ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL.XVIII, 36 SECTIO EE 2000

Zakład Fizjologii Zwierząt Wydziału Weterynaryjnego Akademii Rolniczej w Lublinie
Instytut Żywienia Zwierząt Wydziału Biologii i Hodowli Zwierząt Akademii Rolniczej w Lubilnie

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Effects of Dietary Phytase Supplementation on Growth and Development of Limb Bones in Broiler Chickens

Wpływ dodatku fitazy do diety na wzrost i rozwój kości kończyn kurcząt brojlerów

Most of the phosphorus in feed of vegetable origin is found in bonds of phytic acid (1, 2, 3, 4, 5, 6-hexa-dihydrophosphate-myo-inozytol) with metal ions, sugars or protein (N e l s o n et al. 1968, E e c k h o u t et al. 1994). Monogastric animals utilize dietary phytate phosphorus in a small degree because of lack of phytase, an enzyme that hydrolizes the estric bonds of phytic acid (C r o m w e l l 1993).

Deficiency of phosphorus in the organism, being a consequence of mal absorption, or too low a supply of this element in feed has an influence on the development of the skeletal system, egg laying and endurance of egg shells. An inadequate level of P in feed can lead to rickets, osteomalacia, osteoporosis and osteodystrophy, which are the result of irregular growth and development of chondral and osseous tissues leading to handicaps or irregular ossifications and also demineralization of bones (Q i a n et al. 1996).

The aim of this investigation was to estimate the influence of dietary supplementation of phytase (Natuphos 5000, BASF, Germany) on the growth and development of the organism and of the skeletal system of broiler chickens fed a diet with a lowered content of total phosphorus.

MATERIAL AND METHODS

Investigations were carried out on 360 STARBRO broiler chickens, kept under standard conditions and fed diet 1 (Table 1) till the 21st day of life, containing 5.9 mg/g of total phosphorus and 2.6

mg/g of phytate phosphorus (crude protein 23.3%, crude fat 2.6%, crude fiber 3.2%), and later were fed diet 2 (Table 1) composed of total and phytate phosphorus at a level of 5.5 mg/g and 2.5 mg/g (crude protein 19.2%, crude fat 3.1%, crude fiber 2.6%).

Table 1. Composition of experimental diets

Contents	Diet 1	Diet 2
Corn meal	59.5%	69.5%
Soybean meal	31%	21%
Meat meal	4%	4%
Yeast fodder	2%	2%
Dicalcium phosphate	0.5%	0.5%
Limestone	1.5%	1.5%
Sodium chloride	0.5%	0.5%
Premix DKA (starter or grower)	1%	1%

The chickens were divided into four groups: control and 3 experimental ones. Chickens in the experimental groups from the first day of life till the 28^{th}, 42^{d} and 56^{th} days, respectively, received diets supplemented with phytase (Natuphos 5000, BASF, Germany) in quantities of 500, 750, 1000 FTU/kg of fodder. A unit of phytase activity was defined as that activity which liberates one micromole of inorganic phosphorus per minute from 0.0015 m Na-phytate at 37^{0}C and at *pH* 5.5 under standard conditions.

In weeks 4, 6 and 8 the chickens of every group (n = 10 females/10 males) having similar body weight on average (nearing to group average) were slaughtered.

Body weight of chickens and feed conversion were examined. The content of total phosphorus of excrement, assembled over a period of 3 days at the 4^{th}, 6^{th} and 8^{th} weeks of life was also analysed using spectrocolorimetry method with ammonium molybdate. After slaughtering, limb bones – humeri and femora – were isolated. Mass and length of bones were measured. An Instron Universal Technic Machine and the three-point bending test (Model 4302, Instron Corp., USA) were used for bone breaking strength determination at a loading rate of 10 mm/min, and the physical parameters of bone – ultimate stress (N-Newton) causing disintegration of bone structure, elastic force at point of elasticity (N-Newton) and stiffness (N/mm-Newton/millimetre) were calculated as outlined by Ferretti et al. (3).

The results were analysed statistically using t-Student test at different levels of significance of $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

From estimates of chickens at the age of 4, 6 and 8 weeks old it appeared that in every experimental group the body weight of females and males was greater than in controls (Table 2). The average increase of body weight of experimental chickens between days 1 and 28, 28 and 42, 42 and 56 was greater than in controls. In the 4^{th} week of life the highest value of average increase of body weight was noted in the group fed on the diet supplemented with phytase in quantities of 1000 FTU/kg of feed. In the 6th week of life the greatest increases in body weight, in both males and females, was noted in those receiving 750 FTU/kg of feed and was about 21% greater in females and 31% greater in males than in controls. Between days 42 and 56 the greatest increase in body weight was in hens and amounted to aproximately 13% more than in control chickens and was noted in those fed a diet supplemented with 500 FTU/kg of feed. Instead, the greatest increase of body mass – 21% was noted in males fed on the diet supplemented with 1000 FTU/kg of feed (Table 2).

