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THE EFFECTS ON TIBIA AND FAECES MINERAL LEVELS OF MICROBIAL PHYTASE AND 1,25-DIHYDROXYCHOLECALCIFEROL SUPPLEMENTATION TO BROILER CHICKEN DIETS CONTAINING DIFFERENT LEVELS OF CALCIUM

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The aim of this study was to examine the effects of adding microbial phytase and 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] to diets containing 1:1 or 1:2 proportions of Ca:total(t) P, on the levels of minerals in tibia and faeces. A total of 144 day-old male broiler chicks (ISA 220) were divided into six groups of eight chicks each. A 42-day experiment was repeated three times. The basal diet was supplemented with calcium, phosphorus, microbial phytase (0 and 600 PU/kg) and 1,25-$(OH)_2D_3$ (0 and 5 µg/kg). On the 3rd and 6th weeks of the experiment, the left tibia and faeces of the chicks were collected. In feed, tibia and faeces, the level of calcium was determined by colorimetry, phosphorus by spectrophotometry; manganese, copper and zinc using an atomic absorption spectrophotometer. The differences between the mineral levels of the groups were found to be statistically, significant ($p<0.05$). Thus, three weeks after adding phytase and 1,25-$(OH)_2D_3$ the levels of phosphorus, manganese and zinc where afected. Adding phytase and 1,25-$(OH)_2D_3$ increased the amount of phosphorus retained in the organism.

Key words: Ca:tP ratio, chicks, 1,25-$(OH)_2D_3$, phytase, tibia minerals

INTRODUCTION

In cultivated soil, the amount of organic phosphorus is 30-80% of the total phosphorus. Myoinositol hexaphosphate (phytic acid) salts and derivatives constitute 50% of the organic phosphorus. Myoinositol phosphates are commonly found in soil as a result of their low solubility, solid phase related constancy and high stability (Günter and Nelemans 1993).

The availability of phosphorus in raw materials of plant origin like soybean and corn is only approx. 30-40% because around 70% is in the form of phytic acid (Denbow *et al.* 1998). The digestion and utilization of phytate phosphorus is very low compared to other sources of phosphorus, so, inorganic phosphorus is added to diets for monogastric animals and this increases the cost of the diet (Huyghebaert 1996). Obtaining sources of phosphorus is a problem in many

countries and they make a great amount of contamination environmentally, such as in the Netherlands (Biehl and Baker, 1997; Simons *et al.,* 1990).

Microbial phytase, myoinositol hexaphosphate phosphohydrolase, can catalyse the separation of inorganic ortho-phosphate from the phytate molecule. Adding microbial phytase to rations increases the utilisation of phytate phosphorus in pigs and poultry. This leads to approx. 30% less phosphorus in the faeces, less phosphate pollution in water and soil and increased utilisation of calcium, magnesium and trace elements (Broz *et al.*, 1994, Zyla and Koreleski, 1993).

Microbial phytase has been added to diets containing small amounts of phosphorus. It was reported that, the amount of calcium in the feed affects in the availability of inorganic phosphorus (dicalcium phosphate, DCP) and phytin phosphorus, and the phytase activity (Qian *et al.*, 1996, Sebastian *et al.*, 1996b). Moreover, it was observed that the addition of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) and derivatives greatly increased the utilisation of phytate phosphorus (Mitchell and Edwards, 1996a; Qian *et al.*, 1997). For this reason, in this study the aim was to examine the effects of phytase and 1,25-$(OH)_2D_3$ on the utilisation of other minerals as well as calcium.

MATERIALS AND METHODS

Animals and diets

A total of 144-day-old male broiler chickens of ISA 220 breed obtained from a commercial breeder farm were used in this investigation. The chickens were separated into six groups containing eight chickens each and the experiment was repeated three times. The chickens were housed in metabolic cages (33 cm x 33 cm x 40 cm) heated with air-conditioning for 42 days.

A basal diet containing maize and soybean meal was prepared in accordance with the initial and later growth requirements of the broilers (NRC, 1994). A broiler starter diet was given in the first three weeks of the experiment and from the beginning of the fourth to the end of the sixth week a grower diet. Natuphos (600 PU/kg) produced by BASF was added to the ration for Group 3 and Group 6, and Rovimix 1,25-$(OH)_2D_3$ (5 μg7kg) produced by Roche was added to the feed for Group 2, Group 3, Group 5 and Group 6. The Ca:tP proportion was 1:1 for Groups 1, 2 and 3 and 2:1 for Groups 4, 5 and 6.

