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Effect of Adding Phytase to Sheep Rations on *in vitro* Disappearance of Dry Matter and Organic Matter & Growth Performance

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Abstract: The current study aimed to improve the utilization of phosphorus from different feedstuffs that contain high levels of phytate phosphorus by adding different levels of phytase enzyme. Two experiments were carried out to evaluate the effects of phytase supplementation on *in vitro* degradation of ingredients (1st experiment), *in vivo* performance of growing Rahmani lambs (2nd experiment). 1st experiment, dry matter and organic matter disappearance (*IVDMD and IVOMD*) were determined for undecorticated cottonseed meal (UCSM), wheat bran (WB), rice bran (RB) and the concentrate feed mixture (CFM) that was composed of (46.5% yellow corn, 20% undecorticated cottonseed meal, 30% wheat bran, 1% sodium chloride, 2% limestone and 0.5% vitamins and minerals mixture). These four ingredients that contained high phytate phosphorus were incubated separately with phytase enzyme (Nutrase P 500®) at 5 levels (0, 500, 1000, 1500 and 2000 IU/ Kg DM) for estimation of *IVDMD* and *IVOMD*. Adding phytase enzyme at levels of 500 and 1000 IU/ Kg DM showed the highest values of *IVDMD* and *IVOMD* for all feed types. The difference between the two levels of phytase were not significant (P>0.05). In 2nd experiment, 30% Berseem hay 70% CFM basal diet with no enzyme (G_1), basal diet plus 500 IU phytase/ Kg ration (G_2) and basal diet plus 1000 IU phytase/ Kg ration (G_3). The results showed that a significant (P<0.05) differences in final body weight gain and feed conversion were observed among the experimental groups. The highest values were recorded by lambs in G_2 compared to other groups. These results indicate that phytase supplementation to growing lamb rations improved their performance and economic efficiency.

Key words: Phytase Supplementation · Sheep · *IVDMD* · *IVOMD* · Growth Performance

INTRODUCTION

Phosphorus is one of the major minerals needed by the body in large quantities and it is a natural element found in rocks, soils and organic materials. A significant portion 50-75% of total phosphorus in mature cereal grains, legumes and oilseeds bind phytic acid and form phytate-bound P [1] reduces P availability but also other important minerals such as Ca, Mg, Zn and Fe. In addition, phytic acid, proteins and digestive enzymes including pepsin, trypsin and amylase making them less soluble [2-4].

Because P is an important mineral with numerous functions in the body including, cell membrane structure (Phospholipids), energy transfer, structure of nucleic acids and as an important constituent of bone, it is provided in livestock diets and therefore, it is presented in manure [5, 6].

The importance of using P within ruminants is the maintenance and replication of ruminal microorganisms, extremely low dietary P can inhibit microbial growth leading to reduce protein and energy supply to the host animal [7-9]. Moreover, phosphorus is an important constituent of bone. It act as an important P reservoir when body requirements temporarily exceed dietary intake [6].

Nutrient management plans are developed to balance land application of nutrients and plant nutrient requirements. More recent work has focused on managing the herd and feeding programs to minimize dietary

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P excretion. If the availability of P in cereal grains and their by-products is increased, then total dietary P would be reduced, which would correspondingly decrease fecal P excretion to decrease the environmental pollution problem [10-13].

Before P from phytate can be absorbed by the small intestines the phytate molecule must be hydrolyzed by the enzyme phytase. Unlike ruminants, non ruminants have a limited ability for phytase production in the digestive tract. Adeola *et al.* [2] showed that dietary supplementation with phytase in pigs resulted in linear increases in weight gain and feed efficiency.

The maintenance requirements of phosphorus in sheep is 36 mg/ kg of live body weight, this requirements increases in some form of production such as growth, reproduction and lactation [14].

