Bulgarian Journal of Agricultural Science, 24 (No 2) 2018, 310–316
Agricultural Academy

THE EFFECTS OF PHYTASE IN NITROGEN AND PHOSPHORUS EXCRETION OF LAYING HENS FED REDUCED PROTEIN LEVELS

SKENDER MUJI; MUHAMET KAMBERI*; ALLTANE KRYEZIU**; RAGIP KASTRATI; NURIDIN MESTANI
University of Prishtina,"Hasan Prishtina", Faculty of Agriculture and Veterinary, 10000 Prishtinë, Republic of Kosovo

Abstract

Muji, S., M. Kamberi, A. Kryeziu, R. Kastrati and N. Mestani, 2018. The effects of phytase in nitrogen and phosphorus excretion of laying hens fed reduced protein levels. *Bulg. J. Agric. Sci.*, 24 (2): 310–316

Poultry are fed higher levels of dietary protein than most other farm animals. Due to the limited potential to use plant phosphorus, inorganic sources of this mineral are usually used. Though nitrogen (N) and phosphorus (P) are essential for all animals' proper performance, they are also main pollutants responsible for environmental eutrophication. The reduction of protein level and addition of (NATUPHOS BASF®) phytase in diets of laying hens was studied as a possible way to reduce N and P excretion. Corn-soybean meal laying-hen diet with two crude protein (CP) (14% and 17%) and two phytase levels (0 and 600 FTU kg^{-1}) was fed during eight weeks. Supplemental phytase decreased the total content of excreta N (1.07±0.05, 0.96±0.03, 1.24±0.02, and 1.07±0.06, for hens fed 14% CP- phytase, 14% CP + phytase, 17% CP – phytase, and 17% CP + phytase, respectively). Total excreta P was also affected (1.68±0.02, 1.54±0.04, 1.61±0.03, 1.58±0.03). The experiment's results indicate that the addition of 600 FTU to corn soybean meal-based layer diets containing 14% CP may significantly decrease the levels of total excreta N and P without any adverse effects on laying hens' performance.

Key words: laying hens; phytase; nitrogen; phosphorus; pollution

Introduction

Laying hens' high egg output requires a proper nutrient supply. Nitrogen (proteins) and phosphorus are of main macronutrients to support laying hens' overall performance. Poultry are fed rations containing mainly plant feedstuffs (cereals and oilseed meals) with most of the phosphorus present in the form of phytates. Because poultry lack proper degrading enzymes, phytates are considered very strong anti-nutritive factors due to their chelating abilities, not just for phosphorus but also for other nutrients (Pereira et al., 2017). That is why the use of feed enzymes in poultry diets to enhance nutrient utilisation and performance has become commonplace (Selle and Ravindran, 2007). Rations with lower protein content may not only contribute to lower feed prices but also the excretion of nitrogen, phosphorus and other potential environment pollutants (Nahm and Carlson, 1998) may be lower. The vast majority of nitrogen, expelled via the excreta, originates from feed but may also come from endogenous sources of microorganisms and the digestive tract. Non-digestible feed nitrogen and endogenous nitrogen, is excreted mainly as true proteins. Nitrogen in the urine is usually excreted as urea in cattle, sheep and pigs, and as uric acid in poultry. After excretion, faeces and urine are usually blended and the degradation of nitrogen-containing compounds is due to the presence of a large number of microorganisms in the faeces. Ammonia is the main product of decomposition and can be converted into nitrates and nitrites during the phase of deposition or after its use as fertilizer. It is potential source of pollution and is directly related to the nitrogen content in the faeces (Summers 1993; Meluzzi et al., 2001). Improving the efficiency of phytic phosphorus

Corresponding author: muhamet.kamberi@uni-pr.edu
**These author contributed equally to this work

reduces the need to add phosphate to the diet but can also lower phosphorus content in feed without damage to health and poultry production indicators (Van der Klis et al., 1997; Keshavarz, 1998). Two of the main sources of pollution of agricultural origin are nitrogen and phosphorus in excreta. The presence of these nutrients in manure and slurry contribute to their build up in the soil and via leaching, they pollute water sources (Williams, 1996) creating serious problems in many areas of intensive animal production. Approximately 32% of feed nitrogen is lost to the atmosphere as ammonia N, while another 43% remains in the manure (Patterson and Lorenz, 1997). Phosphorus is a limiting nutrient in algal growth and undigested dietary P, excreted through poultry manure, can stimulate algal growth that depletes dissolved oxygen in surface water (Scott et al., 1999). Faecal phosphorus consists of undigested portions of phytate-bound and non-phytate P from plant sources, undigested portions of P from animal by products and mineral supplements and surplus amounts of bio available P in excess of animal needs. That is why a successful solution to the overall problem of P excretion must consider each of these factors as an area for improvement.

