Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Afro Quarantelli, Antonio Cacchioli, Simone Romanelli, Federico Righi, Irene Alpigiani & Carlo Gabbi (2007) Effects of different levels of dietary biotin on the performance and bone structure of broilers, Italian Journal of Animal Science, 6:1, 5-7, DOI: 10.4081/ijas.2007.5

To link to this article: https://doi.org/10.4081/ijas.2007.5

Effects of different levels of dietary biotin on the performance and bone structure of broilers

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Paper received May 23, 2006; accepted October 15, 2006

ABSTRACT

We evaluated the effects of different levels of biotin on broilers performances and bone growth. Biotin was added at concentrations of 100, 200, 300, 400, 500 μg/Kg to a corn and soybean diet for yellow skin broiler production during the whole production cycle. Biotin at dosages of 200, 300, 400 μg/Kg increased growth rate, and, regardless of dosage, feed conversion rate in the second and third period of growth. Femur and tibiotarsus volume was slightly reduced while the bone mineral content of the same bones showed an increase as a result of biotin supplementation. Any particular dose-response effect was recorded with regard to plasma mineral content and ALP activity.

Key words: Broiler, Biotin, Mineral bone density.

RIASSUNTO

EFFETTI DI DIFFERENTI LIVELLI DI BIOTINA SULLE PERFORMANCES E SULLA STRUTTURA DELLE OSSA DEL POLLO DA CARNE

Sono stati studiati gli effetti di diversi livelli di biotina, aggiunta in ragione di 100, 200, 300, 400, 500 μg/Kg ad una razione a base di mais e soia per la produzione del pollo a cute gialla nei confronti della stessa razione non integrata per tutta la durata del ciclo produttivo. La biotina alle dosi di 200, 300, 400 μg/Kg di mangime ha migliorato la velocità di crescita e, a prescindere dal dosaggio, l'indice di conversione nella seconda e terza fase del ciclo di allevamento. Il volume del femore e del tibiotarso sono risultati tendenzialmente ridotti mentre il contenuto minerale degli stessi è risultato incrementato dalla supplementazione con biotina. Nessun particolare effetto dose-dipendente è stato registrato relativamente al contenuto di minerali del plasma ed alla attività plasmatica dell'enzima ALP.

Parole chiave: *Pollo da carne, Biotina, Densità minerale ossea.*

Introduction

Biotin is an essential coenzyme for all known organisms. Its physiologically active form is linked to enzymes of great metabolic importance like biotin carboxylase and biotin decarboxylase and seems to be a key-enzyme in important processes like gluconeogenesis and fatty acids and protein synthesis, controlling scleroprotein production. Because of its functions, this vitamin contributes to such important processes as growth, skin regeneration, bone development and reproduction, increasing feed conversion in animals (McMahon, 2002).

Man and mammals, like birds, do not have the capability to synthesize this vitamin which is provided, under physiological conditions, by gut flora microbial synthesis (Payne *et al.*, 1974) and diet. On the basis of microbiological tests, biotin appears to be present in variable and often low concentrations in animals and plant tissues (Balnave and Pearce, 1976; Scholtyssek and Seemann, 1988). In particular, high levels of biotin have been observed in organs such as liver and kidney while low biotin concentration has been observed in meat, vegetables and fruits (Payne *et al.*, 1974). In general, biotin bioavailability is less than 50% ranging from the apparent 100% of corn to less than 5% of wheat. This parameter, which has been demonstrated to be a limiting factor for absorption in broilers (Brewer and Edwards, 1972), is influenced by technological factors, such as harvest time, working processes and storage conditions (Whitehead *et al.*, 1985; Blair and Misir, 1989; Oloyo and Ogunmodede, 1991; Oloyo, 1994; Dilger *et al.*, 2004). In addition, biological factors can reduce biotin absorption: proteinous substances produced by fungi mould (*Saccaromyces avidinii*), can bind biotin which then becomes unavailable for the organism; some aflatoxins increase the biotin requirement (Buenrostro and Kratzer, 1983), while avidin of egg albumen completely binds the vitamin. Frigg (Frigg, 1976, 1984) and Whitehead (1984) showed that avian species are not able to absorb wheat biotin, while they can metabolise the biotin of barley, sorghum, oat, bran and manioc; biotin in oil seeds and in dehydrated alfalfa shows high absorption levels.