Recent research has focused on ways to improve phytate utilization by ruminants and other livestock species therefore; the main objective of this study was to evaluate the effect of adding different levels of phytase enzyme on the *in vitro* dry matter and organic matter disappearance (*IVDMD* and *IVOMD*) of different feedstuffs that contains high levels of phytate-phosphorous. Also to study the response of sheep to rations contained different levels of phytase.

MATERIALS AND METHODS

This work was carried out at the Agricultural Experimental Station, Faculty of Agriculture, Cairo University. Giza, Egypt. Laboratory analyses were carried out at the Laboratories of Animal Production Department, National Research Centre (NRC), Dokki, Giza, Egypt.

Phytate phosphorus content in Table 1. To decide the most effective level of phytase addition to the following feedstuffs: undecorticated cottonseed meal (UCSM), wheat bran (WB), rice bran (RB) and the concentrate feed mixture (CFM) that is composed of: 46.5% yellow corn, 20% undecorticated cottonseed meal, 30% wheat bran, 1% sodium chloride, 2% limestone and 0.5% vitamins and minerals mixture.

Five levels of phytase were added to each feed type (0, 500, 1000, 1500 and 2000 IU phytase /Kg DM, Table 1). Also, composition of the *in vivo* trials and the experimental groups is shown in Table 1.

Phytase enzyme (Nutrase P 500®) contained 500 U/ g activities (Produced by NUTREX NV, Belgium) were used. One unit of phytase is defined as the amount of enzyme that liberates 1µmol of phosphate/min from sodium phosphate under the assay conditions.

Table 1: Phytate phosphorus content of various feeds and calculated CFM, *In vitro* experimental treatments and composition of the *in vivo* trials of the experimental groups.

Feed	1- Phytate phosphorus content of various feeds: Phytate P, % of P content				Citation
Corn	63.8%				Morse *et al.* [1]
	66%				Guyton [18]
Wheat bran	78.2%				Morse *et al.* [1]
	67%				Guyton [18]
Rice bran	80.7%				Morse *et al.* [1]
Cottonseed meal	69.4%				Morse *et al.* [1]
	71%				Guyton [18]
Concentrate feed mixture	66.00%				-
2- *In vitro* experimental treatments					
Feeds type	T_1	T_2	T_3	T_4	T_5
Wheat bran (WB)	WB_1	WB_2	WB_3	WB_4	WB_5
Rice bran (RB)	RB_1	RB_2	RB_3	RB_4	RB_5
Undecorticated cottonseed meal (UCSM)	$UCSM_1$	$UCSM_2$	$UCSM_3$	$UCSM_4$	$UCSM_5$
Concentrate feed mixture (CFM)*	CFM_1	CFM_2	CFM_3	CFM_4	CFM_5

3- Composition of the *in vivo* trials and the experimental groups.		
G_1	G_2	G_3
70% CFM + 30% B H Basal diet (BD)	Basal diet + 500 IU Phytase	Basal diet + 1000 IU Phytase

*CFM: Concentrate feed mixture composed of: 46.5% yellow corn, 20% undecorticated cottonseed meal, 30% wheat bran, 1% sodium chloride, 2% limestone and 0.5% vitamins and minerals mixture.

Evaluation of Phytase Efficiency in *IVDMD* and *IVOMD*: Laboratory trials were carried out to choose the best level of incubation phytase at different levels 0, 500, 1000, 1500 and 2000 IU with tested materials includes UCSM, WB, RB and CFM by evaluate *IVDMD* and *IVOMD* for these tested materials.

The procedure was done according to the applied method of Tilley and Terry [15], modified by Norris *et al*. [16] with the modification of adding the buffer and urea solution as described by Naga and El-Shazly [17]. Rumen liquor was collected from rams kept on Berseem hay (BH) ration approximately 2 hrs after the morning feeding and moved directly to the laboratory in separate warmed oxygen-free plastic jars. Rumen liquor contents were then strained through two layers of cheese-cloth and the obtained liquor was used for the *In vitro* studies.

