Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 1, 2013, pp. 5-10

THE QUALITY OF EGGSHELLS IN HENS THAT PRODUCE EGGS FOR CONSUMPTION (GOC) AND WERE FED WITH DIFFERENT LEVELS OF CALCIUM, PHOSPHORUS AND PHYTASE

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ABSTRACT. The experiments were performed on Lohmann Brown Classic hens hybrid, fed five formulas (NC) containing different calcium and phosphorus levels, with or without phytase addition: NC1 and NC2 were commercial formulas; NC3 did not include any mineral phosphorus but was added Phyzyme (500FTU/kg NC), NC4 contained monosodium phosphate, while NC5 contained monocalcium phosphate. The density of the eggshells was different between the experimental groups; NC3 achieved an aP (available phosphorus) concentration of 0.30% due to phytate hydrolisis by Phyzyme. In comparison to phosphorus from monosodium phosphate, the aP from NC3 showed a biological value of 91.19%. The calcium and phosphorus content of eggshells in formulas NC3, NC4 and NC5 did not show any statistically significant differences ($p>0.05$). In the group fed the NC3 formula, phosphorus excreta was up to 20% lower than in the other experimental groups. The serum alkaline phosphatase levels did not show any statistically significant differences between groups. There is a positive correlation ($p<0.005$; $r=0.871$) between the level of serum alkaline phosphatase and the phoshorus content of eggshells in group NC3, while serum phosphorus and phosphorus content in eggshells do not correlate statistically significant ($p>0.005$; $r=-0.763$), where the significance threshold is $r=0.811$.

Keywords: laying hens, Ca, P, phytase, eggshell

INTRODUCTION

Unjustified supplementation with Ca and P of the laying hens food for obtaining the certainty of egg shell resistance determines significant economic losses and unjustified increase in the level of environmental pollution with phosphorus (Spears, 1996, Kornegay and Verstegen, 2001, NRC, 1994). Different experiments have shown the increase of aP from PP using different phytase sources (Stahl, 2011, Wu et. al, 2004 and 2006, Ravindran et al., 2000, Zyla et. Al., 2000, Augspurger et. Al., 2003)

This paper has proposed testing minimum levels of Ca and P from food to maintain the quality of eggshells by phytase supplementation and the reduction of phosphates added to food.

MATERIALS AND METHODS

Experiments were made on six laying hens, Classic Lohmann Brown hybrid, aged 32-42 weeks housed individually in battery type BPC4 cages providing them with an area of 0.22 m² / hen. Five mixed fodder recipes were tested, of which two were commercial recipes (NC 1 and NC 2) purchased from production farms and 3 mixed fodder recipes produced in laboratory and structured on corn and soybean meal where the calcium content was constantly kept at 3.43%, while the total phosphorus was different, as follows:

- NC 3 recipe with 0.12% non-phytic phosphorus (NPP) was not supplemented with mineral phosphates, but it was added 500 FTU (Phyzyme 2400 FTU) for phytic phosphorus availability;
- NC 4 recipe with 0.25% non-phytic phosphorus, of which 0.13% mineral phosphorus provided from monosodium phosphate, being considered as having 100% biological value of phosphorus. For this reason, NC 4 recipe was used as a reference for estimating the biological value of free phosphorus and of phosphorus released from phytate from NC 3 recipe and respectively for eggshell quality.
- NC 5 recipe with 0.25% non-phytic phosphorus of which 0.13% phosphorus was provided from monocalcium phosphate.

Based on previous results revealed that the real values of available phosphorus (aP) are higher than those calculated, the biological value of available phosphorus (aP) from NC 3 recipe being 91.19%, NC 3 recipe having a real content of available phosphorus (aP) of 0.30%, sufficient for an optimal metabolic intake of phosphorus (Roșu et. al., 2012).

Parameters determined:

- average weight of eggs;
- feed conversion ratio;
- density of eggs;
- calcium and phosphorus content of shell eggs;

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Article received: November 2012; published: February 2013

- nutritive material balance of calcium and phosphorus;
- correlation between plasma levels of alkaline phosphatase and the plasma level of Ca and P, respectively with Ca and P from egg shells.

