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RESPONSE OF GROWING CALVES TO DIETS CONTAINING DIFFERENT LEVELS OF EXOGENOUS ENZYMES MIXTURE

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SUMMARY

In a feeding trial lasted 154 days sixteen crossbred steers with an average body weight 163.5 ± 1.07 kg were divided randomly into four equal experimental groups to investigate the effects of feeding exogenous enzymes mixture (cellulase, xylanase, alpha-amylase and polyglacturinase) at different levels on steers performance, nutrients digestibility and rumen functions Animals were fed a concentrate feed mixture (CFM) at 2% of body weight and offered green fodder (Pearl millet) *ad lib*. Enzymes (*Trichoderma reesei* F-418 fermented with sugar beet pulp (SBP) were added at 0.0, 0.2 %, 0.4 and 0.6% (g/kg) of the CFM, respectively. Daily dry matter intake (kg/day, g/kg $W^{0.75}$ and kg/100 kg BW) total digestible nutrient intake (kg/day) and digestible crude protein intake (g/day) were not significantly (P>0.05) affected by enzymatic supplements. However, with 0.6% SBP treatment diet, total digestible nutrients intake (g/kg$W^{0.75}$ and kg/100 kg BW) and digestible crude protein intake (g/kg$W^{0.75}$ and g/100 kg BW) were significantly increased when compared to other treatment diets. Digestibilities for all nutrients were improved with enzymatic supplements. Total digestible nutrients and digestible crude protein values were increased with increasing the levels of enzymatic supplements. Inclusion of exogenous enzymes in the diets had no significant effect (P>0.05) on ruminal pH but significantly affected (P<0.05) ruminal ammonia nitrogen (NH_3-N) and ruminal total volatile fatty acids (TVFA's) concentrations. Final body weight, total body weight gain, average daily gain and feed efficiency (kg gain/ kg intake) were not significantly affected (P>0.05) by enzymatic supplements. Increasing the level of exogenous enzymes supplementation in the diet tended to improve final weight gain, total body weight gain, average daily gain and feed efficiency (kg gain/ kg intake of DM) compared with control diet In conclusion, supplementation of exogenous enzymes to bulls rations significantly improved (P<0.05) all nutrients digestibility coefficients but had no effects on feed intake and growth performance when steers fed on rations containing Pearl millet.

Keywords: *exogenous enzymes, calves, growth performance, digestibility, ruminal fermentation.*

INTRODUCTION

In Egypt, animals are suffering from shortage of feeds especially during summer season. Most of animals feeding in this period depend on grains, concentrate mixture and agricultural residues. The rising costs of feeds (grains and proteins supplements in particular) have led to significant increases in animals feed cost in recent years. Forages are usually the cheapest ingredients in animals ration. Expansion in cultivation of marginal soils is considered one of the most effective solutions to overcome feed shortages. Many

attempts were carried out to improve the quality of local species of green forages and to introduce new green forage species in marginal soils especially in summer season (Mousa *et al.*, 1995; Geweifel, 1997 and Khinizy *et al.*, 1997).

Ibrahim *et al.* (2008) estimated the average fresh yield of Pearl millet by 12.6 tons/ feddan. Also, the same authors reported that the values of in situ dry matter disappearance (*ISDMD*) and in situ organic matter disappearance (*ISOMD*) of Pearl millet were 57.68 and 58.43%, respectively. While, TDN and DCP of Pearl millet were 62.84 and 5.05% in first cut and 66.65 and 9.29% in the second cut, respectively.

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 lignin. These animals do not produce the enzymes responsible for degradation of lignocelluloses but are dependent on associated microbial populations. Exogenous polysaccharides 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.*, 2000).

Interest in applying exogenous enzymes to ruminant diet has increased recently due to enzyme-mediated increase in feed digestion under *in vitro* (*Bowman et al.*, 2002 and Kung *et al.*, 2002) and *in vivo* (El-Kady *et al.*, 2006) conditions.

Factors affecting enzyme effectiveness are diet composition, type of enzyme preparation used, complement of enzyme activities, level of enzyme provided, enzyme stability and method of application (Bowman *et al.*, 2002).

