Effect of different phosphorus levels on the performance and egg quality of laying hens fed wheat- and maize-based diets

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ABSTRACT: The effects of diet type (wheat- or maize-based) and concentration of available phosphorus (AP; about 4, 3 and 2 g/kg) on the parameters of hen performance and egg quality as well as shell calcium (Ca) and phosphorus (P) contents were examined. Two experiments were carried out in which 240 and 120, respectively, older ISA Brown hens were housed in enriched cages. The interaction of diet type and AP concentration was ascertained for all evaluated characteristics except the amount of Ca and P deposited in shells in the first experiment. In the second experiment, the interaction of diet type and AP concentration was found for feed intake, egg weight, shell thickness and weight as well as the albumen quality parameters. Furthermore, Ca deposition in shells increased ($P < 0.001$) with the wheat diet. Hens fed a maize-based diet ($P < 0.001$) laid heavier eggs. The highest level of AP (4.1 g/kg) in the wheat-based diet significantly ($P < 0.001$) decreased albumen height, albumen index and Haugh units (HU). These trends were the same in both experiments. The results indicate that 0.27% AP in wheat-based diet and 0.30% AP in maize-based diet are adequate for hens with the intake 115 g of feed with 3.5% of Ca without a negative impact on performance or egg quality.

Keywords: available phosphorus; brown-egg laying hen; wheat; maize

A high or low level of available phosphorus (AP) in a laying hen's diet may adversely affect the bird's performance and reduce the eggshell quality (Harms, 1982; Bar and Hurwitz, 1984). Nys (1999) reported that 0.3% AP allows normal performance and bone integrity of hens. Excess dietary P is not only detrimental to the bird, but also it reduces the availability of other divalent cations and phytin phosphorus by reducing phytate hydrolysis (Ballam et al., 1985), which subsequently leads to environmental pollution. The amount of phosphorus in the eggshell is around 22 mg. In 1994, the National Research Council (NRC) estimated a requirement of 250 mg of AP per hen per day. This value was significantly lower than the preceding recommendation of 350 mg AP published by the NRC (1984). The dietary calcium (Ca) requirement was also reduced from 37.5 to 32.5 g/kg. Summers (1995) suggested that a maize-soybean meal diet containing 0.2% AP gave similar performance in layers up to 32 weeks of age as a similar control diet containing 0.4% AP. In layers older than 32 weeks, although the shell quality and average egg weight were similar, egg production was significantly reduced with the lower P diet. No differences between the 0.4 and 0.3% AP diets were noted for any of the production variables measured. Composition of the diet, rearing method, age of the bird and season are known to influence the P requirement (Rao et al., 1999). The required level

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of P for caged layers is known to be higher than on litter (Mathur et al., 1982). This finding is consistent with the need for Ca (Lichovníková and Zeman, 2008). Generally, for hens on wheat-based diets, the P requirement is lower than for those on other cereal based-diets (Salman et al., 1969), which was assumed a consequence of the endogenous phytase content. Usayran and Balnave (1995) described that the total phosphorus requirement for laying hens (New Hampshire × White Leghorn) fed on wheat-based diets containing 11.5 MJ of metabolizable energy (AME), 160 g crude protein and 35 g Ca/kg, may be lower than 3.2 g/kg. Further studies revealed that by-products of cereals, such as wheat bran (Eeckhout and Depaepe, 1994) and rye bran (Viveros et al., 2000), are rich in phytase. A study by Yao et al. (2007) demonstrated the positive effects of 0.19% inorganic P together with 10% wheat bran in a laying hen diet on egg yield, feed conversion, crude protein and total P utilisation compared to 0.19% inorganic P alone. The use of phytase in layer diets reduces the requirement for inorganic P and the costs associated with it. However, the effects of phytase in layer diets are complicated by the intimate link between Ca and P metabolisms. When formulating rations based on wheat for laying hens, the endogenous phytase should be considered (Scott et al., 1999).

The objective of the present study was to compare the effects of wheat-based and maize-based diets containing three levels of phosphorus on the production parameters of layers.

