



The Excretion of Ca, Mg, Zn and Cu Through Excreta of Laying Hens Fed Two Different Levels of Protein with and without Phytase

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 10/07/2018 Accepted : 09/11/2018</p> <p>Keywords: Manure Mineral excretion Laying hens Phytase Ca, Mg, Zn, Cu.</p>	<p>An 8-week experiment was conducted to study the effect of added Natuphos[®] 5000 phytase in corn-soybean meal-based diets on laying hens fed different levels of crude protein (CP) (14 and 17%). Two levels of phytase enzyme were used: 0 and 600 Phytase Units (FTU)/kg feed. The experiment used 144 Hisex Brown laying hens in a 2 × 2 factorial design. Four treatments and three replicates per treatment with 12 hens per replicate were used. Egg production was recorded daily while egg weight was assessed on 13th and 14th day of each two-week period to calculate total egg mass. Total excreta were collected and approximately 10% of the amount was used for analysis after drying in a forced draft oven. The results showed no significant effect of added phytase on excreta Calcium (Ca) content, but significantly lower Magnesium (Mg) content was observed with phytase (3.54, 2.48, 3.13 and 2.75 % for hens fed 14% CP no phytase, 14% CP + phytase, 17% CP no phytase, and 17% CP + phytase, respectively). Added phytase also significantly decreased Mg excretion measured as grams/kg of egg mass (21.43, 12.47, 16.76 and 14.75 g/kg egg mass for hens of respective dietary treatments. Phytase had a strong effect on Zink (Zn) levels with 438.96, 369.17, 434.38 and 374.58 mg Zn/kg dry excreta of hens. Similar results were observed with Cu. Added phytase significantly reduced the excreta content and the excretion of Cu. The results of this experiment indicate that adding 600 FTU to laying hen diets containing 14% CP decreases the excretion of Mg, Zn and Cu without any adverse effects on the egg mass produced of laying hens.</p>



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Introduction

As a result of fast growth and a high degree of industrialisation, a major problem faced by the poultry industry is the accumulation of large amounts of waste, which may pose disposal and pollution problems unless environmentally and economically sustainable management technologies are put in place (Bolan et al., 2010). Although the use of poultry excreta as a source of nutrients for plants is common agricultural practice to manage the manure produced from poultry, the use of large amounts in small areas of land can lead to a build-up of nutrients. Manure also contains appreciable quantities of potentially toxic metals, such as arsenic, copper and zinc (Bolan et al., 2004). The form in which these minerals are excreted (bound in phytate) represents a potential environmental threat as they can accumulate in the soil and water agglomerations (Owen, 1994;

Williams, 1996; Zanini and Sazzad, 1999). As a result of increasing legal restrictions in many countries of the world, intensive livestock producers are required to reduce phosphorus excretion in the faeces of animals (Mc Knight, 1999). Rations with a lower protein and phosphorus content contribute to lower feed prices, but also result in the excretion of nitrogen, phosphorus and other potential pollutants of the environment (Nahm and Carlson, 1998). Another feasible alternative to reduce the negative environmental impacts of poultry manure, especially when it is meant to be used as a fertiliser, is the use of the enzyme phytase (Casartelli et al., 2005). It has been shown that the use of phytase may not only release P but also other nutrients including energy and protein (Silversides and Hruby, 2009), which makes it possible to reduce the mineral and protein ingredients in the complete

feed. Kamberi et al. (2017) reported lower N and P excretion from laying hens fed low available phosphorus (0.12%) when this diet is supplemented with 600 FTU/kg. Meluzzi et al. (2001), in experiments with Hy-Line Brown hens fed a diet with 17%, 15% and 13% CP and an adequate supply of amino acids, also reported 50% reduced nitrogen excretion via the faeces when the feed contains a low amount of protein. Nevertheless, they recognise that the desired poultry performance could only be maintained during the eight weeks thanks to the healthy condition of the pullets during rearing period. In a recent study done by Muji et al. (2018), it was demonstrated 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. Decreasing phytate levels and increasing the bioavailability of various minerals, such as iron, manganese, and zinc, would provide safer and more nutritious feed (Sümengen et al., 2012) and possibly reduce their faecal excretion.

In a study with broilers, Shelton and Southern (2006) went even further, stating that phytase may be able to completely replace the trace mineral premix without compromising the growth performance of broilers. However, further studies should be conducted to determine the interaction of phytase from cereal and microbial sources in the performance and nutrient utilisation in poultry (Yao et al., 2007).

Based on these not fully consistent findings on the effect of phytase on mineral retention, the objective of this experiment was to study the effect of this enzyme and lower protein content of laying hens diet on the content and the excretion of Ca, Mg, Zn and Cu through excreta.

