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The excretion of Ca, Mg, Zn and Cu via excreta of laying hens fed low phosphorus diets and phytase

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Abstract. An 8–week experiment was conducted to study the effect of adding phytase (Natuphos® 5000 BASF) to low and normal available phosphorus diets of laying hens on the excreta content and excretion of Ca, Mg, Zn and Cu. A total of 144 Hisex Brown laying hens that were 22 weeks old at the start of the experiment were randomly assigned to four dietary treatments. Treatments included three replicates (12 hens each) or 36 hens per treatment in total. Four corn-soybean meal-based diets were formulated to contain two levels of available phosphorus (AP; 0.12 and 0.46%) and two phytase levels (0 and 600 FTU kg^{-1}). The results showed that there was no significant effect of added phytase on excreta Ca and Mg content ($P > 0.05$), but there was a significant effect of the dietary treatment on the content of Zn ($P = 0.0075$) and Cu ($P = 0.0002$). In terms of the excretion of these minerals, the dietary treatment had no effect on Ca and Zn excretion and a borderline effect ($P = 0.0522$) on Mg excretion measured as the amount of the mineral excreted per egg mass produced is observed. The results however showed a very strong effect of all three factors (available phosphorus, phytase and their interaction) on Cu excretion. The results indicate that adding 600 FTU to the corn-soybean meal laying hen diet with 0.12% or 0.46% AP beneficially affects the content and the excretion of Ca, Mg, Zn and Cu. Therefore, we can conclude that a laying hen diet containing 0.12% available phosphorus and 600 FTU during the first production cycle may not only satisfactorily support hens' performance but will also beneficially affect the environment.

Key words: excretion, manure, minerals, pollution.

INTRODUCTION

From the environmentalists' point of view, animal production is one of the industries, faced with complaints about the possible negative implications on the environment. In this context, intensive animal production is a potential source of global pollution through waste, liquids, gases and odours. It has been estimated that 250,000 tons of manure P are produced annually, which contributes to water pollution (Kazempour & Jahanian, 2017). Waste products from livestock systems can directly affect water resources and the atmosphere in the whole process of food production (Owen, 1994). Phosphorus is an expensive element in poultry feed; however, it is regularly given to poultry to cover their needs owing to the inefficient use of phytate P in plant ingredients used to feed them. Phytate P is the major natural storage form of phosphorus in animal feed (cereal grains, legumes, and oilseeds) (Sumengen et al., 2012). There is a danger of pollution of land from the excreta of animals owing to the addition of minerals such are copper and zinc, which are used in animal feed to improve their performance growth (Pallauf & Rimbach, 1997). Minerals are able to rapidly bind to phytic acid and form a phytate–mineral complex that can be resistant to hydrolysis by phytase of animal, vegetable or microbial origin (Maenz et al., 1999). Decreasing phytate levels may improve the bioavailability of various minerals, such as iron, manganese and zinc. Another tool to overcome such problems and also contribute efforts to improve the nutritive worth of feedstuffs (Slominski, 2011) is the use of exogenous enzymes to monogastric diets (Asmare, 2014) among which phytase was proven to additionally benefit the release of many nutrients other than P, which makes possible the reduction of the amount of high value ingredients in the complete feed (Silversides & Hruby, 2011). Common levels of phytase used in laying hen diets vary from were 300 FTU and 3% calcium in hens' diets improves the digestibility of P and significantly reduces the excretion of P. The addition of Ca increases the digestibility and reduces the excretion of calcium and N (Schwarz, 1994; Mc Knight, 1996; Vargas-Rodrigez et al., 2015). These authors pointed out the role of phytase in improving the relative retention time of Ca in male chickens as a consequence of Ca release from Ca-phytate complexes. Similarly, the results of many studies showed that the addition of phytase in diets with different levels of Ca was effective in maintaining the growth parameters in chickens and improving the uptake of macro and micro minerals. They found that the best retention of Ca was observed when low–Ca diet (0.6%) was supplemented with phytase which is well recognized tool to degrade phytate in animal feed (Dersjant-Li et al., 2015). In his research on the impact of the addition of phytase in broiler corn–soybean meal diets, Nelson (1994) showed significant improvement in retention of Zn and Cu. The author assumed that such a high relative Zn retention improvement (62.3%), relative to a phosphorus–deficient diet and without the addition of phytase, may result in higher digestibility of zinc complexes within phytate-mineral complexes. The concentration of Zn in the bones of chickens fed a diet with a high content of Zn can serve as a useful criterion for assessing the bioavailability of zinc from inorganic sources (Sandoval et al., 1997). Aoyagi & Baker (1995) reported that, despite expectations, the addition of 600 FTU microbial phytase per kg of feed had no positive effect on the efficiency of Cu uptake from soy or cotton pellets. They explained this as a possible influence of phytase leading to the increased utilisation of zinc, which can have an antagonistic effect on the efficiency of Cu absorption. Swiatkiewicz et al. (2001) ran an experiment to investigate the utilisation of Zn from organic and inorganic sources in chicks from 4 to 28 days of age. They found that the utilisation of Zn from organic sources (Zn complexed with amino acids) was better in the diet without phytase.