MATERIAL AND METHODS

Two experiments were carried out using ISA Brown laying hens (Hendrix Genetics) at 49 and 47 weeks of age, respectively. Both experiments lasted 12 weeks. Hens were housed in three-floor enriched colony cages in an environmentally controlled room at the Poultry Farm of the Institute of Animal Science, Prague. Hens were obtained from a commercial farm three weeks prior to the experiment and received only the experimental diet. The cages conform with the European Council Directive (No. 1999/74 EC). The cage floor area without nest was 7 560 cm^2, and there were additional 1 200 cm of feeder, 3 nipple waterers and 150 cm of perch. The lighting was via fluorescent lights. The light intensity was 6.9 to 8.2 lx in the central storey. The photoperiod was 16 h light: 8 h dark. The diets were formulated to contain (per kg): 11.5 MJ of AME$_N$, 165 g crude protein and 35 g Ca. All diets contained coarse limestone (0.80–2.00 mm) and fine limestone (0.09–0.50 mm) at a ratio of 35:65%. Three wheat-based diets (N1 to N3) and three maize-based diets (N4–N6) were identically differentiated in phosphorus concentration and were offered *ad libitum*. The protocol was approved by the Ethical Committee of the Institute of Animal Science.

Experiment 1

Two hundred and forty hens were placed in six dietary treatments. Each treatment was replicated four times with ten hens per cage replication. Diets were prepared to have identical AP contents. The AP contents in three wheat-based diets and three maize-based diets, calculated using analytically determined total phosphorus (TP) in the individual ingredients and data for AP calculation from Zelenka et al. (2007), were 4, 3 and 2 g/kg (Table 1).

Eggs were collected daily. Laying performance and feed intake were calculated weekly on a per cage basis. The eggs used for measuring physical parameters of eggs were collected every other week for three consecutive days. Four eggs were taken at random from each cage every day. Analyses of the Ca and P content of eggshells were conducted three times during the experiment (eight eggs per cage, collected in the 3rd, 7th and 11th week).

Experiment 2

In the second experiment 120 hens were studied. The number of hens per cage was reduced by half from the number in Experiment 1 to make it possible to collect all the eggs laid on three consecutive days every two weeks for laboratory measurements of physical traits. The experiment was designed so that the graded content of total dietary phosphorus was similar in the wheat-based and maize-based diets (Table 2), accounting for the increased concentration of AP in wheat diets due to higher activity of endogenous phytase in wheat. The differentiation in dietary phosphorus levels was greater than in the first experiment. The other methodological requirements were consistent with Experiment 1.

Table 1. Feed mixture composition in Experiment 1

Ingredient (g/kg)	N1	N2	N3	N4	N5	N6
Wheat	510	510	510	–	–	–
Wheat bran	20	20	20	–	–	–
Maize	147	150	151	579	585	582
Rapeseed meal	–	–	–	70	50	70
Soybean meal	193	192	193	202	218	202
Lucerne meal	–	–	–	20	20	20
Rapeseed oil	25	25	25	25	25	25
Dicalcium phosphate	17.5	11.5	4.5	–	–	–
Monocalcium phosphate	–	–	–	14	10	5
Sodium chloride	2	2	2	2	2	2
Limestone[1]	78	82	87	82	84	88
L-lysine	1	1	1	–	–	–
DL-methionine	1.5	1.5	1.5	1	1	1
Vitamin-mineral premix[2]	5	5	5	5	5	5
Nutrient content (g/kg)						
Dry matter	894.7	893.9	893.7	893.6	892.1	892.2
AME_N (MJ/kg)	11.4	11.4	11.5	11.4	11.5	11.4
Crude protein	165.4	165.3	165.8	165.1	165.8	165.3
Calcium	35.3	35.2	35.2	35.0	35.0	35.8
Total phosphorus	7.2	6.0	4.6	7.0	5.9	4.9
Available phosphorus	4.1	3.1	2.1	3.9	3.0	2.0