Biotin deficiency in broilers is a cause of skin diseases such as dermatitis and the softening hard tissue in claws and beaks with subsequent bone deformities or loss of plumage (Harms and Simpson, 1975; Harms *et al.*, 1977; Summers *et al.*, 1978; Stock and Latshaw, 1981). Particularly damaging for the poultry industry are symptoms connected to growth reduction and low feed conversion rate (Ferguson *et al.*, 1978), with decreased productive and reproductive performance. When environmental conditions are not optimal, vitamin deficiency leads to Fatty Liver and Kidney Syndrome (FLKS) with sudden death in poultry (Watkins and Kratzer, 1987; Bain *et al.*, 1989; Watkins *et al.*, 1989, 1997). This disease is characterised by a dramatic reduction of the glucose plasma level between meals which contributes to the damage of brain tissues (Payne *et al.*, 1974; Balnave *et al.*, 1977, 1980; Pearce and Balnave, 1978; Whitehead and Randall, 1982; Watkins, 1989; Watkins *et al.*, 1991). This reduction in glucose plasma level depends on the decrease in activity of the biotin-dependent enzyme pyruvate carboxylase, which leads to gluconeogenesis blockage. Frigg (1976) showed the positive effects of biotin supplementation on the reduction of the occurrence of biotin deficiency symptoms and FLKS insurgence, such as on feed conversion rate.

Commercial conditions of meat-type chickens (Wilson, 1969; Novelli and Giusti, 1992; Giordani *et al.*, 1993) make vitamin supplementation necessary in order to prevent skeletal developmental anomalies to which fast growing strains have a tendency (Orr *et al.*, 1984; Acar *et al.*, 1991).

The objectives of this study are to evaluate the effects of different doses of supplemental dietary biotin on broilers' productive perfor-

mances, bone mineral metabolism and skeletal morphological development and to identify the best vitamin supplementation to improve the feed conversion rate and growth rate.

Material and methods

The trial was carried out in an experimental livestock of 12 boxes (1 to 12) of 18 square meters of surface. A total of 2,160 male chicks (Ross 508) were divided in 6 groups with two replicates of 180 birds. The animals of the Group 1 (control group - boxes 1 and 7) were fed with three pelleted complete feed of first, second and third period without biotin supplementation. Birds in Groups 2, 3, 4, 5 and 6 (experimental groups – remaining boxes) were fed the same feedstuff, supplemented with biotin at doses of 100, 200, 300, 400 and 500 µg/kg, respectively.

Feedstuffs, containing corn and soybean, were formulated for yellow skin broiler production (Table 1).

Table 1. Composition of the diets.

	Diets		
	0-21 d	22-41 d	42-61 d
Ingredients (%):			
Corn meal	56.850	59.635	62.610
Soybean meal 48 c.p.	34.400	28.000	24.300
Corn gluten feed	2.500	3.500	3.500
Fat (tallow and pork fat)	2.000	5.000	5.800
Dicalcium phosphate	2.150	2.000	2.000
Calcium carbonate	0.850	0.750	0.750
DL methionine	0.150	0.115	0.090
Lysine HCl	0.300	0.200	0.150
Sodium chloride	0.200	0.200	0.200
Sodium carbonate	0.100	0.100	0.100
Mineral-vitamin-premix	0.500	0.500	0.500
Composition by analysis (%):			
Moisture	12.57	12.24	12.14
Crude protein	22.62	20.40	18.90
Ether extract	4.55	7.55	8.49
Crude fibre	3.12	2.93	2.84
Ash	5.90	5.36	5.11
Lysine	1.368	1.126	0.990
Methionine	0.500	0.457	0.418
Methionine+ Cystine	0.867	0.801	0.742
Met. Energy (*) Kcal/kg	3017	3255	3306

(*) Estimated content.