Feeding Trial: The best two exogenous phytase levels obtained from the results of the *in vitro* study with control were used to formulate three rations and fed to young lambs with uncompleted functional rumen to study the effect of phytase levels on their performance included average daily gain, feed intake and feed conversion.

Animals and Diets: Fifteen male Rahmani lambs, about 4 months old, with average initial live body weight of 20 kg were used in this experiment. Lambs were randomly divided into three experimental groups, 5 lambs each. Experimental animals were weighed every 14 days (2 weeks) in the morning before offering feed and water (Fasting).

The growth trial lasted for 119 days (17 weeks) and the experimental groups were assigned as: basal diet with no enzyme (G_1), basal diet plus 500 IU phytase per kilogram of diet (G_2) and basal diet plus 1000 IU phytase per kilogram of diet (G_3), as illustrated in Table 3. The phytase enzyme was mixed with the CFM.

Management: Lambs were kept in a separate shaded pen and adapted for the tested ration (Basal diet) for 10 days before starting the feeding trial. Ration was offered in two portions, CFM at 8.00 am while, BH was offered at 12.00 pm and fresh water was available all the time for all experimental groups.

Ration was formulated to meet the lamb's nutrient requirements according to the NRC [14], both of consumed rations and refusals (If any) were daily recorded. Ration requirements were adjusted every 14 days (2 weeks) during the experimental period according to the changes of animal's body weights. Data of live body weights and feed intake were recorded and used to calculate the average daily gain and feed conversion.

Chemical Analysis: Composite samples of experimental diets were ground and analyzed for determine the contents of dry matter (DM), organic matter (OM), ash, crude protein (CP), crude fiber (CF), ether extract (EE), phosphorus (P) and calcium (Ca) according to the Official Method of Analysis [19]. Nitrogen free extract (NFE) was calculated by the difference using the following equation: NFE = 100 – [Moisture + CP + CF + Ash + EE %].

Economic Evaluation (Feed Cost of One Kg Gain): The relation between feed costs and gain was calculated for the different experimental animal.

The general equation by which the profit of each feeding trial was calculated. The profit above feeding = price of body weight gain – price of total feeding

The ingredients cost according to 2013 prices were 2550 LE/ ton for CFM, 1500 LE/ ton for BH and 20 LE/ kg for phytase enzyme, the price of one kg of live body weight (LBW) was considered 36 LE.

The equation by which the economic efficiency was calculated is:

Economic Efficiency = profit feeding cost/ price of total feeding.

Statistical Analyses: Data obtained from this study were statistically analyzed by SPSS [20]. One way ANOVA procedure was used to analyze the data of the effect of phytase supplementation with different levels on *IVDMD* and *IVOMD* and performance data 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 level and e_{ij}= the random error associated with the j^{th} test under the i^{th} treatment.

Significant differences among treatment means were separated by Duncan's multiple range test [21] with a 5% level of probability.

RESULTS AND DISCUSSION

Chemical Composition: Chemical composition (on DM basis) of concentrate feed mixture (CFM), Berseem hay (BH) and the calculated chemical composition of the basal diet (BD) used in this study are shown in Table 2.

Table 2: Chemical composition of concentrate feed mixture (CFM), Berseem hay (BH) and the calculated composition of the basal diet (BD).

Item (%)	CFM	BH	BD *
DM	90.72	93.90	91.67
Chemical composition on DM basis			
OM	89.37	88.06	88.98
CP	14.15	11.80	13.45
EE	3.34	2.47	3.08
CF	10.11	34.38	17.39
NFE	61.77	39.41	55.06
Ash	10.63	11.94	11.02
Ca	0.84	0.98	0.88
P	0.50	0.28	0.43

* BD: 70% concentrate feed mixture + 30% Berseem hay.

Table 3: *In vitro* dry matter and organic matter disappearance (*IVDMD* and *IVOMD*).

