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Research Article
Effect of Xylanase and Phytase Supplementation on Goat's Performance in Early Lactation

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Abstract

Background and Objectives: Supplementing diets of dairy animals with phytase and xylanase can enhance phosphorus availability and fiber degradation in the rumen and positively affect animal's health and productivity. *In vitro* and *in vivo* trials have been conducted to define the optimal addition level of xylanase and phytase to lactating Baldi goat's rations and investigate effects of these enzymes on animal's nutrients digestibility, blood chemistry, milk production and milk composition. **Materials and Methods:** *In vitro* batch culture technique was used to evaluate the effect of phytase and xylanase supplementation at different levels (0, 1, 2 and 3 g kg^{-1} DM) on rumen fermentation characteristics. Eighteen early lactating Baldi goats were randomly assigned into three groups and fed 4% dry matter according to their body weight. The first group was fed control ration (35% yellow corn, 20% corn stalks, 20% berseem hay, 12.5% soybean meal and 12.5% wheat bran), the second group fed control ration+Penizyme at 2 g kg^{-1} DM (R_1), while the third group fed control ration+Phtase-Plus® at 1 g kg^{-1} DM (R_2). **Results:** Xylanase and phytase supplementation increased the *in vitro* DM and OM degradability and ruminal NH_3-N and total volatile fatty acids (TVFA) concentrations, with no effect on total gas production (TGP) volume. All nutrients digestibility (except CP), blood serum glucose concentration, milk production and milk components yields were increased for enzymes supplemented goats than control. **Conclusion:** Inclusion of xylanase and phytase in lactating goat's rations improved their productive performance with no deleterious effects on their health.

Key words: Xylanase, phytase, lactating goats, milk production, nutrients digestibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

At the present time, cereal grains and grains by products represent at least 50% of dairy animal's rations in Egypt. In view of the high xylan and phytate phosphorus content of these feedstuffs, limitations in fibers and phosphorus utilization can occur in the rumen[1,2]. It is worth to mention that, xylan is a fiber polysaccharide composed of more than 10000 xylose units linked by 1,4-β-linkages[3]. While, phytate is an organic complex (myo-inositol hexakisphosphate) generally regarded as the primary storage form of phosphorus (P) in plants[4]. Also, phytate has anti nutritive properties as it has the ability to reduce mineral's (i.e., P, Ca, Mg, Zn and Fe) availability and bind with proteins and digestive enzymes (i.e., amylase, pepsin and trypsin) to make them less soluble[5,6].

It was reported that dairy cows are able to utilize 98% of phytate- P present in their diets[7], but, in another report it was pointed out that only 67% of phytate- P has been hydrolysed[1]. Also, it was stated that around 80% of grain's phytate were hydrolysed to inorganic P by dairy cows[8]. However, slow or incomplete digestion of dietary fibers and the deficiency of phosphorus (P) bioavailability can significantly influence animal performance and increase the cost of production[4,9]. Therefore, the search for tools able to increase nutrients bioavailability for farm animals is very important approach.

The use of enzymatic preparations as a feed supplements have attracted a lot of interest at last decade[10,11]. Phytate P can be hydrolysed to inorganic P and myo-inositol phosphate esters by using phytase. Phytase belongs to a class of enzymes that enables dephosphorylation of phytate in the feed before ingestion or in the digestive tract of the treated animals[12,13]. Similarly, xylanase is the main enzyme of cell wall degrading enzymes that can hydrolyses xylan into soluble sugars[14]. Supplementing diets of ruminants with phytase and fiber degrading enzymes can improve feed utilization and animal performance by enhancing P availability and fiber degradation *in vitro*[14-18], *in situ*[19,20] and *in vivo*[4,21-25]. The proposed mode of action of xylanase and phytase in ruminants including partial hydrolysis of dietary phytate and xylan before ingestion, increase ruminal total xylanase and phytase hydrolytic activity by synergism with endogenous enzymes and improve ability of ruminal micro-organisms to attachment and access of the feed particles[8,9].