Sebastian et al. (1996) reported the ineffectiveness of phytase in improving the retention of Mg, Zn, Fe and Mn, but did not have a suitable explanation for it. They note only that their results correspond with the results of Roberson & Edwards (1994), who found that the addition of phytase had no effect on the retention of Zn in broilers. Liu et al. (2015) mention that standard (500 FTU kg^{-1}) and elevated (1,000 FTU kg^{-1}) phytase inclusions in diets with reduced nutrient specifications have the capacity to enhance the performance of broilers and compensate for these reductions. Viveros et al. (2002) reported that chickens fed a low phytic phosphorus diet exhibited lower retention of Mg and Zn by 26.9% and 88.6%, respectively, in the third week and 24.5% and 91.2% in the sixth week of feeding as compared to normal diets. The possible explanation for this is that the wide Ca to P ratio in low–phosphorus diets causes increased pH in the intestine and reduces the digestibility of the mineral fractions. Therefore, the addition of phytase increases the retention of Zn and Mg, despite their adequate levels in feed. They believe that the increased retention of Zn may be a result of its higher availability from mineral–phytate complexes.

The use of large quantities of animal excreta as fertiliser for many years may undoubtedly result in high concentrations of nitrogen, phosphorus and potassium in soil and water (Webb & Archer, 1994). Calcium is the most important mineral in the diet of all living organisms. The amount of endogenous calcium excreted via the excreta largely depends on the efficiency of absorption of this mineral. Increasing the Ca content in the diet from 3 to 4% may lead to an increase in the total non-phytic P in hens' faeces. Laying hens fed diets containing 4% Ca and 0.64% P from 50 to 72 weeks of age had 28.4% more P excreted in the form of phytate (Scheideler & Sell, 1987). There are several papers reporting beneficial effects of different levels of phytase in performance of laying hens. Englmaierova et al. (2015) found that supplementation with 350 FTU microbial 3-phytase per kg in a low-P diet not only improved the digestibility of minerals but also led to a change in the microflora of the digestive tract. In a meta–analytical study done by Ahmadi and Rodehutscord (2012) analysing 14 experiments phytase levels from 150–500 FTU per kg were used in diets containing between 0.1–0.45% non-phytate phosphorus levels. Kim et al. (2017) compared the super dosing effect of phytase (10,000; 20,000 and 30,000 FTU) added to negative control diet with 0.26% non–phytate phosphorus (NPP) diet with positive control diet containing 0.38% NPP. The found that 20,000 FTU were effective only in hen-day egg production (%) but no effect was observed in all other productive and egg quality parameters.