Feeds type	Experimental treatments T_1	T_2	T_3	T_4	T_5	SEM
1- *In vitro* dry matter disappearance (*IVDM*D)						
WB	51.08^{c}	62.96^{a}	63.82^{a}	61.50^{ab}	58.15^{b}	1.07
RB	34.02^{d}	41.02^{a}	41.76^{a}	39.85^{b}	35.18^{c}	0.61
UCSM	36.38^{c}	47.03^{a}	47.64^{a}	46.75^{ab}	44.57^{b}	0.84
CFM	58.22^{d}	65.87^{ab}	67.34^{a}	64.80^{bc}	64.00^{c}	0.62
2- *In vitro* organic matter disappearance (*IVOM*D)						
WB	61.90^{c}	66.50^{b}	70.49^{a}	69.75^{a}	69.58^{a}	1.01
RB	54.91^{b}	59.58^{a}	59.82^{a}	55.01^{b}	55.00^{b}	0.80
UCSM	48.78^{b}	57.84^{a}	56.88^{a}	56.13^{a}	50.32^{b}	1.08
CFM	70.65^{c}	77.48^{a}	78.25^{a}	72.30^{bc}	72.01^{bc}	1.08

a, b, c and d Means values in the same row with different super scripts differ significantly (P<0.05).

SEM: standard error of the means. WB: Wheat bran; RB: Rice bran; UCSM: undecorticated cottonseed meal; CFM: concentrate feed mixture. T_1: control (Without adding phytase). T_2: control + 500 IU of phytase; T_3: control + 1000 IU of phytase. T_4: control + 1500 IU of phytase and T_5: control + 2000 IU of phytase/ kg DM.

Chemical composition of CFM and BH showed a comparable DM composition. Results showed that CFM contained higher level of phosphorus and lower content of calcium compared to the BH. The calculated composition of the basal diet showed that it contained about 13.5% CP, 17.4% CF, 11% ash and 0.43% P. While, calculated phytate content of CFM was 66.00%.

***In Vitro* DM and OM Disappearance:** The effect of phytase levels on *in vitro* dry matter and organic matter disappearance (*IVDMD* and *IVOMD*) is presented in Table 3.

Values of dry matter significantly (P<0.05) increased with phytase supplementation until it reached its highest levels at T_3 (1000 IU of phytase).

Increasing the phytase level from 500 IU (T_2) to 1000 IU (T_3) increased the *IVDMD* of all feeds, but the differences were not significant (P>0.05). However, increasing the phytase level from 1000 IU (T_3) to 1500 IU (T_4) significantly (P<0.05) decreased the *IVDMD* of all feeds except for WB and UCSM. Moreover, increasing the phytase level from 1500 IU (T_4) to 2000 IU (T_5) decreased significantly (P<0.05) the *IVDMD* of RB.

Similar trend was also, observed in *IVOMD* data. Adding phytase increased significantly (P<0.05) the *IVOMD* of all feeds compared to control T_1 except (T_4 and T_5) of RB and CFM and (T_5) of UCSM, the differences were not significant. So the optimal enzyme concentration to 1000 IU was suitable and for 1500 IU or 2000 IU was less efficient.

Furthermore, increasing the phytase level from 1000 IU (T_3) to 1500 IU (T_4) significantly (P<0.05) decreased the *IVOMD* of RB and CFM. Moreover, increasing the phytase level from 1500 IU (T_4) to 2000 IU (T_5) significantly (P<0.05) decreased the *IVOMD* of UCSM.

Adding phytase enzyme at the 1000 IU/ Kg DM level (T_3) showed the highest *IVDMD* and *IVOMD* values for all feeds except for UCSM. However, the differences between the two levels of phytase (T_2 and T_3) were not significant (P>0.05) for the *IVDMD* and *IVOMD* values of all feeds.

