ISSN 1330-7142
UDK = 636.085.1

IMPACT OF PHYTASE SUPPLEMENTATION ON THE CALCIUM AND PHOSPHOROUS RETENTION IN HIGH PRODUCING LAYERS

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Original scientific paper

SUMMARY

The calcium and phosphorous retention of layers was evaluated at different production levels (45 - 75 - 95 % of final production peak) with Hy-Line Brown hybrid layers during the first twelve weeks of the laying cycle. 32 layers evenly distributed over 4 treatments (Trts). The Ca content of the diets was identical across all Trts (32,5 g/kg), while P contents and phytase activities differed. In the first Trt the P content of the diet was 2.5 g/kg non-phytate P, without any phytase added. In the second Trt the P content of the diet was reduced by 40 % compared to Trt 1 (1.5 g/kg non-phytate P) without phytase supplementation. In the third and fourth Trts the P content of the diets was the same as in 2 (1.5 g/kg non-phytate P) but the diets were supplemented with phytase (3-phytase produced by trichoderma reesei) at a rate of 250 PPU/kg (Trt 3) and 500 PPU/kg (Trt 4), respectively. According to the results the different P content of the diets affected (P≤0.05) the rate of Ca retention at all trial phases. In the case of reduced P level diets the different phytase inclusion rates did not influence the amount of Ca retained by the birds (P≥0.05). Within the same Trt Ca retention increased (P≤0.05) with increased production intensity. At reduced P content phytase improved (P≤0.05) the P retention of the layers at all trial phases. There was no difference between both phytase application rates. As a result of phytase supplementation P retention reached the P retention of positive control birds (Trt 1) in all trial phases.

Key-words: phytase, calcium, phosphorous retention, layers

INTRODUCTION

A considerable increase in the performance of layer hybrids have been seen in the last 15 years. While the results of an international performance test concluded in 1994 showed that the output of medium weight layer hybrids was 270 eggs/year or 17.2 kg of eggmass/year (Heil and Hartmann, 1994), the annual output of present day medium weight hybrids is in excess of 310 eggs or 20 kilograms (Castillo et al., 2004; Bogenfürst and Sütő, 2007). This will justify the setting up of studies the results of which can contribute to P requirements determination of these hybrids more accurately. A further good reason necessitating a more accurate definition of layer hen P requirements is the environmental aspect as, at present, the average P retention hardly reaches 20% over the entire production cycle (Oloffs et al., 1997). When applying the current recommendations the average phosphorous intake of a layer during the 12 months production cycle is 185 gram, of which 148 gram is excreted into the environment. The rate of P excretion can be reduced through applying more accurate P requirements and improving the availability of native P. Availability can be enhanced not only by the proper choice of dietary ingredients but also by the incorporation of an industrially produced phytase enzyme in the diet (Oloffs et al., 1997; Rodehutscord et al., 2002).

OBJECTIVES

The trials conducted with medium weight layer hybrids during the first half of their laying cycle were aimed at exploring how the calcium and phosphorous retention of layer hens develop with various production intensities (level of production: 45%, 75%, 95%) – beside the same level of calcium supply and a varying level of P and phytase supply.