Since the addition of microbial phytase to plant derived feed ingredients fed to poultry improved growth performance and the retention of minerals such are P, Ca, Zn and Cu (Singh, 2008), the objective of the experiment reported herein was to specifically focus on the effect of the addition of phytase on excreta content and excretion of Ca, Mg, Zn and Cu from laying hens fed low phosphorus diet.

MATERIALS AND METHODS

Animals and diets

The experiment reported here lasted 8 weeks and covered the first egg production cycle (22–30 weeks). The trial included 144 Hisex Brown laying hens aged 22 weeks at the beginning of the experiment. Prior to experiment, laying hens were prepared during two weeks. During this period, uniform groups in body weight (P = 0.6972) and egg production (P = 0.7747) were formed. During the pre–experimental period, the hens were fed standard mixtures for this category of laying hens formulated according to NRC (1994). Individual treatments consisted of 36 hens (three replicates with 12 hens). Diets were based on corn and soybean meal, which are raw materials known to contain low levels of available phosphorus.

Two basal diets are formulated (mixtures A and B) to contain all the necessary nutrients in accordance with the recommendations of NRC (1994), except total and available phosphorus (Table 1). Two main ingredients used to formulate diets were corn and soybean meal which are known to contain not only small amounts of phosphorus, but also in the form of phytate. Kiarie et al. (2015) concluded that supplemental phytase was effective in improving the nutritional value of corn- and soy-based diet formulated to be suboptimal in terms of available Ca and P. therefore, in a diet A, no Dicalcium phosphate was used to ensure low phosphorus content. The whole amount of feed produced is divided into two parts. First half was used as treatment 1, while second part of the same mixture (treatment 2) was afterward supplemented with 12 grams of phytase per 100 kg feed similar to that used by Silversides & Hruby (2009) to ensure 600 FTU kg^{-1}. Applying the same procedure, the Diet B was used to prepare treatments 3 and 4. Therefore, four dietary treatments with two levels of Available phosphorus (0.12% and 0.46%) and two levels of Natuphos 5000 phytase (0 and 600 FTU[1] kg^{-1}) were used in this experiment. The enzyme used in this experiment was a product of BASF Corporation–Canada with a guaranteed activity of 5,000 FTU kg^{-1} and was derived from the fermentation of *Aspergillus niger*. Analysis of Ca, Mg, Zn and Cu in the feed and faeces was done using atomic absorption spectroscopy (Blanusa & Breski, 1981).

Table 1. The composition and nutritional value of experimental diets

Raw materials	Diet A (%)		Diet B (%)	
	TREATMENTS			
	0.12%AP no Phytase	0.12% AP + 600 FTU2	0.46% AP no Phytase	0.46%AP + 600 FTU
Ground yellow Corn	61.53	61.53	59.25	59.25
Soybean meal, 44% SP	26.64	26.64	27.08	27.08
Sunflower oil	2.00	2.00	3.00	3.00
Limestone	8.45	8.45	7.37	7.37
Dicalcium phosphate	0.00	0.00	1.92	1.92
Kitchen salt	0.30	0.30	0.3	0.3
Mineral Vitamin premix	1.00	1.00	1.00	1.00
DL–Methionine	0.08	0.08	0.09	0.09
Natuphos^R Phytase	–	0.012	–	0.012
TOTAL	100.00	100.00	100.00	100.00
Calculated nutrient content of diets				
ME; MJ kg^{-1}	12.17		12.25	
Crude protein, %	17.00		17.00	
Ca, %	3.30		3.30	
Total P, %	0.35		0.70	
Available P, %	0.12		0.46	
Lysine, %	0.88		0.88	
Met + Cys, %	0.64		0.64	
Mg, %	0.12		0.12	
Zn, mg	85.21		85.13	
Cu, mg	15.31		15.36	

[1] FTU stands for Phytase Unit and is defined as the amount of enzyme needed to release one micromole of inorganic phosphorus per minute from an excess of sodium phytate at 37 °C (98.6°F) at a pH of 5.5.

