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Influence of using Pectinase Enzymes in the Ration on Nutrient Digestibility, Blood Chemistry, Milk Composition and Economics of Lactating Buffaloes

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Abstract | The present study was performed to evaluate the effects of pectinase enzymes inclusion in the ration on lactating buffaloes performance, nutrient digestibility, blood chemistry and yield and composition of milk. The experiment conducted twelve lactating Egyptian buffaloes having their 3rd to 5th lactation and weighed 480± 8 kg in average. After 20 days of parturition, animals were randomly assigned into three groups, four animals per each group, the first group was fed on ration of 50% concentrates feed mixture, 20% Egyptian clover, 20 % sugar beet pulp and 10% dried orange by-products (Control ration). The second group (R1) was fed control ration supplemented with the locally produced pectinase enzyme at level of 3g /kg DM (dry matter), while the third group (R2) was fed control ration supplemented with commercial pectinase enzyme at level of 3g /kg DM. The result revealed that, R1 and R2 rations significantly (P≤0.05) increased DM, OM (organic matter), CP(crude protein) and CF (crude fiber) digestibility compared to the control one. (total digestible nutrients), SV (starch value) and DCP (digestible crude protein) % compared to control one. Also, actual milk yield and average 4% fat corrected milk yield were increased (P≤0.05) by R1 and R2 rations compared to control one. There were insignificant (P>0.05) increase in milk composition percentages in treated groups. In addition, control ration significantly decreased (P≤0.05) daily feed conversion of DM and DCP compared to R1 and R2 rations. Blood serum metabolites for enzymes treated animals showed higher glucose and total protein concentrations than those of the control with no side effects on animals health. Economic analysis revealed the R1 as a best ration for lactating buffaloes. From the results, it could be concluded that pectinase enzyme inclusion in ration is beneficial to improve the performance of lactating buffaloes.

Keywords | Lactating buffaloes, Digestibility, Milk yield, Milk composition

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INTRODUCTION

Their is a wide gap between ruminant's needs for feeds and available traditional feeds in Egypt. (Azzaz et al., 2018). Agricultural and industrial by products as non traditional feed resources can play vital role to overcome this gap (Azzaz et al., 2017). These abundant by-products are mostly leave in the road sides or may be burnt in the field lead to pollution in environment and increase health risks. In spite of nutritional merits of these by-products, use it as ingredients in formulated ruminant's diets is still low (Ismail et al., 2018). However, the nutritional issues of agro industrial by-products includes low-protein, low-digestibility coefficients, high crude fiber, and containing some anti-nutrients factors (Azzaz et al., 2013; Aboul-Fotouh et al., 2016). Thus, to increase the digestibility of these by-products, it is important to destroy the combined nature of the tissues of lignocellulose and reduce the harmful effects of anti-nutrient agents. There have been efforts to do that by biological processing (Azzaz et al.,

2015, 2016a; Abd El-Tawab et al., 2019). Biological processing of some agricultural by products becomes necessary in order to dissolve lignocellulytic materials into lignin, cellulose, and hemicellulose and improve crude protein content. It is known that, biological treatments can be performed by microbial enzymes Khattab et al. (2019). Pectinase is a group of enzymes which catalyze break down of the glycosidic bonds of the galacturonic acid long chain residues in the pectin rich plants (El-Garhy et al., 2020). Several studies on enzyme supplementation to dairy animals ration had been shown increase in milk yields of 5–25% (Murad and Azzaz, 2010, 2011; Azzaz et al., 2012, 2013, 2019; Kholif et al., 2018). It is well known that pectin constitutes the main component of cell wall of orange peel, sugar beet pulp and pomegranate peel. These by-products become essential sources for livestock feeding in Egypt. Therefore, this study was carried out for investigating the impact of adding pectinase enzymes to lactating buffaloes ration on nutrients digestibility, milk yield and composition, feed efficiency and some blood parameters. Also, simple economical evaluation of the tested rations was carried out.

MATERIALS AND METHODS

The present study was carried out at farm and laboratory of Animal Production Department, Faculty of Agriculture, Fayoum University, Egypt.

Enzymes sources

The pectinase enzyme was produced locally (El-Garhy et al., 2020) from *Penicillium chrysogenum.* Each gram contains 200 units of pectinase. Alternatively, a commercial pectinase enzyme (SMIZYME® from AGRI-VET company, Egypt) was purchased, with similar concentration as produced locally (200 unit of pectinase per gram).

