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Effect of Phytase Supplementation on Blood Chemistry and Milk Composition of Lactating Buffaloes

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Abstract

The impact of adding a laboratory-produced phytase to lactating buffaloes diets compared to commercial phytase (Axtra® PHY) on the nutrients digestibility, fecal excretion of calcium (Ca), phosphorus (P), magnesium (Mg) and some blood biochemical parameters, as well as milk yield composition was evaluated. Twelve lactating buffaloes were randomly divided into three groups, four animals each, using a complete random design. The entire experimental period was 105 day. The experimental groups were: G_1 (control): buffaloes fed the experimental ration (ER, 60% concentrate feed mixture : 40% berseem hay) without enzyme, G_2: buffaloes fed ER plus 1200 IU of the commercial enzyme and G_3: buffaloes fed ER plus 1200 IU of the produced enzyme (PE). Phytase supplementation from both sources resulted in significant improvement ($P<0.05$) in the digestion of dry matter (DM), crude fiber (CF), nitrogen free extract (NFE), the total digestible nutrients (TDN) and digestible crude protein (DCP) values compared to the G_1. The fecal excretion of Ca, P and Mg were significantly decrease ($P<0.05$), while blood serum total protein, albumin, globulin, glucose, Ca and inorganic phosphorus (P_i) values were significantly increase ($P<0.05$) when buffaloes fed diets supplemented with exogenous phytase (G_2 and G_3) compared to the G_1. Also, buffaloes fed the phytase supplemented rations (G_2 and G_3) showed a significant increase ($P<0.05$) in milk yields and milk percentages of total protein, fat, lactose, ash, total solids, solids not fat and P, however, there were no significant differences in all parameters ($P>0.05$) between the two sources of phytase, while a diet supplemented with the laboratory-produced phytase showed the highest economic value. It could be concluded that supplementation of phytase (Axtra® PHY and PE) at 1200 IU/kg of ration tended to increase nutrients digestibility, improve milk yields and composition and decrease fecal excretion of Ca, P and Mg in lactating buffalos.

Keywords**:** Buffaloes; Phosphorus; Phytase; Nutrients digestibility; Milk yield and milk composition

1. Introduction

A large portion (60-80) % of total phosphorus (P) present in legumes, grains and oilseeds is found as phytate-bound P. Also known as phytic acid, an organic complex that is commonly considered as the main storage form of P in plants. Furthermore, phytic acid reduces the availability of P and other important minerals such as calcium and magnesium **[1]**.

Ruminants break down phytate through the effect of their ruminal produced phytase. Therefore, the common thought is that ruminants can make use of nearly all of the phytate phosphorus (Pp) that exists in grains. However, ruminal hydrolysis of phytate is not the same among feedstuffs, and it is influenced by several factors such as grain type, processing

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methods, and ruminal outflow rates. Moreover, modern dairy rations contain large amounts of high-phytate grains and by-products feed ingredients (such as wheat bran and cotton seed meal) **[2]**. Increased Pp intake together with high dry matter intake may limit ruminal Pp hydrolysis by reducing the duration of its exposure to microbial phytase. Besides, a saturation of ruminal phytase activity may occur in high-grain (high-phytate) diets. High-grain diets also are associated with reduced secretion of saliva, possibly decreasing salivary P available for microbial use and absorption in the small intestine **[3]**

Repeated application of manure to agricultural land can lead to a build-up of P in the soil over an extended period of time. Excess P is released into the environment contaminating nearby surface waters and causing eutrophication. Eutrophication kills aquatic life and makes water unsuitable for consumption and recreational purposes. Therefore, phosphorus is one of the key polluting nutrients from animal agriculture as it is an important contributor to both water and soil pollution **[4]**.

To help decrease nutrient runoff, nutrient management plans are required. The purpose of nutrient management plans is to balance nutrients that are brought onto the farm with plant nutrient requirements. Finding a way to reduce phosphorus losses resulting from intensive animal-feeding operations into surface water has been an important subject **[5].** Therefore, recent work has focused on managing the herd and feeding programs to help minimize nutrient excretion on the farm and reduce dietary P excretion. One of the many strategies used to reduce nutrients excretion is using feed additives such as enzymes **[6-7]**. Phytase enzyme is the primary enzyme responsible for increasing the digestibility of dietary phytate phosphorus. This experiment was carried out to investigate the impact of adding 1200 IU of phytase enzyme from two different sources (the commercial phytase enzyme; Axtra® PHY and the laboratory produced phytase enzyme; PE) on nutrients digestibility, fecal Ca, P, Mg excretion, some blood biochemical parameters, milk yield and its composition in lactating buffaloes.