Statistical methods

For statistical processing of the data, the GLM procedure of SAS (1985) was used. One way Analysis of Variance was used to find whether a significant probability value ($P < 0.05$) exists. Duncan's New Multiple-Range Test was used as a post hoc test to compare individual means and determine to what extent they differ.

RESULTS AND DISCUSSION

There are many confirmatory studies on the efficacy of phytase enzyme for maintaining optimum economical poultry production and laying hen performance (Shet et al., 2017), egg quality (Kim et al., 2017), nitrogen and phosphorus excretion (Kamberi et al., 2017), suggesting that this enzyme may be considered and eco-friendly feed additive to enhance the nutritive quality of phytate (Park et al. 2012). However, publications on the effect of phytase used with low phosphorus diets on Ca, Mg, Cu and Zn content and their excretion as the amount per egg mass produced are scarce.

Content and Total Calcium Excretion

Results of egg mass produced and the content of minerals in excreta of minerals included in this study are presented in Table 2.

Table 2. Results of egg mass produced and mineral content, % (Mean ± SEM[1])

Treatments AP[2] and Phytase	Egg mass, kg per hen	Mineral content in dry faeces Ca, %	Mg, %	Zn, mg kg^{-1}	Cu, mg kg^{-1}
0.12 AP/0FTU[3]	1.84 ± 0.169^{b}	7.90 ± 0.31	2.54 ± 0.15	387.92 ± 15.92^{a}	38.11 ± 0.74^{a}
0.12 AP/600FTU	2.84 ± 0.057^{a}	7.12 ± 0.26	2.06 ± 0.07	338.54 ± 8.90^{b}	28.64 ± 1.31^{b}
0.46 AP/0FTU	2.89 ± 0.064^{a}	7.58 ± 0.85	2.36 ± 0.17	402.08 ± 8.33^{a}	37.70 ± 0.78^{a}
0.46 AP/600FTU	2.67 ± 0.098^{a}	7.36 ± 0.26	2.08 ± 0.09	353.54 ± 3.61^{b}	29.19 ± 1.28^{b}
Pr > F	0.0003	0.7128	0.0699	0.0075	0.0002
Main affect					
Available Phosphorus, (%)					
0.12	2.34 ± 0.238^{b}	7.51 ± 0.25	2.30 ± 0.13	363.23 ± 13.72	33.37 ± 2.22
0.46	2.88 ± 0.053^{a}	7.47 ± 0.41	2.22 ± 0.11	377.81 ± 11.59	33.45 ± 2.02
Added phytase (FTU kg^{-1})					
0	2.37 ± 0.249^{b}	7.74 ± 0.41	2.45 ± 0.11^{a}	395.00 ± 8.63^{a}	37.90 ± 0.49^{a}
600	2.85 ± 0.051^{a}	7.24 ± 0.17	2.07 ± 0.05^{b}	346.04 ± 5.45^{b}	28.91 ± 0.83^{b}
Analysis of variance					
	Pr > F	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*
AP	0.0010	0.9418	0.5371	0.1899	0.9462
Phytase	0.0019	0.3347	0.0155	0.0013	0.0001
AP and Phytase	0.0013	0.4601	0.5735	0.9684	0.0699

Values within the same column with different superscript differ significantly ($P < 0.05$); [1]SEM – Standard Error of Mean; [2]AP – Available Phosphorus; [3]FTU Phytase Unit.

As shown in Table 2, hens fed low available phosphorus with the addition of phytase (treatment 2) managed to maintain comparable egg production with hens fed normal AP.

The average values of calcium in dry excreta of laying hens fed the different treatments did not show statistically significant differences between treatments with different levels of AP, so it can be concluded that phytase did not influence the content of this element in dry hen excreta. However, considering the results of the total amount of excreted calcium (g) in relation to the egg mass (kg) produced, it can be clearly seen that the use of phytase had an impact in reducing the amount of excreted calcium.