Colombatto *et al.* (2003) stated that fibrolytic enzymes enhanced the fermentation of cellulose and xylan by a combination of pre and post incubation effects. Exogenous fibrolytic enzymes might enhance attachment and/or improve access to the wall matrix by ruminal microorganisms and by doing so, accelerate the rate of digestion (Nsereko *et al.*, 2000b). Digestibility of CF increases more with the addition of a certain level of enzyme mixture (probably about 2 g/kg feed) than with lower or higher levels (Yang *et al.*, 1999 and Beauchemin *et al.*, 2000).

Adding fibrolytic enzymes to grass hay before feeding beef steers was found to increase dry matter intake (DMI), rate of passage and digestibility of dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Lewis *et al.*, 1996). Beauchemin *et al.* (1999) reported a reduction in ruminal retention time of particles with enzyme supplementation leading to greater feed intake.

The main objectives of this study were to evaluate growth performance, nutrients digestibilities and ruminal fermentation of crossbred steers fed on Pearl millet and supplemented with a mixture of enzymes.

MATERIALS AND METHODS

This study was conducted at El-Nubaria Experimental Station at El-Hussein Village of El-Bostan. Province El-Nubaria area is a new reclaimed land in the western desert of Egypt. Chemical analyses were undertaken at laboratories of Animal Production Department, National Research Centre, Dokki, Giza, Egypt.

Biologically treated sugar beet pulp:

Microorganisms:

Trichoderma reesei F-418 was obtained from Microbial Chemistry Department, National Research Centre, Dokki, Giza, 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 governorate, Egypt.

Preparation of fungal inoculums:

The fungal inoculums was prepared in 250 ml conical flasks containing 50 ml medium contain (g/L) peptone, 5.0; yeast extract, 3.0; malt extract, 3.0 and sucrose,10.0. The flasks were sterilized 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 hours. The fungal mycelial were used to inoculated the experimental flasks at 10% (V/W).

Experimental flasks:

Five hundred conical flasks containing 25g sugar beet pulp (SBP) moisturing at solid: liquid ratio 1: 2 with salt medium contain (g/L) urea, 5; ammonium sulphate, 75; potassium sulphate, 5 and magnesium sulphate, 0.125 in 0.05 ml citrate buffer (pH 5.2). The flasks were autoclaved at 121 °C for 30 min. The cooled sterilized flasks were inoculated with above inoculums and then incubated under static condition (30 °C) for 72 hours. The fermented substrate was then used as inoculums for the following containers.

Scaling up methodology of fungal biomass:

The treatment was scaled up in 20 L flasks each containing 400 ml SBP moistened with above basal liquid medium at solid to liquid ratio of 1: 2 (The moistened SBP was sterilized in heating bags at 121 °C for 30 min). The flasks were then inoculated by the above growing fungal spores at 10% (V/W). The inoculated flasks were incubated at room temperature (28-34 °C) for 5 days according to Abedo *et al*, (2005).

Harvesting:

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

Enzymes assays:

Cellulase as carboxymethyl cellulase (CMCase) was assayed according to Mandls *et al.* (1974). One unit of cellulase activity is defined as the amount of enzyme liberated 1μmol glucose per hour under standard assay conditions. The activity of xylanase was determined according to method described by Bailey (1985). One unit of xylanase activity is defined as the amount of enzyme liberated 1 μmol xylose per hour under standard assay conditions. Polyglacturinase activity was determined according to the method of Hancook *et al.* (1964) and Valsangiacomo and Gessler (1992). One unit of polygalacturinase activity is defined as the amount of enzyme liberated 1 μmol galacturonic acid per hour under standard assay conditions and a-amylase activity was determined according to the method of Degtyarev *et al.* (1989). One unit of a-amylase activity is defined as the amount of

enzyme liberated 1 µmol maltose per hour under standard assay conditions.

enzyme	IU/g fermented SBP
Cellulase	114.2
Xylanase	662.1
a-amylase	1065.1
Polyglacturinase (pectinase)	216.2

Feeding trial:

Experimental animals:

Sixteen crossbred steers with an average body weight of 163.5 ± 7.09 kg were divided into four experimental groups, each of 4 steers. Animals were housed in semi opened pens where they were individually fed.