Materials and Methods

Four diets (treatments) with two levels of protein (14% and 17%) without and with the added Natuphos 5.000 phytase in the form of (0 and 600 FTU/kg) is used in the experiment. Diets were formulated to contain all the necessary nutrients in accordance with the recommendations of NRC (1994), except for the level of CP (Table 1). Diets were based on corn and soybean meal, while mineral feedstuffs used were limestone and dicalcium phosphate (DCP 18% P, 24% Ca). Feed formulation was done using UFFDA software (Used Feed Formulation Done Again), a program from the University of Georgia, USA (Pesti and Miller, 1993).

The experiment included 144 Hisex Brown laying hens aged 33 weeks at the beginning of the experiment. Individual treatments consisted of 36 hens (three replicates of 12 hens). A 2-week preparatory experimental period was applied during which standard commercial laying hen diet was used and uniform replicate groups in terms of body weight and egg production were formed ($P>0.05$). Feed and water were offered ad libitum during the preparatory period and the entire 8-week experimental period.

Table 1 Composition and calculated nutrient value of experimental diet

Feedstuffs	Ration A, %		Ration B, %	
	Treatment			
	1	2	3	4
Corn	70.64	70.64	59.25	59.25
Soybean meal, 44% CP	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 (18% P; 24% Ca)	2.06	2.06	1.91	1.91
Salt (NaCl)	0.30	0.30	0.30	0.30
Vitamin + Mineral- Premix*	1.00	1.00	1.00	1.00
DL-Methionine	0.05	0.05	0.09	0.09
Natuphos ^R Phytase	-	+	-	+
Total, %	100.00	100.00	100.00	100.00
Nutritional value				
ME; MJ/kg	12.13		12.25	
Crude protein, %	14.00		17.00	
Ca, %	3.30		3.30	
Total P, %	0.70		0.70	
Available Phosphorus, %	0.43		0.47	
Lysine, %	0.67		0.88	
Methionine + Cystine, %	0.53		0.64	
Na, %	0.14		0.14	
Mg, %	0.11		0.12	
Zn, mg	85.46		85.13	
Cu, mg	13.19		15.36	

ME-Metabolic Energy, MJ- Mega Joule, Ca-Calcium, P-Phosphorus, Met + Cys-Methionine + Cystine, Na- Sodium, Mg- Magnesium, Zn- Zink, Cu- Copper, *Content of 1 kg Vitamin + Mineral Premix: Vitamin A 1.000.000 I.U., Vitamin D3 200.000 I.U., Vitamin E 1.500 mg, Vitamin K3 200 mg, Vitamin B1 150 mg, Vitamin B2 400 mg, Vitamin B6 200 mg, Vitamin B12 1.200 µg, Niacin 2.000 mg, Kalcijum pantotenat 500 mg, Biotin 10.000 µg, Folic acid 40.000 µg, Choline chloride 40.000 mg, Vitamin C 2.000 mg, Iron 4.000 mg, Copper 800 mg, Manganese 8.000 mg, Zink 6.000 mg, Iodine 60.000 µg, Selenium 15.000 µg, Cobalt 20.000 µg, Methionine 30.000 mg, Antioxidant BHT 10.000 mg

The enzyme used is a product of BASF Corporation-Canada with a guaranteed activity of 5000 FTU/kg derived from the fermentation of *Aspergillum niger*. Total manure collection method was used during the entire experiment to assess the amount of manure produced. Egg mass was calculated from number of eggs and their weights. The amount of excreted minerals (ME) was calculated using following formulae:

$$\text{ME (g/kg)} = \frac{\text{Mineral content (\%)} \times \text{Amount of manure (g)}}{\text{Amount of eggs (kg)}}$$

Dried manure samples (2 g) are weighed and a wet digestion procedure is applied using 20 ml of HNO₃ + H₂SO₄ + HClO₃ in the ratio 20:2:1. Determination of Ca, Mg, Zn and Cu in the feed and faeces was done by atomic absorption spectroscopy using a method described by Blanaša and Breški, (1981). The following wavelengths are used for the respective minerals: Ca 422.6; Mg 285.2; Zn 213.8; Cu 324.8 nm (FAO, 2011).

Statistical analysis: Raw data were statistically processed using the GLM procedure of SAS (1985). Two way Analysis of Variance is used to find whether a significant probability value exists with alfa level of 0.05 as the borderline for significance. Duncan's New Multiple-Range Test was as a post hoc test to compare individual means and determine to what extent they differ.