[1]diets contained 65% fine limestone and 35% coarse limestone

[2]vitamin-mineral premix provided per kg of diet: retinyl acetate 2.5 mg, vitamin D_3 1 000 IU, vitamin E 15 mg, niacin 20 mg, Ca pantothenate 6 mg, thiamine 1.5 mg, riboflavin 4 mg, pyridoxine 2 mg, folic acid 0.4 mg, biotin 0.06 mg, cobalamin 0.01 mg, choline Cl 250 mg, butylated hydroxytoluene 27 mg, Mn 60 mg, Zn 50 mg, Fe 30 mg, Cu 6 mg, I 0.7 mg, Co 0.3 mg, Se 0.2 mg

Laboratory analyses

The shell breaking strength was determined on the vertical axis using Instron 3360 equipment (Instron, Canton, MA, USA). The eggshell thickness (the average of both ends and in the middle, without shell membranes) was measured with a micrometer. The albumen was measured as described by Keener et al. (2006). The Haugh units (HU) were calculated as indicated by Haugh (1937). The shells were weighed after drying at 105°C. The feed was analysed as described previously (Marounek et al., 2008). Total P in the feed and in dried eggshells was determined using a vanadate-molybdate reagent after the ashing of dry samples at 550°C (AOAC procedure 965.17). Calcium was assayed using atomic absorption spectrometry performed with a Solaar M6 instrument (TJA Solutions, Cambridge, UK). The samples were mineralised by heating with a mixture of HNO_3 and H_2O_2.

Statistical analysis

The experiment was conducted using a 2 × 3 full factorial design. The main effects were type of diet (C), concentration of phosphorus (P) and the interaction between these two factors (C × P). The data

Table 2. Feed mixture composition in Experiment 2

Ingredient (g/kg)	N1	N2	N3	N4	N5	N6
Wheat	510	510	510	–	–	–
Wheat bran	152.5	155	155	–	–	–
Maize	20	20	20	585	591.5	590
Rapeseed meal	–	–	–	70	50	70
Soybean meal	193	192	194	202	218	202
Lucerne meal	–	–	–	20	20	20
Rapeseed oil	25	25	25	25	25	25
Dicalcium phosphate	18	8.5	4	–	–	–
Monocalcium phosphate	–	–	–	15	7.5	3
Sodium chloride	2	2	2	2	2	2
Limestone[1]	72	80	82.5	75	80	82
L-lysine	1	1	1	–	–	–
DL-methionine	1.5	1.5	1.5	1	1	1
Vitamin-mineral premix[2]	5	5	5	5	5	5
Nutrient content (g/kg)						
Dry matter	890.8	889.2	892.2	895.0	894.7	895.0
AME_N (MJ/kg)	11.5	11.5	11.5	11.5	11.6	11.6
Crude protein	165.9	165.7	166.6	165.6	166.4	166.0
Calcium	35.1	35.7	35.5	34.9	35.4	35.5
Total phosphorus	7.5	5.6	4.7	7.5	5.6	4.6
Available phosphorus	4.1	2.7	2.0	4.1	2.5	1.6

[1]diets contained 65% fine limestone and 35% coarse limestone
[2]vitamin-mineral premix provided per kg of diet: retinyl acetate 2.5 mg, vitamin D_3 1 000 IU, vitamin E 15 mg, niacin 20 mg, Ca pantothenate 6 mg, thiamine 1.5 mg, riboflavin 4 mg, pyridoxine 2 mg, folic acid 0.4 mg, biotin 0.06 mg, cobalamin 0.01 mg, choline Cl 250 mg, butylated hydroxytoluene 27 mg, Mn 60 mg, Zn 50 mg, Fe 30 mg, Cu 6 mg, I 0.7 mg, Co 0.3 mg, Se 0.2 mg

were analysed by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of the SAS software (SAS, 2001). All differences were considered non-significant at $P > 0.05$. The results in the tables are presented as the means and standard error of the mean (SEM).