The feeding trial lasted for 154 days, offered and refused feeds were recorded daily. The experimental animals were individually weighed bi-weekly before feeding at 8.00 a.m. Offered feeds were weekly adjusted according to changes of body weights.

Feeds and feeding:

Steers were randomly assigned to receive one of the four tested rations. Calves fed on the tested feed mixture at 2% level of their live body weight, while the forage Pearl millet (*Pennisetum glaucum*) was offered *ad lib (please edit as in abstract)*. Experimental rations were offered twice daily in two equal portions at 8.30 a.m. and 2.30 p.m. The fermented SBP was supplemented at 0.0, 0.2, 0.4 and 0.6% (g/kg) to concentrate feed mixture (CFM) and mixed well before feeding.

Animals were raised under hygienic and managerial conditions. Fresh water and mineral blocks were available at all time through the experimental period. Feed intake and body weight changes of the animals were biweekly recorded during the experimental period. Chemical analysis and cell wall constituents (%) of feed ingredients are presented in Table (1).

Digestibility trials:

At the end of the experimental period, four animals from each group were used to determine nutrients digestibility coefficients. A grab sample method was applied at which acid insoluble ash (AIA) was used as an internal marker according to Van Keulen and Young (1977) for determining nutrients digestibility according to the following formula:

$$\text{Digestion coefficient of nutrient} = 100 - \frac{[100 \times \%\ \text{AIA in feeds} \times \%\ \text{Nutrient in feces}]}{[\%\ \text{AIA in feces} \times \%\ \text{Nutrient in feeds}]}.$$

Samples of feces were taken for five days from each animal and sprayed with 10 % sulphuric acid and 10 % formaldehyde solutions and dried at 60 °C for 24 hrs. Samples were mixed and stored for chemical analysis. Composite samples of feeds and feces were finely ground prior to analysis. The nutritive values expressed as the total digestible nutrient (TDN) and digestible crude proteins (DCP) of the experimental rations were calculated.

Table (1): Chemical analysis and cell wall constituents of feed ingredients

Items	Feed ingredients					
	Yellow corn	UCSM	Wheat bran	Berseem hay	CFM*	PMF
Dry matter	91.30	87.88	90.20	94.13	90.84	15.70
Chemical analysis (%) on DM basis						
Crude protein	9.30	24.82	14.00	15.84	14.58	8.94
Crude fiber	2.30	27.75	11.22	24. 97	13.21	33.84
Ether extract	3.50	2.71	3.00	1.75	2.88	0.97
Nitrogen free extract	83.70	38.92	60.08	47.31	64.08	43.00
Ash	1.20	5.80	11.70	10.13	5.25	13.25
Cell wall constituents (%) on DM basis						
NDF	32.63	50.63	44.21	46.25	39.91	65.80
ADF	22.45	36.18	32.16	33.28	28.29	50.30
ADL	2.13	20.46	4.05	6.85	7.62	4.48
Hemicellulose	10.18	14.45	12.05	12.97	11.62	15.50
Cellulose	20.32	15.72	28.11	26.43	20.67	45.82

*The Concentrate feed mixture (CFM) contained: 42% yellow corn, 25% undecorticated cotton seed meal, 16% wheat bran, 14% Berseem hay, 2% limestone and 1% common salt.
Undecorticated cotton seed meal = UCSM, Pearl millet Fodder = PMF, NDF: neutral detergent fiber; ADF: acid detergent fiber and ADL: acid detergent lignin.
Hemicellulose = NDF – ADF.
Cellulose = ADF – ADL.

Rumen fluid:

At the end of the feeding trial, ruminal fluid samples were collected from all animals using stomach tube. Samples were collected before the morning feeding and 3 hrs post feeding. Samples were filtered through four layers of cheesecloth. The samples were used for ruminal parameters determination.

Analytical procedures:

Representative samples of feed ingredients and experimental rations were analyzed for DM, CP, CF, EE and ash according to A.O.A.C. (1995) methods. Neutral detergent fiber, ADF and ADL were analyzed according to Goering and Van Soest (1970).

Ruminal pH was immediately recorded using digital pH meter. Ruminal ammonia nitrogen (NH_3–N) concentrations were determined according to Conway (1962). Ruminal total volatile fatty acids (TVFA's) concentrations were determined according to Kromann *et al.* (1967).