Results and Discussion

Calcium content and excretion: The average egg mass produced and the mineral content measured therein is presented in Table 2 and 3. As can be clearly seen, with the exception of the hens fed the first treatment (low in CP), the mass of eggs produced was not significantly different among other treatments. However, it is important to emphasise that hens fed the lower level of

CP supplemented with phytase produced slightly more eggs than the hens fed 17% CP with or without phytase. Different levels of CP and Phytase as well as their interaction did not affect the Ca content in dried faeces. From the results, it can be clearly seen that the excreta calcium content of hens fed different CP levels was almost the same irrespective of the CP content of the diet. The amount of minerals excreted expressed as the amount in grams per kg of egg mass was determined during this study. The hens given the low CP treatment without phytase excreted more Ca than the hens given all other treatments, but the difference is not significant (P>0.05).

According to Lei and Stahl (2000), animals have lower requirements for Ca when phytase is added to their diet as it increases the retention of Ca owing to the increased utilisation of phosphorus. Parr (1996) also supports the above statement, suggesting that the use of phytase is equivalent to a dietary reduction of 0.3 g of Ca for a laying hen consuming 100 grams of feed per day. In a study with piglets, Pallauf et al. (1994) found that a significant improvement (about 10% higher) in Ca utilisation was observed as a result of phytase addition compared with the control group fed no phytase. However, Musilova et al. (2017) found no effect of 300 FTU/kg of exogenous phytase on the daily calcium intake, the amount of absorbed calcium, the content of calcium in ileal digesta, and on the prececal digestibility of calcium.

Magnesium content and excretion: Different level of dietary of CP (14% and 17%) and phytase in the diet resulted in significantly different (P<0.05) Mg content of the dried excreta. There was no effect of the CP level on the Mg content in the excreta but a very strong effect of phytase (P=0.0004) and CP x Phytase interaction was found (P=0.0249). The hens with no phytase in a diet contained significantly more Mg in excreta compared with those supplemented with 600 FTU/kg (3.34 vs. 2.61%).

Table 2 The effect of CP and Phytase levels on mineral content of manure (Mean±SEM¹)

Treatment	CP, % ² / (FTU) ³ level	Mineral content in dried manure			
		Ca, %	Mg, %	Zn, mg/kg	Cu, mg/kg
1	14/0	8.67±0.50	3.54±0.19 ^a	438.96±5.66 ^a	38.77±3.27
2	14/600	8.41±0.70	2.48±0.14 ^c	369.17±21.81 ^b	30.49±2.48
3	17/0	8.62±0.40	3.13±0.03 ^b	434.38±8.49 ^a	38.00±5.96
4	17/600	8.51±0.28	2.75±0.07 ^c	374.58±10.53 ^b	28.17±0.50
P value		0.9818	0.0015	0.0081	0.1717
Main affect		Crude Protein (%)			
14		8.54±0.39	3.01±0.26	404.06±18.58	34.63±2.61
17		8.57±0.22	2.94±0.09	404.48±14.67	33.09±3.46
		Phytase units (FTU/kg)			
0		8.65±0.29	3.34±0.13 ^a	436.67±4.68 ^a	38.39±3.05 ^a
600 (12g/kg feed)		8.46±0.34	2.61±0.09 ^b	371.88±10.90 ^b	29.63±1.24 ^b
Analysis of variance		P value	P value	P value	P value
CP		0.9647	0.5969	0.9755	0.6810
Phytase		0.7161	0.0004	0.0011	0.0372
CP × Phytase		0.888	0.0249	0.7135	0.8361

Values within the same column with different superscript differ significantly (P<0.05), ¹SEM- Standard Error of Mean, ²CP-Crude Protein, ³FTU Phytase Unit, Ca-Calcium, Mg- Magnesium, Zn- Zink, Cu-Copper

Table 3 The effect of CP and Phytase levels on mineral excretion, (Mean±SEM¹)

Treatment	CP, % ² / (FTU) ³ level	Egg mass, kg/hen	Mineral content in dried manure			
			Ca, %	Mg, %	Zn, mg/kg	Cu, mg/kg
1	14/0	2.53±0.12 ^b	52.57±3.95	21.43±1.59 ^a	265.23±3.99 ^a	23.20±1.75 ^a
2	14/600	3.12±0.32 ^a	42.09±3.42	12.47±0.76 ^c	185.59±8.86 ^c	15.42±0.83 ^b
3	17/0	2.94±0.78 ^a	46.34±3.85	16.76±0.38 ^b	229.31±12.83 ^b	20.24±2.74 ^{ab}
4	17/600	2.97±0.28 ^a	45.08±1.55	14.75±0.63 ^{bc}	201.05±10.03 ^{bc}	15.04±0.68 ^b
P value		0.0027	0.1422	0.001	0.0016	0.0264
Main affect		Crude Protein (%)				
14		2.82±0.14	47.33±3.31	16.95±2.15	225.41±18.33	19.31±1.94
17		2.96±0.37	45.71±1.04	15.75±0.56	215.18±9.64	17.64±1.72
		Phytase units (FTU/kg)				
0		2.74±0.112 ^b	49.46±2.36	19.09±1.27 ^a	247.27±10.03 ^a	21.72±1.60 ^a
600 (12g/kg feed)		3.04±0.373 ^a	45.59±1.81	13.60±0.67 ^b	193.32±6.91 ^b	15.23±0.49 ^b
Analysis of variance		P value	P value	P value	P value	P value
CP		0.1078	0.5833	0.2452	0.3119	0.3562
Phytase		0.0030	0.0726	0.0004	0.0005	0.0053
CP × Phytase		0.0055	0.1432	0.0067	0.0267	0.4719