The use of microbial phytase in poultry diets can be used as a tool to reduce the excretion of nitrogen. In a series of published papers, microbial phytase demonstrated the ability to hydrolyse phytic acid and thus increase the availability of related nutrients therein. In digestibility trials of P with laying hens, Waldroup (1999) conclude that 300 FTU kg are equivalent to 1.0 gram P of monocalcium phosphate that can be comparable with 0.1 gram of P per day, per hen.

The positive effects of the use of phytase on the performance of poultry can be seen not only in a reduced need for inorganic phosphorus but also in reducing the need for other nutrients (minerals, proteins and amino acids) due to an increase in their metabolic availability and digestibility (Yi et al., 1996; Sebastian et al., 1996; Keshavarz, 2000; Ravindran et al., 2000; Lei, Stahl, 2000). This proves that phytase can have a positive effect on the release of proteins captured to protein-phytate complex and improve the digestibility of amino acids. (Hsu et al., 1998; Ravindran et al.; 1999; 2000 and Nahm, 2002) reported that the level of nitrogen excretion has a decreasing tendency in lower protein diets, indicating a better utilisation in comparison with diets with a higher amount of protein. In future, economic reasons will also lead to the need to reduce nitrogen excretion. Under these conditions, it will be necessary to reduce the level of total protein in the diet (Huyghebaert 1996; Leeson et al., 1998). As reported by Pereira et al. (2017), inclusion of the phytase enzyme (500 FTU/kg) ensured comparable egg-quality results of hens fed dietary crude protein levels of 17 and 15%. These authors do not recommend diet with 13% crude protein with or without phytase inclusion for commercial brown-egg layers. Meluzzi et al. (2001), in experiments with Hi Line Brown hens, fed a diet containing 17%, 15% and 13% crude protein and an adequate supply of amino acids, reported 50% reduced nitrogen excretion via the faeces when the diet contained low protein. Nevertheless, they recognised that the poultry's desired performance could be maintained for only eight weeks, thanks to the pullets' good condition during the breeding period. Keshavarz and Jackson (1992) have tried to find an answer concerning the effect of various levels of crude protein (CP) in feeds for pullets up to 18 weeks (16%, 13.5% and 11.5% CP) and for laying hens (14%, 13% and 12% CP) through the addition of certain essential amino acids. They concluded that it is possible to maintain a satisfactory performance in growing chickens, despite low levels of protein, if a required amount of amino acids is offered: this could not be achieved with hens fed a standard diet. However, in experiments with White Leghorn hens, Jacob et al. (2000) stated that it is possible to reduce the level of total protein from 17% to 13.5% without adverse effects on the egg production and feed conversion with a substantial reduction of nitrogen excretion via excrement. In an experiment with broilers, the same authors, (Jacob et al., 2000b), noted that the addition of a phytase, or phytase in combination with pentosanase, in a diet positively influences the daily nitrogen excretion. When phytase was added in the diet with 22.5% protein, with the same content of non-phytate phosphorus (0.45% and 0.60%), reduction of nitrogen excretion of 9% and 22% was observed. Sebastian et al. (1996) noted that the optimum retention of nitrogen was partly a result of the decomposition of the protein-phytate complex after addition of the phytase. This study's objective was to evaluate the influence of phytase on decreasing the content and total excretion of nitrogen and phosphorus from the excreta of laying hens fed rations with different levels of crude protein content

Materials and Methods

Animals and diets

In this experiment, 144 Hisex Brown laying hens, aged 33 weeks at the start of the experiment, were used. The preparatory period has lasted two weeks and it is used to form uniform groups ($p > 0.05$) in body weight and egg production. During the pre-experimental period, the hens were fed a standard diet for this category. Experimental treatments consisted of 36 hens (three replicates with 12 hens). In the experimental period, four diets (treatments) with two levels of crude protein (14 and 17%) and two levels of Natuphos 5000 phytase (0 and 600 FTU kg) were used. Diets (Table

1) were formulated to contain all necessary nutrients (NRC, 1994). Diets based on corn and soybean meal, known as raw materials with low content of available phosphorus, were formulated using UFFDA software from the University of Georgia, USA (Pesti and Miller, 1993).