Experimental procedure

Clean water and feed was always provided for the birds. On the 21st and 36-42nd days of the study, the faeces were collected and weighed in aluminum foil on the same day, they were dried in an oven at 60 ^{o}C. After 48 hours, they ground in a mixer and heated in an oven at 600^{o}C to remove organic matter.

On the 21st and 42nd days of the experiment, the left tibia of the slaughtered birds were collected, and, after separation from skin and muscles, they were boiled in distilled water for three minutes to remove the remaining soft tissues. After extraction in ethanol-benzene solution, they were dried, cleaved and the fat re-

moved with petroleum ether in a Soxhlet apparatus. The bones were dried for 4-6 hours in an oven and then ashed at 600°C.

Methods of analysis

All diets were chemically analysed for content of dry matter, crude protein, crude fiber, 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. The composition of the diets and the calculated and measured contents are presented in Table 1. In the tibia, feed and faeces, calcium was determined using a colorimetric method, phosphorus using a spectrophotometer (AOAC 1984) and manganese, zinc and copper using a UNICAM 929 atomic absorption spectrophotometer (Slavin, 1968). Faeces were analysed after digestion with a mixture of sodium nitrate and perchloric acid (Schuhknecht and Schinkel, 1963).

Statistical analysis

The data obtained for phosphorus, calcium, manganese, zinc and copper levels in tibia and faeces were analysed by SAS (1990) with a General Linear Models procedure for ANOVA, for each experiment. Differences between means were analysed with Duncan' s multiple gaps test. The significant difference statements were based on the possibility $p<0.05$, unless explained in another way.

RESULTS

At 21 days old, the mean live weights of the birds were found to be significantly higher in Group 1, Group 2 and Group 3 than those in the other groups ($p<0.05$) Table 2. Tibia weight was significantly lower in control Group 4 than in the other groups ($p<0.05$). Phosphorus content in Group 3 and Group 6 fed phytase (19.98%, 12.19%), and copper content in Group 2 and Group 3 (6.14 ppm, 5.84 ppm) were higher than for the other groups ($p<0.05$). No significant differences between the levels of crude ash, calcium, manganese and zinc in tibia were observed ($p>0.05$). In the 6th week of the investigation, the mean live weight of the birds in Group 3 was significantly higher than for the others ($p<0.05$) Table 3. Tibia weight was significantly lower in Group 4 than for the others ($p<0.05$). Phosphorus content in Group 3 (12.39%), copper in Group 4 and manganese in Group 6 were found to be higher than the respective mean values for the other groups ($p<0.05$). No significant differences between the mean levels of crude ash, calcium and zinc in tibia were observed between the groups ($p<0.05$).

At 21 days old, faeces dry matter in Group 2 (48.7%) and the crude ash value in Group 5 (17.64%) were found to be significantly higher than in the others, (Table 4). Faecal calcium in Group 4, Group 5 and Group 6 (24.07%, 21.93%, 19.88%), phosphorus in Group 2 (4.50%), manganese in Group 2 (1053.45 ppm), zinc in Group 2 and Group 3 (1310.15 ppm, 1312.33 ppm), and copper in Group 1, Group 2 and Group 3 (445.47 ppm, 437.63 ppm, 433.36 ppm) were also higher than in the other groups respectively. No significant difference between the

Table 1.Composition of the experimental diets (%)