The first goal for dairy herd's breeders is to provide their animals with adequate amount of nutrients to optimize their animal's performance at an economic cost. Feeding excessive amounts of phosphorus should be avoided because it is costly and environmental pollutant and it may affect milk production adversely[26]. In contrast, insufficient intake of P can negatively affect fiber digestion, protein degradation and microbial protein synthesis in the rumen[4]. Therefore; the optimal level for enzymes addition to dairy animal's diets is very important issue.

In this study, the focus has been on: (1) Defined the optimal level of xylanase and phytase enzymes addition to ruminant's rations through (*in vitro*) trial. (2) Investigated the impact of these enzymes addition to Baldi goat's rations on animal's nutrients digestibility, blood chemistry, milk production and milk composition.

MATERIALS AND METHODS

This study was carried out at a private farm (Alsttar farm for animal production), Khatatba, Menofia governorate, Egypt. The entire experimental period was extended from January 2-March 3, 2018. This experimental research has been conducted according to the experimental and ethical rules of the National Research Centre of Egypt.

Xylanase and phytase sources

Penizyme: A laboratory produced xylanase (22.88 IU g^{-1}) from *Penicillium chrysogenumin* dry form by using insoluble-starch as a carrier material. The produced enzyme activity was determined according to method of Bailey *et al.*[27]. One unit of xylanase activity was defined as the amount of enzyme that liberates 1 µmol of reducing sugars equivalent to xylose per minute under the assay conditions[27].

Phytase-Plus®: A commercial dry form of phytase enzyme source (500 IU g^{-1}) produced by Baytara for pharmaceuticals technology, Sadat Industrial city under license of VTR© Guangdong VTR Bio-Tech Co., Ltd.,-China. A mixture of wheat bran and calcium carbonate were used as carrier materials for the enzyme activity. One unit of phytase is defined as the amount of enzyme that liberates 1 µmol of phosphate per minute from sodium phosphate under the assay conditions[15].

***In vitro* study:** A 400 mg of total mixed ration (TMR) consisted of 35% yellow corn, 20% corn stalks, 20% berseem hay, 12.5% soybean meal and 12.5% wheat bran was accurately weighed into125 mL incubation vessels and separately supplemented with solution of Penizyme and Phtase-Plus® at different levels (0, 1, 2 and 3 g kg^{-1} DM). Each vessel was filled with 40 mL of mixture of 1:3 (v/v) rumen fluids: buffer solution[28]. After 24 h of incubation, all vessels were filtered in fiber filter bags 25 micron porosity (ANKOM-USA). The residues in the bags were dried at 70°C in oven for 48 h to

analyse dry matter (DM) and organic matter (OM) digestibility. Rumen fluid pH was measured using pH-meter. Overall volume of the produced gases was determined using Hohenheim Syringes (100 mL)[29]. Quantitative analysis of ammonia concentration was carried out by a modified Nessler's method[30]. The total volatile fatty acids (VFA) were determined[28].

Digestibility and lactating trails: Eighteen early lactating baladi goats weighed on average (26±0.5 kg) were used in the present study. Goats were randomly divided after a week of parturition into three groups. The first group was fed the control ration (35% yellow corn, 20% corn stalks, 20% berseem hay, 12.5% soybean meal and 12.5% wheat bran). The second group was fed control ration+Penizyme at 2 g kg^{-1} DM (R_1), while the third group was fed control ration+Phtase-Plus® at 1 g kg^{-1} DM (R_2). The goats were fed dry matter according to 4% of their body weight and the entire experimental period was 63 days. The feed ingredients and the chemical composition of the experimental control ration are shown in Table 1.

Determination of digestion coefficients: During the last 7 days of each month of the experimental period, fecal grab samples were collected in cloth bag connected to the animal back at 1 pm from 3 animals of each group. The dried feces from each animal were mixed and ground to pass a 1 mm sieve in a feed mill for chemical analysis. The digestibility coefficient of nutrient was calculated according to the following equation[31]:

$$\text{Digestion co-efficient} = 100 - \left[100 \times \frac{\text{Indicatorin feed (\%)}}{\text{Indicatorin feces (\%)}} \times \frac{\text{Nutrientin feces (\%)}}{\text{Nutrientin feed (\%)}}\right]$$

Feed and fecal analysis: Feed stuffs and fecal samples were analyzed according to the AOAC[32] methods to determine dry matter (DM), crude protein (CP), ether extract (EE) and ash contents. Organic matter (OM) contents were calculated by difference. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined[33].