Table 2. The body weight (g) of female and male chickens after phytase supplementation (FTU/kg feed) (M±SE)

Age	Females				Males			
	Control	500	750	1000	Control	500	750	1000
4 weeks	706 a 32.7	781 b 13.8	787 B 13.3	863 C 11.2	714 A 19.1	779 B 6.2	861 C 14.8	922 C 30.1
6 weeks	1450 a 63.8	1611 b 23.5	1691 C 27.5	1670 B 24.1	1478 A 41.3	1761 B 50.6	1861 B 33.6	1808 B 28.0
8 weeks	2160 a 49.8	2414 b 49.2	2286 ab 63.1	2447 B 43.9	2323 a 73.7	2549 b 29.0	2805 C 80.0	2842 C 66.3

Means with different letters are significantly different, abc – p≤0.05, ABC – p≤0.01.

The increases of body weight in chickens in each period of growth were connected with specific feed conversion (Table 3). In the first 21 days of life in experimental groups, feed intake calculated per kilogram of body weight gain was greater. Older chickens receiving a diet with phytase showed better feed conversion.

These results, concerning the body weight increase of animals supplemented with phytase, comply with reports of other authors. According to S c h ö n e r et al. (7) the increase of body weight of chickens, depending on the addition of phytase, takes place first of all by increase of feed intake, but according to S i m o n s et al. (8) mainly by better utilization and better feed conversion.

Table 3. Effect of dietary phytase supplementation (FTU/kg feed) on feed conversion (kg/kg) in chickens

Age	Control	500	750	1000
1-21st day	2.06	2.27	2.14	2.21
22-56th day	3.51	2.3	2.96	2.7
Total	2.7	2.44	2.7	2.41

Table 4. The content of phosphorus in excrement (% of sample)

Age	Control	500	750	1000
4 weeks	1.25	1.17	1.26	1.15
6 weeks	1.6	1.47	1.58	1.33
8 weeks	1.77	1.9	2.27	2.19

The content of total phosphorus in the excrement of control and experimental chickens at 4, 6 and 8 weeks of life is presented in table 4. In 4 and 6 weeks of life in experimental chickens the content of phosphorus in excrement was lower in comparison with controls. Instead, in 8 week of life an addition of phytase to the diet caused a higher content of phosphorus in excrement.

Bone mass of femora and humeri showed greater values in experimental chickens in relation to controls. These changes referred both to chickens in 4, 6, and 8 weeks of life. In 4 and 8 weeks of life the highest skeletal mass, confirmed statistically, occurred in males and females, and appeared in chickens fed the diet supplemented with 1000 FTU/kg of feed (Tables 5 and 7) , but in 6 week with 750 FTU/kg (Table 6).

Growth of bone mass of experimental chickens was also related to an increase in bone length. In 4 and 8 weeks of life the greatest length of femora and humeri, in both males and females, occurred in those fed on the diet with the addition of 1000 FTU/kg (Tables 5 and 7). In 6 weeks of life the greatest length of bones in females was noted in the groups receiving phytase in quantities of 750 and 1000 FTU/kg, and in males fed on fodder with the addition of phytase in quantities of 1000 FTU/kg (Table 6).

Analysis of physical parameters of limb bones in chickens of 4 weeks old showed the existence of differences in strength of femur and humerus in males and females in each group. In females the highest values of ultimate stress, elasticity and stiffness of humerus (Table 8) was in the group receiving 1000 FTU/kg of fodder. The greatest values of ultimate stress and stiffness of femur were determined in the group with phytase in the amount of 750 FTU/kg of feed, while the highest elasticity was in the group with 1000 FTU/kg.

The highest values of ultimate stress and elasticity of humeri were in males fed on the diet supplemented with 1000 FTU/kg (Table 8). Femora were characterized by the highest value of physical parameters in groups receiving increased supplementation of phytase to fodder, attaining the highest values in males with 1000 FTU/kg.