Excreta collection and analysis

During the entire experiment, all excreta produced each day was collected and mixed thoroughly. Approximately 10% was dried at 70°C for 12 hours, using a forced-air dryer, followed by other 12 hours at room temperature. To avoid N losses during drying, two grams of fresh excreta were taken every 13th and 14th day of the experimental phase to determine the content of total N using the Kjeldahl method (AOAC, 2000). The determination of total phosphorus was conducted spectrophotometrically, using the ammonium vanadate-molybdate method (ISO 6491, 1998). The amount of excreted N and P was calculated from the total amount of excreta produced and put in relation with the total egg mass expressed as grams of mineral (in dry matter basis) per kilogram of eggs produced. The egg mass was calculated from the total number of eggs laid and their weights.

Statistical methods

The data was statistically analysed by the (SAS Institute's GLM procedure, 1985). When a significant probability value ($P < 0.05$) was detected, means were subjected to Duncan's New Multiple-Range Test.

Results

Nitrogen content and excretion

The level of protein in the mixture has significantly affected average N content in the hens' raw excreta. The faeces from hens fed with lower crude protein content and phytase contained 0.96% total phosphorus, which is 22.58% less compared with treatment fed normal protein content where 1.24% phosphorus was found (Table 2). Feeding hens with mixtures different in available phosphorus and phytase content with the same protein content, despite some reduction, did not result in statistically significant differences in the total excretion of nitrogen expressed in grams per kilogram of egg mass produced (Kamberi et al., 2017). However, when lower amounts of protein were given and phytase was used (Treatment 2), a significant de-

Table 1
Composition and calculative nutritional value of experimental diet

Raw materials and nutrient composition	Mixture A %		Mixture B %	
	TREATMENTS			
	1	2	3	4
Ground corn	70.64	70.64	59.25	59.25
Soybean meal, 44% SP	18.11	18.11	27.08	27.08
Sunflower oil	0.49	0.49	3.00	3.00
Limestone	7.35	7.35	7.37	7.37
Dicalcium phosphate	2.06	2.06	1.91	1.91
Kitchen salt	0.3	0.3	0.3	0.3
Mineral-Vitamin premiks	1.00	1.00	1.00	1.00
DL-Methionine	0.05	0.05	0.09	0.08
Natuphos^R Phytase	–	+	–	+
Total	100.00	100.00	100.00	100.00
Nutritional value				
ME, MJ kg^{-1}	12.13		12.25	
Crude protein, %	14.00		17.00	
Ca, %	3.30		3.30	
Total P, %	0.70		0.70	
Available P, %	0.428		0.47	
Lysine, %	0.671		0.88	
Met + Cys, %	0.531		0.64	
Na %	0.145		0.142	
Mg, %	0.11		0.12	
Zn, mg	85.46		85.13	
Cu, mg	13.19		15.36	

crease of nitrogen in hens' raw excreta, at all stages of the experiment, was observed.

Adding phytase demonstrated statistically significant effects in the third and fourth phase, since the nitrogen content in the treatment 2 was significantly lower than in treatment 1. The use of phytase has also affected the reduction of nitrogen in the faeces of hens fed with 17% crude protein. Thus, in the third and fourth phase, the excreta of hens fed with a diet without phytase contained 1.08% and 1.50% of nitrogen, compared with treatment 4 hens, where 0.88% and 1.28% nitrogen is found. The average values of nitrogen content in fresh faeces were 1.7%, 0.96%, 1.27% and 1.07%, respectively, for treatments 1-4.