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MATERIAL AND METHODS

Retention studies were set up during the 1st, 2nd and 12th week of production with the level of egg production being 45 %, 75 %, 95 %, using 32 layer hens (Hy-line, brown) per treatment. During the course of the trial the birds were housed in metabolic crates (4 birds/crate, 650 cm^2/hen). Experimental diets were formulated on a corn–soya basis taking into account the NRC (1994) nutrient requirements. The effect of 4 treatments were examined during the studies. The calcium level of the diets was identical across all treatments, while P level and phytase activity varied. In treatment "A" the dietary P level was in accordance with the NRC (1994) recommendations (2.5 g/kg non-phytate P). The diet contained no added phytase enzyme. In treatment "B" the dietary P level was reduced by 40 % compared to the NRC (1994) requirement (1.5 g/kg non-phytate P) and it was formulated without phytase supplementation. Dietary P levels in treatments "C" and "D" were the same as in treatment "B" (1.5 g/kg non-phytate P), but these diets were supplemented with a phytase enzyme (3-phytase produced by Trichoderma reesei) at the rate of 250 PPU/kg (Treatment C) and 500 PPU/kg (Treatment D), respectively. Diets were provided in mash form and fed ad libitum. Basal diets contained per kg of diet 12.0 MJ AMEn, 158 g crude protein, 8.8 g lysine, 7.8 g methionine + cystine, 38.5 g calcium and either 4.7 g/kg total P (treatment A) or 3.7 g/kg total P (treatments B, C and D). The trials consisted of 4-days collection periods while the experimental diets were fed continuously. Daily feed intakes and quantities of the collected excreta were weighed with gram precision during the collection periods. The nutrient content of experimental diets and the Ca and P contents of the excreta samples were determined in accordance with the AOAC (1989) procedures. Experimental data were subjected to ANOVA (SAS, 2004). In the case of a significant treatment effect, the statistical reliability of differences between treatments was verified by Tukey's test (SAS, 2004).

RESULTS AND DISCUSSION

Changes in the calcium retention of the birds are shown in Table 1. According to our data birds in the positive control group retained the most calcium (1707 mg/day) during **the 45 % production level** (Phase 1). Negative control birds retained 29.8 % less calcium in comparison ($P \leq 0.05$). In treatments with reduced P content and including phytase supplementation (treatments C and D) the calcium retention of the birds was the same as the value measured for the negative control group ($P \geq 0.05$). When the Ca retention was related to the calcium intake of the birds (%), the highest level was found for the positive control group (42.0 %) again (data are not shown in table). In contrast, this percentage was an average 31 % for the other treatments. This finding is also remarkable because the Ca level of the trial diets and the Ca intake of the birds was the same (the relevant data are not expressed in tabular form). The higher Ca retention measured for treatment A can probably be attributed to the different calcium and phosphorous ratio of the diets. That is, in case of a large Ca/P ratio the absorption of Ca and P will be less favourable. This has already been clearly demonstrated for pigs (Quian et al., 1996). In poultry, however, due to the joint excretion of urine and faeces there are hardly any data available that were aimed at determining the digestibility of nutrients and minerals. In their broiler trials, however, Tossenberger et al. (1999) showed, that the different dietary calcium and phosphorous levels influence the availability of Ca and P, both. **At 75 % production level of the birds** (phase 2) birds of the positive control group retained the most calcium (2314 mg/day) again. In comparison to that the Ca retention of birds in the negative control group (treatment B) was 1865 mg per day, which corresponds to a 18.1 % lower Ca retention ($P \leq 0.05$). Although the Ca retention of birds fed the diets supplemented with 250 PPU/kg phytase (treatment C) increased (2067 mg/day), it still did not reach that of the positive control birds. The highest phytase dosage resulted in no further increase of the Ca retention. The Ca retention of birds fed diets containing the same Ca levels, but with a reduced P content (treatments B, C and D) was similar ($P \geq 0.05$). Although the retention measured for the birds fed the phytase supplemented diets (treatments C and D) was 7.9% higher than that of their negative control peers, this difference was not significant ($P \geq 0.05$). **At 95 % production level of the layers** the Ca retention of the positive control birds (treatment A) was 2708 mg/day. The negative control layers, however, retained only 2386 mg Ca per day, which means 11.9% lower retention ($P \leq 0.05$). In treatments B, C and D (diets with reduced P content) the Ca retention of the birds was the same ($P \geq 0.05$) – similar to that found in the 1st and 2nd phase of the trial.