RESULTS

Experiment 1

The results of Experiment 1 are presented in Table 3. All the investigated parameters except shell Ca and P content showed a significant interaction component (C × P). Hen-day egg production, egg number per hen per week, egg weight and feed conversion ratio (FCR) were depressed at the highest levels of TP and AP (7.2; 4.1 g/kg) in the wheat-based diet. Also, the P by cereal type of diet interaction was significant ($P < 0.001$) for egg production, with a higher value for the highest level of AP in the maize diet and higher values for medium and low AP levels (3.1 and 2.1 g/kg) in the wheat diets. The eggshell quality measurements indicated small differences among the groups; however, the C × P interactions were significant. Albumen height, albumen index and HU were increased at

Table 3. Results of Experiment 1

Parameter	Levels of phosphorus						SEM	Probability		
	high		medium		low			component	phosphorus	C × P
	wheat	maize	wheat	maize	wheat	maize				
Hen-day egg production (%)	79.7	88.3	88.0	84.8	88.0	85.5	0.05	NS	NS	< 0.001
Egg number/hen/week (pcs.)	5.4	6.0	5.9	5.9	6.2	5.9	0.08	NS	NS	0.024
Egg weight (g)	67.4	69.9	68.2	68.7	67.3	68.1	0.12	< 0.001	0.002	0.001
Feed intake (g/hen day)	115	119	110	114	117	112	0.5	NS	< 0.001	< 0.001
Feed conversion ratio (kg feed/kg eggs)	2.35	2.09	2.00	2.08	2.11	2.10	0.035	NS	0.032	< 0.001
Shell breaking strength (g/cm^{-2})	3 830	3 894	3 822	3 787	3 832	3 879	18.6	0.027	NS	0.021
Shell thickness (μm)	351	353	359	353	352	360	1.0	NS	NS	0.010
Shell weight (g)	6.9	7.1	7.0	6.9	6.9	7.1	0.02	< 0.001	NS	< 0.001
Shell percentage (%)	10.2	10.1	10.3	10.1	10.2	10.4	0.02	NS	0.006	0.001
Albumen height (mm)	6.9	7.4	7.1	7.7	7.2	7.1	0.16	< 0.001	0.001	< 0.001
Albumen index (%)	8.4	8.9	8.5	9.5	8.7	8.5	0.04	< 0.001	0.001	< 0.001
Haugh units	80.2	83.0	81.3	84.6	82.3	81.6	0.18	< 0.001	0.007	< 0.001
Shell Ca content (g/kg DM)	393	393	393	393	393	393	0.1	NS	NS	NS
Shell P content (g/kg DM)	1.4	1.3	1.4	1.4	1.3	1.3	0.01	NS	0.035	NS

Table 4. Results of Experiment 2

Parameter	Levels of phosphorus						SEM	Probability		
	high		middle		low					
	wheat	maize	wheat	maize	wheat	maize		component	phosphorus	C × P
Hen-day egg production (%)	78.8	78.2	79.0	78.9	75.4	76.4	0.64	NS	NS	NS
Egg number/hen/week (pcs.)	5.5	5.5	5.5	5.5	5.3	5.4	0.05	NS	NS	NS
Egg weight (g)	65.9	67.6	65.0	68.3	66.5	67.7	0.14	< 0.001	NS	0.004
Feed intake (g/hen day)	120	118	115	113	122	112	0.7	< 0.001	0.012	0.033
Feed conversion ratio (kg feed/kg eggs)	2.34	2.33	2.29	2.14	2.48	2.20	0.025	0.003	NS	NS
Shell breaking strength (g/cm^{-2})	3 822	3 561	3 989	3 733	3 729	3 586	17.7	< 0.001	< 0.001	NS
Shell thickness (μm)	339	327	330	337	335	327	1.0	0.014	NS	< 0.001
Shell weight (g)	6.7	6.7	6.6	6.9	6.7	6.7	0.02	0.003	NS	< 0.001
Shell percentage (%)	10.1	9.9	10.2	10.1	10.1	9.9	0.02	< 0.001	0.011	NS
Albumen height (mm)	6.7	7.3	7.7	7.6	7.3	7.4	0.03	0.004	< 0.001	< 0.001
Albumen index (%)	7.9	8.7	9.8	9.2	8.8	8.9	0.04	NS	< 0.001	< 0.001
Haugh units	79.4	82.7	86.4	84.2	83.1	83.3	0.17	NS	< 0.001	< 0.001
Shell Ca content (g/kg DM)	394	394	396	394	395	393	0.2	< 0.001	NS	NS
Shell P content (g/kg DM)	1.5	1.5	1.4	1.4	1.4	1.4	0.01	0.024	< 0.001	NS