Blood sampling and analysis: Blood samples were taken from jugular vein of 3 animals each group through the last 3 days of each month of the experimental period. At about 4 h after morning feeding the blood samples were collected in glass tubes and left to coagulate at room temperature. Serum was separated by centrifugation at 4000×g/20 min and kept frozen at -20°C for later analysis. The measured blood parameters (urea, AST, ALT, etc...) were determined[34].

Sampling and analysis of milk: Goats were milked by hand twice a day at 8:00 am and 8:00 pm by milking one teat while, the other one was left to the kid for suckling. Samples of milk were collected immediately from each animal after morning and evening milking and milk yield was recorded. Milk samples were analyzed for total solids, fat, true protein and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Q3 183 Electric, Hillerød, Denmark) according to AOAC[32], procedures. Solids-not-fat (SNF) was calculated. Fat corrected milk (4% fat) was calculated by using the following equation[35]:

$$FCM = 0.4\ M + 15\ F$$

where, M is the milk yield (g) and F is the fat yield (g).

Table 1: Chemical composition of feed ingredients and the calculated total mixed ration (on DM basis)

	Feed ingredients (g kg^{-1} DM)					
Items	Corn grain	Soybean meal	Wheat bran	Corn stalks	Clover hay	TMR (calculated)
DM	914.60	930.60	931.20	924.00	920.00	921.64
OM	985.50	935.60	945.30	940.00	870.00	942.04
CP	82.50	410.00	128.00	27.00	171.00	135.73
EE	52.90	33.00	27.30	17.00	40.00	37.45
Ash	14.50	64.40	54.70	60.00	130.00	57.96
NDF	84.00	119.00	369.00	698.00	410.00	312.00
ADF	22.40	72.00	114.60	428.00	270.00	170.77
ADL	2.50	1.30	29.40	208.00	60.00	58.31
NFC	766.10	373.60	421.00	198.00	249.00	456.86
Cellulose	19.90	70.70	85.20	220.00	210.00	112.45
Hemicellulose	61.60	47.00	254.40	270.00	140.00	141.24

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, NFC: Non fiber carbohydrate and TMR: Total mixed ration (Corn grain 35%, Soybean meal 12.5%, Wheat bran 12.5%, Corn stalks 20% and Clover hay 20%)

Table 2: Effect of xylanase and phytase supplemented rations on *in vitro* rumen fermentation characteristics

Parameters	Enzymes addition level (g kg^{-1}) DM	Ruminal parameters					
		DMD (%)	OMD (%)	PH	TGP	NH_3-N	TVFA
Control	0	58.07^{c}	64.18^{c}	6.68^{a}	137.33	1.08^{b}	7.03^{c}
Penizyme	1	58.63^{c}	64.74^{c}	6.64^{b}	137.67	1.14^{b}	7.70^{b}
	2	60.80^{b}	66.91^{b}	6.62^{b}	137.33	1.99^{a}	8.43^{a}
	3	58.34^{c}	64.45^{c}	6.62^{b}	136.33	1.23^{b}	8.10^{ab}
Phtase-plus®	1	64.44^{a}	70.55^{a}	6.63^{b}	138.67	2.30^{a}	8.40^{ab}
	2	60.29^{c}	66.40^{c}	6.60^{b}	137.67	1.12^{b}	8.27^{ab}
	3	60.11^{c}	66.22^{c}	6.60^{b}	137.00	1.05^{b}	8.20^{ab}
MSE±		0.77	0.77	0.01	0.19	0.12	0.11

DMD (%): Dry matter degradability, OMD (%): Organic matter degradability, TGP: Total gas production (mL/24 h), NH_3-N: Ammonia-Nitrogen (μmol L^{-1}), TVFA: Total volatile fatty acids (mEq dL^{-1}) and MSE±: Mean of standard error. *Means with different letter (a, b, c) in the same column are significantly different at p<0.05. 1, 2 and 3 refers to amount of enzymes by grams which added to each kilogram of animal's rations on dry matter basis

