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INVESTIGATION INTO THE PHOSPHORUS METABOLISM IN RED-MINI-ROCK HENS FED ON DIFFERENT AVAILABLE PHOSPHORUS DIETS

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Abstract

This paper aimed the particularities of the phosphorus (P) metabolism in 40-week-old Red-Mini-Rock hens subject of different available phosphorus (aP) supplemented diet feeding. Six diets containing 4.0, 3.5, 3.0, 2.5, 2.0 and, respectively, 1.5 g aP / kg diet of food were tested. The experimental feeding lasted five weeks. Research on the effect of different levels of aP in the diet on the metabolism of P in Red-Mini-Rock layers allowed the identification of particularities related to the ability to absorb, to store in bones and to release for egg formation of the P. The mobilization of the P from the bone is amplified as the dietary supplement decreases, increasing the loss of soluble P through manure. The total P content of the blood serum decreases with the level of aP supplementation but the variations are not linear. The total P content of the eggs increases only with high levels of aP. Food intake and laying rate are not affected by different levels of aP supplementation. In conclusion, a supplement of 2.0-2.5 g aP/kg diet of diet seems to better meet the metabolic requirements of Red-Mini-Rock hens from 40 to 45 week of age.

Key words*: hen, diet, phosphorus metabolism.*

INTRODUCTION

Phosphorus is an essential element for the animal organism being involved in many physiological processes. 80% of phosphorus is stored in bones in the form of hydroxyapatite, along with calcium, whose bone deposits amount to 90% of the total calcium in the body (Proszkowiec-Weglarz & Angel, 2013). The growth rate of the chicken and laying rate of the laying hens has increased in recent decades. These gains in production efficiency are coupled with metabolic disorders and increased mortality. Such rapid growth requires adequate nutritional supply and, in the case of rapid bone growth, adequate calcium and phosphorus supply. The deficiency or excess of one of these elements interferes with their homeostasis metabolism (Kebreab et al., 2005).

Birds' feed diets are supplemented with mineral phosphorus in the form of monocalcium phosphate and dicalcium phosphate to compensate for the loss of phosphorus in the diet in the form of indigestible phytate. On the other hand, the aim of the work of many researchers is focused on reducing phosphorus supplementation in the diet. Many investi-gations have been conducted to determine the exact phosphorus requirements of laying hens (Keshavarz & Nakajima, 1993; Van der Klis et al., 1997; Punna & Roland, 1999; Bar et al., 2002; Sohail & Roland, 2002; Snow et al., 2004; Ahmadi & Rodehutscord, 2012). According to Lambert et al. (2014) hens raised in alternative systems, especially domestic ones, can better tolerate lower levels of phosphorus in the diet, thus reducing expenses on the one hand and reducing digestive losses of phosphorus added to diets on the other. The reasoning of Lambert et al. (2014) is based on the observation that hens reared in alternative systems have better bone development and appear to utilize dietary phosphorus more efficiently. A reduction in dietary phosphorus in these birds would not have negative consequences on egg quality. Proszkowiec-Weglarz & Angel (2013) studied

the metabolism of phosphorus and calcium in broiler breeders (Proszkowiec-Weglarz & Angel, 2013).

Phosphorus supplementation levels in laying hens' diets are still under discussion; one of the reasons being the insufficient knowledge of the metabolic pathways of this element in the bird organism. This paper aims to investigate the ability to absorb, retain in the body and eliminate phosphorus from various sources in 40-week-old Red-Mini-Rock laying hens.

MATERIALS AND METHODS

Experimental design. Six hen groups of 40-week-old Red-Mini-Rock each one were constituted and noted from P1 to P6. Each group had a number of 60 hens and was housed in 4.2/5.4 m cages. The six layers groups were fed on diets (Table 1) containing different quantities of available phosphorus as follows (in g/kg diet): P1 = 4.0, P2 = 3.5, P3 = 3, P4 = 2.5, P5 = 2.0 and P6 = 1.5. No artificial phytase was added in any diet. The birds were fed *ad libitum* and had free access to water. The light schedule was 16.5 hours a day, from 5:00 A.M. to 10:30 P.M. The housing rooms were provided with wooden slatted floors to allow the collection of manure. During the five-week experimental period, egg production and feed consumption were monitored and manure was collected to determine the phosphorus content removed by manure. At the end of the five-week monitoring period, blood was sampled from the axillary vein to perform biochemical determinations. Excreta were collected per cage in the last three days of the experimental period. Serum was immediately removed from the blood samples as appropriate. Serum samples were stored at −20°C until biochemical processing. Five birds from each group were also slaughtered for bone and ileal content sampling. For this, the laying hens were euthanized by an intracardial injection with an