As presented in the second part of the Table 2, where the amount of nitrogen expressed in grams per kilogram of

Table 2

The effect of CP and phytase levels on Nitrogen content (%) and excretion (Mean ± SEM)

Treatment	CP and Phytase level	Nitrogen content in dry faeces, (%)			
		Phase 1	Phase 2	Phase 3	Phase 4
1	14%CP/0FTU	0.99±0.07	1.17±0.09	0.93±0.07[ab]	1.20±0.10[b]
2	14%CP/600FTU	0.93±0.09	1.03±0.04	0.79±0.06[b]	1.09±0.03[b]
3	17%CP/0FTU	1.18±0.02	1.18±0.05	1.08±0.08[a]	1.50±0.08[a]
4	17%CP/600FTU	0.95±0.14	1.19±0.07	0.88±0.01[ab]	1.28±0.09[ab]
Pr > F		0.2378	0.3436	0.0475	0.0354
Main effect					
Crude Protein CP (%)					
14		0.96±0.05	1.10±0.05	0.86±0.05	1.14±0.05[b]
17		1.07±0.08	1.18±0.04	0.98±0.06	1.39±0.07[a]
Added phytase (FTU kg^{-1})					
0		1.09±0.05	1.18±0.05	1.01±0.06[a]	1.35±0.09[a]
600		0.94±0.07	1.11±0.05	0.83±0.03[b]	1.19±0.06[b]
Analysis of variance					
		Pr > F	Pr > F	Pr > F	Pr > F
CP	1	0.2590	0.2408	0.0861	0.0151
Phytase	1	0.1412	0.3428	0.0201	0.0758
CP * Phytase	1	0.3353	*0.2985*	*0.6148*	*0.4995*
Treatment	CP and Phytase level	Excretion of N (g DM) per kg of egg mass			
		Phase 1	Phase 2	Phase 3	Phase 4
1	14%CP/0FTU	9.45±0.12	10.68±0.34[a]	9.97±0.39[a]	10.28±0.96
2	14%CP/600FTU	8.13±0.75	6.11±1.36[b]	8.28±0.34[b]	8.70±0.32
3	17%CP/0FTU	8.68±0.93	8.37±0.82[ab]	8.10±0.31[b]	8.89±0.62
4	17%CP/600FTU	9.23±0.78	8.78±0.29[ab]	7.95±0.37[b]	7.83±0.27
Pr > F		0.5811	0.0279	0.0126	0.1099
Main effect					
Crude protein, (%)					
14		8.79±0.45	8.40±1.20	9.13±0.44	9.49±0.57
17		8.96±0.55	8.57±0.40	8.03±0.22	8.36±0.38
Added phytase (FTU kg^{-1})					
0		9.07±0.45	9.53±0.65[a]	9.04±0.47[a]	9.58±0.60
600		8.68±0.54	7.45±0.86[b]	8.12±0.24[b]	8.26±0.27
Analysis of variance					
		Pr > F	*Pr > F*	*Pr > F*	*Pr > F*
CP	1	*0.8202*	*0.8354*	*0.0147*	*0.1003*
Phytase	1	*0.6007*	*0.0354*	*0.0321*	*0.0618*
CP * Phytase	1	*0.2249*	*0.0165*	*0.0630*	*0.6765*

SEM – standard error of the mean, CP-Crude protein,
Average values within the same column with different superscript differ significantly, df – degrees of freedom

egg mass produced is shown, there is significant reduction in excreted nitrogen. The effect of phytase in reduction of the N was observed after 4 weeks of feeding experimental diets. Hens fed 17% CP excreted 27% more N compared with hens fed 14% CP with the addition of phytase. The difference in the reduction is much more expressed when comparing low CP diets without and with phytase. Hens of treatment 1 excreted 42.79% more nitrogen than hens of treatment 2 when measured as the amount of nitrogen per mass of eggs produced.