Table 3: Effect of xylanase and phytase supplementation on nutrient digestibility and nutritive values of the experimental rations

Items	Control	R_1	R_2	±SEM
Apparent nutrients digestibility (%)				
Dry matter (DM)	70.62^{b}	75.34^{a}	74.97^{a}	0.77
Organic matter (OM)	73.39^{b}	79.76^{a}	78.74^{a}	1.12
Crude protein (CP)	73.14	77.15	76.16	1.11
Ether extract (EE)	79.77^{b}	84.03^{a}	84.00^{a}	0.83
Non fiber carbohydrate (NFC)	74.63^{b}	79.21^{a}	77.41^{a}	0.76
Neutral detergent fiber (NDF)	64.09^{b}	70.71^{a}	70.37^{a}	1.25
Nutritive value (%)				
Total digestible nutrients (TDN)	70.75^{b}	75.81^{a}	74.74^{a}	0.84
Digestible crude protein (DCP)	9.92	10.47	10.33	0.15

Control group: Goats fed control ration (35% yellow corn, 20% corn stalks, 20% berseem (clover) hay, 12.5% soybean meal and 12.5% wheat bran). R_1: Goats group fed control ration+xylanase (Penizyme) at 2 g kg^{-1} DM. Group was fed control R_2: Goats group fed control ration+phytase (Phtase-Plus®) at 1 g kg^{-1} DM. *Means with different letter in the same row are significantly different at p<0.05

Statistical analysis: Data obtained from this study were statistically analyzed by IBM SPSS Statistics for Windows[36] using the following general model procedure:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where, Y_{ij} is the parameter under analysis of the ij vessel of *in vitro* trial or goats of digestibility and lactation trials, μ is the overall mean, T_i is the effect due to treatment on the parameter under analysis, eij is the experimental error for ij on the observation, the Duncan's multiple range tests was used to test the significance among mean s using probability level less than 0.05 (p<0.05) for significance expression[37].

RESULTS

***In vitro* trial:** Data of Table 2 showed that xylanase and phytase supplementation increased the *in vitro* dry matter (DMD %) and organic matter (OMD %) degradability for the treated rations than that of the control. Also, ruminal ammonia-nitrogen (NH_3-N) and total volatile fatty acids (TVFA) concentrations take the same trend of the ration's degradability. Penizyme (2 g kg^{-1} DM) and Phytase-Plus® (1 g kg^{-1} DM) supplementation levels gave the highest (p<0.05) values of DMD (%), OMD (%), NH_3-N and TVFA for rations treated with xylanase and phytase, respectively. Ruminal pH recorded the highest (p<0.05) value by the control ration, while no significant differences were detected between all of the tested rations in the ruminal total gas production (TGP) volume.

Apparent nutrients digestibility: The goats fed enzymes supplemented rations (R_1 and R_2) showed significant increase (p<0.05) for most of nutrients digestibility coefficients and total digestible nutrients (TDN) than those fed the control ration (Table 3), but, no significant differences were found between all goat's groups in CP digestibility and digestible crude protein (DCP) values.

Blood serum parameters: Xylanase and phytase supplemented goats (R_1 and R_2) had higher (p<0.05) serum glucose concentration than those of the control (Table 4). While no significant change were detected between all goat's groups in creatinine, urea, cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values.

Table 4: Effect of xylanase and phytase supplementation on goat's blood parameters

Items	Control	R_1	R_2	±SEM
Glucose (mg dL^{-1})	61.83[b]	72.00[a]	71.33[a]	1.76
Creatinine (mg dL^{-1})	0.92	0.95	0.90	0.05
Urea (mg dL^{-1})	35.00	36.17	37.33	1.05
Cholesterol (mg dL^{-1})	75.83	77.83	76.67	2.59
AST (U mL^{-1})	28.33	29.33	28.00	1.50
ALT (U mL^{-1})	22.67	24.67	23.83	1.04

Control group: Goats fed control ration (35% yellow corn, 20% corn stalks, 20% berseem (clover) hay, 12.5% soybean meal and 12.5% wheat bran). R_1: Goats group fed control ration+xylanase (Penizyme) at 2 g kg^{-1} DM. Group was fed control R_2: Goats group fed control ration+phytase (Phtase-Plus®) at 1 g kg^{-1} DM. *Means with different letter in the same row are significantly different at $p<0.05$