RESULTS AND DISCUSSIONS

Regarding the egg production, in all experimental recipes, the laying rate was 100% in both phases of adjustment and of measurements. In comparison, in the data sheet of Lohmann Brown Classic hybrid, the laying rate varies between 94.90% and 92.80% for the hens aged 32-42 weeks. We believe that the obtained performance of 100% laying, is due to absence of hall stress registered in production farms.

The egg mass according to the hybrid data sheet varies between 58.8 g at 32 weeks and 59.8 g at 42 weeks.

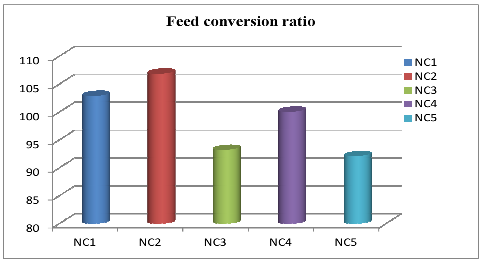

Fig. 1. Feed conversion ratio of combined fodder / kg egg mass expressed in percentage compared with NC_4 at laying hens

At experimental variants average egg weight was of 66.44±3.03 g (NC 1), 65.72± 2.61 g at NC 2 group, 66.88±2.18 g at NC3; 68.27±3.07 g at NC 4, and 69.77±3.93 g at NC 5. Within each experimental recipe, there were recorded significant differences ($F = 19.22$, $P<0.01$) between the weight of eggs produced by hens.

The recipe effect on the average weight of eggs, compared to NC 4, was not statistically significant between groups. The comparison between all groups together shows that egg weight was lower at NC 1 ($P <0.05$) and at NC 2 ($P <0.01$) than NC 5. Average weight of eggs at NC 3 does not differ significantly from statistical point of view compared with other groups.

It means that the level of 0.12% nPP with phytase supplementation does not negatively influence egg production or egg weight.

The calculation of feed conversion ratio of mixed fodder in condition of absence the significant difference of average egg weight between groups revealed that the lowest feed conversion ratio (kg combined fodder / kg egg mass) was obtained at NC 5 (1.62 kg NC / kg egg mass) and at NC 3 (1.64 kg combined fodder / egg mass).

Compared with NC 4, feed conversion ratio at NC 3 was 6.82% lower. Along with reducing costs with combined fodder, the reduction of the feed conversion ratio may enter into economic calculation of egg production and of reducing the environmental pollution.

Average weight of eggshells and their proportion from fresh egg weight are presented in tables 1 and 2.

Table 1

Average weight of eggshells

Specification	NC1	NC2	NC3	NC4	NC5
G_1	6.30	6.26	6.15	6.69	6.43
G_2	6.86	6.73	6.99	7.29	7.32
G_3	5.91	6.26	6.24	6.56	6.26
G_4	5.96	5.98	6.30	6.46	6.29
G_5	5.65	6.11	5.87	5.95	5.92
G_6	5.91	6.60	6.38	6.79	6.92
X	6.10	6.32	6.32	6.62	6.52

Table 2

The proportion of eggshells from eggs weight at GOC

Specification	NC1	NC2	NC3	NC4	NC5
G_1	9.57	9.48	9.22	9.74	9.18
G_2	9.54	9.39	9.76	9.47	8.93
G_3	8.99	10.10	9.85	9.89	9.83
G_4	9.96	9.20	9.18	9.59	9.25
G_5	8.94	9.75	9.09	9.60	9.45
G_6	8.56	9.75	9.62	9.94	9.57
X	9.20	9.61	9.46	9.70	9.37

It was found that in each experimental variant there were no significant differences in the proportion of shells from eggs weight produced by hens in the group.

Compared to recipe NC 4, it was found that only at NC 1, the eggshells proportion is significantly lower ($p<0.05$). Comparison of eggshells proportion between all groups showed significantly different statistical values only between NC 4 and NC 1, NC 1 fodder having the lowest calcium content (3.16%).