Experimental treatments with different levels of available phosphorus and phytase had a statistically significant effect on the total calcium excretion via faeces only in the last stage of the experiment when the hens fed with the low–AP treatment excreted the largest amounts of calcium per kg of egg mass produced (57.19 g).

Hens fed treatment two (low AP + phytase) excreted 21.99 g of Ca for each kg of eggs while hens receiving treatments 3 and 4 excreted about the same amount of calcium (18.99 and 18.75 g kg^{-1} of eggs). The average values for excretion of this element found during this experiment suggest that the minimum amount excreted was seen in hens receiving treatment 2 (35.87 g kg^{-1} of egg mass) compared to all other treatments (57.32, 36.05 and 40.27 g kg^{-1} of egg mass for treatments 1, 3 and 4, respectively).

Studies of Chung et al. (2012) showed that apparent retention of Ca was imporved when dietary phytase was supplemented to low-phosphorus maize–soyabean meal diet for broiler. However Rabie et al. (2015) found no effect of either non–phytate level or phytase on Ca retention in Dokki-4 Laying Hens fed diets containing 0.4%, 0.3%, and 0.2% NPP without or with the addition of 0.05% microbial phytase.

The content of Ca in the excreta of laying hens increases linearly with the increase of dietary Ca (Pelicia et al., 2009).

According to Lei & Stahl (2000), animals require less Ca when phytase is added as a result of increased Ca retention, which can also lead to increased phosphorus utilisation as a direct effect of increased Ca utilisation. It should be noted that, according to Parr (1996) in experiments with laying hens, the use of phytase requires the adjustment of the Ca amount owing to its higher utilisation after phytate degradation. This adjustment is equivalent to the reduction of 0.3 g of Ca for layers that consume 100 grams of feed per day.

Content and Total Excretion of Magnesium through Excreta

After just 14 days of feeding hens different available phosphorus and phytase diets, an impact on the content of magnesium in dry excreta was observed, which was also seen in the third phase. The highest content of magnesium in the excreta (3.08%) was found in hens fed treatments 3 and 1 (2.97%). These treatments contained different amounts of AP but no phytase was added. The excreta of laying hens fed treatment 4 contained 2.55% Mg, while hens fed treatment 2 had the lowest Mg content of 1.91% in the excreta. Despite differences in the excreta of Mg content between treatments during this experiment (seen as main effect), there was no significant effect of different treatments on Mg content in the excreta of hens fed different AP level but there was a significant effect of phytase seen as the means effect.

The results of this research show that there is no significant effect of various levels of available phosphorus on the content of Mg in dry excreta. However, hens fed a diet deficient in AP after phytase addition excreted, on average, significantly less magnesium in relation to the egg output. The amount of excreted magnesium was the highest from hens fed treatment 1 during all phases of the experiment. For the other treatments, except for the lower values, the content of magnesium in the excreta tended to be lower as the experiment progressed. The levels of excreted Mg from treatments 2, 3 and 4 were significantly lower in the third and fourth stage. Magnesium excretion from the laying hens fed treatment 1 was 16.77 g and 19.37 g kg^{-1} egg mass produced during the third and fourth stage, respectively. Although hens that received treatment 2 were offered the same dietary AP level, they excreted significantly less Mg: 10.42 vs. 18.30 g kg^{-1} egg mass compared with those that received treatment 1. This can only be attributed to the addition of phytase. There was no difference in Mg excretion between treatments 3 and 4 with sufficient AP levels, despite the addition of phytase.

No recent publication on the effect of phytase on Mg excretion was found, however, in a study done by Rabie et al. (2015), who investigated the effects of different NPP levels and phytase, no effect of dietary level of phosphorus or the addition of phytase was observed in terms of Mg retention. This may suggest no effect on excretion also.