During the trial, daily health status was observed, while at the end of each period (21st and 41st days) and before slaughtering (61st day), live weight and feed intake were evaluated. At the same times, 10 birds, randomly chosen in each group, were sacrificed. Before slaughtering, blood samples were collected for calcium, phosphorus, magnesium, chlorine and alkaline phosphatase (ALP) determination; analyses were performed with commercial kits.

From each animal, femur, tibiotarsus and fibula were isolated. A careful morphological examination to evaluate cartilage integrity and bone linearity was performed on the right limb of each bone segment; length, volume, craniocaudal and laterolateral diameter measurements, and densitometrical analysis were also carried out. Measurements were performed on right femur and tibiotarsus assuming that no significant differences with the contralateral bones are valuable. Length was evaluated using a particular caliper, whose arms were elongated to improve the instrument's stability on the plane and to

Figure 1. Chicken femur: computerized bone mineralometry.

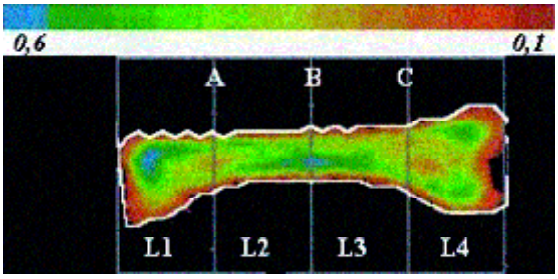
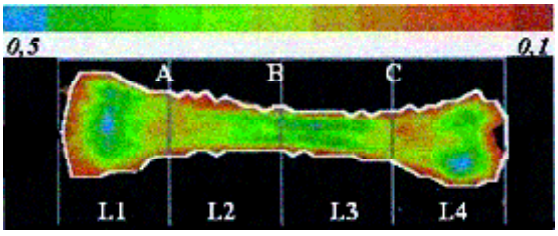


Figure 2. Chicken tibiotarsus: computerized bone mineralometry.



increase the contact surface with bone epiphysis. Bones were leant with their back surface and their axis parallel to the axis of the calliper. Volume was measured evaluating the increment of liquid level after complete immersion of bones in a graduated cylinder filled with distilled water.

In order to determine laterolateral and cranial to caudal diameters, bone widths were taken with the same calliper at A, B and C lines, located in a proximal to distal way, at 1/4, 1/2 and 3/4 of the bone length, respectively (Figure 1 and 2).

Mineral bone density analysis was performed by an X-ray producing system DEXA (Onyango *et al.*, 2003; Hester *et al.*, 2004) with a Samarius filter (Unigramma Plus/P Compact). This procedure was performed with bones leant against their back surface. Mathematical elaboration of the difference between the initial energy supplied from the instrument and the energy taken after the bone segment is processed represents the measurement of the average mineral density (Total BDM, gr/cm^2), and the partial mineral density, of the four areas (Partial BMD: L1, L2, L3 and L4) in which the instrument divides each bone in a proximodistal way automatically; also the instrument provides the average mineral content (BMC, g) and the segments area (cm^2) (Figure 1 and 2).

Graphical elaboration was performed by the software GraphPad Prism version; statistical analysis was carried out by variance analysis and by Tukey test. Statistical analysis was performed on the other data from the sample birds of each group at the different experimental periods, comparing control group with groups fed increasing levels of biotin.

Table 2. Productive parameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
BW at 21 d	g	663^a	642^b	635^b	636^b	622^b	618^b	6.547
FCR 1-21 d	"	1.369	1.368	1.361	1.363	1.364	1.334	
BW at 41 d	"	2300^b	2383^b	2507^a	2487^a	2398^a	2356^b	32.190
FCR 1-41 d	kg	1.911	1.777	1.754	1.761	1.784	1.780	
BW at 61 d	g	3206^b	3290^b	3486^a	3387^a	3358^a	3309^b	38.843
FCR 1-61 d	kg	2.010	1.964	1.966	1.962	1.953	1.984	
Mortality rate	%	5.00	4.72	5.55	4.44	5.28	4.72	

a, b: P<0.05; (*) µg/kg of diet.

Results and discussion

Productive parameters and bone mineral metabolism

At the dosages tested, biotin supplementation appears to be safe. During the trial both control and experimental chickens did not show any syndrome referable to the treatment. Biotin supplementation did not modify mortality rate, which fluctuated around 5% for both control and experimental groups.

