.206^{a}	11.216^{a}	11.233^{a}	±1169.316
TDMI, g/kg BW	30.33	29.97	30.50	30.20^{ns}	±0.427
C/R ratio	2.03	2.07	1.96	2.01^{ns}	±0.085
TDMI of body wt, %	3.03	2.99	3.05	3.01^{ns}	±0.041
Total DM intake $g/w^{0.75}$	130.42	131.68	133.59	132.71^{ns}	-
Nutrient digestion coefficient					
DM	65.04^{b}	66.22^{b}	67.93^{b}	73.32^{a}	±3.407
OM	65.63^{b}	67.72^{b}	67.44^{b}	74.41^{a}	±3.578
CP	61.81^{c}	64.74^{b}	64.13^{b}	71.02^{a}	±3.636
EE	62.70^{c}	67.58^{b}	67.97^{b}	77.01^{a}	±5.489
CF	61.03^{b}	64.79^{b}	63.34^{b}	68.75^{a}	±3.089
NFE	63.58^{c}	67.39^{b}	66.39^{b}	75.47^{a}	±4.704
Nutritive value on (DM basis) %					
TDN	58.64^{c}	62.11^{b}	61.18^{b}	67.62^{a}	± 3.589
DCP	7.87^{b}	8.25^{b}	8.15^{b}	9.03^{a}	± 0.438
E/P	7.45	7.53	7.51	7.60^{ns}	± 0.153

a,b and c Mean in the same row with different superscripts differ ($P < 0.05$) ns = not significant

In contrast, no effects of enzymes on diet digestibility have been found by Hristov *et al*. (2000) in fattening cattle and by Flores (2004) in dairy ewes. These contrasting results can be attributed to factors as enzyme type, level of supplementation particle size of forage (so surface area available for enzymatic attach and/or enhanced accessibility to substrate), method of enzyme application and the level of production of the experimental animals could be potential reasons for increasing digestibility (Beauchemin *et al*., 2003; Yu *et al*., 2005).

2. Blood constituents. There was no significant difference among different experimental animal groups concerning all blood serum parameters tested (Table III). William (1997) reported that the normal level of total protein and albumin in blood of domestic animals has ranged between 6 to 8 and 3.5 to 4 g/100 mL, respectively. On the other hand all values of GOT and GPT are within the normal range found by Ahmed (1997), Baraghit *et al*. (2003) with native buffalo calves. Also serum urea and creatinine concentration is a useful indicator of glomerular filtration (Ismail, 1999). Generally, all these parameters were within the normal range of buffaloes blood as reported by Baraghit *et al*. (2003), Svozil *et al*. (1989) found that enzyme preparation had no adverse effect on the blood biochemistry or rumen content but reduced the retention time of liquid digesta in the digestive tract.

Hristov *et al*. (2000) found that when cannulated heifers were fed a diet of 85.5% rolled barley grain and 14% barley silage (DM basis), and once daily they were given intraruminal doses of 0, 100, 200 or 400 g of a preparation containing carboxymethylcellulase and xylanase activities. Enzyme treatment did not affect urinary excretion of allantoin and uric acid, or concentrations of glucose and urea in blood. However Rao *et al*. (2004) found that the performance of broiler chicks fed diets containing pearl millet (*Pennisetum glaucum*) with and without enzymes containing amylase, hemicellulase, cellulase, proteinase and beta-glucanase. improve the immunological traits, reduces total cholesterol and protein accretion increased in the tissue of broilers.

In conclusion, enzymes treatment can help in improving the use of forage in ruminant diets and consequently encourage the positive animal performance without adverse effect on blood biochemistry, which can reflect on decreasing the financial pressure on the farmer. Further research is required to define the ideal conditions for the exogenous enzymes and its level to optimize this synergistic interaction.

3. Growth performance. Effect of treatment with different doses of enzymes on diet DM intake and growth performance are shown in Table IV. There were no effects of enzymes on DM intake throughout the experimental period. These results are agreement with previous studies, which found no effect of adding different levels of enzymes on feed intake of lactating cows (Rode *et al*., 1999; Lewis *et al*., 1999; Zheng *et al*., 2000), lactating sheep (Bouattour, 2004; Flores, 2004) and lactating goats (Titi & Lubbadeh, 2004; Gonzjlez, 2004). In contrast, others have reported increased feed intake of dairy cows and feedlot cattle supplemented with cellulase enzymes (Lewis *et al*., 1999; McAllister *et al*., 1999). As stated by Beauchemin *et al*. (2003), the effects of exogenous fibrolytic enzymes on DM intake appear to differ among enzyme products and therefore, some but not all enzyme mixture may increase feed intake. Bendary *et al*. (2002) found that the microflora of Buffalo rumen were more efficient in degradation of plant tissue than that of cow and the results obtained indicated that DM, CF fractions and nutrients disappearance percentage of some natural cellulosic materials were more pronounced when samples were incubated in buffalo rumen than in cow rumen. However, Dular and Sangwan (2004) found that *in vitro* DM digestion and nitrogen metabolism by rumen anaerobic fungi from buffalo enhanced DM degradation and rumen protein nitrogen constituent than cattle fungi.