Content and Excretion of Zinc through Excreta

In this study, the content of zinc in the excreta (%) was analysed and the total excretion of this mineral, expressed in mg per kg of egg mass, is calculated. The results of this study indicate that there was no significant difference in the content of zinc in dry excreta regardless of different dietary treatments. However, the Zn content was observed to decrease with the duration of the experiment. However, there were differences in the average values during the whole experiment, particularly in the excreta of treatments with the addition of phytase (treatments 2 and 4). Phytase addition resulted in less zinc in the dry faeces compared with the treatments without phytase (treatments 1 and 3). The effects of Available phosphorus and phytase were significant only in the fourth phase of the experiment where hens given 0.12% AP excreted significantly less Zn (295.83 mg kg^{-1} egg mass) compared with treatment with sufficient AP where excretion of 362.50 mg kg^{-1} of egg mass was observed. The amount of excreted zinc was about 22% lower.

From the data, it can be seen that the highest amount of excreted Zn in milligrams per kilogram of produced eggs was obtained with treatment 1 during all phases of the experiment. Laying hens fed treatment 2 (ow in available phopshorus with the addition of phytase) excreted less Zn over the entire experiment (32.7%, 6.5% and 4.88%) compared with treatments 1, 3 and 4, respectively.

It is already proven that dietary supplementation of Zn (especially in organic form) increases its bioavailability resulting in lower excretion (Yenice et al., 2015). Our results are generally consistent with the results of other authors stating that the addition of phytase in diets with low available P, increases its utilisation in swine and poultry owing to the high concentration of phytic acid. Lei & Stahl (2000) and Pallauf et al. (1994) found that the addition of phytase in diets based on wheat, peas and barley (rich in phytic acid) improved utilisation of phytate phosphorus and also increased the utilisation of Zn and Mg in piglets. Through in vitro experiments, Pallauf & Rimbach (1997) concluded that adding phytase at 500 FTU kg^{-1} of diet led to an increase in utilisation of Zn by 31% compared to the control group (5%). Zacharias et al. (2002) stated that the liberation of zinc from the phytate complex may have an antagonist effect on copper utilisation though Ao et al. (2009) found that the antagonism between Zn and Cu occurred only if inorganic forms of these 2 minerals were included in a chick diet. Research done by Mohanna & Nys (1999) also showed that the addition of phytase in food reduced the antagonistic effects of phytate on zinc bioavailability. Adding 800 FTU microbial phytase per kg of feed in a corn–soybean meal–based diet for broilers allow a limited reduction of the content of Zn in the diet (14 mg kg^{-1}).

Table 3. The effect of AP and phytase levels on excretion, (Mean ± SEM[1])

Treatments AP[2] and Phytase	Mineral excretion, g kg^{-1} egg mass			
	Ca, g	Mg, g	Zn, mg	Cu, mg
0.12 AP/0FTU	57.32 ± 11.29	18.30 ± 2.93^{a}	271.88 ± 36.64	45.99 ± 4.73^{a}
0.12 AP/600FTU	35.87 ± 2.96	10.42 ± 0.15^{b}	182.87 ± 13.83	19.79 ± 1.82^{b}
0.46 AP/0FTU	36.05 ± 5.97	11.70 ± 0.68^{b}	195.48 ± 12.26	25.74 ± 0.72^{b}
0.46 AP/600FTU	40.27 ± 3.58	11.70 ± 1.92^{b}	192.25 ± 18.42	21.84 ± 1.27^{b}
Pr > F	0.1611	0.0522	0.0760	0.0004
Main affect				
Available Phosphorus, (%)				
0.12	46.59 ± 7.09	14.36 ± 2.20	227.38 ± 26.51	32.89 ± 6.28^{a}
0.46	38.16 ± 3.25	11.70 ± 0.91	193.86 ± 9.92	23.79 ± 1.09^{b}
Added phytase, (FTU kg^{-1})				
0	46.68 ± 7.43	15.00 ± 1.99^{a}	233.68 ± 24.30	35.86 ± 5.01^{a}
600	38.07 ± 2.30	11.06 ± 0.91^{b}	187.56 ± 10.51	20.812 ± 1.09^{b}
Analysis of variance				
	Pr > F	*Pr > F*	*Pr > F*	*Pr > F*
AP	0.2497	0.1748	0.1745	0.0087
Phytase	0.2404	0.0581	0.0744	0.0005
AP x Phytase	0.0956	0.0584	0.0929	0.0029

