NUTRITIONAL AND METABOLIC PARAMETERS IN LAYING HENS FED WITH DIFFERENT LEVELS OF CALCIUM, PHOSPHORUS AND PHYTASES

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Abstract

The experiment was made on a Lohmann Brown classic hybrid from 32 to 42 weeks of age. There were used feeds containing various levels of Ca, P or phytase: NC1 and NC2 were commercial recipes, NC3 was supplemented with Phyzyme (500 TFU/kg), NC4 was supplemented with monosodium phosphate and NC5 with dicalcium phosphate according to nutrient requirements (NRC 1998). There was tested the possibility to use the phytase included at a level of 0.12%nPP to make available enough phytic phosphorous to satisfy the birds requirements. (0.25% nPP).
When included at a level of 500 FTU/kg feed, Phyzyme hydrolyzed 75% of the phytic phosphorous contributing to a 0.30% aP in the feed.
The biological value of the phosphorous in NC3 was of 91.19% and it did not influence either the eggs production or the eggs' quality.

Keywords*: laying hens, phytase, egg production,*

INTRODUCTION

Food standards for P at laying hens decreased up to 0.25% (NRC 1994) but it was experimentally shown that the level of P can decrease up to 0.11% nPP associated with food supplementation with phytase.
Aim of this study was to determine phosphorus digestibility and its metabolic utilization at laying hens fed with different levels of calcium and phosphorus in food.

MATERIALS AND METHODS

Experiments were made on six laying hens (GOC) which were fed according to five mixed fodder recipes which contained different amount of total phosphorus (tP), nephites phosphorus (nPP) and available phosphorus (aP), with or without phytase (table 1).

Table 1

The quality condition of combined fodder with different levels and supplementary sources of P at GOC

Specification	NC1	NC2	NC3	NC4	NC5
	0,36 nPP	**0,41 nPP**	**0,12 nPP**	**0,25 nPP**	**0,25 nPP**
Calculated nutritional values					
EM (Kcal)	2790	2773	2794	2783	2773
PB (%)	16,85	16,94	16,85	16,85	16,85
Ca (%)	3,50	4,18	3,43	3,43	3,43
tP (%)	0,75	0,76	0,358	0,505	0,505
nPP (%)	0,36***	0,41***	0,126	0,251****	0,251****
Methionine	0,39	0,41	0,37	0,37	0,37
Lysine	0,75	0,81	0,82	0,82	0,82
Threonine	0,64	0,66	0,58	0,58	0,58
Phytase (FTU/kg)	1000*	300**	504***	-	-

* Natuphos 5000TFU/g
** Physine XP 2400 TPT/g
*** P supplemented from monocalcium phosphate
**** P supplemented from monosodium phosphate

NC 1 and NC 2 recipes were commercial ones used in chicken farms, Lohmann Brown-Classic hybrid. Recipes NC 3, NC 4 and NC 5 had the same structure corn/soybeans; At NC 3 there was no supplementation with fodder phosphates, at NC 4 the supplementary monosodium phosphate with P's biological value of 100% was used as a reference for calculating the biological value of P in the other recipes.

Clasic Lohmann Brown hybrid hens were introduced in the experiment at the age of 32 weeks and they were housed individually in battery type BPC4

cages providing them with an area of 0.22 m²/hen. To get accommodated, hens were fed for 10 days with each recipe, then followed by three days of measurements when there were measured the amounts of given fodder and unconsumed fodder debris to determine the quantity of ingested fodder and feces production. The fodder samples, the unconsumed remains and fecal samples were collected for 3 days to determine the content of dry matter (D.M.), calcium and phosphorus. During these three days of measurements, eggs were collected from each hen, they were weighed and the lay percentage was calculated.

On the morning of the third day of measurements, immediately after laying of eggs, blood was collected from axillary vein from each hen to determine seric level of calcium and phosphorus.

The chosen time for blood collection was immediately after the eggs laying because reference can be given by taking into consideration that the formation of egg shell causes fluctuations of calcium and phosphorus levels. In this way, it can be obtained information regarding the metabolic status of calcium and phosphorus in comparison with the level of Ca and P from the diet.