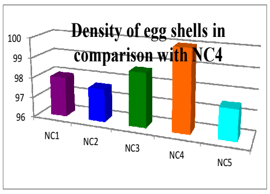

Fig. 5 Density of egg shells (%) in comparison with NC4

Egg shell quality was assessed by measuring the density of egg shells (mg/cm^2), average values of the density were: 76.385±3.55 mg/cm^2 at NC 1; 76.11±5.20 mg/cm^2 at NC 2; 76.93±1.66 mg/cm^2 at NC 3; 77.975±2.31 mg/cm^2 at NC 4 and 77.262±2.56 mg/cm^2 at NC 5.

It was observed that by the variance analysis for eggshells density, there are significant differences between hens fed with the same fodder (F = 2.71). Compared with NC 4 recipe, no statistically significant differences of eggshells density were recorded in any group. Also there are no statistical significant differences between groups.

That means the levels of calcium and phosphorus available in all recipes have been sufficient to ensure the quality of eggshells. It is disclosed that NC 3 recipe, at 3.34% calcium and 0.12% free phosphorus, which by presence of phytase at 500 FTU / kg level brings 0.30 available phosphorus in food, do not determine alteration of eggshells and also that food supplementation with phytase in recipes built on corn-soybean meal does not require supplementary feeding with mineral fodder phosphates.

From variation analysis of calcium content of eggshells from hens fed with the same fodder, there were found significant variations (F = 4.54) at hens fed with the same fodder.

Compared with NC 4 group, calcium content at NC 1 was significantly higher ($p<0.05$). Between groups, it was observed a significantly higher calcium content in NC 1 than in NC 2 ($p<0.01$) and in NC 4 ($p<0.05$).

At NC 2, calcium content of eggshells is significantly lower than at NC 3 ($p<0.05$) and at NC 5 ($p<0.01$). At NC 3, calcium content of eggshells is higher than at NC 4 and lower than at NC 5 without statistical significance.

Regarding phosphorus content of eggshells, there were no significant differences between hens from the group, and compared with NC 4, there are no significant differences in phosphorus content of eggshells. Also, there are no statistical significant differences from comparison to other groups, each to each.

Regarding the material nutritive balance of calcium and phosphorous, the calculation was based on calcium and phosphorus eliminated through eggshell, by measuring calcium and phosphorus content of the shells. Loss of calcium and phosphorus through the egg content was calculated based on the proportion and average content of calcium and phosphorus in the albumen and yolk at an egg with an average weight of 60 g according to the literature data provided by the USDA, National Nutrient Database for Standard Reference (2012).

Table 3

Nutritive balance of calcium at GOC fed with different levels of calcium and phosphorus

Specification	NC1				NC2				NC3			
	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%
G1	2.79	2.23	0.56	20.07	4.14	2.25	1.89	45.65	1.89	2.16	-0.27	-14.28
G2	2.42	2.71	-0.29	-11.98	4.35	2.08	2.27	52.18	1.99	2.42	-0.43	-21.61
G3	2.42	2.61	-0.19	-7.85	4.27	2.29	1.98	46.37	2.74	2.34	0.40	-14.60
G4	3.73	2.42	1.31	35.12	2.45	1.79	0.66	26.94	1.80	2.19	-0.39	-21.66
G5	2.03	2.23	-0.20	-9.85	3.45	1.98	1.47	42.61	3.25	2.08	1.17	36.00
G6	2.17	2.38	-0.21	-9.68	4.13	2.22	1.91	46.25	3.91	2.86	1.05	26.85

Specification	NC4				NC5			
	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%
G1	3.81	2.53	1.28	33.59	2.76	2.39	0.37	13.40
G2	1.69	2.76	-1.07	-63.31	3.76	3.13	0.63	16.75
G3	3.88	2.07	1.81	46.65	2.90	2.82	0.08	2.76
G4	3.12	2.11	1.01	32.37	3.03	2.20	0.83	27.39
G5	2.60	2.33	0.27	10.38	3.58	2.18	1.40	39.11
G6	3.14	2.42	0.72	22.93	2.93	2.55	0.38	12.97