Table 1. Structure and composition of the diets used in the experiment (calculated values)

Ingredient (g/kg)	P1	P2	P3	P4	P5	P6
Wheat	515	515	514	515	520	525
Maize	85	85	87	65	65	57
Rapeseed meal	70	70	70	69	50	70
Soybean meal	175	177	178	200	218	202
Lucerne meal	20	20	20	20	20	20
Rapesead oil	27	25	25	25	25	25
Dicalcium phosphate	17,5	11,5	4,5	-	-	-
Monocalcium phosphate	-	-	-	15	10	5
Sodium chloride	2	2	2	2	2	2
Limestone[1]	81	87	92	82	83	87
L-lysine	1	1	1	1	1	1
DL-metionine	1.5	1.5	1.5	1	1	1
Vitamin - mineral premix	5	5	5	5	5	5
Nutrient contents						
Dry matter	876.4	886.5	889.5	885.5	872.4	886.5
AME_N (MJ/kg)	10.2	9.6	11.03	11.3	12.4	10.5
Crude protein	160.6	162.20	168.3	165.7	169.4	165.4
Calcium	35.0	33.6	35.9	34.9	35.4	35.4
Total phosphorus	7.6	6.7	6.4	5.3	4.6	3.0
Available phosphorus	4.0	3.5	3.0	2.5	2.0	1.5

Note: Diets contained 65% fine limestone and 35% coarse limestone

euthanasia solution (T61). In order to determine the phosphorus content eliminated by the eggs, 60 eggs were sampled from each of the six experimental layer groups.

Analysis. The eggshell was separated, weighed and used to determine the total phosphorus in the shell. The shell, bone, ileal content and manure samples were calcined at 550°C ± 5°C and then used to determine the phosphorus in them. A spectrophotometric method described by Manta et al. (1974) was used to determine the total phosphorus content from mineralized samples of eggshell, bone, ileal content and manure. A standard curve was drawn using a standard solution of 1 mg phosphorus/mL. For this 4.387 g of potassium dihydrogen phosphate p.a. were dissolved in 1000 mL of water. The curve will be linear for concentrations between 0 and 40 µg/ml. The optical density was measured at 430 nm using a blank solution obtained by adding 10 ml of molybdovanadate reagent to 10 ml of water. The amount of phosphorus in the test sample was determined using the calibration curve. The results were expressed as a percentage of the sample. A spectrophotometric method described by Kuttner & Cohen (1927) was also used to determine the phosphorus content of serum samples, using a standard phosphate solution (0.4394 g of dried monopotassium phosphate in 1 liter of distilled water) (normal values range from 2.8 to 4.5 mg/dL of serum in adult hens). The same method was addapted to determine the soluble phosphorus in manure.

Statistics. Data are expressed as the mean and SEM calculated using the GLM procedure in the SAS statistical package (version 9.4; SAS Institute Inc., Cary, NC). One-way ANOVA was used to compare mean between groups. Tukey *post hoc* test was performed to determine which experimental groups differed significantly from the control group. The Kruskal–Wallis non parametric test was used to analyze the effects of experimental diets on phosphorus contents in bone, eggshell, and blood plasma. Differences were considered significant at $P<0.05$. Correlations of phosphorus supplement levels (independent variable) with the investigated dependent variables were determined using Pearson's r values.

RESULTS AND DISCUSSIONS

The analysis of the data presented in Table 2 shows that food intake was not linked with the levels of available phosphorus supplementation (r = +0.12) and the same values of daily consumption were not significantly changed during the experimental period (P>0.05, which is accord with Lambert et al. (2014) and Lordelo et al. (2017). However, the total weight of the egg followed a downward trend, directly correlated with the dietary level of the available phosphorus supplement (r = -0.49). The egg weight loss was statistically significant (P< 0.05) and amounted to a total of 3.3%, which in absolute terms was 1.9 g/egg/period. A decrease in egg weight in chickens fed on diets supplemented with available phosphorus reported Lambert et al. (2014) in Dekalb White laying hens. According to these authors, at weeks 36-45, Dekalb White hens fed on 3.2 g rP/kg (rP = retainable phosphorus) of diet had a lower egg weight when compared to hens fed 2.8 g rP/kg of diet, which is not in agreement with our results. But at 55-65 weeks of age, egg weight of hens fed 2.8 g rP/kg was significantly higher than hens fed 2.6 g rP/kg, which is in agreement with our results.