	Starter groups						Grower groups					
Ingredients	1	2	3	4	5	6	1	2	3	4	5	6
Maize	51.30	51.30	51.30	50.70	50.70	50.70	61.00	61.00	61.00	60.40	60.40	60.40
Soybean meal	41.00	41.00	41.00	40.00	40.00	40.00	31.00	31.00	31.00	30.00	30.00	30.00
Sunflower oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.80	0.80	0.80	0.80	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.60	0.60	0.60	2.20	2.20	2.20	0.70	0.70	0.70	2.30	2.30	2.30
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vit. and min. premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase, PU/kg	–	–	600	–	–	600	–	–	600	–	–	600
1.25-$(OH)_2D_3$, μg/kg	–	5.00	5.00	–	5.00	5.00	–	5.00	5.00	–	5.00	5.00
Analysed values												
Dry matter	87.70	88.11	87.69	87.14	87.31	87.48	88.47	88.59	88.53	88.54	88.58	88.60
Ash	4.62	4.60	4.70	6.22	6.28	6.22	4.48	4.45	4.53	5.63	5.94	5.92
Crude protein	23.03	22.84	23.08	23.20	23.04	22.94	19.91	19.94	19.78	19.95	19.98	20.08
Total fat	7.18	6.91	7.20	6.87	7.16	7.40	7.42	7.49	7.74	7.71	7.50	7.31
Crude fiber	3.78	3.59	3.47	3.68	3.65	4.36	3.04	3.24	3.14	3.06	3.05	3.10
Calcium	0.72	0.73	0.71	1.38	1.40	1.41	0.70	0.68	0.69	1.39	1.40	1.41
Total phosphorus	0.73	0.74	0.72	0.73	0.71	0.72	0.72	0.70	0.70	0.70	0.71	0.70
Zinc, ppm	33.20	32.86	32.11	33.40	33.19	31.44	37.12	37.71	35.06	36.07	35.14	35.66
Manganese, ppm	49.37	46.16	41.45	50.26	50.65	48.53	50.19	52.13	51.85	51.90	50.79	52.48
Copper, ppm	11.65	10.81	10.12	11.49	10.36	10.92	23.61	20.50	20.47	24.46	22.94	21.14
Calculated analysis												
Metabolizable energy, MJ/kg	12.99	12.98	12.97	12.98	12.99	12.96	13.20	13.25	13.24	13.22	13.22	13.21
Nitrogen free extract	49.09	50.17	49.24	50.14	47.18	46.56	53.62	53.47	53.34	52.19	52.11	52.19

[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 and 1,25-$(OH)_2D_3$ on body weight gain and tibia minerals in the 3rd week

	Group 1– control		Group 2 + D_3		Group 3 + D_3		Group 4–control		Group 5 + D_3		Group 6 + D_3 and phytase	
	x	SE	x	SE	x	SE	x	SE	x	SE	x	SE
Weight gain, g	594.20^{a}	24.12	595.40^{a}	28.90	606.80^{a}	22.43	426.40^{b}	8.72	438.40^{b}	47.81	466.60^{b}	20.52
Tibia gain, g	1.41^{a}	8.04	1.33^{a}	9.01	1.22^{ab}	8.61	0.91^{c}	3.50	1.01^{bc}	9.31	1.24^{ab}	0.10
Crude fiber, %	41.55	1.92	40.65	1.08	40.66	1.17	42.99	0.66	41.97	1.23	43.75	1.05
Ca, %	40.72	0.61	41.06	1.98	41.21	1.31	39.90	0.72	41.33	1.16	38.65	0.94
P, %	10.98^{bc}	0.43	10.25^{bc}	0.30	19.98^{a}	1.60	8.87^{c}	0.47	10.23^{bc}	0.94	12.19^{b}	0.82
Mn, ppm	4.46	0.37	5.30	0.86	5.81	0.15	5.55	0.58	5.84	0.34	5.96	0.18
Zn, ppm	554.97	22.72	583.08	39.44	594.38	15.61	570.83	6.65	567.13	15.29	552.49	11.01
Cu, ppm	3.14^{b}	0.38	6.14^{a}	1.08	5.84^{a}	0.79	3.57^{b}	0.36	3.84^{b}	0.62	2.51^{b}	0.30

a,b, cAverage 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 and 1,25-$(OH)_2D_3$ on bodi weight gain and tibia minerals in the 6th week

	Group 1–control		Group 2 + D_3		Group 3 + D_3 and phytase		Group 4–control		Group 5 + D_3		Group 6 + D_3 and phytase	
	x	SE	x	SE	x	SE	x	SE	x	SE	x	SE
Weight gain, g	1770.00^{ab}	115.76	1798.00^{ab}	92.27	1900.00^{a}	52.44	1561.20^{b}	55.82	1548.80^{b}	103.06	1588.00^{b}	78.13
Tibia gain, g	4.90^{a}	0.35	4.52^{ab}	0.45	5.02^{a}	0.15	3.39^{c}	0.13	3.77^{bc}	0.25	4.00^{bc}	0.18
Crude fiber, %	41.81	2.81	43.59	1.98	40.70	2.15	43.45	1.29	40.62	0.65	41.04	2.04
Ca, %	52.56	2.03	54.57	4.67	47.03	5.62	47.63	5.08	55.66	4.77	58.08	3.64
P, %	11.04^{ab}	0.34	10.99^{ab}	0.80	12.39^{a}	1.03	9.45^{b}	0.81	10.47^{ab}	0.41	11.20^{ab}	0.99
Mn, ppm	4.97^{b}	0.95	3.71^{c}	0.34	4.22^{c}	0.60	4.68^{b}	0.47	6.25^{ab}	0.63	7.13^{a}	0.45
Zn, ppm	315.47	29.03	280.83	15.53	285.64	10.68	289.65	16.15	309.55	8.84	289.05	12.28
Cu, ppm	3.31^{ab}	0.50	2.14^{b}	0.43	2.27^{b}	0.17	4.08^{a}	1.03	3.13^{ab}	0.16	2.05^{b}	0.36

