Retention of cadmium in the tissues of broiler chicks by dietary supplemental microbial phytase

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ABSTRACT: The objective of this study was to investigate the effect of Ca : total(t) P ratio, vitamin C and microbial phytase on broiler performance and cadmium retention of broiler. In experiment, 288 day-old male broiler chicks (Cobb) were randomly assigned to 12 treatment groups, 3 replicates of 8 chicks each. The study was carried out for 42 days. The basal diet supplemented calcium, phosphorus, cadmium (0.5 and 5 mg/kg), zinc (20 mg/kg), vitamin C (0 and 1 g/kg) and microbial phytase (0 and 600 PU/kg feed). Differences among diets fed to individual experimental groups affect either body weight gain or feed intake and conversion after the 3 weeks and at the end of the experiment ($P < 0.05$). Cadmium and microbial phytase supplement to diet caused a significant increase of cadmium concentration in the tissues examined. There were significant differences in tissues concentrations of cadmium ($P < 0.05$) among the groups fed diets supplemented. In conclusion, addition of 600 PU feed of phytase per kg of diet compensates this effect and lowers the cadmium burden by up to 60%.

Keywords: chicks; cadmium; Ca : P ratio; liver; kidney; phytase; performance; vitamin C; tibia

After numerous examples of alteration of cadmium uptake due to dietary composition behavior of cadmium at various steps of the human food chain became important. If supplemental intakes may be protect cadmium toxicity can be enhanced by a deficiency of certain nutrients. Many examples of effects of essential minerals and dietary components on cadmium toxicity and cadmium carryover have been reviewed (Fox, 1974).

Phytic acid acts as an antinutritive factor in monogastrics due to its chelation of mono and divalent trace minerals such as Cu, Zn, Mn, Mg, Fe, Ca and K (Aoyagi and David, 1995; Yi *et al*., 1996). Microbial phytase as a dietary additive was firstly evaluated by Nelson *et al*., (1971). In previous experiments it has been demonstrated that a diet rich in phytate or supplemented with microbial phytase considerably improves the utilization of phosphorus, calcium and zinc in chickens (Schoner *et al*., 1991; Aoyagi and David, 1995; Sebastian *et al*., 1996; Yi *et al*., 1996; Aksakal and Bilal, 2002).

The objective of this experiment was to investigate the effect of Ca : total(t) P ratio, vitamin C and microbial phytase on broiler performance and cadmium retention in kidney, liver and tibia of broiler fed corn-soybean meal diets.

MATERIAL AND METHODS

Animals and experimental design

In this research, 288 day-old male broiler chicks (Cobb) were purchased from a local hatchery. The chicks were separated into twelve groups (A, B, C, D, E, F, G, H, I, J, K, L). There were three replicates with eight chicks for each dietary treatment and placed individually in metabolic cages (33 cm × 33 cm × 40 cm). The broilers were kept under continuous lighting and warm room heating with temperatures maintained at approximately 31°C for the 1st wk, 29°C for the 2nd wk, 27°C for the 3rd wk, and 24°C from 4th wk to the end of the experiment. Each cage was equipped with nipple drinkers and tube feeder.

Two basal diets were formulated for the requirements as established by the NRC (1994). The broilers

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were fed starter diet to 3 wk of age and a grower diet to 6 wk of age. The composition of diets, calculated and analysed contents of nutrients are presented in Table 1. The basal diets supplemented with calcium, phosphorus, cadmium, zinc, vitamin C (K and L groups) and microbial phytase. Table 2 shows cadmium concentrations of experimental diets. To have 600 PU/kg diet phytase in the diets (B, D, F, H, J, L groups) 120 mg Natuphos®-BASF (*Aspergillus niger*, containing 5 000 PU/g phytase activity) was used. For zinc, zinc acetate has added to diet (all groups) 67.11 mg/kg since it contains 35.64% zinc. Food in mash form and drinking water were provided *ad libitum*.

In experiment, individual body weights of chickens were recorded on days 1, 21, and 42. Feed intake was determined per replicate group (days 0 to 21 and 0 to 42). Feed conversion ratio (FCR) was calculated as feed consumed per unit of gain. In experiment, four chicks from each treatment (288 chicks, total) were killed by cervical dislocation at 21 d of age and 42 d of age, and left tibia, liver, and kidney were removed and frozen for subsequent mineral analysis. The left tibia of each bird was removed and cleaned from adhering tissue. The percentage of tibia ash was determined according to the AOAC methods (1984). Tibia samples were dried to a constant weight at 100°C and then ash in a muffle furnace at 600°C for 4 hours. Tibia, liver and kidney were analysed after washing with a mixture of sodium nitrate and perchloric acid.