Table 5: Effect of xylanase and phytase supplementation on goat's milk yield and milk composition

Items	Control	R_1	R_2	±SEM
Milk production (g day^{-1})				
Milk yield	285.35[b]	347.22[a]	329.86[ab]	14.03
4% FCM yield	271.20[b]	346.87[a]	322.79[ab]	12.32
Total protein yield	9.42[b]	12.39[a]	11.31[ab]	0.48
Fat yield	10.47[b]	13.87[a]	12.72[ab]	0.55
Lactose yield	13.60[b]	17.03[a]	15.73[ab]	0.66
Ash yield	1.90[b]	2.41[a]	2.18[ab]	0.08
Total solids yield	35.40[b]	45.70[a]	41.95[ab]	1.65
Solids not fat yield	24.93[b]	31.83[a]	29.22[ab]	1.20
Milk composition (%)				
Total protein	3.34	3.57	3.53	0.07
Fat	3.92	4.13	4.04	0.16
Lactose	4.77	4.96	4.84	0.09
Ash	0.68	0.70	0.67	0.01
Total solids	12.71	13.36	13.08	0.29
Solids not fat	8.79	9.23	9.04	0.15

Control group: Goats fed control ration (35% yellow corn, 20% corn stalks, 20% berseem (clover) hay, 12.5% soybean meal and 12.5% wheat bran). R_1: Goats group fed control ration+xylanase (Penizyme) at 2 g kg^{-1} DM. Group was fed control R_2: Goats group fed control ration+phytase (Phtase-Plus®) at 1 g kg^{-1} DM. *Means with different letter in the same row are significantly different at $p<0.05$

Milk yield and its composition: Milk composition of supplemented goats with xylanase and phytase were not affected significantly compared with goats of the control (Table 5), but goats fed xylanase supplemented ration (R_1) had higher ($p<0.05$) milk, 4% fat corrected milk (FCM) and all milk components yields than those fed the control. The goats fed ration supplemented with phytase (R_2) showed numerical (but not significant) increase in milk and its components yields compared with those of the control. Also, there were no significant differences between goats fed enzymes supplemented rations (R_1 and R_2) in milk and its component's yields.

DISCUSSION

The resulting improvement in the *in vitro* ruminal parameters after enzymes supplementation may be due to highly hydrolytic effect of xylanase on the ration's hemicellulose and positive impact of phytase on ration's phosphorus bioavailability. The availability of P and simple carbohydrates may lead to increase in microbial colonization of feed particles and consequently more DM and OM digestion and increase TVFA's and microbial protein production. In this concern, it has been reported that phytase addition to ruminant's rations can positively affect the P-utilization (*in vitro*)[38]. Also, it was found that total bacterial count were higher in rumen liquor of goats and steers treated with xylanase than untreated[18]. The reduction of ruminal pH values after enzymes supplementation maybe due to higher TVFA's production in response to enzymes treatment. In this context, it has been reported that *in vitro* ruminal pH decreased and TVFA's and NH_3-N concentrations increased with xylanase addition[14]. While, maize stover treated with fibrolytic enzymes had no effects on final ruminal pH values, but increase significantly TVFA's production *in vitro*[39]. Generally, ruminal gas production seems to be related to the ration's chemical composition especially fiber contents[40]. This may give an explanation for non-significant change of TGP after xylanase or phytase supplementation. Similarly, positive effects of phytase and xylanase supplementation on *in vitro* DM and OM digestion have been noticed[15,18]. Increase DM, OM, EE, NFC, NDF and TDN digestibility for goats fed enzymes treated rations (R_1 and R_2)