Values within the same column with different superscript differ significantly ($P < 0.05$); [1]SEM – Standard Error of Mean; [2]AP – Available Phosphorus, [3]FTU – Phytase Unit.

Content and Excretion of Copper through Excreta

The results show that lower copper content was found in the excreta of hens at all stages of the experiment when diets were supplemented with 600 FTU. The highest concentration of Cu in dry excreta was found in hens of treatment 1 (fed low AP) and treatment 3 (normal AP) were 38.11 mg Cu kg^{-1} and 37.70 mg Cu kg^{-1} of dry excreta is observed. This content is significantly different with treatments where phytase is added indicating no effect of AP level. The addition of phytase significantly decreased Cu content of dry excreta although the dietary Cu levels were the same for all treatments. In a study with broilers, Skrivan et al. (2006) found that Cu concentration of the excreta is strongly related with its content in a diet and will linearly increase with dietary Cu supplementation. In a diets containing dietary Cu concentration in a range from 9.2 to 243.7 mg Cu kg^{-1} DM, they observed an increase of Cu in excreta from 25.3 to 396.8 mg kg^{-1} DM.

Copper excretion (mg kg^{-1} egg mass) in the excreta of hens fed diets deficient in AP with added phytase (treatment 2) was significantly lower (24.85% on average) than for hens receiving treatment with the same content of phosphorus without phytase and 24.03% lower than in treatment with the appropriate amount of phosphorous (treatment 3). In particular, it is quite obvious that the lower phosphorus content resulted in higher excretion of Cu after calculating the egg mass produced. Differences in the amount of excreted copper started to be statistically significant after the third stage of the experiment (27.64, 10.11, 16.48 and 9.35 mg kg^{-1} egg mass) and also continued during the fourth phase (108.17, 30.83, 44.34 and 35.87 mg kg^{-1}egg mass) for respective treatments. There was also a significant difference in the average values of Cu excretion (45.99, 19.79, 25.74 and 21.84 mg kg^{-1}egg mass) for treatments 1, 2, 3 and 4, respectively. Seen as the main effect, phytase is found to significantly ($P = 0.0005$) affect the amount of copper excreted. There is significant effect of available phosphorus, phytase and AP × phytase interaction ($P > 0.05$). Hens fed low phopshorus diet and those without the addition of phytase excreted more Cu (9.1 and 15.05 mg kg^{-1}egg mass respectively). Chung et al. (2013) found better retention of Cu from low phosphorus diets of broilers after phytase supplementation. The rate of mineral excretion is related to the level and the form of trace mineral supplementation (Yenice et al., 2015). Same authors reported lower Cu excretion particularly if the mineral is given in organic form as a result of better bioavailability. However, the amount of excreted Cu was linear with its dietary level.

CONCLUSIONS

The addition of 600 FTU to a corn–soybean meal laying hen diet containing either 0.12% or 0.46% AP, in general, was beneficial in terms of the content and excretion of Ca, Mg, Zn and Cu. The effect was more pronounced for Cu content and excretion, especially when phytase was added to the low–available phosphorus diet, with a reduction observed for all minerals. Thus, it can be concluded that a laying hen diet containing 0.12% AP and 600 FTU during the first production cycle may not only satisfactorily support hens' performance but will also beneficially affect the environment.

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