Statistical analysis:

Data collected for feeding and digestibility trials were subjected to statistical analysis as one way analysis of variance .Ruminal parameters data were subjected to statistical analysis as two factors factorial analysis of variance using SAS (1998) examined the effects of dietary treatments. Duncan's Multiple Range Test (Duncan, 1955) was used to separate means when the dietary treatment effect was significant.

RESULTS AND DISCUSSION

Composition, chemical analysis and cell wall constituents:

Results of chemical analysis and cell wall constituents of feed ingredients and experimental rations are shown in Table 1. The results were in the same trend with those obtained by Ibrahim *et al.* (2008) who found that the Pearl millet CP content ranged from 6.74 to 10.81%; CF from 23.62 to 25.57%; ADF from 49.68 to 50.76%; hemicellulose from 14.93 to 16.26 % and cellulose from 45.70 to 46.02 %, respectively. Chamberlain and Robertson (1992) and Sheperd and Kung (1996) reported that addition of enzymes causes a substantial amount of hydrolysis of the plant cell wall and consequently fiber content should be decreased. Enzyme treatments degrade the cell wall content of grass and mixed grass and legume silage.

Digestion coefficients and nutritive values:

The results of digestion coefficients and nutritive values of experimental rations are presented in Table (2). Results showed that inclusion of exogenous enzymes in the diet significantly (P<0.05) improved digestibilities for all nutrients (DM, OM, CP, EE, CF and NFE) relative to control and was. Supplementing the exogenous enzymes at the 0.6% level showed the highest values of nutrients digestibilities

Table (2): Digestion coefficients and nutritive value of experimental rations fed to steers

Item	Treatment diets				SEM
	0.0% SBP	0.2% SBP	0.4% SBP	0.6% SBP	
1- Nutrient digestion coefficient					
DM	67.23^{c}	67.39^{c}	70.22^{b}	73.89^{a}	0.85
OM	67.26^{c}	67.84^{c}	70.38^{b}	74.23^{a}	0.85
CP	62.70^{c}	64.43^{bc}	66.96^{b}	72.20^{a}	1.14
EE	63.98^{c}	64.16^{c}	69.02^{b}	74.66^{a}	1.35
CF	62.86^{c}	63.87^{bc}	66.45^{b}	70.57^{a}	0.94
NFE	67.62^{b}	68.08^{b}	69.75^{b}	74.63^{a}	0.91
2- Nutritive values (DM basis) %					
TDN	61.39^{b}	61.98^{b}	63.97^{b}	68.55^{a}	0.89
DCP	7.99^{b}	8.16^{b}	8.49^{b}	9.14^{a}	0.14

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05), SBP: Fermented sugar beet pulp.

Nutritive values (TDN and DCP) of the tested diets were improved with addition of the exogenous enzymes. Results showed that TDN and DCP values were increased with increasing the level of supplementation of exogenous enzymes in the diets. Total digestible nutrient was improved by 0.96, 4.20 and 11.66% with addition of exogenous enzymes in the rations at 0.2, 0.4 and 0.6%, respectively compared to control diet. Similar results were reported by Mohamed *et al.*, 2005 and El-Kady *et al.*, 2006. They reported significant increases in DM, OM, NDF, ADF and hemicellulose digestibilities regardless level of

forage in animal diets when exogenous enzymes as cellulase and xylanase were supplemented in animal diets.

Mora-Jaimes *et al.* (2002) noted that a-amylase from *B. Licheniformis* and glucoamylase from *Aspergillus niger* could be used as additives to improve ruminal digestibility of sorghum grain starch. Moreover, Gutierrez *et al.* (2005) reported that 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.

Gado *et al.* (2007c) indicated that treatments with cellulase enzyme (15%) resulted in higher digestibility ($p< 0.05$) compared with the other treatments.

Gado *et al.* (2007d) noted that addition of cellulase enzyme to bagasse caused the highest DMD and OMD while the untreated bagasse silage had the lowest value. Also, they concluded that enzymatic treatments of bagasse silage improved its chemical composition, DMD and OMD through its effect on cell wall structure.