Phosphorus content and excretion

Since the most known effect of phytase is related to phosphorus release from phytate complex, the content and the excretion of P from hens fed different CP but same dietary P was also investigated (Table 3). Results show that

Table 3
The effect of CP and phytase levels on phosphorus content (%) and excretion (Mean ± SEM)

Treatment	CP and Phytase level	Phosphorus content in dry faeces (%)			
		Phase 1	Phase 2	Phase 3	Phase 4
1	14%CP/0FTU	1.62±0.01	1.61±0.08	1.68±0.03	1.80±0.05[a]
2	14%CP/600FTU	1.58±0.05	1.21±0.16	1.59±0.02	1.80±0.04[a]
3	17%CP/0FTU	1.46±0.19	1.56±0.07	1.62±0.01	1.80±0.04[a]
4	17%CP/600FTU	1.51±0.03	1.60±0.03	1.62±0.07	1.60±0.05[b]
Pr > F		0.484	0.0567	0.4515	0.0297
Main effect					
Crude Protein CP (%)					
14		1.59±0.02	1.41±0.12	1.64±0.03	1.80±0.03
17		1.49±0.06	1.58±0.03	1.62±0.03	1.70±0.05
Added phytase, (FTU kg^{-1})					
0		1.54±0.07	1.59±0.05	1.65±0.02	1.80±0.03
600		1.54±0.03	1.40±0.11	1.61±0.03	1.70±0.05
Analysis of variance					
		Pr > F	Pr > F	Pr > F	Pr > F
CP	1	0.1886	0.1150	0.6766	0.0526
Phytase	1	0.9642	0.0982	0.2932	0.0526
CP * Phytase	1	0.4533	0.0577	0.2602	0.0591
Treatment	CP and Phytase level	Excretion of P (g DM) per kg of egg mass			
		Phase 1	Phase 2	Phase 3	Phase 4
1	14%CP/0FTU	9.45±0.12	10.68±0.34[a]	9.97±0.39[a]	10.28±0.96
2	14%CP/600FTU	8.13±0.75	6.11±1.36[b]	8.28±0.34[b]	8.70±0.32
3	17%CP/0FTU	8.68±0.93	8.37±0.82[ab]	8.10±0.31[b]	8.89±0.62
4	17%CP/600FTU	9.23±0.78	8.78±0.29[ab]	7.95±0.37[b]	7.83±0.27
Pr > F		0.5811	0.0279	0.0126	0.1099
Main effect					
Crude protein, (%)					
14		8.79±0.45	8.40±1.20	9.13±0.44	9.49±0.57
17		8.96±0.55	8.57±0.40	8.03±0.22	8.36±0.38
Added phytase, (FTU kg^{-1})					
0		9.07±0.45	9.53±0.65[a]	9.04±0.47[a]	9.58±0.60
600		8.68±0.54	7.45±0.86[b]	8.12±0.24[b]	8.26±0.27
Analysis of variance					
		Pr > F	Pr > F	Pr > F	Pr > F
CP	1	0.8202	0.8354	0.0147	0.1003
Phytase	1	0.6007	0.0354	0.0321	0.0618
CP *Phytase	1	0.2249	0.0165	0.0630	0.6765

SEM – standard error of the mean, CP – Crude protein,
Average values within the same column with different superscript differ significantly, df – degrees of freedom

laying hens of the first treatment, except low production performance, contained more total phosphorus in their excreta compared with all other treatments during all phases of the experiment. At the end of the experiment, average P concentration of excreta of hens fed low protein and no phytase was significantly higher than of all other treatments (14% CP and phytase or 17% CP without and with phytase). However excreta of hens fed 14% CP and phytase contained less P. The amount of P excreted via excreta of hens fed low CP and no phytase was also significantly higher than of all other treatments since in average they excreted (10.9 grams of P per every kg) of egg mass produced. Hens of treatments 3 and 4 were fairly uniform in terms of excreted total phosphorus with a slight average decrease in the final phases of the experiment. Feed mixtures, into which 600 FTU kg phytase was added, resulted in the excretion of significantly lower amounts of total phosphorus. Though protein level did not significantly affected the amount of excreted P calculated per kg of egg mass, treatments without phytase however excreted 9.30 g P kg of eggs in average which is 12.58% more than from treatments without phytase.