Table 4

Nutritive balance of phosphorus at GOC fed with different levels of calcium and phosphorus

Specification	NC1				NC2				NC3			
	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%
G1	0.38	0.05	0.33	86.84	0.69	0.03	0.66	95.65	0.26	0.04	0.22	84.61
G2	0.57	0.05	0.52	91.23	0.71	0.04	0.67	94.37	0.25	0.05	0.20	80.00
G3	0.54	0.02	0.52	96.29	0.75	0.02	0.73	97.33	0.30	0.03	0.27	90.00
G4	0.44	0.03	0.41	93.18	0.55	0.03	0.52	94.54	0.27	0.02	0.25	92.59
G5	0.37	0.04	0.33	89.19	0.66	0.03	0.63	95.45	0.34	0.03	0.31	91.18
G6	0.49	0.02	0.47	95.92	0.73	0.04	0.69	94.52	0.41	0.04	0.37	90.24

Specification	NC4				NC5			
	Digested	Egg shell	Retained in shell (g)	%	Digested	Egg shell	Retained in shell (g)	%
G1	0.60	0.03	0.57	95.00	0.44	0.03	0.41	93.18
G2	0.30	0.04	0.26	86.66	0.34	0.04	0.30	88.23
G3	0.53	0.04	0.49	92.45	0.43	0.04	0.39	90.70
G4	0.50	0.03	0.47	94.00	0.43	0.04	0.39	90.70
G5	0.38	0.04	0.34	89.47	0.51	0.04	0.47	92.16
G6	0.51	0.04	0.47	92.16	0.37	0.04	0.33	89.19

Thus, the egg albumen content is 61.5% with 7 mg% calcium and 15 mg% phosphorus; the yolk is 29% from egg and it contains 129 mg% calcium and 390 mg% phosphorus.

Tables 3 and 4 present balance data of calcium and phosphorus in relation to eggshell and calculated balance which includes calcium and phosphorus content in the albumen and yolk.

Table 5

Quantities of average calcium and phosphorus eliminated by egg at GOC

Specification	Egg mass without shell (g)	Ca*(g)	P*(g)
NC_1	60.13	0.025	0.074
NC_2	59.40	0.024	0.072
NC_3	60.56	0.025	0.074
NC_4	61.65	0.025	0.075
NC_5	63.25	0.026	0.077

**Calculated using a 0.041% Ca and 0.122% P content in the albumen and yolk. The albumen is 61.5% from the egg weight and contains 7 mg% Ca and 15 mg% P. The yolk is 29 mg% from the egg and contains 129 mg% Ca and 390 mg% P. Percentage values of material nutrient balance are presented in Table 6.*

Table 6

Ca and P balance at GOC in relation to elimination of Ca and P through eggshell and respectively the calculation of Ca and P content in albumen and yolk

Specification	Balance Ca(%)		Balance P (%)	
	Egg shell	Whole egg	Egg shell	Whole egg
NC_1	6.18	5.21	93.48	77.39
NC_2	44.74	44.10	95.59	85.00
NC_3	10.00	9.04	90.00	65.33
NC_4	22.04	21.22	91.49	75.53
NC_5	19.30	18.48	90.48	79.74

It was observed that from digestible calcium, the calcium quantity retained in the body is highly variable (between 5.21% at NC 1 and 44.10% at NC 2). The retained calcium is stored in bones and it can be mobilized in alimentary deficiency of calcium. At NC 1, it was realized the smallest reserve of calcium, the dietary intake being the lowest (3.16% Ca in NC) suggesting that smaller proportions of calcium in the diet on long-term lead to negative balance at hens, affecting the egg shells.