Methods of analysis

All diets samples were chemically analysed for the dry matter content, the crude protein, calcium and total phosphorus (AOAC, 1984), the laboratory of Animal Nutritional Diseases Department of the Faculty of Veterinary Medicine, University of Istanbul. Cadmium concentrations in diets, liver, kidney, and tibia ash were analysed with an atomic absorption spectrophotometer (Varianspectra AA, 220 fast sequential), the laboratory of Chemistry

Table 1. Composition of experimental diets

Groups	A, B, E, F, K, L		C, D, G, H		I, J	
Ingredients (%)	Starter	Grower	Starter	Grower	Starter	Grower
Corn	49.71	59.50	50.09	59.89	50.48	60.29
Soybean meal	40.00	30.00	40.0	30.0	40.00	30.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Calcium carbonate	0.74	0.92	1.07	1.24	0.68	0.84
Monocalcium phosphate	1.66	1.69	0.95	0.98	0.95	0.98
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	0.07	0.07	0.07	0.07	0.07	0.07
DL-Methionine	0.21	0.21	0.21	0.21	0.21	0.21
Corn starch	1.66	1.66	1.66	1.66	1.66	1.66
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME (joule/kg)	12.95	12.95	12.95	13.16	13.16	13.16
Analyzed values (g/kg)						
Dry matter	888.0	891.5	892.0	888.5	890.0	889.0
Crude protein	199.5	201.5	230.0	199.0	229.5	198.5
Calcium	8.8	8.9	9.1	8.7	7.8	7.4
Total phosphorus	6.6	6.6	5.1	4.8	4.9	5.1

[1]Composition of premix/kg: vitamin A, 1 204 mg; cholecalciferol, 25 mg; vitamin E, 4.5 mg; riboflavin, 2.25 mg; niacin, 15.0 mg; d-pantothenic acid, 4.0 mg; folic acid, 25 mg; vitamin B_{12}, 5 mg; choline chloride, 200 mg; thiamine, 0.5 mg; biotin, 25 mg; ethoxyquin, 12.5 mg; menadione sodium bisulfite, 1.25 mg; pridoxine, 50 mg; manganese, 24.9 mg; zinc, 22 mg; iodine, 2 mg; iron,13.6 mg; and copper, 1.6 mg

Table 2. Cadmium concentrations in experimental diets

Groups	A, B, C, D		E, F, G, H, I, J, K, L,	
	Starter	Grower	Starter	Grower
Supplemental $CdCl_2$ (mg/kg)	0.91		9.1	
Cadmium (mg/kg)	0.52	0.49	5.25	5.36

Department of the Faculty of Engineering, University of Istanbul (AOAC, 1984).

Results are presented as means with their standard errors. All data were analysed by analysis of variance. Differences between means were analysed using the Tukey test. The calculations were performed using SPSS (1999) program.

RESULTS

Initial body weight, at days 21 and 42, weight gain, feed intake and FCR data are presented in Table 3. The differences between groups of body weight gain values were significant at days 21 and 42 except for initial body weight values ($P < 0.05$). Body weight gains were significantly higher in B, F, L groups (508, 519, 532) given microbial phytase at day 21 and in B, L groups (1 757, 1 783) at day 42 than other groups. Feed intakes were significantly higher in F, L groups (955, 970) at day 21 and in L group (3 100) at day 42 than other groups ($P < 0.05$). FCR in B, F, L groups (respectively, 1.80, 1.84, 1.82) were better than C, G, H groups (1.97, 1.98, 1.98) at day 21 and in B, F, K, L groups (1.70, 1.76, 1.77, 1.73) were better than C, D, G, H, I groups (1.89, 1.82, 1.91, 1.87, 1.88) at day 42 ($P < 0.05$).