The experimental animals

Twelve lactating Egyptian buffaloes (in their 3rd to 5th lactation seasons and weighed 480±8 kg in average) were used in this study. Twenty days after parturition, buffaloes were randomly assigned to three groups, four animals per each group by using complete randomized design. The experimental period was 70 days.

The tested rations

The buffaloes were individually fed rations of concentrate: roughage at ratio of 1:1 on DM basis. The first animal group was fed on ration of 50% concentrates feed mixture, 20% Egyptian clover, 20% sugar beet pulp and 10% dried orange by-products (control ration). The rations R1 and R2 were supplemented 3 g /kg (DM basis) of locally produced and commercial pectinase enzyme respectively. The dose of pectinase enzyme for supplementation was selected from a recent study (El-Garhy et al., 2020). Animals were fed to cover their nutritional requirements according to Shehata (1971). The composition of tested rations is shown in Table 1.

Table 1: Composition of the tested rations of lactating buffaloes (on DM basis).

Item	The tested rations		
	Control	R1	R2
Concentrate feed mixture*	50	50	50
Sugar beet pulp	20	20	20
Egyptian clover	20	20	20
Dried orange by-products**	10	10	10
Pectinase enzymes	----	3 g of the produced enzyme / kg DM	3g of MIZYME®/kg DM

* Formulation of concentrates feed mixture on DM basis was 55% yellow corn, 21.5 % wheat bran,20 % soya bean meal,3.5 % feed additives (feed additives composed of 1.5% limestone,0.5% dicalcium phosphate, 0.2% yeast,0.3% bicarbonate,0.5% premix and 0.5% NaCl); ** Dried orange by-products comprised of peel and pulp (membrane and seeds) after juice is extracted from the fruit.

Digestibility experiment

Digestibility experiment was carried out at the end of the lactation experiment; feeding values and nutrient digestibilities were determined by using acid insoluble ash (AIA) technique according to Van Keulen and Young (1977). Feces samples were collected daily per each animal for seven days, dried over night at 65 °C in hot air oven, weighted, ground through 1mm screen, then complete drying was undertaken at 105 °C for 3 hr, then weighted and stored in tight bottles until analyzed for chemical analysis.

Milk production

The technique of hand milking had been used to calculate milk production. Buffaloes had been milked twice daily at 6:00 am and 6:00 pm by milking half udder while, the other half udder left to young buffalo calves for suckling as pointed out by Farag (1979). Daily milk yield and total milk yield was recorded for each animal in the experiment for 70 days.

Analytical procedures

Analysis of feeds and feces

Chemical analysis of fodder and feces samples were performed to calculate the percentage of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content according to methods of AOAC (1995). The nitrogen free extract (NFE) was determined by difference (OM- (CP+EE+CF). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent Lignin (ADL) were calculated in feeds and feces as reported by Goering

and Van Soest (1970).

Milk samples and analysis

Samples of daily milk (100 ml each) were collected at 6:00 am and 6:00 pm and mixed for each group in the trial. It had been kept frozen at (-20 ˚C) until the chemical analysis had executed. Milk samples had been analyzed for total solids, fat, protein, and lactose by using infrared spectrophotometry (Milkotester LM2, Belovo, Bulgaria). The ash content of milk was determined after heating a milk sample in a muffle furnace at 550 ˚C for 8 h.

Fat corrected milk (4% FCM) was determined by using the following equation as showed by Gaines (1928).

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

Where: M= milk yield (g/d); F= fat yield (amount of fat = M x fat %).

Blood samples analysis

Blood samples were taken from jugular vein of 4 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 Xg /20 min. and kept frozen at -20°c for later analysis. Serum protein, albumin, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, creatinine and cholesterol concentration were determined using specific kits (Stanbio Laboratory, Boerne, TX, USA) following manufacturer instructions.

Economical evaluation

Economical returns of the tested rations were determined assuming that the price of one kg of actual milk was 12 L.E. The cost of one ton DM of concentrate feed mixture (92.37% DM), Egyptian clover (13 % DM), Sugar beet pulp (89.5 DM) and orange by-products (93.4% DM) were 4600, 300, 3400 and 700 L.E., respectively. Also, price of one kg of the produced enzyme was 80 L.E. and price of one kg of SMIZYME® was 250 L.E.