Femora and humeri of control and experimental chickens showed significant differences of physical parameters also at 6 weeks of life (Table 9). In females the

Table 5. Mean values of mass (g) and length (mm) of femur and humerus in chickens at 4 weeks of life after phytase supplementation (FTU/kg feed)

		Females				Males			
		femur		humerus		femur		humerus	
		mass	length	mass	length	mass	length	mass	length
4 weeks	Control	4.7 a	56.21 a	3.7 a	52.26 a	5.2 a	57.13 a	3.8 A	52.26a
	500	5.2 b	58.74 b	3.9 a	53.61 ab	5.5 a	57.84 a	3.8 A	52.53 a
	750	5.2 b	58.1 ab	3.9 a	53.51 ab	6.0 B	58.68 b	4.4 B	54.32 b
	1000	5.4 b	59.25 b	4.1 a	54.66 b	6.4 b	61.28 c	4.6 B	56.73 c

Means with different letters are significantly different, abc – p ≤ 0.05, ABC – p ≤ 0.01.

Table 6. Mean values mass (g) and length (mm) of femur and humerus in chickens at 6 weeks of life after phytase supplementation (FTU/kg feed)

		Females				Males			
		femur		humerus		femur		humerus	
		mass	length	mass	length	mass	length	mass	length
6 weeks	Control	8.3 A	70.04 a	6.0 a	65.12 a	10.2 a	71.71 a	7.2 a	65.12 a
	500	9.5 B	74.32 b	6.7 b	67.84 b	12.2 a	76.62 b	8.5 b	69.84 b
	750	10.4 C	76.94 c	7.1 C	69.62 c	12.7 a	79.34 b	8.8 B	72.34 b
	1000	10.0 CB	76.9 bc	7.0 bc	69.59 bc	12.4 a	78.49 b	7.8 a	70.97 b

Means with different letters are significantly different, abc – p ≤ 0.05, ABC – p ≤ 0.01.

Table 7. Mean values of mass and length of femur and humerus in chickens at 8 weeks of life after phytase supplementation (FTU/kg feed)

		Female				Male			
		femur		humerus		femur		humerus	
		mass	length	mass	length	mass	length	mass	length
8 weeks	Control	13.1 a	85.57 a	8.8 a	78.3 a	15.6 a	85.71 a	10.6 a	78.96 a
	500	13.9 ab	86.29 ab	10 b	79.19 ab	17.1 b	87.1 a	11.3 ab	80.23 a
	750	13.6 ab	86.74 ab	9.2 ab	77.81 a	19.4 C	93.11 b	12.3 b	83.17 b
	1000	14.6 b	88.46 b	9.8 b	80.62 b	20.0 C	94.03 b	13.3 C	84.31 b

Means with different letters are significantly different, abc – $p \leq 0.05$, ABC – $p \leq 0.01$.

Table 8. Mean values of ultimate stress (N), elastic force (N) and stiffness (N/mm) of femur and humerus in chickens at 4 weeks of life after phytase supplementation (FTU/kg feed)

		Females						Males					
		femur			humerus			femur			humerus		
		ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness
4 weeks	Control	80 a	48 a	48 a	86 A	54 a	60 A	73 a	45 a	42 A	85 A	49 A	63 A
	500	101 b	63 b	53 b	95 AB	58 a	68 AB	97 b	63 b	48 AB	89 A	52 A	66 A
	750	122 C	74 B	65 C	110 B	63 a	84 B	113 BC	70 B	67 B	122 B	75 B	90 B
	1000	120 BC	79 B	58 bc	112 B	64 a	88 B	138 C	88 C	69 BC	142 B	84 B	84 B

Means with different letters are significantly different, abc – $p \leq 0.05$, ABC – $p \leq 0.01$.

Table 9. Mean values of ultimate stress (N), elastic force (N) and stiffness (N/mm) of femur and humerus in chickens at 6 weeks of life after phytase supplementation (FTU/kg feed)

		Females						Males					
		femur			humerus			femur			humerus		
		ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness
6 weeks	Control	124 a	71 a	73 A	117 a	63 a	87 a	114 A	73 a	64 A	107 A	64 A	72 A
	500	162 b	95 b	107 B	128 a	80 b	97 ab	182 B	111 B	110 CB	186 B	102 B	116 B
	750	163 b	96 b	112 B	156 b	87 B	107 ab	167 B	91 b	107 B	187 B	101 B	123 B
	1000	196 C	111 B	120 B	162 b	94 B	114 b	185 B	104 B	128 C	160 B	93 B	104 B

Means with different letters are significantly different, abc – $p \leq 0.05$, ABC – $p \leq 0.01$.