Discussion

Normal diets for laying hens are composed of quite a high protein amounts. As reported by Paterson and Lorenz (1993), 43.2% of the total amount of nitrogen from feeds can be excreted via faeces. As a way to reduce nitrogen excretion to between 18%-35% (in laying hens) and 10%-27% (in broilers), (Nahm, 2002) suggests the use of synthetic amino acids in meals with reduced protein content. Enzyme phytase is also used as a dietary feed additive and with the breakage of phytate complex, not only increases the digestibility of phytate P but positively influences the digestibility of other nutrients (Englmaierova et al., 2012), including proteins (Dersjant-Li, 2015). The use of phytase in the diet of laying hens with different levels of protein has significantly affected the total nitrogen excretion. Diets with 14% CP and 600 FTU added phytase, despite better egg production, resulted in 20.85% less nitrogen excreted per kg of egg mass. Phytase reduced the excretion of nitrogen by 11.75% in diets with 17% crude protein. The main mean effects of protein levels show a reduction in nitrogen excretion of diets with the use of phytase for 4.17g and 16.32% for each kilogram of egg mass. Similar to this, Jacob et al. (2000b) reported significant reduction of nitrogen excretion with the addition of phytase in the diets of White Leghorn laying hens at reduced protein level from 17% to 13.5%.

Yao at al. (2007) has found similar results and reported the enhanced utilisation of total phosphorus (tP) ($p < 0.001$) and CP (P = 0.060) in treatment with 500 FTU, compared to the control diet.

According to the results of this research, phytase was effective in reducing the total phosphorus content of excreta from laying hens fed different protein diets, despite the same phosphorus content of the diet. Hens fed with 14% protein with added phytase excreted 8.33% less phosphorus compared with hens fed 17% protein without phytase, although differences were not significant. Mixtures used in this experiment contained the same amount of total phosphorus. However, results show some influence of the treatment (levels of crude protein and phytase) on the total phosphorus excretion in dry faeces expressed in grams per kilogram of egg mass.

Our results do generally agree with other authors' results. According to Heinzl (1996), the daily amount of phosphorus discharged from 0.46 g hen^{-1} (in the diet without phytase) can be reduced to 0.28 g if 300 FTU/kg are used. The same author notes that the total annual excretion of 1000 hens fed diets without phytase is 385 kg P and can be reduced to 234 kg if phytase is added. Nahm (2002) also reported that the use of phytase can reduce phosphorus excretion by 25%-35% from broilers and 25%-60% from pigs.

Conclusion

Results of this short eight week experiment, demonstrate that a significant decrease of nitrogen (20.20%) and a noticeable decrease of phosphorus excretion (8.20%) of laying hens and satisfactory performance may be achieved if diet with low (14%) CP content is complemented with 600 units of phytase per kg of the diet.

References

AOAC 984.13, 2000. Protein (crude) in animal feed and pet food, copper catalyst Kjeldahl method. Gaithersburg, MD, USA.

Dersjant-Li, Y., A. Awati, H. Schulze and G. Partridge, 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J. Sci. Food Agr.,* **95**: 878-896.

Englmaierova, M., G. Dlouha, M. Marounek and M. Skrivan, 2012. Efficacy of contrast levels of non-phytate phosphorus and *Aspergillus niger* phytase in hens fed wheat-maize-based diets. *Czech. J. Anim. Sci*., **57**: 499-505.

Heinzl, W. 1996. Technical specifications of Natuphos: phytic acid and phytate. *BASF*, Technical symposium, January, Atlanta., 21-36.

Hsu, J.C., C.Y. Lin and P.W.S. Chiou, 1998. Effects of ambient temperature and methionine supplementation of low protein diet on the performance of laying hens. *Anim. Feed Sci. Technol.,* **74**: 289-299.