Mahrous and Abou Ammou (2005) found that biological treatments increased ($P<0.05$) digestibility coefficients of DM, OM, CF and NFE than the untreated rice straw. The same authors stated that biological treatments (fungi, yeast or combined fungi + yeast) are good methods for improving digestibility of low quality roughages (rice straw) and without any hazard on animal health.

The improvement in fiber fraction digestibility as a result of biological treatments may be due to the effect of the cellulase enzyme of fungi, which may be responsible for the stepwise hydrolysis of cellulose to glucose (El-Shafie *et al.*, 2007) and could be attributed to the retention time of different fiber in the rumen; length of time that fiber is exposed to the fibrolytic process, rate of particle size reduction, particle density and rate of digestion (Nsereko *et al.*, 2000 a and b).

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

Gado *et al.* (2007b) noted that biological treatments by *Cellulomonas cellulasea* and *Ruminococcus albus* as silage improved nutritive value of corn stalks. Abd El-Galil (2000) referred that the biological treatments of bagasse increased DCP from 20.5 % to 55.87% and TDN values increased from 46.5 % to 68.9 %. Moreover, Gado *et al.* (2007c) showed that feeding on biologically treated bagasse was affect on TDN values (need editing, not sure what they mean) compared with untreated.

Subhash *et al.* (1991) found that treated rice straw with *Aspergillus niger* or *Trichoderma viride* or a mixed culture did not improve the nutritive value of rations. Similarly results were obtained by El-Ashry *et al.* (2001) and El-Sayed *et al.* (2002) on goats fed roughages treated with *Trichoderma viride*.

Abdel Gawad *et al.* (2007) found that feeding fibrolytic enzymes at2%/ Kg DM or 2g/h/d improved the nutritive value of rations containing 30% roughage.

Feed intake:

Daily dry matter and nutrient intakes by steers are presented in Table 3. The results revealed that the concentrate and roughage intake and consequently total dry matter intake were not significantly different (P>0.05) between treatments. However, the 0.4 and 0.6% diets tended to insignificant (P>0.05) increase CFM, roughage and total dry matter intakes compared to control and 0.2% diets.

Daily dry matter intake (kg/day, g/ kg $W^{0.75}$ and kg/100 kg BW), total digestible nutrient intake and digestible crude protein intake (kg/day) were not significantly (P>0.05) different between treatment diets. However, the 0.6% diet significantly increased total digestible nutrient intake as g/kg$W^{0.75}$ and kg/100 kg BW and digestible crude protein intake as g/kg$W^{0.75}$ and g/100 kg BW in comparison with the other treatments. The similar feed intake among treatment diets might indicate that exogenous enzymes had no effects on palatability. These results are agreement with previous studies, which showed no effects of adding different fiberlytic enzymes on feed intake of lactating cows (Lewis *et al.*, 1999; Rode *et al.*, 1999 and Zheng *et al.*, 2000) and lactating goats (Gonzijlez, 2004 and Titi and Lubbadeh, 2004).

Table (3): Dry matter, TDN and DCP intake by animals in experimental groups.

Item	Treatment diets				SEM
	0.0% SBP	0.2% SBP	0.4% SBP	0.6% SBP	
Av. Body weight(kg)*	251.59	251.95	254.44	257.53	7.89
Metabolic body size**	63.17	63.24	63.71	64.29	1.44
Dry matter intake (kg / day) of:					
CFM	4.880	4.837	4.963	5.010	0.16
Roughage	2.533	2.552	2.579	2.619	0.08
Total DM intake	7.413	7.389	7.542	7.629	0.24
Concentrate (%)	65.83	65.46	65.80	65.67	---
Roughage (%)	34.17	34.54	34.20	34.33	---
Feed intake as:					
1- Dry matter					
kg/ day	7.413	7.389	7.542	7.629	0.24
g/ kg $W^{0.75}$	117.35	116.84	118.38	118.67	1.00
kg/ 100 kg BW	2.95	2.93	2.96	2.96	0.01
2- TDN					
kg/ day	4.551	4.580	4.825	5.230	0.17
g/ kg $W^{0.75}$	72.04^{b}	72.42^{b}	75.73^{b}	81.35^{a}	1.14
kg/ 100 kg BW	1.81^{c}	1.82^{c}	1.90^{b}	2.03^{a}	0.02
3- DCP					
g/ day	592.30	602.94	640.32	697.29	22.30
g/ kg $W^{0.75}$	9.38^{c}	9.53^{bc}	10.05^{b}	10.85^{a}	0.17
g/ 100 kg BW	235.42^{c}	239.31^{c}	251.66^{b}	270.76^{a}	3.60

* Av. Body weight, kg = Initial weight + Final weight / 2.