Values within the same column with different superscript differ significantly ($P < 0.05$), ¹SEM- Standard Error of Mean, ²CP-Crude Protein, ³FTU-Phytase Unit, Ca-Calcium, Mg- Magnesium, Zn- Zinc, Cu-Copper

The different levels of CP in the diets used in this experiment influenced not only the content but also significantly ($P=0.001$) affected the amount of Mg excreted via the faeces expressed as grams per kg of egg mass. Laying hens fed a diet low in protein excreted significantly more Mg, but after the addition of phytase the Mg excretion was the lowest of all treatments. However, Pallauf et al. (1994) pointed out that the addition of 600 FTU phytase in piglets had a significant positive effect on magnesium uptake from the feed, an increase of 38.5% compared to 24.9% in the control group.

Zinc content and excretion: Adding phytase to the diets with 14% and 17% protein was very effective at reducing the zinc content in the faeces compared to the other treatments. The difference in Zn content of the dry excreta started to become apparent after the first 2 weeks of feeding different CP and phytase diets. No mean effect of the CP level in Zn content of the excreta was found but over the entire course of the experiment the treatments supplemented with phytase contained significantly less Zn ($P < 0.05$). In absolute terms, the phytase treatments (2 and 4) contained 65 mg less Zn than treatments 1 and 3 (no phytase). Regarding the excretion of Zn, a similar trend was observed. The laying hens fed diets with phytase excreted significantly less Zn, irrespective of CP content. Zn excretion with the second treatment was much lower than from all treatments, but what is of most interest is when compared with treatment 3, which is considered as the standard diet for this category of layers. Each hen that received the second treatment produced 180 g more eggs (Table 2) and excreted 95 mg less Zn than hens that received treatment 2. 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 and Edwards (1994), according to whom the addition of phytase had no effect on the retention of Zn in broilers. On the contrary, experimental results from Yi et al. (1996) suggest that microbial phytase is effective at improving the efficiency

of Zn retention from low-Zn corn and soybean meal-based diets. Results reported by Patterson and Lorenz (1997) suggest that about 0.9 mg of zinc may be liberated per 100 FTU phytase added in the range of 150–600 FTU/kg in the diet.

Copper content and excretion: The different levels of protein and phytase also affected the Cu content of the excreta (Table 2) and its excretion (Table 3). The hens fed the same CP diets (no phytase) compared with those supplemented with Phytase had less Cu in their dried excreta. While the CP level (seen as a mean effect) did not affect the Cu content, the addition of 600 FTU significantly reduced the Cu content of the dry excreta. No effect of CP × phytase was observed. When it comes to the excretion of Cu, a significant effect of the treatment was observed ($P=0.0264$). No significant difference was observed in diets with different CP levels without phytase, but the hens fed the diet low in CP and phytase excreted significantly less Cu compared with those fed the diet with the standard level of CP and no phytase. Therefore, the level of CP itself had no impact on the amount of copper excreted via faeces. However, the addition of phytase at 600 FTU/kg feed had a significant impact on the amount of excreted copper. Thus, the hens fed the treatment with phytase via faeces excreted less copper in all stages of the experiment, with statistically significant differences in the first and second phase, as well as the average values for the whole experiment as compared to treatment without phytase. Decreased excretion of copper in the dry faeces of hens fed a diet supplemented with phytase is in accordance with the results reported by Adeola (1996), who demonstrated in experiments with different levels of Cu and two levels of phytase in mixtures that this enzyme was effective in increasing the metabolic efficiency of copper and zinc.

Conclusions

The results of this 8-week experiment indicate that added 600 FTU to laying hen diets containing 14% CP was beneficial in terms of decreasing the excretion of Mg,

Zn and Cu without any adverse effects on the performance of laying hens from 33 to 41 weeks of age. Results demonstrate that hens fed standard diets containing 17% CP without phytase excreted more Ca, Mg, Zn and Cu compared with the hens fed same diet with phytase.

Main conclusion of this study is that laying hens can be fed diets with lower crude protein content (14% CP) and excrete less minerals maintaining comparable egg mass with hens fed standard diets when supplemented with 600 FTU/kg of feed.

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