was may be due to break down of ration's anti-nutritional factors (phytate and compacted cell wall fibers), beside liberation of more phosphorus and soluble carbohydrate for the action of rumen microflora. Absence of the enzymes effect on the CP digestibility is may be due to low degradability of corn protein (zein) in the rumen[35]. The current positive nutrients digestibility results are supported by findings of many researchers[4,9,10,23]. The elevation of blood glucose concentrations due to enzymes supplementation was may be attributed to higher nutrients digestibility (Table 3) which may let for more blood glucose circulation in goat's body. In this concern, many studies stated increase animal's blood glucose concentration after their feeding on fiber degrading enzymes[9,40]. However, it was also reported that no marked effect of phytase addition to diets on sheep's blood parameters[4]. The marked increase in milk and its component's yields by enzymes treated goats is probably due to higher production of TVFA's especially propionate and NH_3-N in their rumen, higher nutrients digestion and higher blood glucose concentration, which may lead for more glucogenic precursor's delivery to the mammary gland[11]. It was stated that fibrolytic enzymes addition to goat's rations have been associated with improved of microbial protein synthesis and increase efficiency of diet's energy utilization[11]. This may gave an explanation for higher milk fat and protein yields in goats of R_1. The positive impact of fiber degrading enzymes on milk and its components production has been recorded[9,11,22,23]. While, many researchers demonstrated that addition of phytase to lactating animals rations has no significant impact on the milk production or milk components yields[21,41,42]. It was obvious from the results of this study that phytase and xylanase addition to lactating goat's diets can improve animal's performance; so more studies are recommended in this topic for find out the relation (synergism or antagonism) between action of xylanase and phytase as feed additives.

CONCLUSION

It could be concluded that inclusion of xylanase and phytase in lactating goat's rations positively affect all rumen fermentation characteristics (*in vitro*) and improved most of nutrients digestibility coefficients and milk production by treated goats than those of control with no deleterious effects on goat's health.

SIGNIFICANCE STATEMENT

This study discovered the effectiveness of the xylanase enzyme produced by aerobic fungi in the degradation of feed fibers in anaerobic media like rumen. This can be beneficial for the researchers and industry men as well and gives a greater opportunity to expand production of these enzymes on a commercial scale to be used in several areas including animal nutrition.

REFERENCES

1. Godoy, S. and F. Meschy, 2001. Utilisation of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating goats fed on different forage to concentrate ratios. Reprod. Nutr. Dev., 41: 259-265.
2. Azzaz, H.H., T.A. Morsy and H.A. Murad, 2016. Microbial feed supplements for ruminant's performance enhancement. Asian J. Agric. Res., 10: 1-14.
3. Kalim, B., N. Böhringer, N. Ali and T.F. Schäberle, 2015. Xylanases–from microbial origin to industrial application. Br. Biotechnol. J., 7: 1-20.
4. Ahmed, S.M., S.M. Allam, H.A.A. Omer, R.R. Elelaime and N.A. Hassaan, 2014. Effect of adding phytase to sheep rations on digestibility and bioavailability of phosphorus and calcium. Global Vet., 13: 844-855.
5. Garrett, J.B., K.A. Kretz, E. O'Donoghue, J. Kerovuo and K. William *et al*., 2004. Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric-feed supplement. Applied Environ. Microbiol., 70: 3041-3046.
6. Van Doorn, D.A., H. Everts, H. Wouterse and A.C. Beynen, 2004. The apparent digestibility of phytate phosphorus and the influence of supplemental phytase in horses. J. Anim. Sci., 82: 1756-1763.
7. Clark, Jr. W.D., J.E. Wohlt, R.L. Gilbreath and P.K. Zajac, 1986. Phytate phosphorus intake and disappearance in the gastrointestinal tract of high producing dairy cows. J. Dairy Sci., 69: 3151-3155.
8. Kincaid, R.L., D.K. Garikipati, T.D. Nennich and J.H. Harrison, 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J. Dairy Sci., 88: 2893-2902.
9. Azzaz, H.H., H.A. Murad, A.M. Kholif, T.A. Morsy, A.M. Mansour and H.M. El-Sayed, 2013. Increasing nutrients bioavailability by using fibrolytic enzymes in dairy buffaloes feeding. J. Biol. Sci., 13: 234-241.
10. Azzaz, H.H., A.M. Kholif, H.A. Murad, M.A. Hanfy and M.H. Abdel Gawad, 2012. Utilization of cellulolytic enzymes to improve the nutritive value of banana wastes and performance of lactating goats. Asian J. Anim. Vet. Adv., 7: 664-673.
11. Azzaz, H.H., E.S.A. Farahat and H.M. Ebeid, 2017. Effect of partial replacement of corn grains by date seeds on rahmani ram's nutrients digestibility and Nubian goat's milk production. Int. J. Dairy Sci., 12: 266-274.