the high (3.9 g/kg) and medium (3 g/kg) AP dietary levels in the maize-based diets. The shell phosphorus deposition was higher at the medium P levels for both cereal diets.

Experiment 2

The effects of the levels of P and different grain-based diets on layer performance and quality of eggs are summarised in Table 4. Egg production remained unaffected by the composition of the diets. The maize-based diet produced a higher weight of eggs ($P < 0.001$). The egg weight showed a significant C × P interaction ($P = 0.004$). Significant C × P interactions were also noted in feed intake ($P = 0.033$), shell thickness ($P < 0.001$), shell weight ($P < 0.001$), albumen height ($P < 0.001$), albumen index ($P < 0.001$) and HU ($P < 0.001$). The shell Ca deposition increased with all wheat-based diets, and the shell P concentration improved with P in diets. The highest albumen height, albumen index and HU were observed at the medium P levels for both cereal diets. The shell breaking strength in the framework of grain type diet also corresponded with the medium P levels.

DISCUSSION

Diets for laying hens on farms producing table eggs usually contain high levels of P and often phytase, which can make things worse. The 1994 NRC recommendation for this category of hens specified less AP and Ca than its 1984 recommendation. However, the values recommended by 1994 (NRC) are so low that they should be considered a minimum for the ideal nutrient-balanced diet.

Inadequate dietary P results in low feed intake and poor performance (Owings et al., 1977). Excess P reduces feed intake, egg production and eggshell quality (Vandepopuliere and Lyons, 1992). A high content of AP in the wheat-based diet (4.1 g/kg) reduced egg production in first experiment, and a significant C × P interaction was observed. The same dietary treatment decreased the weight of eggs, and significant effects of the type of diet, AP concentration and C × P interaction were documented. Similarly, the weight of eggs decreased in the low AP (2.1 g/kg) wheat diet. In addition, Harms (1982) and Bar and Hurwitz (1984) reported a decline in performance at high and low dietary P concentrations. The maize-based diet with a high content of AP (3.9 g/kg) did not correspond to a decrease in egg production or egg weight.

Egg production was the same (88%) in the case of wheat-based diets containing 0.31 and 0.21% AP in Experiment 1. By contrast, 0.20% AP in the same type of diet resulted in hen-day egg production of only 75% compared to 79% production for the diet containing 0.27% AP in Experiment 2.

The maize-based diets containing 0.3% AP were associated with a non-significant decrease in egg production and a significant reduction in shell breaking strength, but these eggs had the highest HU (Experiment 1). Further reduction of AP in the maize diet to 0.25% did not lead to a decline in the laying rate. Egg weight, shell breaking strength and HU were the highest for all three maize diets in Experiment 2. Feed intakes of maize-based diet at the medium P levels per hen per day were 114 and 113 g, respectively. Thus, we agree with the recommendation of Nys (1999) that the 0.3% AP ensures normal performance of laying hens. Both wheat and maize diets with low AP (0.21% and 0.20% in Experiment 1 and 0.20% and 0.16% in Experiment 2) reduced the weight of eggs and HU and maintained the shell breaking strength (Experiment 1) or decreased egg production and shell breaking strength (Experiment 2).

In conclusion, the results presented here allow us to assume that for brown laying hens from 47 weeks of age, the feed intake of 115 g per hen per day with a dietary Ca content of 3.5% is sufficient to completely 0.27% AP in wheat-based diet and 0.30% AP in maize-based diet without added phytase.

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