2. Experimental

2.1. Experimental animals

Lactating buffaloes (n = 12), averaging 560 Kg ± 0.5 at the start of the trial, were used in the present experiment. Buffaloes were randomly assigned, after 3 months of parturition, to one of the experimental groups (four animals each) using a complete random design. The entire experimental period was 105 day with the first 2 weeks serving as an adaptation period. Milk samples were taken after this adaptation period each week up to the end of experimental period.

2.2. Experimental ration

Ration was formulated to meet the buffalo's nutrient requirements **[8]**, concentrate feed mixture (CFM) was used at 60% of total requirements and berseem hay (BH) was used at 40%. The experimental groups were: experimental ration (ER) without enzyme (G_1), ER plus 1200 IU of the commercial enzyme (Axtra® PHY) per kilogram of diet (G_2) and ER plus 1200 IU of the laboratory produced enzyme (PE) per kilogram of diet (G_3). Enzymes preparation and assay were explained by noha *et al.* **[9].** The commercial enzyme produced by Danisco Animal Nutrition, UK, and distributed by Multi Vita Co. for Animal Nutrition, Second Industrial, 6 October Governorate, Giza, Egypt. This phytase feed enzyme, is extracted from a *Buttiauxella* species bacterium and is expressed in a *Trichoderma reesei* fungus and including 6000 unit/g of phytase. The laboratory produced phytase enzyme contains 2000 unit/g of phytase.

2.3. Feeding management

Buffaloes were fed individually and diets were offered twice daily at 6:00 am. and 6:00 pm (with each milking) in two portions, concentrate feed mixture (CFM) was offered during milking, while berseem hay (BH) was offered within 3 hours after milking. Fresh water was available all the time for all experimental groups. Enzymes were introduced to each animal of the second and third groups twice daily mixed with the CFM. Formulation of CFM and the chemical composition (%, on dry matter basis) of the CFM, BH and ER are given in **Tables (1 and 2)**, respectively.

Table (1): Ingrediets (%) of the concentrate feed mixture (CFM) of the expermintal ration.

Ingredients	Content
Undecorticated cottonseed meal	12.5
Yellow corn	52.2
Soybean meal	12.5
Wheat bran	21.0
Limestone	0.80
Sodium chloride	0.50
Vitamins and minerals mixture*	0.50

* Each 3 kg Vitamins and Minerals mixture contains: Vit. A 12500000 IU, Vit. D3 2500000 IU, Vit. E 10,000 mg, Manganese 80000 mg, Zinc 60,000 mg, Iron 50000 mg, Copper 20000 mg, Iodine 5000mg, Cobalt 1000 mg and carrier (CaCo3) add to 3000g. (Produced by Agri-Vet Company).

Table (2): Chemical composition (%, on dry matter basis) of the concentrate feed mixture (CFM), berseem hay (BH) and the experimental ration (ER).

Item	CFM	BH	ER*
Moisture	11.07	10.24	10.74
Chemical composition % on DM basis:			
OM	96.05	93.21	94.91
CP	16.51	14.18	15.58
EE	4.24	0.74	2.84
CF	14.64	43.41	26.15
NFE	60.66	34.88	50.35
Ash	3.95	6.79	5.09
Ca	0.14	1.40	0.64
P	0.51	0.22	0.39
Mg	0.26	0.19	0.23

*ER: 60% concentrate feed mixture (CFM) + 40% berseem hay (BH).

