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The Effects of Microbial Phytase to Low-Protein Diets Supplemented with Individual Amino Acids on Performance and Carcass Characteristics in Broilers

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Abstract: A 6 weeks experiment with 450 unsexed, one day-old broilers was carried out to determine the effects of adding phytase to low-protein diets supplemented with individual amino acids on performance, carcass traits and some organ weights. The chicks were fed with diets containing 22 and 20% Crude Protein (CP; control) for the 0-3 and for the 4-6 weeks of period, respectively. During the 0-3 and 4-6 weeks of periods, the other groups were fed with low protein diets (20 and 18% CP, respectively) containing lysine, methionine and threonine (EAA) levels of 100, 110, 120, or 130% of NRC recommendation. The diets were then supplemented with (500 FTU kg^{-1} of diet; Natuphos, BASF, Germany) and without additional microbial phytase. This resulted in 4×2 factorial arrangements with four levels of and two levels of phytase (0 and 500 FTU kg^{-1}) and so a total of 9 treatments with a control group were tested. Each treatment was replicated 3 times with each replication consisting of 15 chicks. All other nutrient contents of diets were met the requirements by NRC recommendation and were contained 3200 kcal ME kg^{-1}. The effects of interactions consist of EAA and phytase levels on body weight gain, feed intake, feed conversion ratio, carcass traits except for wings weight and relative organs weight had no significant ($p>0.05$). There were no main effects of dietary EAA levels on body weight gain, feed intake, feed conversion ratio, all measured carcass traits and relative organs weight, except for pancreas weight, in the experiment. As a main factor, effects of dietary phytase levels on all parameters had no significant. Also, there was no significant difference in terms of performances, carcass traits and organ weights (body weight (%)) between control diet and low-CP diets. These data suggest that broilers fed low CP, EAA supplemented diet (lysine, methionine, threonine levels of 100% NRC recommendation) have performance and carcass traits similar to broilers fed higher level of CP recommended by NRC.

Key words: Broiler, carcass, low-protein, performance, phytase, amino acids

INTRODUCTION

Poultry rations are based largely on grains and oilseed meals. Unfortunately, approximately two thirds of phosphorus in cereal grains and oilseed meals are present in the form of phytate, which is the mixed salt of phytic acid and which are not available for poultry and pigs. Also, phytate constitutes insoluble complexes with various cations such as Ca, Mg, Mn, Cu, Fe, Zn (Nelson and Kirby, 1987) and protein and starch (Thompson and Yoon, 1984). The phytate-protein complexes may reduce the utilization of the protein and amino acids (Cheryan, 1980). Dietary supplemental phytase improved utilization of protein (Officer and Batterham, 1992) and nitrogen retention (Farrell *et al*., 1993) in broiler chicks.

Formulating broiler diets on a digestible amino acid basis and utilizing the economically feasible commercial amino acid supplements (lysine, methionine and threonine) result in diets marginally reduced in Crude Protein (CP) that support equal broiler growth to diets containing higher CP and excess amino acids (Kidd *et al*., 2002). There is a common agreement that supplementing diet with methionine, the first limiting amino acid and lysine, the second limiting amino acid, allows the reduction of CP levels to a point (Lipstein and Bornstein, 1975). The former reduction in dietary CP typically renders isoleucine (third limiting) as the fourth limiting-critic amino acid in broiler diet containing threonine (Kidd *et al*., 2004).

The performance of chicks was significantly influenced by the dietary CP regimen (Deschepper and Degroote, 1995; Yi *et al*., 1996; Biehl and Baker, 1997; Sebastian *et al*., 1997).

The objective of this study was to investigate effect of low-protein diets supplemented amino acids and phytase on performance, carcass traits and relative organs weight in broiler chicks.

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MATERIALS AND METHODS

A total of 405-days-old mixed sex broiler chicks were used in this experiment. The chicks were fed with diet containing 22% CP (control) for the 0-3 weeks of period and 20% CP for the 4-6 weeks of period according to NRC (1994) recommendations. For the 0-3 and 4-6 weeks of periods, low protein diets (20 and 18% CP, respectively) contained NRC recommended levels of lysine, methionine and threonine EAA at 100, 110, 120, or 130% of NRC. The diets were then supplemented with microbial phytase (0 and 500 FTU kg^{-1} of diet; Natuphos, BASF, Germany) This resulted in 4×2 factorial arrangement with four levels of EAA (100, 110, 120 and 130% of NRC) and two levels of phytase (0 and 500 FTU kg^{-1}) and so a total of 9 treatments diets were used in the experiment (Table 1).