Cadmium and microbial phytase supplement to diet caused a significant increase of cadmium concentration in the tissues examined (liver, kidney, tibia, see Table 4). There were significant differences in tissues concentrations of cadmium ($P < 0.05$) among the groups fed diets supplemented. Tibia ash amounts were significantly higher in B, E, F, L groups (47, 46, 47, 47) at day 21 and in F group (48) at day 42 than other groups ($P < 0.05$). Tibia cadmium amounts were significantly higher in G, I groups (208, 210 and 194, 185) at days 21 and 42 than other groups ($P < 0.05$). Liver cadmium amounts were significantly higher in G group (2 613 and 2 666) at days 21 and 42 than other groups ($P < 0.05$). Kidney cadmium amounts were significantly higher in G group (2 939 and 2 956) at days 21 and 42 than other groups ($P < 0.05$).

DISCUSSION

The results of research show us that adding cadmium to broilers diet effects their performance in a negative way also in addition to this low level of phosphorus in diet effects the situation in negative way. But by addition of phytase to the diet effects weight gain, food intake and feed conversion in a positive way its proved that. Addition of vitamin C with phytase to diet takes performance to accepted level. Korol *et al*. (1995) phytase add effects the performance in a positive way. During the research weight gain is obtained in order 1 653, 2 025, 2 168 g and feed conversion is informed in order as 2.14, 1.89, 1.78. Dietary microbial phytase supplementation in broiler chicks is also common in the literature. Aksakal and Bilal (2002) found that dietary supplement of 600 PU/kg microbial phytase, resulted in higher performance broilers. Zobac *et al.* (1995) reported that the addition of enzymatic preparation Natuphos significantly positively affected body weight gain and tibia Ca and P level. Sebastian *et al*. (1996) showed that addition phytase to the corn-soy bean diet including various levels of Ca, resulting in increases in feed intake and body weight gain in broilers.

Korol *et al*. (1995) found that cadmium concentrations in the tissues were 0.043, 0.044 and 0.056 mg/kg. According to Guillot (1995), phytase did not increase cadmium retention, but decreased cadmium in liver and kidneys. This seems to be supported by the significant effects in some organs and tissues on supplementary cadmium in this study as similar to Bokori *et al*. (1996). In many researches (Rambeck and Kollmer, 1990; Rambeck and Guillot, 1996; Rambeck *et al*., 1996) which has made with nearly same big ions of calcium and cadmium its informed that deficiency of calcium higher the retention of cadmium. And also unsuitable rate of Ca : tP, makes a negative effect in the situations that calcium in take is not enough the own effect of phosphor has investigated in few research. In quails when phosphorus is lowered from 10 to 6 g/kg, the accumulation of cadmium in

Table 3. The effect of phytase, zinc, vitamin C and cadmium supplementation of corn-soybean diets on the broiler performance (n = 12, means ± SE)

Groups	Dietary Treatment						Initial body weight (g)	0 to 21 d			0 to 42 d		
	Phytase	Ca	P	Zn	Vit. C	Cd		Feed intake (g)	Weight gain (g)	FCR	Feed intake (g)	Weight gain (g)	FCR
	PU/kg	g/kg	g/kg	ppm	g/kg	ppm							
A	0	9	6.5	20	0	0.5	47 ± 1.09	790 ± 10.21^{cd}	417 ± 20.67^{bc}	1.89 ± 4.37^{cd}	2 575 ± 81.40^{bcd}	1 431 ± 27.87^{c}	1.79 ± 1.20^{cde}
B	600	9	6.5	20	0	0.5	46 ± 0.91	915 ± 23.42^{ab}	508 ± 17.19^{a}	1.80 ± 1.77^{e}	2 990 ± 95.41^{ab}	1 757 ± 52.15^{a}	1.70 ± 2.85^{g}
C	0	9	5	20	0	0.5	47 ± 1.25	620 ± 21.70^{e}	315 ± 12.11^{de}	1.97 ± 2.61^{a}	1 770 ± 210.73^{e}	931 ± 50.00^{d}	1.89 ± 1.35^{a}
D	600	9	5	20	0	0.5	46 ± 0.88	825 ± 17.55^{c}	434 ± 18.06^{bc}	1.91 ± 3.02^{abc}	2 580 ± 82.29^{bcd}	1 412 ± 28.01^{c}	1.82 ± 2.95^{bc}
E	0	9	6.5	20	0	5	46 ± 0.81	780 ± 38.31^{cd}	415 ± 20.21^{bc}	1.87 ± 1.13^{cde}	2 620 ± 99.81^{bcd}	1 473 ± 28.61^{bc}	1.81 ± 1.20^{bcd}
F	600	9	6.5	20	0	5	46 ± 0.73	955 ± 27.50^{a}	519 ± 17.96^{a}	1.84 ± 1.93^{cde}	2 960 ± 144.61^{abc}	1 680 ± 42.43^{ab}	1.76 ± 4.59^{ef}
G	0	9	5	20	0	5	46 ± 1.22	575 ± 43.00^{e}	291 ± 24.24^{e}	1.98 ± 8.12^{a}	1 710 ± 136.51^{e}	892 ± 35.55^{d}	1.91 ± 1.10^{a}
H	600	9	5	20	0	5	47 ± 0.91	785 ± 30.30^{cd}	397 ± 20.55^{c}	1.98 ± 2.82^{a}	2 420 ± 170.74^{d}	1 314 ± 69.50^{c}	1.84 ± 1.87^{b}
I	0	7.5	5	20	0	5	46 ± 1.41	735 ± 18.74^{d}	350 ± 20.82^{d}	1.91 ± 2.58^{abc}	1 960 ± 199.52^{e}	1 042 ± 54.55^{d}	1.88 ± 1.47^{a}
J	600	7.5	5	20	0	5	46 ± 1.50	785 ± 19.12^{cd}	421 ± 17.62^{bc}	1.87 ± 3.03^{cde}	2 610 ± 115.70^{bcd}	1 444 ± 28.71^{c}	1.80 ± 1.29^{bcde}
K	0	9	6.5	20	1	5	47 ± 1.71	850 ± 11.00^{bc}	449 ± 10.87^{b}	1.89 ± 2.12^{bcd}	2 550 ± 119.12^{cd}	1 434 ± 36.97^{c}	1.77 ± 1.44^{def}
L	600	9	6.5	20	1	5	46 ± 0.74	970 ± 10.84^{a}	532 ± 11.80^{a}	1.82 ± 2.42^{de}	3 100 ± 35.82^{a}	1 783 ± 25.46^{a}	1.73 ± 3.68^{fg}

