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EFFECTS OF MICROBIAL PHYTASE AND 1,25-DIHYDROXYCHOLECALCIFEROL ON THE ABSORPTION OF MINERALS FROM BROILER CHICKEN DIETS CONTAINING DIFFERENT LEVELS OF CALCIUM

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The aim of this study was to investigate the effect of various calcium: total phosphorus (Ca:tP) ratios due to addition of microbial phytase and 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] on the absorption levels of minerals. In a 42-day experiment repeated three times, 144 day-old male broiler chicks (ISA 220) were divided into six groups of eight chicks each. Diets containing two different (1:1 and 2:1) Ca levels were prepared. Groups 1, 2 and 3 received a diet of 1:1 Ca:tP ratio while Groups 4, 5 and 6 a diet of 1:2 Ca:tP ratio. These diets contained 1,25-$(OH)_2D_3$ and phytase in levels of 5 µg/kg and 600 FYT/kg, respectively. The faeces was collected to analyse the absorption of minerals. At the end of the study, the absorption levels of Ca, P, Zn, Mn and Cu were increased by the addition of phytase enzyme ($p < 0.05$). This effect was obvious in the 3rd week. On the other hand, in the 6th week only Ca and P absorption levels were influenced positively by the addition of phytase enzyme. The results proved the positive effect of phytase, an enzyme which is used for increasing the utilisation rate of phytate P, Ca and other minerals in broilers.

Key words: Broilers, mineral absorption, microbial phytase, 1,25-dihydroxycholecalciferol, Ca:tP ratios

The steady progress of the poultry industry during the last 20 years and the consequent genetic and nutritional improvements have prompted researchers to search for ways that can improve the efficiency of animal metabolism, increase productivity and decrease costs. One of these ways is the use of enzymes. Enzymes can be used to increase productivity, to use available resources more efficiently and to prevent environmental pollution. Studies on the structure and purpose of utilisation of phytate phosphorus, one of these enzymes, and on the possibility to increase the utilisation of phosphorus are not new. What prompts researchers to use this enzyme is the possibility to improve the utilisation of phy-

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tate phosphorus in the feed. The phytase enzyme reduces the amount of P in excreta and, thus, increases its concentration in the body, especially in the bones which is important as far as P is concerned (Broz et al., 1994; Mitchell and Edwards, 1996*a*, 1996*b*). The need for calcium and phosphorus is met through cereals and inorganic sources such as dicalcium phosphate (DCP). A major portion of the P in cereal grains and oilseed meals (2/3) is in the form of phytate phosphorus, an organically bound form of the mineral that is poorly available to monogastric animals. Chickens 0 to 3 weeks of age are lacking or limited in phytase, the enzyme that is necessary for breakdown of the molecule and subsequent release of phosphorus for absorption (Nelson, 1967). High levels of unavailable P are excreted and applied to land, thus causing a potential environmental problem. Addition of phytase enzyme to the feed improves phosphorus utilisation and reduces phosphorus excretion by 30–50% (Schöner et al., 1991; Roberson and Edwards, 1996; Vetési et al., 1998; Ahmad et al., 1999; Yan et al., 2001). This, in turn, reduces pollution on agricultural land and helps improve environmental conscience.

Phytase enzyme activity is closely related to dietary Ca:t (total)P ratios and/or 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$]. There are many studies confirming this point (Mitchell and Edwards, 1996*a*, 1996*b*; Roberson and Edwards, 1996; Qian et al., 1997). A recent study with broiler chickens suggests that supplemental microbial phytase in a low-P diet increased growth and relative retention of total P, Ca, Cu and Zn and improved bone mineralisation (Sebastian et al., 1996*a*; Windisch and Kirchgessner, 1996*a*). Qian et al. (1997) suggested that phytase enzyme, cholecalciferol and Ca:tP are important factors in degrading phytate and improving phytate phosphorus and calcium utilisation in broiler chickens.

This objective of this work was to study the effects exerted by the addition of microbial phytase and 1,25-$(OH)_2D_3$ and by different dietary Ca:tP ratios (1:1 and 1:2) on apparent absorption levels of Ca, P, Zn, Mn and Cu. Furthermore, the interaction of these three factors in broiler chickens was also examined.