Table 1: Composition of experimental diets

		Low CP diets							
		100% NRC		110% NRC		120% NRC		130% NRC	
	Control	0	500	0	500	0	500	0	500
Ingredients (%)	diet	1	2	3	4	5	6	7	8
Starter diets (0-3 weeks)									
Corn	47.70	56.90	56.90	57.00	57.00	57.50	57.50	56.40	56.40
Soybean meal (47.6% CP)[1]	30.60	28.00	28.00	27.50	27.50	26.70	26.70	24.70	24.70
Sunflower meal (32% CP)[1]	9.80	4.50	4.50	4.50	4.50	4.50	4.50	7.00	7.00
Vegetable oil (8800 ME kcal kg^{-1})	8.25	6.64	6.64	6.74	6.74	6.76	6.76	7.10	7.10
Limestone	1.26	1.27	1.27	1.28	1.29	1.28	1.28	1.27	1.27
Dicalcium phosphate	1.61	1.72	1.72	1.72	1.72	1.73	1.73	1.72	1.72
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	-	0.092	0.092	0.228	0.228	0.360	0.360	0.502	0.502
DL-Methionine	0.179	0.214	0.214	0.268	0.268	0.321	0.321	0.365	0.365
L-Threonine	-	0.067	0.067	0.156	0.156	0.249	0.249	0.344	0.344
Phytase (FTU kg^{-1})	-	-	500	-	500	-	500	-	500
Total	100	100	100	100	100	100	100	100	100
Calculated nutrients									
CP (%)	21.98	20.03	20.03	20.08	20.08	20.02	20.02	20.06	20.06
ME, kcal kg^{-1}	3202	3201	3201	3202	3202	3202	3202	3201	3201
Ca (%)	0.999	1.000	1.000	1.007	1.007	1.004	1.004	1.002	1.002
Non-phytate P (%)	0.449	0.451	0.451	0.450	0.450	0.450	0.450	0.450	0.450
L-Lysine (%)	1.119	1.100	1.100	1.221	1.221	1.331	1.331	1.440	1.440
DL-Methionine (%)	0.500	0.500	0.500	0.551	0.551	0.601	0.601	0.650	0.650
Methionine+Cystine (%)	0.902	0.866	0.866	0.914	0.914	0.958	0.958	1.003	1.003
L-Threonine (%)	0.806	0.800	0.800	0.880	0.880	0.960	0.960	1.040	1.040
Grower diets (4-6 weeks)									
Corn	56.50	62.70	62.70	63.20	63.20	63.00	63.00	63.20	63.20
Soybean meal (47.6% CP)[1]	28.00	22.00	22.00	21.80	21.80	21.00	21.00	20.10	20.10
Sunflower meal (32% CP)[1]	6.07	6.50	6.50	5.90	5.90	6.50	6.50	6.92	6.92
Vegetable oil (8800 ME kcal kg^{-1})	6.30	5.38	5.38	5.40	5.40	5.56	5.56	5.61	5.61
Limestone	1.36	1.36	1.36	1.38	1.38	1.38	1.38	1.35	1.35
Dicalcium phosphate	1.13	1.19	1.19	1.21	1.21	1.121	1.121	1.22	1.22
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	-	0.137	0.137	0.250	0.250	0.366	0.366	0.487	0.487
DL-Methionine	0.09	0.109	0.109	0.150	0.150	0.189	0.189	0.229	0.229
L-Threonine	-	0.079	0.079	0.162	0.162	0.245	0.245	0.331	0.331
Phytase (FTU kg^{-1})	-	-	500	-	500	-	500	-	500
Total	100	100	100	100	100	100	100	100	100
Calculated nutrients									
CP (%)	19.98	18.01	18.01	18.02	18.02	18.03	18.03	17.99	17.99
ME (kcal kg^{-1})	3202	3199	3199	3201	3201	3203	3203	3202	3202
Ca (%)	0.900	0.901	0.901	0.911	0.911	0.911	0.911	0.902	0.902
Non-phytate P (%)	0.350	0.350	0.350	0.351	0.351	0.351	0.351	0.351	0.351
L-Lysine (%)	1.018	1.000	1.000	1.100	1.100	1.200	1.200	1.300	1.300
DL-Methionine (%)	0.380	0.380	0.380	0.418	0.418	0.456	0.456	0.494	0.494
Methionine + Cystine (%)	0.751	0.716	0.716	0.749	0.749	0.783	0.783	0.816	0.816
L-Threonine (%)	0.748	0.74	0.74	0.814	0.814	0.888	0.888	0.962	0.962