2.4. Digestion coefficients

Silica was used as an internal marker for determining the digestibility as described by Ferret *et al.* **[10]**. Fecal samples were collected from the rectum of each animal by hand at 12:00 p.m. for three consecutive days (after the morning feeding) twice, the first one was taken after 45 days of the beginning of the experimental period and the second one was taken at the last day of the experimental period. The collected feces were sprayed with 10% sulfuric acid and 10% formaldehyde solutions and dried in a drying oven at 60˚C for 48 hours. Dried samples were pooled together; representative samples were ground, composite by animal, and stored for later chemical analyses. The digestibility coefficient of nutrient was calculated according to the following formula: Digestion coefficient =

$$100 - \left[100 \, x \, \frac{\%\ indicator\ in\ \ feed}{\%\ indicator\ in\ \ feces} \, x \, \frac{\%\ nutrient\ in\ \ feces}{\%\ nutrient\ in\ \ feed} \right]$$

2.5. Feed and feces analysis

Feedstuffs, feeds and feces samples were analyzed according to A.O.A.C. **[11]** methods to determine dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, phosphorus (P), calcium (Ca) and magnesium (Mg) contents. Nitrogen free extract (NFE) was calculated by the difference using the following equation:

$$NFE = 100 - [CP + CF + ash + EE\ \%]$$

*2.6. **Sampling and measurement of blood***

Blood serum samples were taken from the jugular vein, of each buffalo, at 12:00 p.m. (after the morning feeding) at the last day of the experimental period. The samples were collected through a clean dry needle into 10 ml glass tubes and left to coagulate at room temperature. Serum was separated and kept frozen at -20°C for later analysis.

The measured blood serum parameters; total protein, albumin, urea, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using commercial kits according to method of Gornal *et al.* **[12]**, Doumas *et al.* **[13]**, Fawcett and Scott **[14]**, Trinder **[15]** and Reitman and Frankel **[16]**, respectively. All commercial kits were purchased from Biodiagnostic company, Egypt. The globulin values were obtained by subtracting albumin values from total protein values. Albumin/globulin ratio (A/G) was obtained by dividing albumin value on its corresponding globulin value. Calcium and P were determined calorimetrically using commercial kits (Quimica Clinica Aplicada S.A., Spain), as described by Gindler and King **[17]** and El-Merzabani *et al.* **[18]**, respectively.

2.7. Milk sampling and analysis

Buffaloes were milked by hand twice a day (at 6:00 am. and 6:00 pm). Milk samples were taken after the end of the adaptation period weekly up to the end of the experimental period (105 d). Samples of milk were collected immediately from each animal after morning and evening milking and milk yield was recorded. The sample of each animal represented a mixed sample of constant percentage of the evening and morning yield. Milk samples were analyzed for total solids, fat, total protein and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Q3 183 Electric, Hillerød, Denmark). Solids-not-fat (SNF) was calculated. Calcium, phosphorus and magnesium concentration in milk were determined according to A.O.A.C. **[11]** procedures. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines **[19]**:

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

Where: M= milk yield (g) and F= fat yield (g)

2.8. Economical evaluation (feeding cost/kg of milk)

The relation between feed costs and milk yield was calculated for the different experimental groups. The general equation by which the profit (LE) above feeding cost was calculated is:

The profit above feeding cost (LE/head/105 d) = outcome of milk yield – total feeding cost

The ingredients cost according to 2019 prices were 4500 LE/ton of CFM, 1500 LE/ton of BH, 160 LE/kg of the commercial phytase enzyme (Axtra®PHY), and 30 LE/kg of the produced phytase enzyme (PE), the price of one kg of milk was 10 LE.

2.9. Statistical analysis

Collected data were statistically analyzed by SPSS **[20]**. One way ANOVA procedure was used to analyze the data of effect of phytase supplementation to the ration of lactating buffaloes on nutrients digestibility, fecal mineral excretion, blood parameters and milk yield and composition according to the following model:

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

Where:

Y_{ij} = any value from the overall population

μ = the overall mean

T_i = effect of the i^{th} phytase source

e_{ij}= the random error associated with the j^{th} test under the i^{th} treatment

The difference between means was statistically measured for significance at (P<0.05) according to Duncan's multiple range test **[21]**

3. RESULTS AND DISCUSSION

3.1. Digestibility coefficients and nutritive values

The effect of phytase supplementation on nutrients digestibility and nutritive values of the experimental rations are shown in **Table (3)**. The digestion of DM, OM, CP, CF and NFE were improved significantly (P<0.05) in buffaloes fed diets with exogenous phytase (78.53, 80.48, 82.89, 67.02, 87.04% and 79.53, 81.80, 83.80, 67.53, 87.77% for G_2 and G_3, respectively) compared with G_1 (74.20, 77.61, 75.37, 63.47 and 83.69%), with no significant differences (P>0.05) between the two sources of phytase (G_2 and G_3). However, the EE digestibility was not significantly changed (P>0.05) among all of the experimental groups (83.22, 84.19 and 84.84% for G_1, G_2 and G_3, respectively.