Statistical analysis

Statistical analyses were determined by the general linear model procedure mentioned by SPSS (2007) according to the following model:

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

Where; Y_{ij} is the dependent variable, μ is the overall mean, T_i is the impact of treatment and e_{ij} is the residual error. Duncan's multiple test (Duncan, 1955) was performed for separating means.

RESULTS AND DISCUSSION

Chemical composition of feed ingredients

The chemical composition and cell wall constituents (DM basis) of concentrate feed mixture, sugar beet pulp, Egyptian clover and orange by-products used throughout the study are shown in Table 2. The chemical composition of all ingredients indicated a comparable dry matter composition. Orange by-products showed the highest levels of ash content and lower content of CP compared to concentrate feed mixture, Egyptian clover and sugar beet pulp, while sugar beet pulp showed higher levels of crude fiber and NDF compared to other feed ingredients.

Table 2: Chemical composition of feed ingredients (on % DM basis).

Item	CFM*	Egyptian clover	Sugar beet pulp	Orange by-products**
Chemical composition, %				
OM	93.28	84.68	96.19	81.26
CP	15.48	16.56	11.53	6.69
EE	2.87	2.19	0.74	3.43
CF	5.25	20.33	24.80	19.22
NFE	69.68	45.6	59.12	51.92
Ash	6.72	15.32	3.81	18.74
Cell wall constituents, %				
NDF	22.95	49.58	58.85	22.91
ADF	8.74	41.09	31.73	19.59

*CFM: concentrate feed mixture; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ** Dried orange by-products comprised of peel and pulp (membrane and seeds) after juice is extracted from the fruit.

Digestibility and nutritive values

Data presented in Table 3 indicated that, rations supplemented with the produced enzyme (R_1) and SMIZYME® (R_2) significantly ($P \leq 0.05$) increased DM, OM, CP and CF digestibility compared to control one. While, there were insignificant ($P>0.05$) increase between the produced enzyme and SMIZYME® rations concerning DM, OM, CP, EE and NFE digestibilities. Moreover, there were insignificant ($P>0.05$) increase among all the tested rations regarding NFE and EE digestibilities.

The results of current study are in close conformity to the findings of previous studies that reported increase in gastrointestinal tract digestibility of DM and OM following treatment with exogenous fibrolytic enzymes (Rojo et al., 2015; Aboul-Fotouh et al., 2017; Arif et al., 2019; Khattab et al., 2019).

Table 3: Pectinase enzymes effects on digestion coefficients and nutritive values of the rations fed to buffaloes.

Item	Control	R1	R2	± SE
Nutrient digestibilities (%)				
DM	66.18[b]	70.14[a]	70.85[a]	0.83
OM	69.72[b]	73.65[a]	73.99[a]	
CP	62.55[b]	64.73[a]	65.56[a]	
CF	65.50[c]	68.96[b]	71.00[a]	
EE	61.03	62.94	62.80	
NFE	74.11	76.99	77.17	
Nutritive values:				
TDN (%)	66.38[b]	69.02[a]	69.51[a]	
SV (%)	60.20[b]	62.80[a]	63.28[a]	
DCP (%)	9.01[b]	9.32[a]	9.44	

Is may be due to increasing ruminal digestion of NDF fraction following pectinases addition (Yang et al., 1999) and lower digesta viscosity (Hristov et al., 2000). Morgavi et al. (2000) pointed out the synergism between ruminal enzymes and exogenous enzymes by the combined hydrolytic impact in the rumen that showed more effects than that determined from individual enzyme activities. Wang et al. (2001) noted that, supplementation of enzyme enhanced numbers of non-fibrolytic and fibrolytic bacteria in a batch culture with fluid of rumen. Also, stimulatory action of these enzymes showed maximum microbial biomass, that provide more total polysaccharides to digest roughage or improved attachment and colonization to cell of plant wall by microorganisms of rumen (Nsereko et al., 2000) or /and alterations in ruminal fermentation (Nsereko et al., 2002).

The nutritive values of the tested rations expressed as total digestible nutrients (TDN), starch value (SV) and digestible crude protein (DCP) are shown in Table 3.