[1]Analyzed value, [2]Supplied per kilogram of diet, manganese, 100 mg, iron, 60 g, copper, 10 mg, cobalt, 0.20 mg, iodine, 1 mg, selenium, 0.15 mg, Vitamin A, 12.000 IU, Vitamin D_3, 1.500 IU, Vitamin E, 30 mg, Vitamin K, 5.0 mg, Vitamin B_1, 3.0 mg, Vitamin B_2, 6.0 mg, Vitamin B_6, 5.0 mg, Vitamin B_{12}, 0.03 mg, Nicotine amide, 40.0 mg, D-calcium panthotenate, 10.0 mg, folic acid, 0.75 mg, d-biotin, 0.075 mg, choline chloride, 375 mg, antioxidant, 15.0 mg

The chicks were allotted to 9 treatments with three replicate per treatments consisted of 45 chicks. All diets contained 3200 kcal ME kg^{-1}. The experiment was conducted a factorial arrangement in completely randomized design. Water and feed were offered *ad libitum* throughout the experiment. Lighting was treated as a 23 h day^{-1}. Analyzed diet compositions were generally in agreement with calculated diet (Table 1).

Body weight and feed intake were recorded on a pen basis as weekly intervals. Mortality was recorded daily. At the end of the experiment (at 6 weeks of age), randomly selected 4 chicks (2 male, 2 female) from each replicate were slaughtered at a processing plant. After warm washing, draining and cooling, the carcasses were eviscerated.

The dressed and eviscerated weights were recorded before dissecting out the back + breast, thigh, neck and wing. The corresponding organs (liver, pancreas, gizzard and small intestine) were carefully dissected out before weighing. All dissections were done on the same day and by the same person to ensure uniformity of cuts.

The data from the experiments were analysed using the GLM procedure of Minitab Software (Minitab, 2000). The experimental design for amino acids and phytase was a completely randomized factorial (4×2) arrangement of treatments. Separation of main effects of phytase and amino acids and their interaction means differences were determined by Duncan (1955) Multiple Range Test. In addition, the control diet was compared with low-CP diets with a contrast statement using orthogonal contrast. These contrasts were not significant (p>0.05); therefore, all of these contrasts were given as a name of all treatments in tables.

RESULTS AND DISCUSSION

In the present study, broilers were fed either high-CP which was supplemented EAA (methionine, lysine, threonine) equal or above the suggested levels of NRC (1994) and two levels of phytase. The interactions between EAA and phytase levels had no significant effect on Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR) for all age periods (Table 2), carcass parts weight except wings weight (Table 3) and organs weight (Table 4). The highest wings weight was found in group fed with diet 3 (the 110% EAA diet without phytase) and difference among this group and the others, which were fed with diets 4-7 were significant (Table 3, p<0.05). There were no significant differences in terms of performance, carcass parts and organs weight between control group and other treatment groups (p>0.05).

There has been great controversy about the effects of phytase on protein and amino acid digestibility. Pan *et al.* (2002) reported that FCR was not significantly influenced by diets with and without phytase to containing 100 and 110% EAA of NRC (1994) in broilers. Augspurger and Baker (2004) showed that phytase supplementation, even at a very high dose level (500 vs. 10.000 FTU kg^{-1}) did not improve the protein utilization of chick fed low-protein diets containing ingredients first limiting in different amino acids.

Table 2: Body weight gain, feed intake and feed conversion ratio of broiler chickens fed low-protein amino acids and phytase supplemented diets ($\bar{X} \pm S\bar{x}$)