** Metabolic body size = kg $W^{0.75}$.

a, b and c: Means in the same row having different superscripts differ significantly at level (P<0.05).

Rumen fermentation:

The effects of dietary treatments on rumen fluid parameters are shown in Table 4. Feeding the exogenous enzymes to steers had no effect (P>0.05) on ruminal pH but significantly affected (P<0.05) both ruminal NH_3-N and TVFA's concentrations. With increasing level of exogenous enzymes in the diets, the concentrations of ruminal NH_3-N and TVFA's were significantly (P<0.05) increased. The resulted Increase in TVFA's concentrations with the enzymes supplement is correlated with the increase in nutrients digestibilities seen in this study. Briggs *et al.* (1957) noted that an increasing in ruminal TVFA's concentration caused a reduction in ruminal pH value. Ruminal pH is one of the most important factors affecting the fermentation and influences its functions. It varies in a regular manner depending on the nature of the diet and on the time it is measured after feeding and reflects changes of organic acids quantities in the ingesta. The level of NH_3-N and TVFA's as end products of fermentation and breakdown of dietary protein, have been used as parameters of ruminal activity by Abou-Akkada and Osman (1967). Gado *et al.* (2007b and c) indicated that rumen liquor pH values did not differ significantly (P>0.05) among treatments. While, Gado *et al.* (2007b) found that the total volatile fatty acids values for treated corn stalks by *Cellulomonas* (T_1) and *Ruminococcus* (T_2) were higher than that for untreated corn stalks (T_3), but treated stalks by *Ruminococcus* was the highest value. On the other hand the same authors noted that the TVFA's values for treated bagasse by cellulase enzyme, rumen liquor and *Cellulomonas* were higher than that for untreated bagasse. Lewis *et al.* (1996) observed that fibrolytic enzyme treatment significantly decreased ruminal pH and increased TVFA's concentration in the rumen.

Table (4): Effects of treatments diets and sampling time on rumen fluid parameters

Item	Sampling time								
	Before feeding				3 hrs post feeding				
	0.0% SBP	0.2% SBP	0.4% SBP	0.6% SBP	0.0% SBP	0.2% SBP	0.4% SBP	0.6% SBP	SEM
pH	7.04^{ab}	7.03^{ab}	7.09^{ab}	7.19^{a}	6.74^{bc}	6.67^{bc}	6.84^{abc}	6.59^{c}	0.06
NH_3 – N mg / dl	10.8^{e}	10.2^{e}	11.5^{de}	13.4^{d}	17.9^{c}	19.2^{c}	21.53^{b}	25.9^{a}	1.13
TVFA's meq./ dl	10.0^{d}	10.2^{d}	10.6^{d}	10.9^{d}	15.0^{c}	15.4^{bc}	16.0^{ab}	16.8^{a}	0.58

a, b, c, d and e: Means in the same raw having different superscripts differ significantly at level (P<0.05).

Sampling time had significant effects on rumen fluid parameters (Tables 4). Supplementation exogenous enzymes in the diets significantly decreased (P<0.05) ruminal pH at 3 hrs post feeding compared with before feeding. However, it was significantly increase (P<0.05) ruminal NH_3–N and TVFA's concentrations at 3 hrs post feeding in comparison with before feeding. These results were in agreement with those obtained by Salama *et al.* (2007) who found that the level of ruminal TVFA's reached to maximum at 3 hours after feeding for lambs fed *ad libitum*. These results of exogenous enzyme might be related to the more utilization of the dietary energy and positive fermentation in the rumen

It should be noted that, TVFA's concentration in the rumen is governed by several factors such as dry matter digestibility, rate of absorption, rumen pH, transportation of the

digesta from the rumen to the other parts of the digestive tract and the microbial population in the rumen and their activities (Allam *et al.*, 1984).