Materials and methods

Animals and husbandry. A total of 144 chicks (ISA 220, as-hatched, supplied by a commercial breeding company) were used. The chicks were divided into six groups containing eight chicks each and the experiment was repeated three times. The chicks were housed in air-conditioned metabolic cages (33 cm × 33 cm × 40 cm) for 42 days.

Feeding. A basal diet of maize and soybean was prepared, taking into account the young age and the growth requirements of broilers (NRC, 1994). In the first 3 weeks of the experimental period, broilers were fed a starter diet and from the fourth week to the end of the sixth week they received a broiler grower diet.

A phytase enzyme preparation (Natuphos, BASF; 600 FYT/kg) was used in Groups 3 and 6, and Rovimix 1,25-$(OH)_2D_3$ (Hoffmann-La Roche Ltd.; 5 µg/kg) was used in Groups 2, 3, 5 and 6. The Ca:tP ratio of the diets was as follows: Group 1: 1:1, Group 2: 1:1 plus 1,25-$(OH)_2D_3$, Group 3: 1:1 plus 1,25-$(OH)_2D_3$ and phytase enzyme, Group 4: 2:1, Group 5: 2:1 plus 1,25-$(OH)_2D_3$, Group 6: 2:1 plus 1,25-$(OH)_2D_3$ and phytase enzyme.

Experimental procedure. The apparent absorption of minerals was measured from day 16 to 21 and from day 36 to 42 by the collection of all excreta.

Methods of analysis. All diets were chemically analysed for dry matter, crude protein, crude fibre, total fat, ash, calcium and total phosphorus (AOAC, 1984) in the laboratory of the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Istanbul. Mn, Zn and Cu were determined using a UNICAM 929 atomic absorption spectrophotometer (Slavin, 1968). Excreta were analysed after washing with a mixture of sodium nitrate and perchloric acid (Schuhknecht and Schinkel, 1963).

Statistical procedure. The comparison of the consumption levels of Ca, P, Mn, Zn and Cu was based on an analysis performed according to the SAS Institute (1990) for each test using ANOVA. When it was feasible, the average differences were separated by Duncan's multi-interval test. Specified expressions of significant difference are based on the probability of $p < 0.05$.

Results

The composition of the broiler diets is presented in Table 1. The average apparent absorption of minerals in the 3rd and 6th weeks is shown in Tables 2 and 3, respectively.

In the third week, the apparent absorption of Ca was lower in Groups 1 and 4 than in the other groups, while that of P was significantly higher in Groups 3 and 6 ($p < 0.05$). It can be seen that the apparent absorption of Mn was higher in Group 6 than in the other groups, that of Zn was higher in Groups 3 and 6, while that of Cu was significantly lower in Group 4 than in the other groups ($p < 0.05$).

In the sixth week, the apparent absorption of Ca was lower in Groups 1 and 4 than in the other groups, whereas that of P was significantly higher in Groups 3 and 6 ($p < 0.05$). The apparent absorption of Mn was higher in Group 1, that of Zn was higher in Groups 1 and 6, and that of Cu was significantly higher in Group 2 than in the other groups ($p < 0.05$).

Table 1

Composition of the experimental diets (%)