Treatments	EAA (NRC%)	Phytase (FTU kg^{-1})	Body weight gain (g)	Feed intake (g)	Feed conversion ratio (feed:gain)
EEA × Phytase					
Diet 1	100	0	2185±58.5	4365±134.6	2.00±0.01
Diet 2	100	500	2194±42.2	4436±96.5	2.02±0.03
Diet 3	110	0	2160±36.9	4244±120.8	1.97±0.05
Diet 4	110	500	2046±11.1	4104±180.7	2.00±0.08
Diet 5	120	0	2033±16.1	4048±38.6	1.99±0.01
Diet 6	120	500	2188±106.7	4278±179.8	1.96±0.02
Diet 7	130	0	2106±72.7	4173±86.9	1.98±0.05
Diet 8	130	500	2138±45.1	4145±85.0	1.94±0.02
Main effect					
EAA	100		2189±32.4	4401±107.2	2.01±0.02
	110		2103±30.8	4174±102.1	1.99±0.04
	120		2110±59.4	4163±97.0	1.98±0.02
	130		2122±55.1	4159±54.7	1.96±0.03
Phytase	0	2121±27.9	4208±55.7	1.99±0.02	
	500	2141±31.9	4241±72.4	1.98±0.02	
Control diet	100	0	2105±28.7	4048±8.5	1.92±0.03
Effects				**P**	
EAA × phytase			ns	ns	ns
EAA main effect			ns	ns	ns
Phytase main effect			ns	ns	ns
Contrasts					
Control vs. all treatments*			ns	ns	ns

[a, b]Means with different minuscule in the same column are significantly different at p<0.05, ns: non-significant (p>0.05), *Control diet vs. Diet 1, control diet vs. diets 1, 3, 5 and 7, control diet vs. diets 2, 4, 6 and 8 and control diet vs. diets 1-8

Table 3: Carcass traits of broiler chickens fed low-protein amino acids and phytase supplemented diets, g ($\bar{X} \pm S\bar{x}$)

Treatments	EAA (NRC%)	Phytase (FTU kg^{-1})	Carcass	Thigh	Back + breast	Neck	Wings
EEA × Phytase							
Diet 1	100	0	1697±43.3	704±17.4	719±22.6	88±1.7	186±7.1^{ab}
Diet 2	100	500	1732±72.3	736±31.0	721±37.3	90±2.3	186±6.7^{ab}
Diet 3	110	0	1796±125.0	743±53.6	752±65.9	92±2.3	209±18.1^{a}
Diet 4	110	500	1628±30.3	680±10.5	688±15.8	88±0.4	173±4.3^{b}
Diet 5	120	0	1650±104.7	679±41.5	710±53.3	88±5.0	174±7.0^{b}
Diet 6	120	500	1653±43.8	659±22.3	721±15.8	92±4.9	181±2.6^{b}
Diet 7	130	0	1596±46.7	651±21.6	692±17.6	84±4.7	169±6.0^{b}
Diet 8	130	500	1705±40.2	682±15.0	761±24.7	76±8.0	185±2.6^{ab}
Main effect							
EAA	100	-	1715±38.5	720±17.4	720±19.5	89±1.3	186±4.4
	110	-	1712±68.7	712±28.2	720±33.5	90±1.5	191±11.6
	120	-	1652±50.8	669±21.5	715±25.0	90±3.3	178±3.7
	130	-	1650±36.3	667±13.7	727±20.6	80±4.4	177±4.6
Phytase	-	0	1685±43.4	694±18.7	718±20.2	88±1.8	185±6.5
	-	500	1680±24.4	689±12.4	723±26.5	86±2.8	181±2.5
Control diet	100	0	1728±29.7	708±8.7	751±23.9	81±4.1	188±5.4
Effects					**P**		
EAA × phytase	-	-	ns	ns	ns	ns	0.029
EAA main effect	-	-	ns	ns	ns	ns	ns
Phytase main effect	-	ns	ns	ns	ns	ns	-
Contrasts							
Control vs. all treatments*	-	-	ns	ns	ns	ns	ns

a, bMeans with different minuscule in the same column are significantly different at p<0.05; ns: non-significant (p>0.05). *Control diet vs. Diet 1, control diet vs. diets 1, 3, 5 and 7, control diet vs. diets 2, 4, 6 and 8 and control diet vs. diets 1-8

Table 4: Some organs weight of broiler chickens fed low-protein amino acids and phytase supplemented diets, body weight (%) ($\bar{X} \pm S\bar{x}$)