Rations supplemented with pectinase enzymes (R1 and R2) significantly (P≤ 0.05) increased TDN, SV % and DCP% compared to control one. While, there were insignificant increases in TDN, SV and DCP between rations supplemented with pectinase enzymes (R1 and R2). The improvement of nutritive value of rations supplemented with the produced enzyme (R1) and SMIZYME® (R2) may be because of increasing digestibility of nutrients which may be attributed to accumulation of agreat amount of readily fermentable carbohydrate which liberated because of action of pectinase enzymes on pectin of rations. This result is in accordance with those obtained by Kholif et al. (2018), who pointed out that, supplementation of fibrolytic enzymes in animal rations were improved (P≤ 0.05) nutrients digestibility and the nutritive value of the tested rations compared to the control ration.

Milk yield and its composition

Data exhibited in Table 4 showed that, rations supplemented with pectinase enzymes (R_1 and R_2) increased (P≤0.05) actual milk yield and average 4% fat corrected milk yield compared to control one. Control ration recorded the lowest milk yield, being 5.89 Kg/head/day followed by R_1, being 6.61 Kg/h/d. The highest value was detected for R_2, being 6.78 Kg/h/d. Furthermore, there were insignificant increases in actual milk yield and average fat corrected milk yield between rations supplemented with pectinase enzymes (R_1 and R_2).

Table 4: Impact of pectinase enzymes supplemented rations on buffalo's milk yield and composition.

Items	Rations			± SE
	Control	R_1	R_2	
Average actual milk yield (Kg/head/day).	5.89[b]	6.61[a]	6.78[a]	0.16
Average 4% fat corrected milk yield (kg/head/day)	8.108[b]	9.535[a]	9.719[a]	0.25
Milk components (kg/head/day)				
Total solids	0.936[b]	1.091[a]	1.108[a]	0.03
Fat	0.383[b]	0.459[a]	0.467[a]	0.02
SNF	0.552[b]	0.632[a]	0.641[a]	0.02
Total protein	0.201[b]	0.228[a]	0.236[a]	0.01
Lactose	0.302	0.349	0.351	0.01
Ash	0.049	0.055	0.054	0.001
Milk compositions %				
Total solids	15.89	16.51	16.34	0.26
Fat	6.51	6.95	6.89	0.23
SNF	9.38	9.56	9.45	0.11
Total protein	3.41	3.45	3.48	0.04
Lactose	5.13	5.28	5.17	0.08
Ash	0.83	0.83	0.80	0.01
Milk energy Mcal/Kg*	0.99	1.04	1.04	0.09

Average in the same row having different superscripts are differ significantly (P≤0.05) for a, b and c. Each value is a mean of 4 samples. *, Milk energy (Mcal/Kg) was calculated as milk energy = 0.0929 × % fat + 0.0547 × % protein + 0.0395 × % lactose according to the NRC (2001).

Adding SMIZYME® to lactating buffalo's ration (R2) increased milk yield by 15.11% and 4% fat corrected milk yield by 19.87%, while adding the produced enzyme to lactating buffalo's ration (R1) increased milk yield by 12.22 % and 4% fat corrected milk yield by 17.60% compared to control ration. In contrast, there were insignificant increase among all the tested rations in milk compositions percentage, while there were significant (P≤0.05) increase by rations supplemented with pectinase enzymes in milk

component's yields of total solids, SNF (solids not fat), fat and total protein compared to control ration. On the other hand, there were insignificant increase among all the tested rations in milk lactose, ash yields and the values of milk energy. Several studies on enzyme supplementation to dairy animals rations had reported increasing trend in milk yields of 5–25% (Murad and Azzaz, 2011; Azzaz et al., 2012, 2013, 2019; Aboul-Fotouh et al., 2017; Khattab et al., 2019).

The results of fat corrected milk (FCM) yield are in close conformity to the findings which pointed out by Aboul-Fotouh et al. (2017) and Khattab et al. (2019), that 4% FCM yield were higher (P≤0.05) for animals fed on fibrolytic enzymes compared to the control animals. These findings may reflect the impact of pectinase enzymes which are due to a greater amount of digested fiber in the rumen for providing more acetate for synthesis of fatty acid. As shown in Table 4, rations supplemented with pectinase enzymes increased milk compare to control ration, the improvement in milk yield may be because of increasing absorption of nutrients in the gastrointestinal tract, which resulted in gain of more net energy as reported by Kung (2000).