Within the same treatment Ca retention grew significantly (P≤0.05) with the increase of production intensity. The rate of increase for birds fed the diet with a P content according to the NRC requirement (treatment A) was 58.6%, while in case of birds fed the diets with reduced P levels (treatments B, C and D) it exceeded 95% on the average. This suggests that during the first trial phase (45% production) there was enough calcium for eggshell formation even beside a lower level of Ca retention. During the 3rd trial phase (95 % production) however, this Ca level was probably no more sufficient, and thus the birds endeavoured to provide enough calcium for eggshell formation by means of increasing the absorption of calcium and reducing the urinary calcium excretion to the minimum. In the case of higher calcium requirement (start-up of egg production, increase of production intensity) the activity of 1,25-dihydroxy-cholecalciferol increases – primarily in consequence of estrogens – and this reaching the gut mucosa enhances the synthesis of CaBP in the epithelial cells. As a result of more intense CaBP synthesis, the rate of calcium absorption increases (Bar et al., 1992). The increased estrogen and testosterone concentration also causes the level of the parathyroid hormone to increase, which through the increased concentration of the 1,25-dihydroxy-cholecalciferol boosts the rate of Ca resorption in the renal tubules. The increased rate of Ca absorption combined with the lower rate of urinary calcium excretion, occurring simultaneously with the increase in intensity of egg production result in a higher retention of calcium (Wiedeman, 1987).

When the change of P retention was studied **at the 45% production level of the birds** it was found to be the highest in the positive control group (Table 1). In this treatment the layers retained 145 mg/day phosphorous. The P retention of negative control birds was only 74 mg/day. The 49 % lower (P≤0.05) P retention can primarily be attributed to the different P level of the diets and to the difference in P sources.

Table 1. The influence of different levels of P supply on the calcium and phosphorous retention of layers

Item		TREATMENTS*				RMSE**
		A	B	C	D	
1ST PHASE (45 %)						
Ca retention (mg/d)		1707^{aC}	1198^{bC}	1234^{bC}	1184^{bC}	13
P retention (mg/d)		145^{aA}	74^{cB}	107^{bB}	114^{bA}	12
2ND PHASE (75 %)						
Ca retention (mg/d)		2314^{aB}	1895^{bB}	2067^{abB}	2012^{bB}	26
P retention (mg/d)		136^{aA}	84^{cAB}	113^{bB}	118^{bA}	15
3RD PHASE (95 %)						
Ca retention (mg/d)		2708^{aA}	2386^{bA}	2346^{bA}	2326^{bA}	133
P retention (mg/d)		148^{aA}	93^{cA}	129^{bA}	125^{bA}	17
RMSE**	Ca	186	211	192	142	-
	P	19	11	14	15	-

* **A:** diet with recommended P level, without phytase supplementation
B: diet with reduced P level, without phytase supplementation
C: diet with reduced P level, with 250 PPU/kg phytase supplementation
D: diet with reduced P level, with 500 PPU/kg phytase supplementation
** : RMSE : Root Mean Square Error
a,b,c: different superscripts within the same line indicate significant difference (P≤0.05)
A,B,C: different superscripts within the same column indicate significant difference (by elements) (P≤0.05)

The diet of birds in treatment B (reduced dietary P without phytase supplementation) contained 1.5 g/kg non-phytate P, only of which 0.7 g/kg was of inorganic origin. This means that of the 3.7 g/kg total P content of the diet almost 60% (2.2 g/kg) was bound to phytate and thus – in the absence of a phytase enzyme – was indigestible for poultry. On the other hand, the availability of P from inorganic feed phosphates – and thus also from the mono-calcium phosphate used in our studies – is high, and may even reach 80%. The digestibility of dietary P in corn – soy based diets without phytase supplementation, however, will not even reach 15% in adult poultry (Tossenberger and Babinszky, data not published). In treatment C the 250 PPU/kg phytase supplementation resulted in a 44.6% increase in P retention compared to the negative control, up to 107 mg/day (P≤0.05). The further increase of the phytase dosage (treatment D, 500 PPU/kg) did not produce any further increase in P retention (P≥0.05). It should be noted however, that the birds in this treatment retained 54.1 % more P compared to their negative control peers (P≤0.05).