Positive effects of phytase supplementation on DM digestibility have been reported in different studies. Bravo *et al.* [22] noticed that fungal phytase slightly increased DM digestibility when added to the formaldehyde-treated soybean diet in sheep. Furthermore, Colombatto *et al.* [23] suggested that enzymes were able to degrade complex substrate to simpler one, allowing a faster ruminal colonization and fermentation. Also, Yang *et al.* [24] attributed this effect to an increase in microbial colonization of feed particles and speculated that exogenous enzymes may act similarly to primary bacterial colonization.

When determined on isolated bacteria, P requirements are high for cellulolytic (20 mg/ l, Bryant *et al.* [25] and amylolytic bacteria (Up to 16 mg/ l, Caldwell *et al*. [26]. When determined on a mixed population, *in vitro*, the rumen ecosystem appears to be phosphorus dependent for the degradation of cell walls [7]. This is primarily due to the specific phosphorus requirements of cellulose activity [27]. Maintaining a constant rumen inorganic phosphorus concentration appears essential to maintain appropriate diet degradation.

Moreover, *In vitro*, phytase activity of amylolytic rumen bacteria strains is greater than that of the other strains [28]. They explain why the microbes that use starch are able to solubilize inorganic phosphorus from phytate for their own phosphorus requirements, maintaining the homeostasis of ruminal phosphorus, which is beneficial for the host animal as well.

The low response to the low enzyme level is understandable, but that for the higher level is less evident. It may be attributed to the negative feedback inhibition which is one of the classical modes of regulation of enzyme action. This feedback mechanism occurs when enzyme action is inhibited by production of a critical concentration of a product of the enzyme-substrate interaction [29].

Growth Performance: Data in Table 4 represents the effect of phytase supplementation on body gain, dry matter intake and feed conversion of the experimental groups.

A significant ($P<0.05$) differences in final body weight, total body gain, average daily gain and feed conversion ratio were observed among the experimental groups. The highest values were for lambs receiving 500 IU phytase/ Kg DM (G_2), while control group (G_1) recorded the lowest value as shown in Table 4. Increasing phytase level from 500 IU (G_2) to 1000 IU (G_3) decreased the final body weight, total weight gain, ADG and the feed conversion ratio but the differences were not significant ($P>0.05$). The results obtained here are in agreement with those obtained by Gartner *et al.* [30], who stated that live body weight gain and feed efficiency were improved by adding P to beef heifers ration. Also, Shanklin [31] reported increased digestibility in lambs fed phytic acid diets supplemented with phytase. This indicates that there was an increase in ruminal fluid P for these lambs which may have enhanced ruminal microorganism activity. However, the current results are in contrast with the finding of Dilip [32] who reported that addition of exogenous phytase had no significant effect on body gain of lactating cows fed diets containing barley and corn. Furthermore, Shanklin [31] showed that steers fed 0.19 % P diet tended to have a better feed conversion than those

Table 4: Daily gain, dry matter intake and feed conversion ratio of the experimental groups.

Item	G_1	G_2	G_3	SEM
Initial body weight (IBW), Kg	20.20	20.20	20.40	0.21
Final body weight (FBW), Kg	32.83^b	35.56^a	35.25^a	0.38
Total weight gain, Kg	12.63^b	15.36^a	14.85^a	1.16
Average daily gain, Kg	0.106^b	0.129^a	0.125^a	0.01
Dry matter intake (DMI), Kg/ head/ day				
Concentrate	0.630	0.661	0.657	0.10
Berseem hay	0.280	0.293	0.291	0.04
Total	0.910	0.954	0.948	0.14
Intake, Kg/ 100 Kg BW/ day	3.431	3.425	3.407	0.51
Feed conversion ratio (Kg DMI/ Kg gain)	8.58^b	7.40^a	7.58^a	0.14

a, b and c Means in the same row between groups with different super scripts differ significantly ($P<0.05$).

SEM: standard error of means.

G_1: 30% Berseem hay + 70% CFM (Basal diet) without enzyme.