$^{a–g}$Means each colum with no common superscript differ significantly ($P < 0.05$)

Table 4. The effect of phytase, zinc, vitamin C and cadmium supplementation of corn-soybean diets on the tibial bone content of ash, and cadmium retention in kidney, liver and tibia of broiler chickens (n = 12, means ± SE)

Groups	Dietary Treatment						21 d				42 d			
	phytase	Ca	P	Zn	vit. C	Cd	Tibia		Liver	Kidney	Tibia		Liver	Kidney
	PU/kg	g/kg	g/kg	Ppm	g/kg	ppm	% ash	Cd (ng/g)	Cd (ng/g)	Cd (ng/g)	% ash	Cd (ng/g)	Cd (ng/g)	Cd (ng/g)
A	0	9	6.5	20	0	0.5	46 ± 0.58^{ab}	19 ± 2.64^{cd}	243 ± 15.37^{e}	400 ± 7.63^{e}	46 ± 0.85^{abc}	14 ± 0.30^{d}	272 ± 14.70^{e}	408 ± 6.06^{e}
B	600	9	6.5	20	0	0.5	47 ± 0.33^{a}	6 ± 1.85^{d}	98 ± 8.51^{f}	157 ± 6.22^{f}	47 ± 0.34^{ab}	6 ± 0.50^{d}	103 ± 2.60^{f}	159 ± 13.17^{f}
C	0	9	5	20	0	0.5	43 ± 0.58^{cd}	32 ± 2.08^{c}	406 ± 9.20^{d}	595 ± 5.51^{d}	43 ± 0.54^{d}	25 ± 2.30^{cd}	394 ± 7.00^{d}	571 ± 15.13^{d}
D	600	9	5	20	0	0.5	44 ± 0.33^{abc}	15 ± 1.73^{cd}	157 ± 9.30^{ef}	371 ± 8.71^{e}	45 ± 0.30^{bcd}	12 ± 0.50^{cd}	153 ± 18.04^{f}	400 ± 14.43^{e}
E	0	9	6.5	20	0	5	46 ± 0.88^{a}	83 ± 4.90^{b}	$1\,052 \pm 18.94^{c}$	$1\,544 \pm 12.85^{c}$	46 ± 0.31^{abc}	80 ± 0.80^{b}	$1\,035 \pm 15.27^{c}$	$1\,545 \pm 15.37^{c}$
F	600	9	6.5	20	0	5	47 ± 0.33^{a}	34 ± 2.60^{c}	391 ± 18.27^{d}	612 ± 11.56^{d}	48 ± 0.33^{a}	28 ± 1.50^{c}	387 ± 7.23^{d}	588 ± 23.33^{d}
G	0	9	5	20	0	5	42 ± 0.57^{de}	208 ± 6.30^{a}	$2\,613 \pm 13.31^{a}$	$2\,939 \pm 33.54^{a}$	44 ± 0.32^{c}	194 ± 4.30^{a}	$2\,666 \pm 62.64^{a}$	$2\,956 \pm 41.06^{a}$
H	600	9	5	20	0	5	44 ± 0.31^{abc}	83 ± 2.70^{b}	$1\,035 \pm 7.79^{c}$	$1\,575 \pm 8.83^{c}$	45 ± 0.33^{bcd}	75 ± 2.60^{b}	$1\,020 \pm 13.22^{c}$	$1\,552 \pm 17.39^{c}$
I	0	7.5	5	20	0	5	36 ± 0.86^{f}	210 ± 10.00^{a}	$2\,271 \pm 101.37^{b}$	$2\,377 \pm 69.18^{b}$	38 ± 0.57^{e}	185 ± 5.70^{a}	$2\,318 \pm 10.11^{b}$	$2\,425 \pm 26.45^{b}$
J	600	7.5	5	20	0	5	39 ± 0.56^{ef}	83 ± 2.71^{b}	$1\,016 \pm 19.60^{c}$	$1\,542 \pm 12.72^{c}$	40 ± 0.56^{e}	81 ± 3.20^{b}	$1\,015 \pm 15.27^{c}$	$1\,530 \pm 7.60^{c}$
K	0	9	6.5	20	1	5	45 ± 0.80^{ab}	74 ± 5.75^{b}	$1\,011 \pm 8.81^{c}$	$1\,546 \pm 10.69^{c}$	45 ± 0.34^{bcd}	68 ± 1.70^{b}	996 ± 5.92^{c}	$1\,518 \pm 49.00^{c}$
L	600	9	6.5	20	1	5	47 ± 0.30^{a}	30 ± 3.10^{c}	393 ± 11.14^{d}	598 ± 10.14^{d}	47 ± 0.32^{ab}	25 ± 2.00^{cd}	422 ± 9.07^{d}	565 ± 23.09^{d}

$^{a–f}$Means each colum with no common superscript differ significantly ($P < 0.05$)