At 75 % production level of the layer hens P retention was the highest for the positive control birds again (136 mg/day). In the negative control birds (treatment B) P retention reached an average 84

mg/day level, which corresponds to a 24.7% relative P retention. The 38.2% lower P retention ($P \leq 0.05$) is probably attributable to the different P content of the diets and the different source of dietary P (phytate vs non-phytate) – similar to the findings under phase 1. In treatment C (250 PPU/kg phytase supplementation) the level of P retention increased by 34.5 % compared to the negative control up to 113 mg/day ($P \leq 0.05$). The further increase of the phytase dosage (treatment D, 500 PPU/kg) did not produce any further increase in P retention ($P \geq 0.05$).
The level of P retained **at the 95% production level** of the layers was the highest for the birds in treatment A (positive control) similar to the findings of the first two phases. The birds in this treatment retained the average of 148 mg P per day. The P retention of birds in the negative control group (treatment B) (93 mg/day) was 37.2% lower than the retention measured for the positive control group ($P \leq 0.05$). The reduction in absolute terms (mg/day) of P retention by 37.2% ($P \leq 0.05$) can be attributed to the causes discussed above. In treatment C (250 PPU/kg phytase supplementation) P retention increased by 38.7% compared to the negative control, which corresponds to 129 mg retained phosphorous per day. The further phytase supplementation (treatment D, 500 PPU/kg) did not result in any further improvement in the P retention of the layers ($P \geq 0.05$). Within the positive control group the increasing intensity of production was not accompanied by a significant increase of P retention ($P \geq 0.05$). In the birds fed the diets with reduced dietary P (treatments B, C and D), however, an increase was found. The difference was only significant ($P \leq 0.05$), however, between the 45% and 95% levels of production. This signifies that the layer hens need more phosphorous due to the higher egg production, despite the fact that the shell of the average egg contains only 7 mg P beside the 2 g Ca, while the eggmass contains only 73 mg P (Tossenberger and Babinszky, data not published). According to our calculations the available P content of the test diets (A, B, C, D) was 1.4, 0.9, 1.2 and 1.2 g/kg respectively, at the peak of production (95% production level). Our data show, that the feeding of reduced dietary P diets together with a 250 PPU/kg or 500 PPU/kg phytase supplementation resulted in the birds excreting on the average 25 % less phosphorous daily than their positive control peers (these data are not shown in tabular form). In summary it can be concluded that the P retention of layer hens was significantly influenced ($P \leq 0.05$) by the different dietary P level and phytase activity of the diets at all trial phases. As a result of the phytase supplementation the P retention increased significantly ($P \leq 0.05$), but in absolute terms it did not reach the value measured for the positive control birds (treatment A) in any of the trial phases. Within the same treatment retention increased significantly between the 45 % and 95 % levels of production ($P \leq 0.05$) – except for treatment A.

CONCLUSION

The following main conclusions can be drawn from the results of our trial series conducted with layer hens. The Ca retention of layer hens is significantly influenced by the different dietary P level and phytase activity of the diets in all trial phases ($P \leq 0.05$). Within the same treatment Ca retention increases significantly with the increase in production intensity ($P \leq 0.05$). As a result of phytase supplementation P retention increases significantly ($P \leq 0.05$), but it reaches the level of P retention of positive control birds (treatment A) in none of the trial phases. Within the same treatment, the increase of production intensity results in a significant increase of P retention ($P \leq 0.05$) – with the exception of treatment A. Our trial data suggest that the dietary P level of layer diets might be reduced. The rate of this reduction could be determined more accurately in further target-oriented studies.

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(Received on 17 May 2007; accepted on 21 June 2007)