Huyghebaert, G., 1996. The response of broiler chicks to phase

feeding for P, Ca and phytase. *Arch Geflugelkd.,* **60**: 132-141.
ISO 6491, 1998. Animal Feeding Stuffs – Determination of Phosphorus Content – Spectrometric Method, Geneva, Switzerland.
Jacob, J.P., S. Ibrahim, R. Blair, H.Namkung, and I.K.Paik, 2000a. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of broilers. *Asian-Australas J Anim Sci*., **13**: 1561-1567.
Jacob, J.P., S. Ibrahim, R. Blair, H. Namkung and I.K. Paik, 2000b. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of White Leghorn hens. *Asian-Australas J Anim Sci.,* **13**: 1743-1749.
Kamberi, M., S. Muji, A. Kryeziu, R. Kastrati and N. Mestani, 2017. Ecological implications of phytase added to normal and deficient available phosphorus diets of laying hens measured by N and P excretion. *Bulg. J. Agric. Sci.,* **23**: 632-638.
Keshavarz, K., 1998. Investigation on the possibility of reducing protein, Phosphorus, and Calcium requirements of laying hens by manipulation of time of access to these nutrients. *Poult. Sci*., **77**: 1320-1332.
Keshavarz, K., 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poult. Sci.,* **79:** 748-763.
Keshavarz, K. and M.E. Jackson, 1992. Performance of growing pullets and laying hens fed low protein, amino acid-supplemented diets. *Poult Sci*., **71**: 905-918.
Leeson, S., J.D. Summers and L.J. Caston, 1998. Performance of white – and brown- egg pullets fed varying levels of diet protein with constant sulphur amino acids, lysine, and tryptophan. *J Appl Poult Res*., **7**: 287-301.
Lei, X.G. and C.H. Stahl, 2000. Nutritional benefits of phytase and dietary determinants of its efficacy. *J Appl Poult Res*., **17**: 97-112.
Meluzzi, A., F. Sirri, M. Tallarico and A. Franchini, 2001. Nitrogen retention and performance of brown laying hens on diets with protein content and constant concentrations of amino acids and energy. *Br Poult Sci*., **42**: 213-217.
Nahm, K.H., 2002. Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. *Environ. Sci. Technol*., **32**: 1-16.
Nahm, K.H. and C.W. Carlson, 1998. The possible minimum chicken nutrient requirements for protecting the environment and improving cost efficiency. *Asian-Australas J Anim Sci*., **11**: 755-768.
Patterson, P.H. and E.S. Lorenz, 1997. Nutrients in manure from commercial White Leghorn pullets. *J Appl Poult* Res., **6**: 247-252.
Pereira, A., A.Jr. Griep Junqueira, O.M. Jr. de Lima, D.N. Sgavioli and S.S. Moraes da Silva, 2017. Phytase in diets with crude protein levels for commercial layers. *Acta Sci. Anim. Sci*., **39**: 163-168.
Pesti, G.M. and B.R. Miller, 1993. Animal feed formulation- Economics and computer applications. *Springer* Netherlands.
Ravindran, V., S. Cabahug, G. Ravindran and W.L. Bryden, 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broiler. *Poult Sc*i., **78**: 699-706.
Ravindran, V., S. Cabahug, G.P.S. Ravindran Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient igestibility and nutrient retention. *Br Poult Sci*., **41**: 193-200.
SAS Institute, 1985. SAS User Guide: Statistics Version 5 edition. *SAS Institute*, Inc., Cary NC.
Scott, T.A, R. Kampen and F.G. Silversides, 1999. The effect of phosphorus, phytase enzyme and calcium on the performance of layers fed corn-based diets. *Poult Sci*., **78**: 1742-1749.
Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean diets. *Poult Sci*., **75**: 729-736.
Selle, P.H. and V. Ravindran, 2007. Microbial phytase in poultry nutrition. Review. *Anim. Feed Sci. Technol*., **135**: 1-41.
Summers, J.Dj., 1993. Reducing nitrogen excretion of laying hen by feeding lower crude protein diets. *Poult Sci*., **72**: 1473-1478.
Van der Klis, J.D, H.A.J. Versteegh, P.C.M. Simons and A.K. Kies, 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. *Poult Sci*., **76**: 1535-1542.
Waldroup, P., 1999. Nutritional approaches to reducing phosphorus excretion by poultry. *Poult Sci*., **78**: 683-691.
Williams, P.E.V., 1996 Animal production and European pollution problems. *Anim. Feed Sci. Technol.*, **53**:135-144.
Yao, J.H., J.C. Han, S.Y. Wu, M. Xu, L.L.Y.R. Zhing Liu and Y.J. Wang, 2007. Supplemental wheat bran and microbial phytase could replace inorganic phosphorus in laying hen diets. *Czech. J. Anim. Sci*., **52**: 407-413.
Yi, Z., T. Kornegay and D.M. Denbow, 1996. Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. *Poult Sci*., **75**: 979-990.

Received July, 21, 2017; accepted for printing March, 12, 2018