Feed intake and feed conversion

Result of daily feed intake of lactating buffaloes showed that, there were insignificant differences between the tested rations (Table 5). Several previous studies also reported no effects of adding fibrolytic enzymes to animals rations on dry matter intake (Aboul-Fotouh et al., 2017; Tirado-González et al., 2018; Arif et al., 2019; Khattab et al., 2019) while, some studies mentioned a positive impact of fibrolytic enzymes addition in diet with pronounced effects on dry matter intake Kholif et al. (2018).

Results presented in Table 5 indicated that, feed conversion of DM and DCP of control ration was significantly (P ≤ 0.05) decreased compared to (R_1) and (R_2) rations. On the other hand there were insignificant differences between the tested rations in feed conversion of SV and TDN. Also, there were insignificant differences between R1 and R2 regarding feed conversion. The present results agree with findings of Aboul-Fotouh et al. (2017), who pointed out that, rations supplemented with fibrolytic enzymes efficient for feeding than control ration of lactating baldi goats. Moreover Mohamed et al. (2013) reported that, supplementation of fibrolytic enzymes to the early lactating dairy cow rations increased (P≤0.05) feed efficiency compared to control cows. Furthermore Khattab et al. (2019) mentioned that, supplementing ration contains cracked date seed with fibrolytic enzymes increased (P≤0.05) feed efficiency of buffaloes compared to control ration. Such findings support the result of current study.

Table 5: Effect of pectinase enzymes in rations on feed intake and feed conversion of lactating buffaloes.

Items	Rations			± SE
	Control	R_1	R_2	
Average 4% Fat corrected milk yield (kg/head/day).	8.108[b]	9.535[a]	9.719[a]	0.25
Average daily feed intake/head				
DM, kg	12.85	12.78	12.81	0.16
TDN, kg	8.53	8.82	8.90	0.13
SV, Kg	7.74	8.03	8.11	0.15
DCP, kg	1.16	1.19	1.21	0.08
Feed conversion *				
DM/ kg/kg milk	1.58[a]	1.34[b]	1.32[b]	0.12
TDN/ kg/kg milk	1.05	0.92	0.92	0.06
SV/ kg/kg milk	0.95	0.84	0.83	0.09
DCP/ g/g milk	143.1[a]	124.8[b]	124.5[b]	0.14

Average in the same row having different superscripts are differ significantly (P ≤ 0.05) for a and b. Each value is a mean of 4 samples. *, Feed conversion was calculated depend on daily 4% fat corrected milk.

Some blood serum parameters

Effect of pectinase enzymes (the produced enzyme and SMIZYME®) supplemented rations on serum total protein, albumin, urea, creatinine, AST, ALT, glucose and cholesterol of lactating buffaloes are shown in Table 6.

Serum total protein

Impact of pectinase enzymes on concentration of blood serum total protein of lactating buffaloes received the tested rations are shown in Table 6. The values of serum total protein content were 7.13, 8 and 7.8 g/dl, for control, $R_1$and R_2, respectively. Rations supplemented with pectinase enzymes (R_1 and R_2) increased (P≤0.05) serum total protein compared to the control one.

Increasing amount of serum total protein in blood of buffaloes fed rations supplemented with pectinase enzymes compared to control may indicate that these buffaloes cover their protein needs from their tested ration protein which may described by its higher solubility and digestibility compared to control ration protein (Azzaz et al 2016b). Bush (1991) mentioned that, serum total proteins concentration reflects the nutritional status of the animal and it had a positive correlation with protein level of ration. This finding is in line with the result that obtained by Gado et al. (2007), who investigated that, biological treatment of bagasse increased plasma total protein of Baldi goats. Furthermore El-Bordeny et al. (2015) pointed out that, adding fibrolytic enzymes to dairy cows ration showed significant increase (P≤0.05) in serum total proteins. The values of serum total protein were in normal range as recorded by Abd Ellah et al. (2013).

Serum albumin

Impact of pectinase enzymes on concentration of blood serum albumin of lactating buffaloes received the tested rations are shown in Table 6. The overall means of serum albumin content were 3.63, 3.87 and 3.70 g/dl, for control, R_1 and R_2, respectively. Serum albumin concentration showed higher insignificant values by rations supplemented with pectinase enzymes compared to control ration. The increase of serum albumin concentration may be because of higher digestibility of organic matter and crude protein for buffaloes fed rations supplemented with pectinase enzymes compared to control ration. Finally, the values of serum albumin were in normal range as recorded by Abd Ellah et al. (2013).