The peak concentration of NH_3-N at 3 hrs after feeding may be because of degradation of protein and hydrolysis of NPN substances (Reddy *et al.*, 1989). On the other hand Chandra *et al.* (1991) noted that the peak of ruminal NH_3-N at 3 hours after feeding may be due to deamination of amino acids in the rumen.

Gado *et al.* (2007c) noted that, the values of sampling time on ammonia-N concentrations were at the minimum at 0 hrs before feeding and increased to its maximum levels at 4hrs after feeding then values tended to decrease gradually as the time passed up to 6 hrs after feeding.

It is worthy to notice that the balance between NH_3-N and TVFA's concentrations reflect the pH values in the rumen liquor and effect of fungi might be related to the more utilization of the dietary energy and positive fermentation in the rumen (El-Shafie *et al.*, 2007).

Growth performance

Growth performance for steers is presented in Table 5. The results showed that, final weight, total body weight gain, average daily gain (ADG), relative gain, feed intake and feed efficiency (kg gain/ kg intake) were similar ($P>0.05$) among treatment diets.

Increasing level of exogenous enzymes supplementation in the diet tended to ($P>0.05$) increase final weight gain, total body weight gain and ADG. Addition of exogenous enzymes in the diets improved the ADG by 1.23% 4.04 and 7.03% for rations contained enzymes at 0.2, 0.4 and 0.6% respectively, compared to the control diet.

Increasing level of exogenous enzymes supplementation in the diet tended to ($P>0.05$) increase feed efficiency (kg gain/ kg DM intake) compared with the control diet. These results were in agreement with previous studies, which found no effects of adding different levels of enzymes on feed intake of lactating cows (Lewis *et al.*, 1999; Rode *et al.* 1999 and Zheng *et al.*, 2000), lactating sheep (Bouattour 2004 and Flores 2004) lactating goats (Gonzijlez 2004 and Titi and Lubbadeh 2004) and buffalo calves (El-Kady *et al.*, 2006).

As stated by Beauchemin *et al.* (2003) the effect of exogenous fibrolytic enzymes on DMI appear to differ among enzyme products therefore, some but not all enzymes may increase feed intake.

Gado *et al.* (2007a) found that feeding lambs on rations treated with ZAD compound were significantly ($P<0.05$) heavier, grew faster and had higher weight gain than lambs fed control rations.

Kennedy (1987) found that ensiling grass with cellulase or cellulase-hemicellulase enzyme mixtures failed to improve animal performance consistently. Woodford (1978) found that ensiling alfalfa with cellulase-based enzyme mixtures did not improve silage fermentation or animal performance

Aganga and Autlweste (2000) noted that sheep that reared on millet forage had a higher daily weight gain in comparison with sheep fed on veldt grass. Titi (2004) noted that exogenous fibrolytic enzymes resulted in improved ($P<0.05$) feed conversion ratio of fattened Awassi sheep with no effect on feed intake. The same author also, indicated that

fibrolytic enzymes could enhance the growth of fattened lambs and improve their conversion rations mainly through improving digestibility.

Table (5): Growth performance of calves fed the experimental rations

Item	Treatment diets				SEM
	0.0% SBP	0.2% SBP	0.4% SBP	0.6% SBP	
Initial weight	164.00	163.25	163.25	163.75	7.09
Final weight	339.18	340.65	345.63	351.30	8.75
Total body weight gain, kg	175.18	177.40	182.38	187.55	2.30
Average daily gain, kg	1.138	1.151	1.184	1.218	0.02
Relative gain*	106.82	108.67	111.72	114.53	3.21
Feed intake as:					
Dry matter (kg/day)	7.413	7.389	7.542	7.629	0.24
TDN (kg/day)	4.551	4.580	4.825	5.230	0.17
DCP (g/day)	592.3	602.9	640.3	697.3	22.30
Feed efficiency (kg. gain / kg. intake) of :					
Dry matter	0.1535	0.1558	0.1570	0.1597	0.003
TDN	0.2501	0.2513	0.2454	0.2329	0.01
DCP	1.921	1.909	1.849	1.747	0.04

* Relative gain (% of initial weight) = Gain / initial weight x 100.