Despite his positive effect on locomotion, biotin supplementation appeared to have a dose-dependent negative influence on growth rate, especially in the first period of growth. At 21 d of age, the animals of all the experimental groups showed a lower growth rate ($P<0.05$), respectively of -3.17%, -4.23%, -4.07%, -6.19% and -6.79% for Group 2, 3, 4, 5 and 6 in comparison to the control group (Table 2).

During the following periods, biotin at the doses of 200, 300 and 400 µg/kg improved growth rate ($P<0,05$). At slaughtering (61d) differences between control and experimental chickens were +8.73, +5.64 and +4.47 for Group 3, 4 and 5, respectively. There were no significant differences between group 1 and group 2 and 6.

Experimental chickens, independently from biotin supplementation, consumed a quantity of feed constantly lower than those in the control group.

Differences, calculated over the entire rearing period, were of -2.29%, -2.19%, -2.39%, -2.84%, -1.29% for Groups 2, 3, 4, 5 and 6, respectively.

Any particular dose-response effect was recorded with regard to plasma mineral content and ALP activity, the main bone related enzyme. Some significant differences ($P<0.05$) were observed between groups, but the values of all the parameters appeared randomly distributed around those of the control group. The highest Ca and P levels were recorded in the 300 µg/kg supplemented group (Ca: 10.95 mg/dl; P: 9.49 mg/dl), while the lower levels were observed in the 100 and 400 µg/kg supplemented group for P and Ca, respectively (4.44 and 4.94 mg/dl). The highest level of Mg was recorded in the 500µg/kg supplemented group (2.39 mg/dl), while the lowest was found in the 400µg/kg supplemented group (2.30 mg/dl). Cl plasma levels appeared similar for the different groups (Table 3).

Morphological parameters

Length

Femur - Any significant difference was detected in femur length between the experimental groups at the considered growing periods (Table 4).

Table 3. Bone metabolism parameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
Ca	mg/dl	7.00^{c}	5.26^{d}	9.14^{b}	10.95^{a}	4.94^{d}	8.97^{b}	0.921
P	"	6.84^{b}	4.44^{d}	7.29^{ab}	9.49^{a}	5.20^{c}	5.25^{c}	0.630
Mg	"	2.33^{b}	2.28^{c}	2.37^{a}	2.33^{b}	2.30^{c}	2.39^{a}	0.018
Cl	"	384.00	388.33	381.33	386.00	393.67	386.67	0.687
ALP	U/L	3730.00	3437.67	2799.00	2705.00	3274.67	3540.67	184.511

Tested at 25° C; a, b, c, d: P<0.05 (*) µg/kg of diet.

Table 4. Femur–length, volume, total bone mineral density (BMD_t).

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
	21d	54.57	53.07	53.60	51.40	54.17	51.93	0.510
Length (mm)	41d	75.50	77.00	79.87	78.37	78.60	78.13	0.611
	61d	94.33	94.40	95.43	97.10	97.37	90.50	1.019
	21d	3.50	3.83	3.67	3.43	4.00	3.67	0.086
Volume (ml)	41d	8.75	10.83	10.83	10.00	10.50	11.33	0.371
	61d	28.33^{A}	25.67	15.50^{B}	18.33^{B}	16.33^{B}	18.00^{B}	2.191
	21d	0.30^{a}	0.27^{b}	0.26^{b}	0.26^{b}	0.26^{b}	0.25^{b}	0.006
BMD_T (g/cm^2)	41d	0.37	0.37	0.37	0.36	0.39	0.41	0.008
	61d	0.42	0.41	0.43	0.44	0.46	0.42	0.007

a, b: P<0.05; A,B: P<0.01; (*) µg/kg of diet.

Table 5. Tibiotarsus – length, volume, total bone mineral density (BMD_t).