Table 2. Feed intake and laying performances of Red-Mini-Rock hens fed on different supplement levels of phosphorus in diets (data are presented as means of a minimum 5 samples)

Ingredient	P1	P2	P3	P4	P5	P6	SEM	r	P
Food intake (g/cap./day)	92.2	94.3	101.5	88.5	97.0	98.8	12.3	+0.12	0.843
Laying performance (%)	85.6	89.4	87.5	86.0	88.4	82.2	22.3	+0.05	0.808
Feed convertion ratio (g/g)	2.44	2.43	2.50	2.54	2.49	2.61	0.32	+0.22	0.546
Egg weight (g)	59.4	58.9	58.8	58.7	57.3	57.5	7.55	-0.49	0.044
Mortality (%/month)	0.56	0.86	0.88	0.34	0.89	0.66	0.03	-0.05	1.433

Means with the same exponent indicate significant differences (*: < 0.05; **: P < 0.01). All means were compared with the control group.

The analysis of the data presented in Table 3 shows that the different levels of available phosphorus in the diets significantly changed the quantities of absorbed phosphorus, the accumulation in the bone, and the quantities eliminated in the egg and manure, as it follows. The phosphorus content in the feces decreased as the dietary phosphours intake decreased, finding a very close correlation between the level of fecal and dietary phosphorus (r = +0.94). Such a decrease is confirmed by other authors (Snow et al., 2004; Rama Rao et al., 2006; Lambert et al., 2014) but for some ages of the birds only. The exact reason why fecal phosphorus content is not altered by diets supplemented in rP at other ages is not known. There are also large differences between total fecal phosphorus and total ileal phosphorus (insoluble and soluble). Difference in total phosphorus between ileal and faecal digesta is on average 2.02 g/kg. An explanation on the physiologic mechanism of these high difference was done by Lambert et al. (2014) is this difference is due to the large amount of soluble P in faecal digesta. The soluble P fraction is related to the resorption of medullary bone and is excreted by the uric acid fraction in the faeces. There is increased demand for calcium during the period of egg shell formation in the shell gland. Because this usually occurs during the night when supply of calcium from the digestive system is low, a high proportion of shell calcium comes from resorbed medullary bone (Whitehead, 2004). Calcium is stored in bones as calcium phosphate, so both calcium and phosphorus are resorbed at the same time from the medullary bone. Calcium is used for egg shell formation and phosphorus must be excreted by uric acid (Lambert et al., 2014). The rate of absorption of phosphorus and its introduction into the intermediate metabolism depends on the functional capabilities of the digestive tract, knowing that phosphorus absorption occurs throughout the digestive tract, but especially the duodenum and jejunum, and is controlled by transmembrane transporters (Olukosi, 2011).

The level of phosphorus in the bones was highly influenced by the level of phosphorus in the diet (r = +0.55). Phosphorus supplementation in food tends to increase bone deposits. On the other hand, phosphorus in the bones is mobilized daily together with calcium in order to form eggshells in laying hens. Thus, the level of phosphorus and calcium in the bones is the algebric sum of the intervention of two diametrically opposed processes (Li et al., 2016). In our experiments we found a downward trend in phosphorus in the tarsal bones of Red-Mini-Rock hens but the curve of decreasing phosphorus in the bone is not linear. Lei et al. (2011) and Snow et al. (2004) reported an accumulation of dietary retainable phosphorus levels in bone and carcass of Dekalb White and LSL Classic laying hens and this agrees with our results. On the other hand,

Table 3. Phosphorus contents of bone, blood serum, eggshell, and manure of Red-Mini-Rock hens fed on different supplement levels of phosphorus in diets (data are presented as mean of a minimum 15 samples)