Table (3): Effect of phytase supplementation on nutrients digestibility and nutritive values of the lactating buffaloes.

Item	Experimental groups			±SEM
	G_1	G_2	G_3	
***Nutrients digestibility, %*:**				
DM	74.20[b]	78.53[a]	79.53[a]	0.85
OM	77.61[b]	80.48[a]	81.80[a]	0.69
CP	75.37[b]	82.89[a]	83.80[a]	1.36
CF	63.47[b]	67.02[a]	67.53[a]	0.67
EE	83.22	84.19	84.84	0.40
NFE	83.69[b]	87.04[a]	87.77[a]	0.71
Nutritive values, %:				
TDN	75.79[b]	79.64[a]	80.33[a]	0.73
DCP	11.74[b]	12.91[a]	13.06[a]	0.21

[a and b:] Mean values in the same row with different superscripts differ significantly (P<0.05); G_1: 40% berseem hay + 60% CFM (experimental ration, ER) without enzyme; G_2: ER + 1200 IU commercial phytase/kg ration; G_3: ER + 1200 IU produced phytase/kg ration and SEM: standard error of the means.

The nutritive values of the experimental rations expressed as total digestible nutrients (TDN) and digestible crude protein (DCP) take the same trend of the nutrients digestibility. Results showed that phytase supplementation from both sources significantly (P<0.05) improved the TDN and DCP values (79.64, 12.91% and 80.33, 13.06% for G_2 and G_3, respectively) compared with G_1 (75.79 and 11.74%), with no significant differences (P>0.05) between the two sources of phytase.

The current results are in agreement with the findings of Shanklin **[22]** who reported that phytase supplementation (1000 IU/kg ration) to phytic acid diets of lambs improved DM and CP digestibility. Furthermore, Knowlton *et al.* **[23]** concluded that an exogenous phytase and cellulase enzyme formulation (297 g of enzyme formulation/ton of DM fed) reduced the fecal nutrients excretion of lactating cows and tended to increase the fiber and DM digestibility. Ray et al. **[3]** reported that fiber digestibility and apparent digestibility of N tended to decrease linearly with increasing dietary phytate P.

On the other hand, Ahmed *et al.* **[24]** found that neither nutrients digestibility nor nutritive values of mature Rahmani sheep fed 30% berseem hay and 70% CFM were significantly affected by phytase supplementation (500 IU phytase/ Kg ration). However, they observed that increasing phytase level from 500 IU to 1000 IU improved the digestibility values, but the differences were not significant.

The significant improvement (P<0.05) in the nutrients digestibility and the nutritive values of the experimental ration with phytase supplementation in the current study may be due to the improvement in phytate P digestibility leading to an increase in ruminal fluid P which may have enhanced ruminal microorganisms activity. Stimulation of rumen microbial numbers by exogenous enzymes could result in higher microbial biomass, which would provide more activity to digest feedstuffs. The effect of P intake on digestibility was studied by Field *et al.* **[25]**, who reported a decrease of DM digestibility of diets low in P. Durand and Komisarczuk **[26]** summarized digestibility results from various studies in which low-P diets were fed to ruminants. They indicated that the rumen ecosystem appears to be phosphorus dependent for the degradation of the cell wall, thus they suggested lower cell wall digestibility for low-P diets. Ternouth **[27]** suggested that P-deficient diets may affect microbial activity, which in turn would decrease DMI. Also, Wang *et al.* **[28]** reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid.