Data concerning growth performance (Table IV), illustrated that the average initial weight gain of the experimental groups was almost similar. Final weight of calves, total body weight gain and daily gain with enzyme treatments were significantly higher ($P < 0.05$) than that of the control ration. These results indicate that the use of enzyme treatments in concentrate feed mixtures containing different levels of enzymes had a positive effect on daily gain. The overall average daily gain for R_1 was 0.755 g/h/d,

Table III. Blood serum parameters for buffalo calves fed experimental rations

Item	R_1	R_2	R_3	R_4	± SE
Total proteins g/100 ml	6.75	6.80	6.90	6.70^{ns}	±0.102
Albumin g/100ml	3.30	3.45	3.40	3.22^{ns}	±0.146
Globulin g/100 ml	3.45	3.35`	3.50	3.48^{ns}	±0.180
A/G ratio	0.95	1.02	0.97	0.92^{ns}	±0.180
GOT (U/I)	35.00	33.95	32.80	34.60^{ns}	±1.40
GPT (U/I)	23.30	22.70	21.7	22.80^{ns}	±0.82
Urea nitrogen (mg/dl)	50.74	47.78	50.39	48.70^{ns}	±0.180
Greatinine m mal/1	1.44	1.54	1.57	1.48^{ns}	±1.10

ns = non significant differences

Table IV. Growth performance of calves fed the experimental rations

Item	R_1	R_2	R_3	R_4	SE
Av. Dry matter intake (Kg/h/d)					
Concentrate feed mixture	5.08	5.39	5.33	5.53^{ns}	±6.889
Roughage	2.89	3.12	3.05	3.27^{ns}	± 0.499
Total dry matter intake	7.97	8.51	8.38	8.80^{ns}	± 1.367
Initial Wt (Kg)	186.25	184.51	184.75	186.00	±41.316
Final body wt (Kg)	322.13^{b}	359.28^{a}	348.43^{a}	367.35^{a}	±49.252
Total body weight gain (Kg)	135.88^{d}	170.78^{b}	163.68^{c}	181.35^{a}	±17.966
Daily body weight gain (g)	0.755^{b}	0.949^{a}	0.909^{a}	1.008^{a}	±0.110
Relative gain (% of initial wt)	78.68^{b}	96.17^{a}	92.17^{a}	98.22^{a}	±18.507
Feed conversion:					
Kg DM/Kg gain	10.51^{a}	8.94^{c}	9.18^{b}	8.78^{c}	±1.550
Kg TDN/Kg gain	6.16^{a}	5.55^{b}	5.61^{b}	5.96^{a}	±0.866
Kg DCP/Kg gain	0.83	0.74	0.75	0.79^{n}	±0.114

a,b,c and d Means within the some raw different superscripts are different at ($P < 0.05$) ns = not significant differences

which was increased by 25.7, 20.4 and 33.5% for R_2, R_3 and R_4 treatments, respectively. These values of average daily gain for treated buffalo calf groups in the present study are higher than those reported by some investigator in Egypt when buffalo calves fed treated CFM and forage. Baraghit *et al*. (1999) reported 700 g/h/d, El-Shinnawy *et al*. (1999) reported 890 g/h/d, Etman *et al*. (2001) reported 814 g/h/d, Abdel-Baki *et al*. (2003) reported 835 g/h/d, Boraei (2003) reported 760 g/h/d, El-Kholy *et al*. (2003) reported 1003 g/h/d; El-Basiony *et al*. (2003) reported 969 g/h/d. These differences in ADG may be attributed to different plane of nutrition.

Supplementation with exoenzymes showed a significant ($P < 0.05$) improvement in feed conversion as (kg DM/kg gain) and (kg TDN/kg gain). The highest value of (kg DM/kg gain) was recorded with R_4 groups. However, the highest value of (kg TDN/kg gain), were recorded with R_2 and R_3 groups. However, DCp/gain was similar for different dietary treatments. This improvement in TDN/gain of enzymatic treatment might due to the improvement of digestibility. Gado (1999) reported that the concentration of cellulase enzyme had a positive ($P < 0.01$) effect on average body gain of treated bagasse in comparison with un-treated one.

Aganga and Autlwetse (2000) found that sheep fed on millet forage had a higher daily weight gain compared with sheep fed on Veldt grass. Zobell *et al*. (2000) found that feed efficiency showed a trend for improvement during the first 28 days for the enzyme treatment ($P = 0.063$). However, Zahiroddini *et al*. (2004) reported that the feedlot study, DM intake did not differ among treatments, but average daily gain (ADG) by steers fed diet + cellulase and amylase activities were higher ($P = 0.1$), by 4.8 and 7.6%, respectively, than ADG by steers fed control diet. Also feed efficiency of steers fed diet containing enzymes was improved ($P = 0.01$) relative to those fed control diet. Moreover, Titi (2004a) indicated that fibrolytic enzymes increased gain and reduced feed costs as a result of improved digestibility by Awassi lambs. However, Titi (2004b) found that exogenous fibrolytic enzyme resulted in improved ($P < 0.05$) feed conversion ratio of fattened Awassi with no effect on feed intake. Results indicated that fibrolytic enzymes could enhance the growth of fattened lambs and improve their conversion rations mainly through improving digestibility.

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