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

Table 4. Effect of the addition of phytase and 1,25-$(OH)_2D_3$ on faeces minerals in the 3rd week

	Group 1–control		Group 2 + D_3		Group 3 + D_3 and phytase		Group 4–control		Group 5 + D_3		Group 6 + D_3 and phytase	
	x	SE	x	SE	x	SE	x	SE	x	SE	x	SE
Dry matter, %	32.07^{b}	2.62	48.70^{a}	6.27	36.80^{b}	1.01	31.41^{b}	2.24	28.69^{b}	2.47	29.18^{b}	3.54
Crude ash, %	13.08^{c}	0.16	12.59^{c}	0.31	13.25^{c}	0.28	16.34^{b}	0.30	17.64^{a}	0.44	16.70^{ab}	0.66
Ca, %	11.37^{b}	0.42	11.89^{b}	0.26	9.83^{b}	0.35	24.07^{a}	4.38	21.93^{a}	2.24	19.88^{a}	1.07
P, %	3.68^{b}	0.26	4.50^{a}	0.46	3.49^{b}	0.18	3.24^{b}	0.21	3.49^{b}	0.24	3.14^{b}	9.69
Mn, ppm	995.53^{ab}	32.12	1053.45^{a}	28.59	914.75^{bc}	19.24	728.07^{d}	24.85	892.09^{c}	15.50	913.62^{bc}	34.99
Zn, ppm	1166.58^{b}	44.78	1310.15^{a}	31.32	1312.33^{a}	21.09	930.09^{c}	22.43	944.62^{c}	25.52	983.19^{c}	14.90
Cu, ppm	445.47^{a}	5.67	437.63^{a}	15.39	433.36^{a}	3.25	278.93^{c}	8.05	292.15^{c}	9.15	321.21^{b}	11.98

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

Table 5. Effect of the addition of phytase and 1,25-$(OH)_2D_3$ faeces on minerals in the 6th week

	Group 1–control		Group 2 + D_3		Group 3 + D_3 and phytase		Group 4–control		Group 5 + D_3		Group 6 + D_3 and phytase	
	x	SE	x	SE	x	SE	x	SE	x	SE	x	SE
Dry matter, %	34.83	1.95	33.59	2.02	34.74	4.58	25.68	4.79	25.62	1.64	28.66	2.78
Crude ash, %	15.10^{c}	0.28	15.22^{c}	0.30	15.13^{c}	0.42	19.05^{b}	0.46	20.96^{a}	0.69	19.59^{ab}	0.81
Ca, %	17.43^{b}	2.69	14.79^{b}	0.66	15.96^{b}	0.70	23.61^{a}	0.69	22.84^{a}	0.59	24.55^{a}	1.34
P, %	5.30^{a}	0.13	5.37^{a}	0.26	4.82^{a}	0.25	4.24^{b}	0.19	3.47^{c}	0.16	3.72^{bc}	0.13
Mn, ppm	994.96^{a}	29.06	1029.46^{a}	58.62	1000.93^{a}	32.59	820.55^{b}	0.49	796.05^{b}	59.04	773.65^{b}	49.25
Zn, ppm	1107.08^{a}	37.07	1069.22^{a}	34.93	1087.37^{a}	31.43	854.95^{b}	75.99	826.11^{b}	56.69	837.81^{b}	60.14
Cu, ppm	397.93^{a}	3.20	414.26^{a}	11.22	409.12^{a}	11.20	312.78^{b}	10.61	304.94^{b}	11.07	300.55^{b}	5.74