3.2. Fecal excretion of calcium, phosphorus and magnesium

The effect of phytase supplementation on fecal calcium, phosphorus and magnesium excretion are shown in **Table (4).** The fecal excretion of Ca, P and Mg were significantly decrease (P<0.05) for buffaloes fed diets with exogenous phytase (1.92, 0.30, 0.51% and 1.90, 0.29, 0.50% for G_2 and G_3, respectively). Compared with G_1 (2.40, 0.36 and 0.75%), with no significant differences (P>0.05) between the two sources of phytase (G_2 and G_3).

These results are in line with previous outcomes mentioned by Shanklin **[22]** who showed that phytase supplementation (1000 IU/kg ration) with phytic acid diets resulted in an increase in Ca absorption. Also, the same author noticed an increase in P intake, P absorption and P retention in lambs fed cottonseed meal diets with exogenous phytase compared to lambs fed the phytic acid diets. Moreover, the absorption of Mg was lower for lambs fed the low-P or organic P diet compared to inorganic P. While there was no difference in Mg absorption between inorganic P and phytase supplementation. Dilip **[29]** showed that the addition of phytase (427 IU/kg total mixed ration) to diets of lactating cows increased P digestibility and decreased phytate P excretion.

Table (4): Effect of phytase supplementation on fecal calcium, phosphorus and magnesium excretion.

Item	Experimental groups			± SEM
	G_1	G_2	G_3	
***Fecal mineral excretion, %*:**				
Ca	2.40[a]	1.92[b]	1.90[b]	0.09
P	0.36[a]	0.30[b]	0.29[b]	0.01
Mg	0.75[a]	0.51[b]	0.50[b]	0.04

[a and b:] Mean values in the same row with different superscripts differ significantly (P<0.05); G1: 40% berseem hay + 60% CFM (experimental ration, ER) without enzyme; G2: ER + 1200 IU commercial phytase/kg ration; G3: ER + 1200 IU produced phytase/kg ration and SEM: standard error of the means.

Furthermore, Knowlton *et al.* **[30]** observed that addition of a blend of phytase and cellulase (200 g of fibrolytic enzyme formulation and 280 g of phytase/tone of DM fed) to the diets of lactating cows tended to increase apparent P digestibility and decreased fecal P excretion. Brask-Pedersen *et al.* [**31]** investigated the effect of exogenous phytase (1200, 2400 and 3600 IU/kg ration) on phytate P degradation in the rumen of dairy cows. Results showed that exogenous phytase increased rumen degradability of phytate and the high dose resulted in a higher rumen degradability. Rodríguez *et al.* **[32]** noted that the use of exogenous phytase (0, 150, 300 and 450 g phytase enzyme/ton of the diet) improved lambs' weight gain and decreased the concentration of P in feces. They concluded that dose 300 g phytase/ton had the lowest P excretion in manure.

The current results may be due to the enhancement in phytate P digestibility with phytase supplementation leading to an increase in the absorption and retention of P, Ca, and Mg which decrease their excretion in feces. Where other experiments illustrated that there was a linear relationship between P intake and its fecal excretion **[33-34-35]**.

3.3. Blood serum parameters

The effect of phytase supplementation on blood serum parameters of the experimental lactating

buffaloes are shown in **Table (5)**. The phytase treated buffaloes (G_2 and G_3) showed a significant increase ($P<0.05$) in blood serum total protein, albumin, globulin and glucose concentrations than those of the G_1 group (6.57 and 6.63 vs 6.02 g/dl; 4.03 and 4.07 vs 3.84 g/dl; 2.54 and 2.56 vs 2.18 g/dl; 73.55 and 74.18 vs 66.73 mg/dl for G_2 and G_3 vs G_1, respectively), without any significant differences ($P>0.05$) between the two sources of phytase (G_2 and G_3). Blood serum urea, AST and ALT concentrations were not significantly ($P>0.05$) affected by phytase supplementation among all groups (40.43, 40.16 and 40.25 mg/dl; 50.00, 50.33 and 50.67 U/ml; 30.25, 30.50 and 31.00 U/ml for G_1, G_2 and G_3, respectively). Blood serum Ca and inorganic P (P_i) values were significantly increased ($P<0.05$) with phytase supplementation from both sources of phytase (G_2 and G_3) compared with G_1 (10.14 and 10.19 vs 9.64 mg/dl; 5.64 and 5.74 vs 4.86 mg/dl for G_2 and G_3 vs G_1, respectively). There were no significant differences ($P>0.05$) between the two sources of phytase (G_2 and G_3), except for P_i values. The buffaloes of the G_3 group showed the highest P_i value ($P<0.05$) than those of the G_1 and the G_2 groups as a direct impact of the produced phytase supplementation.