Table 6: Impact of pectinase enzymes supplemented rations on some blood parameters of lactating buffaloes.

Items	Rations			± SE	Normal range
	Control	R_1	R_2		
Total protein(g/dl)	7.13[b]	8[a]	7.8[a]	0.17	6.4– 8.5
Albumin(g/dl)	3.63	3.87	3.70	0.06	2.7-4.3
Urea(mg/dl)	33.67	31.7	32	0.47	12.84-57.78
Creatinine(mg/dl)	1.2	0.95	1.1	0.09	0.8–1.9
AST(IU/L)	114	117.3	111.7	4.35	78-132
ALT(IU/L)	39	36	34.8	2.03	13.6-57.2
Glucose (mg/dl)	78.33[b]	85.5[a]	88.1[a]	4.00	37.8–94.9
Cholesterol (mg/dl)	84	83.6	87	5.07	65–220

Average in the same row having different superscripts are differ significantly (P≤0.05) for a and b. Each value is a mean of 4 samples.

Serum urea

Impact of pectinase enzymes on concentration of blood serum urea of lactating buffaloes received the tested rations are shown in Table 6. Urea is the main end product of nitrogen metabolism in ruminants. It is synthesized in the liver and extracted in glomerular. The values of serum urea were 33.67, 31.7 and 32 (mg/dl) for control, R1 and R_2, respectively. Serum urea concentration showed higher insignificant values by control ration compared to rations supplemented with pectinase enzymes. The lower serum urea concentration in the present study may be because of high protein utilization by buffaloes supplemented with pectinase enzymes which may associated with the use of urea for protein synthesis on the hepatic pathway because of a compensation of low protein absorption. The values of serum urea were in normal range as reported by Jhambh et al. (2016).

Serum creatinine

Blood serum creatinine of lactating buffaloes received the tested rations are shown in Table 6. The values of blood serum creatinine were 1.2, 0.95 and 1.1 (mg/dl) for control, R_1 and R_2, respectively. These values of blood serum creatinine were in normal range as reported by Abd Ellah et al. (2013). There were insignificant differences in serum creatinine between the tested rations. Moreover Aboul-Fotouh et al. (2017) found that, no effects (P≥0.05) were observed for blood serum creatinine, when feeding lactating goats with fibrolytic enzyme.

Aspartate aminotransferase (AST)

Blood serum AST of lactating buffaloes received the tested rations are exhibited in Table 6. The AST is the most important indicator for liver activity. There were no significant differences between the rations in the overall means of serum AST. The overall means of serum AST were 114, 117.3 and 111.7 (IU/L) for control, R1 and R_2, respectively. The values of serum AST of the current study were within the normal range as reported by Jhambh et al. (2016). Such finding pointed out that, there were no side effect regarding using the tested pectinase enzymes in lactating buffaloes rations. Azzaz et al. (2012) mentioned that, insignificant differences (P≥0.05) between the rations of lactating goats which contain fibrolytic enzyme compared to the control ration in serum AST. Furthermore Aboul-Fotouh et al. (2017) reported that, no effects (P>0.05) were observed for blood serum aspartate aminotransferase (AST) when feeding lactating goats with fibrolytic enzyme supplementation.

Alanine aminotransferase (ALT)

Blood serum Alanine aminotransferase of lactating buffaloes received tested rations are shown in Table 6. There were no significant differences among the tested rations in the overall means of serum ALT concentration. The values of serum ALT were 39, 36 and 34.8 (IU/L) for control, R_1 and R_2, respectively. The values of blood serum ALT were in normal range as reported by Abd Ellah et al. (2013). Moreover Khattab et al. (2019) pointed out that, buffaloes fed ration containing date seed and fibrolytic enzymes had no significant increase in serum ALT concentration. Finally, the mean values of AST and ALT of the current study showed that, experimental animals were in good health.