kidneys and liver gets higher at an important rate (Fox *et al*., 1980).

In our research its proved that add of cadmium to a diet effects the accumulation of this element in organs in a important rate. Add of vitamin C with microbial phytase enzyme decreases the accumulation. Little is known about the effect of dietary microbial phytase supplementation on the cadmium accumulation in monogastric animals. The levels of nutrients which were changed in an otherwise sufficient chicken diet were iron, selenium, copper, manganese, calcium, phosphorus, zinc, phytate, vitamin C, vitamin E, vitamin $B_{6,}$ vitamin D, fiber content, protein and fat (Rambeck and Kollmer, 1990; Rambeck and Guillot, 1996). Is also find similar result in his researches which were made with broilers that add of vitamin C decreases cadmium retention 40% and the positive effect of phytase enzyme add. Rambeck and Kollmer (1990) was to compare the positive and negative effects of various components of practical feeds on cadmium retention in a single species in one standardized animal model with the intention of working out dietary measures for minimizing cadmium accumulation in the livestock in cases of environmental exposure. They found that supplements of vitamin B_6, vitamin E, manganese and copper did not decrease the concentration of cadmium in the kidney. Adding Fe^{2+} to diet led to a moderate decrease. The most striking protective effects have been obtained with supplements of vitamin C or with selenium. This is in agreement with Fox *et al*. (1980) observed a reduced accumulation of cadmium under the influence of vitamin C in the kidney of Japanese quail fed a very low level of cadmium. Our results and those achieved by Fox *et al*. (1980) with Japanese quail show that a diet supplement vitamin C. Herzig *et al*. (1994) reported dietary supplement of 0.3 mg (per bird) cadmium chloride increased cadmium concentrations in liver and kidneys, but no statistically significant changes were observed in the muscle.

CONCLUSION

This effect of phytase enzyme is recommended that can support by usage of phosphorus and zinc. Similar results were obtained when obtained when cadmium concentration in the kidney and liver were determined. Addition of 600 PU/kg feed of phytase per kg of diet compensates this effect and lowers the cadmium burden by up to 60%.

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