The analytical measurements of substance content of D.M., Ca and P from fodder and feces were effectuated as follows:

- D.M. content was determined by drying in an oven at 105 Celsius degrees;
- Total calcium and phosphorus content was determined after calcination of the samples at 500 °C and colorimetric determination of the spectrophotometer;
- nPP or free P content was determined by *Megazyme* method;
- Alkaline phosphathesis (FAL) was realized from sanguine serum during the third day of measurements with Hospitex Diagnostics kits and reading at 405 nm.
- Phytic phosphorus (PP) was calculated from the difference between total phosphorus and free phosphorus (from which it was subtracted, where appropriate, the intake of

  supplement mineral phosphorus);
- Levels of phosphorus and calcium from sanguine serum was determined colorimetrically (with a-cresolftaleine for calcium and with ammonium molybdate for phosphorus) utilizing the spectrophotometer at 578 nm for calcium and 340 nm for phosphorus using Hospitex Diagnostics kits;

- Statistical calculation of differences between lots was performed by multiple T test and Fischer test for analysis the variation.

RESULTS AND DISCUSSIONS

From the measurements regarding average daily consumption (ADC), it was found that the lowest ADC was recorded at NC 3 recipe (109.83 g/day), consumption which suggests that the absence of fodder phosphate supplements may be the cause of a lower ingestion. The highest ADC was recorded at NC 2 (123,56 g/day).
Statistical calculation shows that between hens fed with the same recipe and also between types of ingested mixed fodder, there are no significant statistical differences. Calcium content, tP, nephites phosphorus and available phosphorus as well as calculated available calcium/phosphorus ratio are presented in table 2.

Table 2

Calcium level and total, nephites and available phosphorus level calculated for experimental fodder recipes

Specification	NC1	NC2	NC3	NC4	NC5
Ca	3,16	4,18	3,43	3,43	3,43
tP	0,62	0,73	0,36	0,50	0,50
nPP	0,36	0,41	0,12	0,25	0,25
aP	0,42	0,46	0,17	0,25	0,23
Ca/P	7,52	9,09	20,18	13,17	14,91

At commercial recipes, the available phosphorus content was calculated by the manufacturers of combined fodder based on an algorithm given by the phytase suppliers.
At NC 4 and NC 5 recipes, the available phosphorus does not contain phosphorus released from phytates. At NC 3, it was estimated that phytase free up 20% of phytic phosphorus.
Based on the mineral chemical composition of ingestion and excretion, there were calculated the amounts of calcium and phosphorus retained in the body and the digestibility coefficients of DM, Ca and P. It was found that between digestibility coefficients of DM there are no significant statistically differences. In fact, weighing hens after each experimental phase (approximately every 14 days), revealed that weight variations were on average approximately (±) 50 g. Concerning digestibility of calcium, the

lowest value was recorded at NC 3 (65.82%), significantly lower than at NC 5 (81.44%), probably due to the lowest level of phosphorus, which is known to promote calcium absorption.
Regarding phosphorus digestibility, the lowest value was recorded at NC1, possibly due to inhibition of phytase through a feed-back mechanism in a high level of free phosphorus. At NC 3 there were no significant statistically differences of phosphorus digestibility comparing to NC 2, NC 4 and NC 5, suggesting that the biological value of Nephites phosphorus and of the phytic one laid off by phytase was high (91.19%) compared to phosphorus from sodium phosphate (100%).
Based on digestibility coefficients of phosphorus and on Nephites phosphorus content, values of real available phosphorus were calculated (table 3).

Table 3

Average digestibility coefficients of dry matter (D.M.), of calcium (Ca) and of total phosphorus (tP) at GOC fed with different levels of phosphorus

Specification	D.M	Ca	tP	nPP	calculated aP	measured aP
NC1	72,17	77,54	62,16	0,36	0,42	0,46
NC2	72,10	73,64	75,55	0,41	0,46	0,68
NC3	71,01	65,82	71,43	0,12	0,17	0,30
NC4	73,96	73,61	78,33	0,25	0,25	0,47
NC5	70,59	81,44	75,00	0,25	0,25	0,42

Compared to values of calculated available phosphorus, it was found that the real available phosphorus was higher in all recipes.
Of interest is the NC 3 recipe, which from the calculated value (0.17 g aP/100g), at an available of 20% from phytic phosphorus under phytase action, it was achieved an availability of 75% ensuring absorption of 0.3 g phosphorus /100g fodder, respectively 0.12 g from nPP and 0.18 g from phytic phosphorus (fig. 1).