Table (5): Effect of phytase supplementation on blood serum parameters of the lactating buffaloes.

Item	Experimental groups			±SEM
	G_1	G_2	G_3	
Total protein (g/dl)	6.02[b]	6.57[a]	6.63[a]	0.10
Albumin (g/dl)	3.84[b]	4.03[a]	4.07[a]	0.04
Globulin (g/dl)	2.18[b]	2.54[a]	2.56[a]	0.07
Albumin / Globulin ratio	1.76[a]	1.59[b]	1.59[b]	0.03
Urea (mg/dl)	40.43	40.16	40.25	0.08
AST (U/ml)	50.00	50.33	50.67	0.57
ALT (U/ml)	30.25	30.50	31.00	0.30
Glucose (mg/dl)	66.73[b]	73.55[a]	74.18[a]	1.26
Ca (mg/dl)	9.64[b]	10.14[a]	10.19[a]	0.09
P_i (mg/dl)	4.86[c]	5.64[b]	5.74[a]	0.14

[a, b and c]: Mean values in the same row with different superscripts differ significantly ($P<0.05$); G_1: 40% berseem hay + 60% CFM (experimental ration, ER) without enzyme; G_2: ER + 1200 IU commercial phytase/ kg ration; G_3: ER + 1200 IU produced phytase/kg ration and SEM: standard error of the means.

The current results are consistent with those of Shanklin **[22]** who concluded that phytase supplementation (1000 IU/kg of diet DM) with organic P diets (phytic acid or cottonseed meal) increased the serum P_i and Ca concentration, but had no effect on blood urea nitrogen for lambs fed the phytic acid diets or the cottonseed meal diets. Dilip **[29]** showed that phytase supplementation (427 IU/kg of diet DM) increased serum P_i, but had no effect on serum Ca concentration in cows fed both diets containing barley or corn. Moreover, Ahmed *et al.* **[24]** found that plasma Ca and Pi values of mature Rahmani sheep fed 30% berseem hay and 70% CFM were significantly increased ($P<0.05$) with phytase supplementation (500 or 1000 IU phytase/Kg ration) and the highest P_i level was observed with 1000 IU/ Kg ration. However, they observed that blood total protein, albumin, globulin, A/G ratio, urea, glucose, ALT and AST values were not significantly affected. Azzaz *et al.* **[36]** reported that xylanase and phytase supplemented goats had higher ($P<0.05$) serum glucose concentration than those of the control group, while no significant change were detected between all goats groups in creatinine, urea, cholesterol, AST and ALT values.

Because blood P_i is affected by dietary P intake **[37]**, the supplementation of exogenous phytase apparently improved the amount of absorbable P_i in the small intestine of the experimental buffaloes. This could be the explanation for the increased values of blood serum P_i in the current study. Furthermore, the positive effect of exogenous phytase supplementation on blood serum total protein, albumin, globulin, glucose and Ca values could be attributed to the improved nutrients utilization through enhancing digestion of the entire diet, as evidenced by increased nutrients digestibility and nutritive values **(Table 3)**.

Although there have been previous works studying the effect of phytase supplementation on the dietary P utilization in ruminants nutrition, very little of these works have included measurements of biochemical blood components instead of focusing on measuring P and Ca. However, the obtained values of blood serum total protein, albumin, glucose, urea, AST, ALT, Ca and P were within the normal range for buffaloes as stated by Neama **[38]**. This indicates that the tested enzymes did not have any negative effects on liver activity or animal health.

3.4. Milk yield and its composition

The effect of phytase supplementation on buffaloe's milk yield and composition are shown in **Table (6)**. The yields of milk (kg/d), 4% fat corrected milk (FCM, kg/d), and other milk components (total protein, fat, lactose, ash, total solids and solids not fat, g/d) were significantly ($P<0.05$) increased by

Table (6): Effect of phytase supplementation on buffaloe's milk yield and composition.