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

groups for mean dry matter in faeces was observed ($p<0.05$) in the 6th week of the experiment (Table 5). Faeces crude ash content was significantly lower in Group 5 (20.96%) ($p<0.05$). Faecal calcium in Group 4, Group 5 and Group 6 (23.61%, 22.84%, 24.55%), phosphorus in Group 1, Group 2 and Group 3 (5.30%, 5.37%, 4.82%), manganese in Group 1, Group 2 and Group 3 (994.96 ppm, 1029.46 ppm, 1000.93 ppm), zinc in Group 1, Group 2 and Group 3 (1107.08 ppm, 1069.22 ppm, 1087.37 ppm), and copper in Group 1, Group 2 and Group 3 (397.93 ppm, 414.26 ppm, 409.12 ppm) were higher than in the other groups respectively ($p<0.05$).

DISCUSSION

The effect on tibia mineral matter content

The amount of tibia ash in broilers fed on low phosphorus diets is affected by 1,25-$(OH)_2D_3$ and phytase suplements. Thus, Mitchell and Edwards (1996b) reported that, the addition of phytase and 1,25-$(OH)_2D_3$ to 0.45% to diets containing phosphorus increased the tibia ash by 45.86 % while addition of phytase and 1,25-$(OH)_2D_3$ to diets containing 0.55% phosphorus increased the tibia ash by 13.40%. Thus, the effect of adding phytase enzyme and 1,25-$(OH)_2D_3$ decreased when the dietary phosphorus level was within the required limit. The fact that the diets used here contained an adequate amount of phosphorus could be the reason why the tibia ash did not increase with the dietary addition of phytase and 1,25-$(OH)_2D_3$. Qian *et al.* (1996) found that phosphorus and calcium accumulation were high, when the Ca: tP proportion was 1.1/1. The best result was obtained when the dietary Ca: tP proportion was fixed to 1.1-1.4/1 and phytase and 1,25-$(OH)_2D_3$, added. Sebastian *et al*. (1996a) have also reported that low phosphorus diets decreased the ash content of tibia. Ahmad *et al*. (2000) showed that microbial phytase improved bone mineralization in broiler chickens, which confirmed earlier studies on phosphorus availability by Simons *et al.* (1990). The increases in the tibia ash of the chickens fed with the phytase supplements were the result of phytase activity. Huyghebaert (1996) explained the differences between tibia and toe ash; at high phosphorus concentrations, the amount of ash increased only in tibia and the effect of the microbial phytase was more evident in the toe ash. However, no biological explanation was made about these two statements. An increase was not reported by Mitchell and Edwards (1996b) that could explain the interaction of phosphorus and phytase in the bone ash at 0.63% or more phosphorus levels. Calcium, phosphorus and phytase have significant interrelationships, which makes it hard to make comments about the data for tibia ash.

Being a strong acid, phytate can decrease the solubility and absorbability of many minerals and form salts with them. When phytate is hydrolysed with phytase, the minerals are released, together with myoinositol and inorganic phosphate. At low calcium levels, the increase in phosphorus retention with added phytase points to a decrease in calcium–phytate complexes; for this reason, it was suggested that more phytate molecules are exposed to phytate hydrolysis (Sebastian *et al.,* 1996b). Broz *et al.,* (1994) reported that adding phytase to low cal-

cium diets for broiler chickens with no inorganic calcium supplement made no difference in the tibia ash calcium concentration. Schöner *et al.* (1993) observed that the increase in calcium retention in broilers fed with high calcium diets decreased with added phytase. Qian *et al.* (1997) showed that, in corn- soybean diets a supplement of 1,25-$(OH)_2D_3$ increased calcium retention whether phytase was present or not. It was found that phytic acid favoured the utilisation of phytate calcium, which might have depended on the reduction of phytic acid with 1,25-$(OH)_2D_3$. Our observations support these findings. Feeding with high levels of calcium decreased the availability of phytate phosphorus. More research is needed to determine the best calcium level in broiler diets containing additional phytase and 1,25-$(OH)_2D_3$.