CONCLUSION

From these results, it can be concluded that supplementation of exogenous enzymes to steers rations as fibrolytic enzymes (your enzymes were a mixture; had amylase enzyme too) significantly improved digestibilities for all nutrient and rumen TVFA's concentrations. Feeding the exogenous enzymes had no effects on feed intake, feed efficiency, or growth performance. . More information is needed concerning the level of exogenous enzymes that is best to use in steers ration supplementation.

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استجابة العجول النامية للعلائق المحتوية على مستويات مختلفة من مخلوط الإنزيمات

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أستخدم فى هذه الدراسة ستة عشر عجل بمتوسط وزن ١٦٣,٥ ± ١,٠٧ كجم والتى قسمت إلى أربعة مجاميع تجريبية متساوية تحتوى كل مجموعة أربعة حيوانات بهدف دراسة تأثير إضافة مخلوط من الإنزيمات المحللة للألياف والمكونة من (إنزيم السليوليز والزيلانيز والألفا أميليز والبوليجلاكتيونيز) على النمو والهضم وتخمرات الكرش فى العجول النامية الخليطة واستمرت تجربة التغذية لمدة ١٥٤ يوم وغذيت الحيوانات بمعدل ٢% من وزن الجسم الحى على العليقة المركزة المحتوية على (صفر ، ٠,٢% ، ٠,٤% ، ٠,٦% من مخلوط الإنزيمات) مع تقديم نبات الدخن كعلف أخضر حتى الشبع وكانت العلائق متقاربة فى محتواها من البروتين والطاقة.

وأظهرت النتائج ما يلى:

* إضافة مخلوط الإنزيمات للعلائق لم يغير مكونات الجدار الخلوى للعلائق المغذاه.

* لم يتأثر معدل استهلاك الغذاء اليومى تأثرا معنويا (عند مستوى ٠,٠٥) بإضافة مخلوط الإنزيمات للعلائق التجريبية ولكن لوحظ أن العليقة المحتوية على ٠,٦% من مخلوط الإنزيمات أدى إلى زيادة معنوية فى الماكول من المركبات المهضومة الكلية والبروتين المهضوم المقدرة على أساس (جم/كجم وحدة حيز جسم تمثيلى ، كجم/١٠٠كجم وزن حى) مقارنة بالعلائق التجريبية الأخرى.

* أدت إضافة مخلوط الإنزيمات للعلائق إلى تحسن فى كل معاملات الهضم وزيادة القيم الغذائية المقدرة فى صورة مركبات مهضومة كلية وبروتين مهضوم.

* لم تؤثر إضافة مخلوط الإنزيمات للعلائق تأثيرا معنويا (عند مستوى ٠,٠٥) على درجة حموضة الكرش pH ومع هذا كان لإضافة مخلوط الإنزيمات تأثيرا معنويا (عند مستوى ٠,٠٥) على كلا من أمونيا الكرش والأحماض الدهنية الطيارة. كما لوحظ أن زمن أخذ عينات سائل الكرش قد أثر تأثيرا معنويا (عند مستوى ٠,٠٥) على كل قياسات تخمرات الكرش موضع الدراسة.

* لم يكن لإضافة مخلوط الإنزيمات للعلائق تأثيرا معنويا (عند مستوى ٠,٠٥) على الوزن الحى النهائى أو الزيادة الكلية فى النمو أو معدل النمو اليومى وكذلك الكفاءة الغذائية المقدرة على أساس (كجم نمو/كجم ماكول) ومع هذا فقد لوحظ أنه بزيادة مستوى الإضافة من مخلوط الإنزيمات كانت النتائج المتحصل عليها تميل إلى تحسن فى الوزن الحى النهائى أو الزيادة الكلية فى النمو أو معدل النمو اليومى وكذلك الكفاءة الغذائية المقدرة على أساس (كجم نمو/كجم ماكول من المادة الجافة) مقارنة بعليقة الكنترول الغير محتوية على مخلوط الإنزيمات.

* من هذه النتائج المتحصل عليها يمكن الإشارة إلى أن إضافة مخلوط الإنزيمات (كإنزيمات محللة للألياف) لعلائق العجول النامية أدت إلى تحسين النمو ومعاملات الهضم.