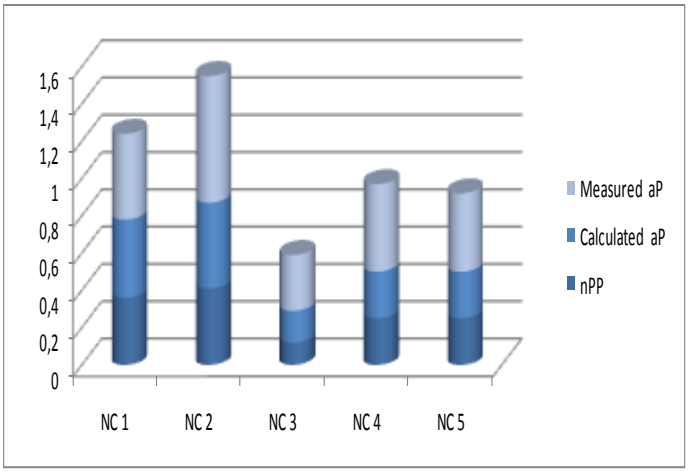

Fig. 1. Nephites phosphorus content (nPP) and real available phosphorus of fodder recipes

Thus, even at a level of 0.12 nPP in diet, by supplementation with 500 FTU phytase Phyzime (2400 FTU / g), it is ensured the need of aP according to NRC regulations (1994).

Values of seric level at calcium and phosphorus are presented in tables 4 and 5.

Table 4

Seric level of calcium (mg%) immediately after laying of eggs at GOC, fed with experimental NC

Specification	NC1	NC2	NC3	NC4	NC5
G 1	26,78	25,25	25,51	26,87	31,87
G 2	25,57	25,55	22,25	26,41	33,72
G 3	23,84	23,13	24,17	23,26	23,95
G 4	21,26	25,01	24,80	27,74	25,44
G 5	23,73	26,79	30,36	28,66	29,90
G 6	23,72	24,71	27,91	27,18	27,69
X	**24,15**	**25,07**	**25,83**	**26,69**	**28,76**

Table 5

Seric level of phosphorus (mg%) immediately after laying of eggs at GOC, fed with experimental NC

Specification	NC1	NC2	NC3	NC4	NC5
G 1	6,14	5,02	4,07	4,04	1,91
G 2	7,40	4,47	2,20	3,86	5,36
G 3	6,05	4,90	3,61	3,85	4,60
G 4	6,40	4,64	4,94	5,23	5,07
G 5	5,70	4,18	4,39	4,17	4,56
G 6	6,03	3,91	3,47	3,88	4,33
X	**6,29**	**4,52**	**3,78**	**4,16**	**3,41**

The obtained results show that in terms of calcium level, in case of all recipes, there are significant differences of calcium level between hens fed with the same level of calcium and phosphorus (F = 4.01). Compared with the NC 4 recipe there are no statistically significant differences. From comparing the calcium level between lots, it was found that at NC 1 and NC 2 lots, the calcium level is significantly lower (P <0.001) than at NC 5. At NC 3 lot, calcium level is significantly lower (P <0.05) compared with NC 5, but it is not significant different statistically from that of NC 4.

In case of phosphorus level, there were significant differences (F = 8.85, P <0.01) between hens at each experimental recipe. Compared with phosphorus level recorded at NC 4, it was found that at NC 1 phosphorus level 1 is significantly higher (P <0.001). Moreover, phosphorus level at hens fed with NC 1 recipe is significantly higher also than of NC 2 (P <0.01) and of NC 3 and NC 5 (P <0.001). Compared with NC 4, NC 3 recipe is of interest, where phosphorus level is not significantly different.

Plasma level of alkaline phosphatase (FAL) involved both in phosphocalcic metabolism in bones but also in eggshell is presented in Tables 6; 7 and 8.

Table 6

Seric level of alkaline phosphatase (ALP) / (U/I) immediately after laying of eggs at GOC

Specification	NC1	NC2	NC3	NC4	NC5
G_1	1047,98	1092,12	1571,35	934,33	762,44
G_2	1751,43	1128,10	1141,96	855,60	1931,32
G_3	1188,37	969,09	1710,16	1573,29	1543,31
G_4	1712,59	2338,32	578,40	820,98	1443,38

G_5	2370,66	1001,68	881,74	970,01	865,68
G_6	3527,57	1172,53	1273,40	1100,22	2066,99
X	1933,10	1283,62	1192,83	1042,40	1435,52

Table 7

Values of correlation coefficients (Sperman) between alkaline phosphatase and different sources of calcium and phosphorus