In this study some differences between the mean copper levels in tibia ash in the 3rd week and between manganese and copper levels in the 6th week were found to be statistically significant ($p<0.05$). No significant differences between the mean zinc levels of the groups were observed. On the basis of present knowledge we cannot explain why the addition of phytase did not increase the retention of manganese and zinc, but these observations partly agree with other published data that show that there is no effect of phytase supplements on zinc retention in broiler chicks (Roberson and Edwards 1996). Sebastian *et al.* (1996a) showed that, no matter what the calcium level was, dietary phytase had no effect on the relative retention of manganese and zinc. It was reported that the absorption of manganese from corn- soybean diets containing high levels of calcium was reduced without phytase or 1,25-$(OH)_2D_3$, because manganese- phytate bonding was increased and the solubility of managnese in the intestine was reduced (Halpin *et al.,* 1986). Roberson and Edwards (1996) showed that 1,25-$(OH)_2D_3$ is more effective than phytase for increasing the utilisation of zinc in chicks. Aoyagi and Baker (1993) observed that the addition of microbial phytase decreased the availability of copper in chickens to 50 %, but increased the relative retention of zinc to 62.3 %. The increase in zinc concentration favoured the intestinal synthesis of metallothionein from cysteine. This metalloprotein binds zinc, copper and other divalent cations. Copper binds to metallothionein more tightly than zinc, so metallothionein acts as a negative regulator of copper absorption.

The effect on faeces mineral matter

At the 3rd week of this study, the excretion of phosphorus increased the faeces dry matter. Therefore, as high dietary calcium concentrations increase the consumption and discharge of phosphorus, evidently they also increase the faeces dry matter ($p<0.05$). Shafey *et al.* (1990) also reported that high dietary calcium concentrations, phosphorus and/or 1,25-$(OH)_2D_3$ increased the discharge of phosphorus and faeces dry matter. Our results confirmed these findings. At both examined time intervals, the differences between mean calcium and phosphorus levels were statistically significant ($p<0.05$) (Table 4, 5). Mitchell and Edwards (1996a) examined a calcium proportion of 1% at different phosphorus levels, with the addition of 5 g 1,25-$(OH)_2D_3$ and 600 FYT phytase enzyme. Phytase independetly decreased the faeces phosphorus content in broiler chicks. In addition to the effect of phytase and a smaller effect of 1,25-$(OH)_2D_3$, faecal phospho-

rus was influenced by the dietary phosphorus. It was observed that faecal phosphorus increased in direct proportion to the dietary phosphorus level. In our study the addition of phytase also improved the utilisation of phosphorus and decreased the amount of phosphorus in the faeces.

There were significant differences between the faeces manganese levels of the groups in both the 3rd and 6th weeks of the study ($p<0.05$). However, phytase did not affect the excretion of manganese in the faeces at the 3rd week, while at the 6th week the increase in dietary calcium decreased the excretion of manganese in the faeces in all three groups.

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EFEKTI MIKROBIJALNE FITAZE I 1,25-DIHIDROKSIHOLEKALCIFEROLA NA NIVO MINERALA U TIBIJI I FECESU BROJLERA HRANJENIH OBROCIMA SA RAZLIČITIM KONCENTRACIJAMA KALCIJUMA

BILAL T i HILKAT AKSAKAL D

SADRŽAJ

Cilj ove studije je bio ispitivanje efekata enzima mikrobijalne fitaze i 1,25 dihidroksiholekalciferola [1,25-$(OH)_2 D_3$] dodatih u hranu za brojlere, koja sadrži kalcijum i fosfor u odnosima 1:1 ili 1:2, na nivo minerala u tibiji i fecesu. U ogledu su korišćeni mužjaci brojlera (ISA 220), stari 144 dana podeljeni u 6 grupa, od po osam jedinki. Eksperiment je trajao 42 dana i ponovljen je pod istim uslovima tri puta. Osnovnoj smeši za brojlere su dodavani kalcijum, fosfor, mikrobijalna fitaza (0 i 600 PU/kg) i 1,25-$(OH)_2 D_3$ (0 i 5 mg/kg). Leva tibija i feces pilića su uzorkovani 3. i 6. nedelje eksperimenta. Nivo kalcijuma u hrani, tibiji i fecesu određivan je spektrometrijskim postupkom. Razlike u nivou minerala između pojedinih grupa su bile statistčki značajne ($p<0.05$). Treće nedelje istraživanja je utvrđeno da suplementacija fitazom i 1,25-$(OH)_2 D_3$ ima uticaja na nivo fosfora, magnezijuma i cinka. Uočeno je da fitaza i 1,25-$(OH)_2 D_3$ smanjuju količinu fosfora u organizmu.