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
	21d	69.60	72.47	71.03	68.73	72.17	69.00	0.661
Length (mm)	41d	107.50	108.33	114.23	113.20	114.50	113.70	1.281
	61d	132.43	135.83	135.10	138.10	140.83	140.43	1.335
	21d	5.50	5.33	5.50	5.00	5.33	6.00	0.134
Volume (ml)	41d	14.25	14.83	18.17	16.50	17.33	16.67	0.608
	61d	25.00	26.00	27.00	26.67	28.33	28.00	0.507
	21d	0.28^{Aa}	0.26^{b}	0.25^{b}	0.25^{b}	0.26^{b}	0.24^{B}	0.006
BMD_T (g/cm^2)	41d	0.37	0.38	0.37	0.37	0.40	0.41	0.007
	61d	0.42	0.42	0.47	0.50	0.47	0.43	0.014

a, b: P<0.05; A, B: P<0.01; (*) µg/kg of diet.

Table 6. Femur – partial bone mineral density (BMD_p).

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
BMD_p L1 (g/cm^2)	21d	0.29	0.28	0.26	0.27	0.27	0.26	0.004
	41d	0.40	0.39	0.42	0.38	0.43	0.44	0.008
	61d	0.46	0.44	0.45	0.49	0.49	0.47	0.008
BMD_p L2 (g/cm^2)	21d	0.33^a	0.30^a	0.28^b	0.29^b	0.29^b	0.27^b	0.009
	41d	0.38	0.40	0.39	0.39	0.42	0.44	0.007
	61d	0.43	0.44	0.47	0.48	0.48	0.44	0.013
BMD_p L3 (g/cm^2)	21d	0.34^a	0.30^a	0.28^a	0.30^a	0.29^a	0.26^b	0.010
	41d	0.36^b	0.39^b	0.36^b	0.37^b	0.40^a	0.41^a	0.010
	61d	0.40	0.41	0.44	0.43	0.45	0.40	0.012
	21d	0.26	0.24	0.23	0.23	0.24	0.23	0.006
BMD_p L4 (g/cm^2)	41d	0.34^b	0.33^b	0.34^b	0.32^b	0.35^b	0.37^a	0.006
	61d	0.40	0.37	0.39	0.40	0.43	0.40	0.008

a, b: P<0.05; A, B: P<0.01; (*) µg/kg of diet.

Tibiotarsus - As observed for femur length any significant difference was registered in tibiotarsus length (Table 5).

Volume

Femur - In the last period of growth (41-61 days) a highly significant difference (P<0.01) between the control group and the experimental group receiving 200 µg/Kg of biotin (giving the lowest volume) was documented. Moreover, significant differences (P<0.05) between the control group and the groups integrated with 300, 400 and 500 µg/Kg of biotin were found. Despite the differences recorded, no specific dose-response effect of biotin on femur volume was observed (Table 4).

Tibiotarsus - The differences found in tibiotarsus volume between groups did not appear to be statistically significant. Nevertheless, a slightly higher volume was observed in the treated groups in comparison to the control group (Table 5).

Total bone mineral density

Femur - During the first experimental period, a significant difference (P<0.05) between total bone mineral content of the control group and the treated groups was registered (Table 4).

In the last interval, a gradual, not significant increase, of the total bone mineral density was observed at 200, 300, 400 µg/Kg of biotin added. A similar result was reported by Schreiweis *et al.* (2003) which observed a variation of vitamin efficiency.

Tibiotarsus - As observed for femur, there is a significant difference (P<0.05) between the control and the treated groups. Moreover, a highly significant difference (P<0.01) was found between the control group and the 500 µg/Kg of biotin integrated group, which shows the lower value (Table 5).

In summary, total mineral density at 21 days for biotin integrated groups was lower compared to the control group, for both femur and tibiotar-

Table 7. Tibiotarsus – partial bone mineral density (BMD_p).