	P1	P2	P3	P4	P5	P6	Mean	SEM	r	P
Ileal P (g/kg DM)	$9.4^{\#;*;\times}$	9.6	8.8	6.8×	6.6*	$4.4^{\#}$	7.6	2.12	+0.99	0.002
Ileal soluble P (g/kg DM)	0.57	0.55	0.58	0.52	0.54	0.54	0.55	0.64	+0.33	0.544
Fecal P (g/kg DM)	$10.3^{e;f}$	10.2	9.3	7.3	6.9^{f}	4.6^{e}	8.1	1.11	+0.88	0.032
Fecal soluble P (g/kg DM)	$2.44^{a;b;c}$	2.44	2.34	2.50^{c}	2.68^{b}	2.78^{a}	2.53	0.54	-0.76	0.000
Bone (g/kg DM)	177.7	178.5	165.5	134.4	133.3	143.1	155.4	65.0	+0.55	0.021
P in blood serum (mg/dL)	4.3	5.2	4.6	4.6	3.0	2.9	4.1	0.54	+0.70	0.029
Egg shell P (g/kg DM)	1.22^{a}	0.95^{a}	1.04	1.00	1.09	0.98	1.04	1.09	+0.21	0.554
Egg albumen P (g/kg DM)	0.77^{a}	0.58^{a}	0.55	0.57	0.62	0.70	0.63	0.65	+0.33	0.433
Egg yolk P	11.11^{a}	10.25^{a}	11.4	9.56	9.99	10.54	10.47	2.22	+0.31	1.320

Values with a common superscript within the same row differ significantly ($P \leq 0.05$)

Punna & Roland (1999) and Sohail & Roland (2002) reported a decrease of the bone mineral density in hens fed on low levels of retainable phosphorus.

Total blood phosphorus ranged from 2.3 to 4.3 mg/dL of serum, following a non-linear downward curve, with a Pearson correlation coefficient of +0.70, which reveals a high degree of synchronization with the level of phosphorus supplementation in the chicken diet (Fig. 1). Klingensmith & Hester (1983) did not find singnificant differences in plasma inorganic phosphorus levels of 90-week-old Leghorn hens fed on different dietary phosphorus supplementation levels (0.2, 0.4 and 0.4% respectively, which is in agreement with our results on 40-week-old Mini-Rock hens, but reported significant differences in high incidence soft-shelled and shell-less layers (6.1 mg/dL) vs. low incidence soft-shelled and shell-less layers (5.2 mg/dL).

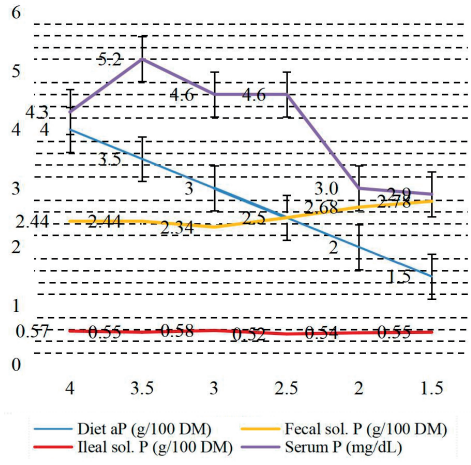

Fig. 1. Evolution of the fecal ileal and serum soluble phosphorus according to diet available phosphours supplementation. Abscissa: g suppl. aP/100 g DM diet; ordinate: P

The phosphorus content of eggs was not influenced by the level of phosphorus in the diet, except for chickens fed on a supplement of 4.0 g/kg diet, whose phosphorus values in shell, yolk and egg white were significantly higher ($P < 0.05$) than of the next experimental fed groups. Lower phosphorus values were found in groups receiving supplements of 2.0 and 1.5 g/kg diet, respectively.

The particularities of breed, age, diet, etc. of the phosphorus metabolism make the recommendations regarding the level of phosphorus supplementation in the diets still far from being in consensus. According to Lambert et al. (2014) a rP level of 2.4 to 2.6 g/kg diet could be sufficient to support the maximal egg number, egg weight, egg mass and feed conversion ratio from 36 to 90 weeks of age. According to Skřivan et al. (2010) the requirements of laying hens would be completely met with 0.27% available phosphorus in wheat-based diet and 0.30% available phosphorus in maize-based diet without added phytase. Regular use of phytase in common recipes in practice remains a method of improving the phosphorus metabolism and reducing the available phosphorus content of the diets, thus reducing mineral supplements, which are more difficult to assimilate metabolically.

CONCLUSIONS

Research on the effect of different levels of phosphorus in the diet on the metabolism of phosphorus in laying hens allowed the identification of particularities related to the ability to absorb, store in bones and release for egg formation. The mobilization of bone phosphorus is amplified as the dietary supplement decreases, increasing the loss of soluble phosphorus through manure. The total phosphorus content of the blood serum decreases with the level of phosphorus supplementation but the variations are not linear. The total phosphorus content of the eggs increases only with high levels of supplementation. Food intake and laying rate are not affected by different levels of supplementation.

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