12. Haefner, S., A. Knietsch, E. Scholten, J. Braun, M. Lohscheidt and O. Zelder, 2005. Biotechnological production and applications of phytases. Applied Microbiol. Biotechnol., 68: 588-597.
13. Woyengo, T.A. and C.M. Nyachoti, 2011. Supplementation of phytase and carbohydrases to diets for poultry. Can. J. Anim. Sci., 91: 177-192.
14. Togtokhbayar, N., M.A. Cerrillo, G.B. Rodriguez, M.M. Elghandour and A.Z. Salem *et al*., 2015. Effect of exogenous xylanase on rumen *in vitro* gas production and degradability of wheat straw. Anim. Sci. J., 86: 765-771.
15. Allam, S.M., S.M. Ahmed, H.A.A. Omer, R.R. Elelaime, M. Farghaly and N.A. Hassaan, 2014. Effect of adding phytase to sheep rations on *in vitro* disappearance of dry matter and organic matter & growth performance. Global Vet., 13: 918-925.
16. Aboul-Fotouh, G.E., G.M. El-Garhy, H.H. Azzaz, A.M. Abd El-Mola and G.A. Mousa, 2016. Fungal cellulase production optimization and its utilization in goat's rations degradation. Asian J. Anim. Vet. Adv., 11: 824-831.
17. Elghandour, M.M.Y., A.E. Kholif, J. Hernández, M.D. Mariezcurrena and S. López *et al*., 2016. Influence of the addition of exogenous xylanase with or without pre-incubation on the *in vitro* ruminal fermentation of three fibrous feeds. Czech J. Anim. Sci., 61: 262-272.
18. Vallejo-Hernández, L.H., M.M. Elghandour, R. Greiner, U.Y. Anele, R.R. Rivas-Cáceres, M. Barros-Rodríguez and A.Z. Salem, 2018. Environmental impact of yeast and exogenous xylanase on mitigating carbon dioxide and enteric methane production in ruminants. J. Cleaner Prod., 189: 40-46.
19. Krueger, N.A., A.T. Adesogan, C.R. Staples, W.K. Krueger, S.C. Kim, R.C. Littell and L.E. Sollenberger, 2008. Effect of method of applying fibrolytic enzymes or ammonia to Bermudagrass hay on feed intake, digestion and growth of beef steers. J. Anim. Sci., 86: 882-889.
20. Phakachoed, N., P. Lounglawan and W. Suksombat, 2012. Effects of xylanase supplementation on ruminal digestibility in fistulated non-lactating dairy cows fed rice straw. Livest. Sci., 149: 104-108.
21. Winter, L., U. Meyer, D. Soosten Von, M. Gorniak, P. Lebzien and S. Dänicke, 2015. Effect of phytase supplementation on rumen fermentation characteristics and phosphorus balance in lactating dairy cows. Ital. J. Anim. Sci., 14: 53-60.
22. Romero, J.J., E.G. Macias, Z.X. Ma, R.M. Martins, C.R. Staples, K.A. Beauchemin and A.T. Adesogan, 2016. Improving the performance of dairy cattle with a xylanase-rich exogenous enzyme preparation. J. Dairy Sci., 99: 3486-3496.
23. Kholif, A.E., A.Y. Kassab, H.H. Azzaz, O.H. Matloup, H.A. Hamdon, O.A. Olafadehan and T.A. Morsy, 2018. Essential oils blend with a newly developed enzyme cocktail works synergistically to enhance feed utilization and milk production of Farafra ewes in the subtropics. Small Rumin. Res., 161: 43-50.
24. Neumann, M., G.F.M. Leão, G.L.D. Vigne, L.C.D. Santos, B.J. Venancio and A. Dochwat, 2018. Xylanase-complex efficacy in high-energy diet for bulls finished in feedlot. Acta Scient. Anim. Sci., Vol. 40. 10.4025/actascianimsci.v40i1.37321.
25. Vallejo, L.H., G. Buendía, M.M.M.Y. Elghandour, D. Menezes-Blackburn, R. Greiner and A.Z.M. Salem, 2018. The effect of exogenous phytase supplementation on nutrient digestibility, ruminal fermentation and phosphorous bioavailability in rambouillet sheep. J. Sci. Food Agric., 98: 5089-5094.
26. Morse, D.H., H.H. Head and C.J. Wilcox, 1992. Disappearance of phosphorus in phytate from concentrates *in vitro* from rations fed to lactating dairy cows. J. Dairy Sci., 75: 1979-1986.
27. Bailey, M.J., P. Biely and K. Poutanen, 1992. Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol., 23: 257-270.
28. Ismail, S.A., A.M. Abdel-Fattah, M.A. Emran, H.H. Azzaz, M.S. El-Gamal and A.M. Hashem, 2018. Effect of partial substitution of ration's soybean meal by biologically treated feathers on rumen fermentation characteristics (*in vitro*). Pak. J. Biol. Sci., 21: 110-118.
29. Navarro-Villa, A., M. O'Brien, S. Lopez, T.M. Boland and P. O'Kiely, 2011. Modifications of a gas production technique for assessing *in vitro* rumen methane production from feedstuffs. Anim. Feed Sci. Technol., 166-167: 163-174.
30. Szczechowiak, J., M. Szumacher-Strabel, M. El-Sherbiny, E. Pers-Kamczyc, P. Pawlak and A. Cieslak, 2016. Rumen fermentation, methane concentration and fatty acid proportion in the rumen and milk of dairy cows fed condensed tannin and/or fish-soybean oils blend. Anim. Feed Sci. Technol., 216: 93-107.
31. Ferret, A., J. Plaixats, G. Caja, J. Gasa and P. Prio, 1999. Using markers to estimate apparent dry matter digestibility, faecal output and dry matter intake in dairy ewes fed Italian ryegrass hay or alfalfa hay. Small Rumin. Res., 33: 145-152.
32. AOAC., 1995. Official Methods of Analysis of AOAC International. 16th Edn. Vol. 1, Agricultural, Chemicals, Contaminants, Drugs. AOAC International, Washington, D.C., USA.
33. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
34. Azzaz, H.H., H.A. Aziz, H. Alzahar and H.A. Murad, 2018. Yeast and *Trichoderma viride* don't synergistically work to improve olive trees by products digestibility and lactating Barki ewe's productivity. J. Biol. Sci., 18: 270-279.
35. Azzaz, H.H., H.M. Ebeid, T.A. Morsy and S.M. Kholif, 2015. Impact of feeding yeast culture or yeast culture and propionibacteria 169 on the productive performance of lactating buffaloes. Int. J. Dairy Sci., 10: 107-116.