Ingredients	Starter groups						Grower groups					
	1	2	3	4	5	6	1	2	3	4	5	6
Maize	51.3	51.3	51.3	50.7	50.7	50.7	61.0	61.0	61.0	60.4	60.4	60.4
Soybean meal	41.0	41.0	41.0	40.0	40.0	40.0	31.0	31.0	31.0	30.0	30.0	30.0
Sunflower oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.8	0.8	0.8	0.8	0.8	0.8	1.0	1.0	1.0	1.0	1.0	1.0
Limestone	0.6	0.6	0.6	2.2	2.2	2.2	0.7	0.7	0.7	2.3	2.3	2.3
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vit. and min. premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phytase, FYT/kg	–	–	600	–	–	600	–	–	600	–	–	600
1,25-$(OH)_2D_3$, µg/kg	–	5.0	5.0	–	5.0	5.0	–	5.0	5.0	–	5.0	5.0
Calculated analysis												
ME, joules/kg	12.9	12.9	12.9	12.9	12.9	12.9	13.2	13.2	13.2	13.2	13.2	13.2
Analyzed values												
Dry matter	87.7	88.1	87.7	87.1	87.3	87.5	88.5	88.5	88.5	88.5	88.5	88.6
Ash	4.6	4.6	4.7	6.2	6.3	6.2	4.5	4.4	4.5	5.6	5.9	5.9
Crude protein	23.0	22.8	23.1	23.2	23.0	22.9	19.9	19.9	19.8	19.9	19.9	20.1
Total fat	7.9	6.9	7.2	6.9	7.2	7.4	7.4	7.5	7.7	7.7	7.5	7.3
Crude fibre	3.8	3.6	3.5	3.7	3.6	4.4	3.0	3.2	3.1	3.1	3.1	3.1
Nitrogen-free extract	49.1	50.8	49.2	50.1	47.2	46.6	53.6	53.5	53.3	52.2	52.1	52.2
Calcium	0.7	0.7	0.7	1.4	1.4	1.4	0.7	0.7	0.7	1.4	1.4	1.4
Total phosphorus	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7

[1]Composition of premix/kg: Vitamin A 10,000,000 IU, vitamin D_3 1,500,000 IU, vitamin E 40,000 mg, vitamin K_3 3000 mg, vitamin B_1 2200 mg, vitamin B_2 4500 mg, niacin 30,000 mg, Cal. D-Pant. 13,000 mg, vitamin B_6 3000 mg, vitamin B_{12} 15 mg, folic acid 1500 mg, biotin 100 mg, choline chloride 250,000 mg, vitamin C 12,000 mg, Mn 80,000 mg, Zn 60,000 mg, Fe 30,000 mg, Cu 5000 mg, I 1000 mg, Co 200 mg, Se 150 mg

Table 2

Effect of the addition of phytase enzyme and 1,25-$(OH)_2D_3$ on the absorption of minerals in the 3rd week (mean ± SE)

	Group 1 Ca:P 1:1	Group 2 Ca:P 1:1 + 1,25-$(OH)_2D_3$	Group 3 Ca:P 1:1 + 1,25-$(OH)_2D_3$ + Phytase	Group 4 Ca:P 2:1	Group 5 Ca:P 2:1 + 1,25-$(OH)_2D_3$	Group 6 Ca:P 2:1 + 1,25-$(OH)_2D_3$ + Phytase
Ca	$40.55^{c} \pm 1.75$	$48.70^{b} \pm 1.72$	$53.31^{ab} \pm 2.17$	$39.42^{c} \pm 1.95$	$51.49^{ab} \pm 1.78$	$56.03^{a} \pm 1.62$
P	$51.42^{d} \pm 1.87$	$57.97^{c} \pm 0.67$	$66.60^{b} \pm 0.95$	$56.61^{c} \pm 0.77$	$59.81^{c} \pm 1.58$	$70.50^{a} \pm 0.80$
Mn	$1.59^{c} \pm 0.27$	$1.50^{c} \pm 0.25$	$3.98^{b} \pm 0.35$	$0.87^{c} \pm 3.04$	$1.07^{c} \pm 2.03$	$5.51^{a} \pm 0.80$
Zn	$11.95^{c} \pm 0.46$	$13.20^{ab} \pm 0.32$	$14.44^{a} \pm 0.11$	$10.76^{c} \pm 1.22$	$12.81^{ab} \pm 0.35$	$14.38^{a} \pm 0.23$
Cu	$13.02^{a} \pm 0.16$	$13.35^{a} \pm 0.45$	$13.15^{a} \pm 9.98$	$9.45^{c} \pm 0.26$	$10.90^{b} \pm 0.34$	$12.77^{a} \pm 0.47$

$^{a, b, c}$Average values marked with different superscripts in the same line are significantly different from each other ($p < 0.05$)

Table 3

Effect of the addition of phytase enzyme and 1,25-$(OH)_2D_3$ on the absorption of minerals in the 6th week (mean ± SE)