Serum glucose

Blood serum glucose of lactating buffaloes received the tested rations are presented in Table 6. The values of serum glucose were 78.33, 85.5 and 88.1 (mg/dl) for control, R1 and R2, respectively. The mean values of serum glucose within the normal range as stated by Abd Ellah et al. (2013). Rations supplemented with pectinase enzymes (R_1 and R_2) increased (P≤0.05) blood serum glucose compared to the control ration. These result on the same trend were reported by the Kholif (2006) who mentioned that, goats fed on treated silage with fibrolytic enzymes had higher

(P≤0.05) values of blood serum glucose compared to control group. Furthermore, Morsy et al. (2016) indicated that, the impact of using two commercial enzyme on rations of Egyptian buffaloes had increased (P≤0.05) concentration of blood serum glucose compared to the control group. Moreover, according to Arif et al. (2019), post-feeding, increasing concentration of blood glucose is due to releasing soluble sugars by the action of exogenous fibrolytic enzymes.Also high production of fermentable carbohydrates and increased proportions of propionate, which may be transformed to glucose, cause increasing concentration of blood glucose in animals. Such findings support the obtained results in the current study.

Total cholesterol

Blood serum cholesterol of lactating buffaloes received the tested rations are shown in Table 6. The values of blood serum cholesterol were 84, 83.6 and 87 (mg/dl) for control, R1 and R2, respectively. There were no significant differences in the overall means of serum cholesterol among all the tested rations. These values of serum cholesterol were in normal range as reported by Jhambh et al. (2016). The result of blood serum cholesterol is on the same trend with Kholif (2012), who noted that, ruminants fed on fibrolytic enzymes had insignificant impact in blood serum cholesterol.

Table 7: Economical evaluation of pectinase enzymes supplemented rations in lactating buffaloes.

Item	Rations		
	Control	R_1	R_2
Milk yield (kg/head/70d)	412.3	462.7	474.6
Dry matter consumed (kg / head /70d)	899.5	894.6	896.7
Fibrolytic enzymes (kg/head/70d)	0	2.73	2.73
Price of one kg DM of the ration, L.E	3.76	4	4.51
Cost of feed consumed (L.E / head / 70 d)	3382	3578	4044
Total revenue, L.E*	4948	5552	5695
Net revenue, L.E**	1566	1974	1651
Relative percentage of net revenue	100	126.05	105.43

*, Total revenue, L.E= Milk yield (kg /head /70 day)×12 L.E (price of one kg buffaloes milk). Each value is a mean of 4 samples; **, Net revenue (L.E. / head/70day)= Total revenue (L.E. / head/70day)- Cost of feed consumed (L.E. / head/70day); All price values are in L.E (Egyptian pound) which is equal to 0.063 USD.

Economical evaluation of the tested rations

The simple economical evaluation of the tested rations fed to lactating buffaloes has been determined and represented in Table 7. The economical values expressed as a net revenue (L.E./head/70d) were increased for lactating buffaloes fed rations supplemented with pectinase enzymes (R1 and R2) compared to control group. The best net revenue (L.E./h/70d) was recorded for lactating buffaloes fed ration supplemented with the produced enzyme (R1) followed by lactating buffaloes fed ration supplemented with SMIZYME® (R2) than control ration. The cost of feed consumed for lactating buffaloes fed ration supplemented with SMIZYME® was higher than the other tested rations due to the high price of commercial enzyme. Moreover Aboul-Fotouh et al. (2017) pointed out that, the economical values were increased for lactating goats fed with the produced enzyme compared to commercial enzyme and control ration. Finally, the superiority of R1 concerning net revenue may due to the reducing cost of the produced enzyme compared to SMIZYME® (commercial enzyme).

CONCLUSION

Additions of the produced enzyme and SMIZYME® to the rations of lactating buffaloes lead to marked increasing most of nutrient digestibilities and improving (P≤0.05) milk yield and 4% fat corrected milk yield. From economical point of view, the produced enzyme ration was the best one. Moreover; these studies recommend producing enzyme and adding it to the ration of lactating buffaloes.

AUTHORS CONTRIBUTION

G.M. El-Garhy: Participating in designing of the farm experiments and revision of the final version of the manuscript.
A.M. Abd El-Mola: Participating in conducting of the farm experiments and conducting of the statistical analysis of the data.
H.H. Azzaz: Participating in designing and conducting of the farm experiments , and participating in editing of the paper.
G.A. Mousa: Doing of the chemical analysis for feed, feces, blood and milk samples and writing and editing of the paper.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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