36. IBM Corporation, 2011. IBM SPSS statistics for windows, version 20.0. IBM Corp, Armonk, New York.
37. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
38. Brask-Pedersen, D.N., L.V. Glitsø, L.K. Skov, P. Lund and J. Sehested, 2013. Effect of exogenous phytase on degradation of inositol phosphate in dairy cows. J. Dairy Sci., 96: 1691-1700.
39. Diaz, A., M.D. Carro, C. Saro, I. Mateos, E. Odongo and M.J. Ranilla, 2013. *In vitro* evaluation of commercial fibrolytic enzymes for improving the nutritive value of low-quality forages. Anim. Nutr. Feed Technol., 13: 461-476.
40. Elghandour, M.M.Y., J.C.V. Chagoyan, A.Z.M. Salem, A.E. Kholif, J.S.M. Castaneda, L.M. Camacho and M.A. Cerrillo-Soto, 2014. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. Ital. J. Anim. Sci., 13: 295-301.
41. Jarrett, J.P., J.W. Wilson, P.P. Ray and K.F. Knowlton, 2014. The effects of forage particle length and exogenous phytase inclusion on phosphorus digestion and absorption in lactating cows. J. Dairy Sci., 97: 411-418.
42. Knowlton, K.F., C.M. Parsons, C.W. Cobb and K.F. Wilson, 2005. Exogenous phytase plus cellulase and phosphorus excretion in lactating dairy cows. Applied Anim. Sci., 21: 212-216.