Item	Experimental groups			±SEM
	G_1	G_2	G_3	
Mikl yields				
Milk yield (kg/d)	6.70[b]	7.49[a]	7.54[a]	0.15
4% FCM (kg/d)	8.94[b]	10.18[a]	10.40[a]	0.24
Total protein (g/d)	228[b]	294[a]	302[a]	11.84
Fat (g/d)	417[b]	479[a]	492[a]	12.39
Lactose (g/d)	324[b]	421[a]	425[a]	17.27
Ash (g/d)	54.27[b]	65.91[a]	67.11[a]	2.09
Total solids (g/d)	1023[b]	1260[a]	1286[a]	42.68
Solids not fat (g/d)	606[b]	781[a]	794[a]	30.53
Milk composition, %				
Total protein (%)	3.40[b]	3.93[a]	4.00[a]	0.09
Fat (%)	6.22[b]	6.40[a]	6.53[a]	0.05
Lactose (%)	4.83[b]	5.62[a]	5.64[a]	0.14
Ash (%)	0.81[b]	0.88[a]	0.89[a]	0.01
Total solids (%)	15.26[b]	16.83[a]	17.06[a]	0.28
Solids not fat (%)	9.04[b]	10.43[a]	10.53[a]	0.24
Ca (%)	0.13	0.15	0.15	0.004
P (%)	0.18[b]	0.24[a]	0.26[a]	0.013
Mg (%)	0.01	0.02	0.02	0.002

[a and b:] Mean values in the same row with different superscripts differ significantly ($P<0.05$); G_1: 40% berseem hay + 60% CFM (experimental ration, ER) without enzyme; G_2: ER + 1200 IU commercial phytase/ kg ration; G_3: ER + 1200 IU produced phytase/ kg ration and SEM: standard error of the means.

phytase supplementation (G2 and G3) compared with G1 (7.49 and 7.54 vs 6.70; 10.18 and 10.40 vs 8.94; 294 and 302 vs 228; 479 and 492 vs 417; 421 and 425 vs 324; 65.91 and 67.11 vs 54.27; 1260 and 1286 vs 1023; 781 and 794 vs 606; for G2 and G3 vs G1, respectively). No significant differences ($P>0.05$) were detected in the milk yields between the two sources of phytase (G2 and G3).

Compared with buffaloes fed the experimental ration without phytase (G_1), buffaloes fed the phytase supplemented ration (G_2 and G_3) showed a significant increase ($P<0.05$) in milk total protein, fat, lactose, ash, total solids, and solids not fat percentages (3.93, 4.00 and 3.40; 6.40, 6.53 and 6.22; 5.62, 5.64 and 4.83; 0.88, 0.89 and 0.81; 16.83, 17.06 and 15.26; 10.43, 10.53 and 9.04% for G_2, G_3 and G_1, respectively). No significant differences ($P>0.05$) between the two sources of phytase (G_2 and G_3) were found.

The percentage of milk P was significantly ($P<0.05$) improved by phytase supplementation (G_2 and G_3) compared to the G_1 group (0.24 and 0.26 vs 0.18; for G_2 and G_3 vs G_1, respectively), with no significant differences ($P>0.05$) between the two sources of phytase (G_2 and G_3). However, the percentages of milk Ca and Mg were not significantly ($P>0.05$) affected by phytase supplementation among all groups (0.13, 0.15 and 0.15; 0.01, 0.02 and 0.02% for G_1, G_2 and G_3, respectively).

The current results are similar to those reported by Kincaid *et al.* **[39]** where they observed that increasing dietary P increased 4 percent fat-corrected milk yield. In another study, milk yield was lower for cows fed 0.24 versus 0.32 or 0.42 percent of dietary P, also milk protein percentage was increased as dietary P increased from 0.24 to 0.32 percent **[40]**. Furthermore, the protein content of milk was higher with 0.45 vs 0.35% of dietary P in the study of Wu and Satter **[41].** However, other studies did not found any effect of dietary P concentration or phytase supplementation on milk yield and its composition **[23 - 30 - 31 - 42]**.