Variant	Character	Calcium Seric level	Phosphorus Seric level	Calcium from shell	Phosphorus from shell
NC1	Alkaline phosphatase	-0,543	-0,429	0,029	-0,486
NC_2	Alkaline phosphatase	-0,029	-0,314	-0,771	0,657
NC_3	Alkaline phosphatase	-0,200	-0,486	0,486	0,257
NC_4	Alkaline phosphatase	-0,314	-0,600	-0,257	0,714
NC_5	Alkaline phosphatase	-0,143	0,371	0,086	0,243

$r_{5\%} = 0,811$; $r_{1\%} = 0,942$; $r_{0,1\%} = 0,974$

Table 8

Values of correlation coefficients (Pearson) between alkaline phosphatase and different sources of calcium and phosphorus

Variant	Character	Calcium seric level	Phosphorus seric level	Calcium from shell	Phosphorus from shell
NC1	Alkaline phosphatase	-0,301	-0,218	0,090	-0,546
NC_2	Alkaline phosphatase	-0,002	0,075	-0,615	0,062
NC_3	Alkaline phosphatase	-0,231	-0,415	0,257	0,522
NC_4	Alkaline phosphatase	$-0,831^0$	-0,471	-0,573	0,871*
NC_5	Alkaline phosphatase	-0,149	0,619	0,331	-0,218

$r_{5\%} = 0,811$; $r_{1\%} = 0,942$; $r_{0,1\%} = 0,974$

It was found that, among laying hens fed with different levels of Ca and P, there are no significant differences in seric level of alkaline phosphatase when consuming any of the combined fodders. Compared with NC 4, it was observed that at NC 1, the alkaline phosphatase level is significantly higher ($p < 0.05$). Between lots there were found statistically significant differences only at NC 1 compared to NC 3 ($p < 0.05$) and NC 4 ($p < 0.005$).
This indicates a much higher osteoclastic activity at NC 1, suggesting a greater mobilization of calcium from the bones on a less calcium background in food. Mineral analysis of bones would support this finding.
Calculation of correlation coefficients (Pearson and Spearman) between alkaline phosphatase and seric levels and respectively in eggshell, were as follows:
At Pearson test:

- correlation between alkaline phosphatase and seric calcium is negative and insignificant at NC 1, NC 2, NC 3, NC 5, being positive ($p < 0.05$) only in NC 4 lot ($r = -0.831$).
- correlation between alkaline phosphatase and seric phosphorus is negative and insignificant in NC 1 - NC 4 lots and at NC 5, correlation is positive but insignificant.
- correlation between alkaline phosphatase and calcium from eggshell was positive at NC 1 and NC 5 and negative at NC 2, NC 3 and NC 4, statistically insignificant.
- correlation between alkaline phosphatase and phosphorus in egg shell was positive ($r = 0.871$) ($p < 0.05$) at NC 4, NC 2 and NC 3 and at NC 1 and NC 5 was negative, without being statistical significant.

It follows from these correlations that the lowest level of calcium in the diet (3.16% at NC 1) determines significant increase of alkaline phosphatase, reflected in plasma level of calcium which is the lowest (24.15 mg%). This suggests mobilization of calcium from bones, without affecting calcium deposit from eggshell.
Seric level of phosphorus is positively correlated with alkaline phosphatase at NC 2 and NC 5 and negatively at NC 1, NC 3 and NC 4 without statistical significance. Instead correlation of alkaline phosphatase with phosphorus from eggshell is statistically significant ($r = 0.871$) ($p < 0.05$) only at NC 4.

CONCLUSIONS

Ensuring a low level of Nephites phosphorus (0.12%) in recipes without mineral phosphate supplement, but supplemented with 500 FTU reduce statistically insignificant the combined

2400 FTU Phyzyme phytase supplementation at 500 FTU / kg combined fodder without mineral phosphates supplement, lays off 75% of phytic phosphorus, increasing the value of free and available phosphorus from vegetal raw materials (corn and soybean meal, 0.12% nPP) at 0.30 available phosphorus (aP) for absorption.

Compared with NC 4, phosphorus level at NC 3 with minimal level of nPP do not differ significantly statistically. Compared to phosphorus from sodium phosphate (NC 4), the biological value of nPP and of aP phosphorus from phytates at NC 3 was 91.19%.

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

Between alkaline phosphatase and seric 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

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