Treatments	EAA (NRC%)	Phytase (FTU kg^{-1})	Liver	Pancreas	Gizzard	Small intestine
EEA × Phytase						
Diet 1	100	0	2.19±0.29	0.26±0.02	2.13±0.17	4.35±0.35
Diet 2	100	500	2.10±0.15	0.22±0.03	2.21±0.04	3.69±0.30
Diet 3	110	0	2.38±0.10	0.25±0.02	2.25±0.07	4.42±0.44
Diet 4	110	500	2.21±0.07	0.26±0.04	2.37±009	4.01±0.13
Diet 5	120	0	2.06±0.18	0.18±0.01	2.48±0.10	3.51±0.11
Diet 6	120	500	2.01±0.14	0.21±0.01	2.07±0.12	3.70±0.10
Diet 7	130	0	1.99±0.16	0.25±0.01	2.34±0.02	3.83±0.31
Diet 8	130	500	2.10±0.08	0.26±0.02	2.18±0.10	4.30±0.14
Main effect						
EAA	100		2.14±0.15	0.24±0.02^{ab}	2.17±0.08	4.02±0.25
	110		2.29±0.07	0.26±0.02^{a}	2.31±0.06	4.22±0.22
	120		2.04±0.10	0.20±0.01^{b}	2.27±0.12	3.61±0.08
	130		2.05±0.09	0.25±0.01^{a}	2.26±0.06	4.06±0.19
Phytase		0	2.15±0.10	0.24±0.01	2.30±0.06	4.03±0.18
		500	2.10±0.05	0.24±0.01	2.21±0.05	3.93±0.11
Control diet	100	0	1.38±0.19	0.24±0.03	2.04±0.13	3.94±0.32
Effects					**P**	
EAA × phytase			ns	ns	ns	ns
EAA main effect			ns	0.032	ns	ns
Phytase main effect			ns	ns	ns	ns
Contrasts						
Control vs. all treatments*			ns	ns	ns	ns

a, bMeans with different minuscule in the same column are significantly different at p<0.05; ns: non significant (p>0.05). *Control diet vs. Diet 1, control diet vs. diets 1, 3, 5 and 7, control diet vs. diets 2, 4, 6 and 8 and control diet vs. diets 1-8

In the present research, the treatments significantly affected wings weight but they did not have any effect on the other parts especially more economical parts such as breast and thigh weight. Therefore, it can be seen that the low-CP, EAA supplemented diets has no weight loss of carcass and it is seen that they bear similarity with control diet including high-CP.

The results of present experiment are in generally agreement with results of other experiments (Aletor *et al*., 2000; Si *et al*., 2001; Jianlin *et al*., 2004; Rezaei *et al*., 2004). In contrast, Dozier *et al*. (2000) also reported that threonine supplementation to low-CP diet improved breast percentage, but had no significant effect on the drumstick and thigh weight.

There were no main effects of dietary EAA levels on BWG, FI and FCR (Table 2), carcass parts weight (Table 3). It has been reported that EAA requirements for better feed efficiency are greater than those maximum

BWG (Thomas *et al*., 1977). There was no effect of dietary EAA levels on organs weight except pancreas weight. Pancreas weight of broilers fed the 120% EAA diet had lowest and was significantly lower than broilers fed the 110 and 130% EAA diet ($p<0.05$, Table 4).

As a main factor, different phytase levels did not significantly influence the BWG, FI and FCR (Table 2), carcass parts and organs weight (Table 3 and 4, $p>0.05$). According to Ravindran *et al*. (2006), although, there was minimum effect in protein and EAA availability when 500 FTU kg^{-1} phytase was added to broiler diets, a significant improvement was observed in diets containing 750 and 1000 FTU kg^{-1} phytase. There has been no significant difference in the digestibility of amino acid with 600 and 1200 FTU kg^{-1} phytase supplementation to diets (Adeola and Sands, 2003). Eeckhout and De Paepe (1994) reported that dietary phytase level should be at least 1000 FTU kg^{-1} in order to prevent the negative effects of phytate on nutrient availability. Growth studies, however, have generally failed to show a positive response to supplemental phytase in chicks fed diets with adequate P (Zhang *et al*., 1999; Peter and Baker, 2001).

The discrepancies in responses often observed in the literature in chicks fed low-CP, amino acids supplemented diets appear to be related to the type and age of the chickens used, the degree of reduction of CP levels, the extend of feeding period, the source and dose of using phytase and the concentration of intact protein source.

CONCLUSION

In this study, the growth performance, carcass traits and organs weight (BW%) observed in the low-CP diet with or without phytase (low approximately 10% than recommended level) that containing the levels of EAA suggested by NRC (1994) was comparable to those observed in the normal-CP maize-soybean meal diet at 42 days. These data support the idea that low-CP diets without phytase depending on the extent to which protein is reduced may support live performance and carcass traits when diets are well balanced for EAA.

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