Since the major proteins of milk (caseins) contain phosphorus, the synthesis and secretion of phosphoproteins are important functions of the lactating mammary gland **[43]**. Several studies on casein biosynthesis have shown that the mammary gland utilizes the P_i of the blood for the formation of casein phosphorus **[43-44-45]**. The increased values of milk protein percentage in the current study with phytase supplementation (G_2 and G_3) could be explained by the increased values of blood P_i and total protein **(Table 5)** as milk protein affected by the availability of blood amino acids and the content of blood P_i.

Furthermore, lactose is the major carbohydrate found in milk of most species. Milk yield greatly depends on mammary lactose synthesis due to its osmoregulation of milk, one that induces mammary uptake of water. Therefore, the rate of lactose synthesis in the epithelial cells of the mammary gland serves as a major factor influencing milk volume production **[46]**. The supply of glucose for lactose synthesis increases dramatically in the mammary gland of lactating animals. It has been estimated that lactating mammary in goats utilizes 60–85% of the total glucose produced in the body **[47]**. Mammary tissue of dairy cows extracts about 20% of glucose from blood **[48]**. Therefore, the significant increase in milk yield and milk lactose percentage for buffaloes fed the exogenous phytase supplemented ration (G_2 and G_3) compared with buffaloes of G_1 group in the current study is probably due to increased nutrients digestibility **(Table 3)** and increased blood glucose values **(Table 5).**

3.5. Economical evaluation (feeding cost/kg of milk)

The economical evaluation of phytase supplementation to the ration of the experimental groups is presented in **Table (7)**. The economic values expressed as the profit above feeding cost (LE/Kg of milk/105 day) for both G_2 and G_3 were

Table (7): Economical evaluation of phytase supplementation to the rations of the experimental groups.

Item	G_1	G_2	G_3
Feed intake (as it is), kg/head/day			
Concentrate feed mixture	10.0	10.1	10.1
Berseem hay	13.5	14.0	14.0
Total	23.5	24.0	24.0
Phytase intake, g/head/day	0	4.80	14.40
Feeding cost, LE/head/day [f]			
Concentrate feed mixture	45.0	45.5	45.5
Berseem hay	20.3	21.0	21.0
Phytase enzyme	0	0.77	0.43
Total	66.3	67.3	66.9
Total feeding cost, LE/head/105 d	6962	7067	7025
Milk yield, kg/head/day	6.70	7.49	7.54
Price of milk yield, LE/head/day	67.0	74.9	75.4
Outcome of milk yield, LE/head/105 d	7035	7865	7917
Profit above feeding cost [h], LE/head/105 d	73	798	892

G_1: 60% CFM + 40% berseem hay (experimental ration, ER) without enzyme; G_2: ER + 1200 IU commercial phytase/kg ration; G_3: ER + 1200 IU produced phytase/kg ration; Phytase activity for the commercial and produced enzymes = 6000 and 2000 IU/g, respectively.

[f] Based on prices of year 2019 (Price of one ton of concentrate feed mixture = 4500 LE, Price of one ton of berseem hay = 1500 LE, price of one Kg of commercial and produced enzymes = 160 and 30 LE, respectively. Price of one Kg of milk = 10 LE).

[h] Profit above feeding cost = Outcome of milk yield - total feeding cost.

greatly increased compared with G_1 (798, 892 and 73 LE/Kg of milk/105 day for G_2, G_3 and G_1, respectively). Buffaloes fed the experimental ration supplemented with the produced phytase (G_3) showed the highest economic value followed by G_2. This result is in good agreement with those of Ahmed *et al.* **[24]**, who found that the profit above feeding cost for the growing sheep's ration improved by adding phytase enzyme. Accordingly, the expansion in the production of phytase locally will lead to improve animal productivity and support the Egyptian economy.

4. Conclusion

It could be concluded that supplementation of phytase (Axtra® PHY and PE) at 1200 IU/kg of ration tended to increase nutrients digestibility, improve milk yields and composition and decrease fecal excretion of Ca, P and Mg in lactating buffalos. Buffaloes fed the experimental ration supplemented with the PE showed the highest economic value.

These results should be considered to make the right decision to expand the local production of such an important enzyme (phytase) to achieve the dual benefit of preventing environmental pollution and supporting the Egyptian economy by maintaining the hard currency stock.

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