Table 10. Mean values of ultimate stress (N), elastic force (N) and stiffness (N/mm) of femur and humerus in chickens at 8 weeks of life after phytase supplementation

		Females						Males					
		femur			humerus			femur			humerus		
		ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness	ultimate stress	elastic force	stiffness
8 weeks	Control	186 a	110 a	140 a	159 a	89 a	136 a	176 a	103 a	111 a	168 A	93 A	121 a
	500	189 ab	106 a	143 a	193 B	101 b	153 a	220 b	121 b	143 b	230 B	127 B	168 B
	750	210 ab	124 a	151 a	184 b	103 bc	141 a	231 B	123 ab	130 ab	238 B	132 B	166 b
	1000	212 b	119 a	138 a	207 B	117 C	158 b	236 B	132 ab	134 ab	242 B	125 B	153 ab

Means with different letters are significantly different, abc – $p \leq 0.05$, ABC – $p \leq 0.01$.

highest values of all analysed parameters of humeri and femora were in the group fed on the diet supplemented with phytase in quantities of 1000 FTU/kg. Results of the analysis of physical parameters of limb bones in males at 6 weeks of life are presented in Table 9.

In females of 8 weeks old humeri showed the greatest values of ultimate stress, elasticity and stiffness in the group receiving 1000 FTU/kg (Table 10). On the basis of femur results, it was ascertained that the highest strength (although not differing statistically) was in females receiving phytase in quantities of 750 and 1000 FTU/kg.

At 8 weeks of life, as at the age of 4 and 6 weeks, humeri shafts and femora shafts of experimental males were characterized by greater values of mechanical parameters in comparison with controls (Table 10). These differences were confirmed statistically. The highest value of ultimate stress of femora and humeri were in chickens receiving the diet with 1000FTU/kg of fodder.

Values of physical parameters of limb bones showed dependence and influence of phytase in fodder on physical parameters. Addition of phytase to the diets to a very essential degree influenced the physical parameters of bones causing higher values of strength, being an expression of development of bones. Femora and humeri in experimental females and males showed higher values of the analysed parameters in comparison with controls. One should also emphasize that bones in males were characterized by considerably greater dynamics and higher values of physical parameters in comparison with females.

Higher strength of bones of broiler chickens, determined on the basis of changes of ultimate stress, occurring after the addition of phytase to the diet of chickens was also ascertained by other authors, Q i a n et al. (6) and P e r n e y et al. (5). However in the accessible literature no reports were found which would describe such large increases in values of the mechanical parameters of bones of broiler chickens, which were found in our investigations.

In sum, the results, especially the evidently profitable effects connected with more rapid growth of chickens, greater body weight gain (5.8-29% greater in comparison with control) and also with increased bone strength (ultimate stress – 13-89% greater in comparison with control) prove the positive influence of dietary phytase supplementation on the growth and development of broiler chickens.

CONCLUSIONS

1. Supplementation of phytase to the diet influences the utilization of feed and also the body weight gain.

2. Addition of phytase to the diet increases the mass, length and physical parameters of bones, causing their higher strength as well.

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STRESZCZENIE

Badania przeprowadzono w celu określenia wpływu dodatku fitazy (Natuphos® 5000, BASF, Niemcy) do diety kurcząt brojlerów na wzrost i rozwój układu kostnego. Fitazę w ilości 500, 750 i 1000FTU/kg paszy stosowano od pierwszej doby życia przez okres 4, 6 i 8 tygodni. Ocenie poddano przyrost masy ciała kurcząt oraz zużycie paszy w przeliczeniu na kilogram przyrostu masy ciała. Oznaczono także zawartość fosforu ogólnego w kale w 4, 6 i 8 tygodniu życia. Po uboju badano masę, długość i parametry wytrzymałościowe – siłę maksymalną, siłę w punkcie przekraczania granicy sprężystości oraz sztywność – kości udowych i ramiennych. Kurczęta otrzymujące paszę wzbogaconą fitazą cechowały się większą masą ciała (o 5,8%-29%) oraz lepszym wykorzystaniem paszy. Dodatek fitazy do diety w istotnym stopniu wpływał na masę (wzrost o 0-28%) i długość (wzrost do 0,5-11%) poddanych badaniom kości. Kości udowe i ramienne zarówno kurek, jak i kogutków grup doświadczalnych cechowały się większymi wartościami analizowanych parametrów wytrzymałościowych – wzrost wartości siły maksymalnej o 13-89% – w porównaniu z grupą kontrolną. Szybki wzrost kurcząt, osiąganie większej masy ciała oraz wzrost wytrzymałości kości dowodzą pozytywnego wpływu fitazy na wzrost i rozwój kurcząt brojlerów.