At NC 2, calcium content of food was in excess (4.18%) according to food standards, the actual value of available calcium being even higher if one takes into account also the calcium laid off by phytase. At NC 3, NC 4 and NC 5 recipes with the same level of calcium in the diet, it was noticed that the smallest reserve of Ca was obtained at NC 3.

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Since calcium absorption is favored in the duodenum by the presence of free phosphorus, at NC 3, it is possible that part of the phosphorus laid off by phytase to have been done postduodenal.

Phosphorus nutritive balance shows that approximately 10% of digestible phosphorus is found in albumen and yolk except at NC 3 where almost 25% of digestible phosphorus is found in albumen and yolk. Elimination of phosphorus through eggshell at NC 3 is up to 5% lower from digestible phosphorus comparing to the other fodder recipes.

Measurement of calcium and phosphorus in bones would be necessary also to laying hens for better understand the metabolism of these minerals at laying hens.

From the analysis of correlation between seric levels of calcium and phosphorus on one hand, and calcium and phosphorus content of eggshell on the other hand, no significant statistical correlations were found. The highest correlation coefficient was found between seric phosphorus and phosphorus from eggshell at NC 3 (r = -0.763) under the statistical significance limit (r = 0.811). Correlations calculations by Spearman test for correlations between alkaline phosphatase and respectively seric level and from eggshell of calcium and phosphorus, as well as correlations between seric level and from shell of calcium and phosphorus, showed no significant statistical correlation.

CONCLUSIONS

Level of 0.12% nPP and respectively of 0.30% aP in NC 3 recipe has not negatively affected egg production nor the average weight of eggs.

Specific consumption at laying hens, fed with different levels of calcium and phosphorus, was lower at NC 5 (1.62 kg NC / kg egg mass) and NC 3 (1.64 kg NC / egg mass).

The proportion of eggshells from average eggs weight was statistically significant ($p <0.05$) lower at NC 1 than NC 4.

Eggshell quality at NC 3, expressed by shells' density was not influenced by the level of available phosphorus in food. At NC3 with 0.12% non-phytic phosphorus, supplementation with 500 FTU made a level of available phosphorus of 0.30%, which is over the level of food standards in phosphorus (0.25% NRC 1994).

Biological value of available phosphorus at NC 3 (from free phosphorus and laid off with phytase) was 91.19% compared with phosphorus from monosodium phosphate (VB% = 100).

Combined fodder recipes on maize-soybean meal with phytase supplementation (500 FTU / kg) and without supplementation of mineral phosphate, provide sufficient phytic phosphorus to ensure resistance to cracking eggshells.

Calcium content of eggshells at NC 3 did not differ significantly from the recipes with the same level of calcium in the diet (NC 4 and NC 5) but with different non-phytic phosphorus intake (0.12% nPP versus 0, 25% nPP at NC 4 and NC 5).

Phosphorus content of eggshells was not statistically significantly different at NC 3 than at other recipes.

Nutritive material balance of calcium at recipes with the same level of calcium (3.43%) at NC 3, NC 4 and NC 5 revealed that the free phosphorus level from food at duodenum level (the place of calcium absorption) influence the amount of absorbed calcium. At NC 3 with the lowest level of free phosphorus, tissue reserve of calcium was lower than NC 4 and NC 5.

Nutritive material balance of phosphorus revealed that phosphorus elimination by eggshell was up to 20% lower at NC 3 comparing with other recipes, but without affecting egg shell quality.

Serum level of alkaline phosphatase was not significantly different from the level of calcium and phosphorus in hens food.

Between alkaline phosphatase and serum calcium, there is a negative and statistically significant correlation only at NC 4 recipe (r = - 0.831); there is a positive and statistically significant correlation ($p < 0.05$) (r = 0.871) between alkaline phosphatase and the level of phosphorus in the eggshell.

Between the serum level and shell level of calcium and phosphorus, there were found no statistically significant correlations at hens fed with different levels of calcium and phosphorus. At NC 3, between serum level of phosphorus and the eggshell level of phosphorus, there is a negative and statistically insignificant correlation (r = - 7.63) at a significance level of r = 0.811.

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