	Group 1 Ca:P 1:1	Group 2 Ca:P 1:1 + 1,25-$(OH)_2D_3$	Group 3 Ca:P 1:1 + 1,25-$(OH)_2D_3$ + Phytase	Group 4 Ca:P 2:1	Group 5 Ca:P 2:1 + 1,25-$(OH)_2D_3$	Group 6 Ca:P 2:1 + 1,25-$(OH)_2D_3$ + Phytase
Ca	$38.47^{b} \pm 2.15$	$43.62^{a} \pm 0.87$	$45.56^{a} \pm 1.00$	$37.91^{b} \pm 2.04$	$46.29^{a} \pm 0.63$	$47.24^{a} \pm 0.66$
P	$45.55^{d} \pm 0.50$	$45.96^{d} \pm 1.86$	$64.23^{ab} \pm 2.03$	$55.94^{c} \pm 1.02$	$61.28^{b} \pm 1.33$	$66.47^{a} \pm 0.67$
Mn	$2.00^{a} \pm 5.83$	$1.84^{ab} \pm 0.11$	$1.78^{abc} \pm 7.08$	$1.50^{c} \pm 8.84$	$1.58^{bc} \pm 0.11$	$1.59^{bc} \pm 0.10$
Zn	$11.25^{a} \pm 0.52$	$9.79^{ab} \pm 0.69$	$9.73^{ab} \pm 0.28$	$8.23^{b} \pm 0.73$	$9.63^{ab} \pm 0.79$	$10.55^{a} \pm 0.76$
Cu	$9.48^{b} \pm 0.43$	$11.06^{a} \pm 0.36$	$8.91^{b} \pm 0.19$	$8.75^{b} \pm 0.65$	$9.52^{b} \pm 0.60$	$10.04^{ab} \pm 0.21$

$^{a, b, c}$Average values marked with different superscripts in the same line are significantly different from each other ($p < 0.05$)

Discussion

From the tables it can be seen that differences in the apparent absorption level of minerals among the 6 groups are statistically significant ($p < 0.05$).

Windisch and Kirchgessner (1996*a*) reported that, at constant P levels, the addition of 0, 200, 400, 600, 900 and 1000 FYT/kg phytase enzyme to broiler diets including 0.62% and 0.79% Ca increased not only the apparent absorption of P (P bound to phytates) but also the bioavailability of Cu, Zn and Mn. This limits the negative effects of unabsorbed P. Studies also show that the addition of phytase enzyme to the diet also reduces the excretion of P. Windisch and Kirchgessner (1996*b*) studied how the bioavailability of Zn, Cu and Mn was affected by the addition of phytase enzyme to broiler feed. They reported that the addition of phytase to the feed increased the apparent absorption levels of Cu, Zn and Mn. It is claimed that the necessary level of elements is surpassed by the use of phytase enzyme. However, they found that higher Ca levels decreased the apparent absorption of Zn and increased the absorption of Cu and Mn. This may be due to the interaction of Ca with the other elements. High dietary Ca content increased the intestinal pH and reduced the apparent absorption of some minerals, thus limiting the utilisation of minerals by the body (Sebastian et al., 1996*b*). Calcium is thought to be a key factor in influencing the activity of mucosal phytase in the small intestine of poultry, and it has been observed that a high Ca:tP ratio markedly decreases phytase activity.

This study shows that supplementation of the feed with phytase enzyme and 1,25-$(OH)_2D_3$ has a favourable effect on the apparent absorption of Ca and P in chicks of 0 to 3 weeks of age. This effect can also be observed in the apparent absorption of Mn, Zn and Cu. Both phytase and 1,25-$(OH)_2D_3$ also improved the apparent absorption of Ca and P between 3 to 5 weeks of age. Like other studies on phytase (Schöner et al., 1991; Broz et al., 1994; Vetési et al., 1998; Ahmad et al., 1999) and phytase plus 1,25-$(OH)_2D_3$ supplementation (Mitchell and Edwards, 1996*a*, 1996*b*; Roberson and Edwards, 1996; Qian et al., 1997), the present work also supports that supplementation of poultry feeds with phytase and 1,25-$(OH)_2D_3$ improves the apparent absorption of Ca, P, Mn and Zn, which is not influenced by the Ca:tP ratio.

Practically, the addition of phytase with 1,25-$(OH)_2D_3$ to broiler diets can decrease the need for added trace elements. These findings support the beneficial effects of using phytase with 1,25-$(OH)_2D_3$.

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