G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration.

Table 5: Economic evaluation of phytase supplementation to the experimental groups.

Item	G_1	G_2	G_3
Feed intake of group (as it is), Kg/ day			
Concentrate	3.473	3.645	3.621
Berseem hay	1.488	1.562	1.552
Total	4.961	5.207	5.173
Phytase intake of group, Kg/ day	0	0.005	0.010
Feeding cost of group, LE/ day [f]			
Concentrate	8.86	9.29	9.23
Berseem hay	2.23	2.34	2.33
Phytase enzyme	0	0.1	0.2
Total feeding cost, LE/ day	11.09	11.73	11.76
Daily gain of group, kg	0.530	0.645	0.625
Price of gain, LE	19.08	23.22	22.50
Profit above feeding cost, LE	8.00	11.50	10.70
Economic efficiency [h]	0.72	0.98	0.91
Relative economic efficiency, % [g]	100	136	126

G_1: 30% Berseem hay + 70% CFM (basal diet) without enzyme. G_2: basal diet + 500 IU phytase/ kg ration.

G_3: basal diet + 1000 IU phytase/ kg ration. Phytase activity = 500 IU/ g.

[f] Based on prices of year 2013 (Price of one ton of concentrate feed mixture = LE 2550, Price of one ton of Berseem hay = LE 1500, Price of one Kg of Phytase enzyme = LE 20 and Price of one Kg of live body weight = LE 36). [h] Economic efficiency = profit above feeding cost/ total feeding cost.

[g] Assuming that the relative economic efficiency of control group (G_1) equals 100.

receiving 0.12 % P diet, but the difference was not significant. An explanation for this improvement in body gain and ADG with phytase supplementation might be due to the increase of propionate in the rumen. Bravo *et al.* [33] reported that phytase supplementation to the concentrate diets of lactating goats had no effect on acetate but it increased propionate and decreased butyrate.

Data of total dry mater intake (DMI) presented in Table 4 indicated that lambs fed the phytase supplemented diets in G_2 (500 IU) and G_3 (1000 IU) tended to have better dry matter intake than those receiving the basal diet (G_1). However, the difference was not significant (P>0.05). The present results are consistent with previous findings of Guyton [18], who reported that phytic acid supplementation did not affect the DMI of dairy cows. Also, Dilip [32] found that addition of exogenous phytase had no significant effect on dry matter intake of lactating cows fed diets containing barley and corn.

However, Ternouth [34] noted that P-deficient diets may have a negative effect on microbial activity, which in turn would decrease DMI. Also, a decrease in DMI was observed when lactating dairy cows were fed diet deficient in phosphorus over two lactation and two dry periods [35]. Moreover, Knowlton *et al.* [12] noticed that DMI tended to decrease when wheat bran was fed as the P supplement in lactating dairy cow diets compared with a diet that was supplemented with mono- or di-calcium phosphate.

Economic Evaluation: The economic evaluation and feasibility of adding phytase enzyme to the growing lamb rations were calculated for two groups supplemented with phytase versus the control group Table 5.

Although the control group (G_1) showed the lowest feeding cost, both G_2 (500 IU phytase/ kg ration) and G_3 (1000 IU phytase/ kg ration) had higher gain over cost and consequently higher profit per Kg gain over feeding cost than G_1. Moreover, the relative economic efficiency was 136% for G_2 and 126% for G_3 versus the control group (G_1) 100%. While the 1000 IU phytase group (G_3) showed a good response to enzyme supplementation in comparison with G_1, it was noticed that the 500 IU phytase group (G_2) was more economically feasible than the other groups. This result should be considered to make the right decision for the application of phytase supplementation to growing sheep rations, with no adverse effect on growth performance.

CONCLUSION

It could be concluded that adding phytase to growing lamb rations improved both growth performance and relative economic efficiency. The recommended dose of phytase enzyme was 500 IU/ Kg ration.

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