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
	21d	0.28[a]	0.27[a]	0.26[a]	0.27[a]	0.27[a]	0.25[b]	0.004
BMD_p L1 (g/cm^2)	41d	0.41	0.41	0.40	0.39	0.4	0.45	0.010
	61d	0.47	0.43	0.47	0.48	0.49	0.46	0.008
	21d	0.29[a]	0.26[a]	0.23[b]	0.24[a]	0.25[a]	0.23[b]	0.008
BMD_p L2 (g/cm^2)	41d	0.33	0.34	0.35	0.35	0.38	0.35	0.010
	61d	0.44	0.41	0.47	0.46	0.45	0.39	0.010
	21d	0.33	0.30	0.27	0.28	0.31	0.27	0.011
BMD_p L3 (g/cm^2)	41d	0.37	0.38	0.35	0.38	0.42	0.40	0.008
	61d	0.45	0.41	0.48	0.46	0.43	0.40	0.018
	21d	0.26[a]	0.24[a]	0.23[a]	0.23[a]	0.23[a]	0.22[b]	0.004
BMD_p L4 (g/cm^2)	41d	0.36	0.36	0.37	0.36	0.38	0.40	0.006
	61d	0.45	0.43	0.47	0.47	0.48	0.44	0.008

a, b: P<0.05; A, B: P<0.01; (*) µg/kg of diet.

Table 8. Femur – laterolateral diameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
Lat-lat A	21d	6.90	6.13	6.60	6.37	6.67	6.67	0.109
diameter (mm)	41d	9.40	9.67	9.97	9.63	9.43	9.77	0.086
	61d	13.30	12.03	12.00	11.77	12.50	11.57	0.255
Lat-lat B	21d	6.43	5.83	6.10	5.87	5.87	5.97	0.093
diameter (mm)	41d	9.30	9.13	10.00	9.67	11.03	10.20	0.282
	61d	11.90	11.50	12.10	12.07	12.40	12.30	0.131
Lat-lat C	21d	7.77	8.43	8.57	8.13	7.50	8.37	0.170
diameter (mm)	41d	12.60	13.07	13.77	13.30	13.50	14.37	0.247
	61d	16.03	14.80	16.30	15.43	15.90	16.67	0.270

(*) µg/kg of diet.

Table 9. Tibiotarsus – laterolateral diameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
Lat-lat A	21d	9.80	6.34	10.07	9.47	10.57	9.07	0.612
diameter (mm)	41d	12.85	13.07	13.67	13.53	13.80	14.07	0.187
	61d	19.37	15.30	16.57	17.33	17.67	16.20	0.573
Lat-lat B	21d	6.93	6.53	6.73	6.43	6.10	6.27	0.124
diameter (mm)	41d	8.65	9.37	9.77	9.87	9.77	9.77	0.190
	61d	12.17	10.90	11.70	12.00	11.20	11.80	0.198
Lat-lat C	21d	9.03	8.53	7.63	7.40	8.03	7.80	0.249
diameter (mm)	41d	10.90	11.47	11.37	11.43	11.90	11.87	0.150
	61d	13.93[a]	11.87[b]	13.13[a]	13.07[a]	13.23[a]	13.27[a]	0.275

a, b: P<0.05; (*) µg/kg of diet.

sus; In the subsequent periods, on the contrary, total mineral content tended to increase, although not significantly, at the doses of 200, 300, 400 mg/kg, particularly on tibiotarsus. On the basis of these data (Tables 4 and 5), biotin integration in the first growing period is supposed to be useless and unsuitable, while during the subsequent growing periods it can also improve bone mineral content, increasing growth rate as anticipated by other Autors (Hamrick, 2003). Trends observed in Ca and P levels support this hypothesis. In fact, at 61 days, an increased Ca (9.14, 10.95, 8.97, respectively, for Groups 3, 4 and 6 vs 7.00 mg/100 ml of the control group) and P (7.29, 9.49, 5.25 for Groups 3, 4 and 6, respectively, vs 6.84 mg/100 ml) content is reported. These data show how vitamin supplementation promotes absorption and bone deposition of Ca and P.

Partial bone mineral density

Femur - Partial bone mineral density gives an estimate of mineral content of the four bone segments from L1 to L4 (Figure 1 and 2). Any significant differences were noticed at the various intervals considered in the different groups on the L1 segment, femoral proximal epiphysis.

As shown in Table 6, at 21 days a significant difference (P<0.05) was registered between the control group and the biotin integrated groups (200, 300, 400 and 500 µg/Kg) in the L2 segment, or femoral diaphysis.

Significant differences were found on the L3 segment at 21 and 41 days between Group 6 and 5 and the other groups; significant differences (P<0.05) were observed also on the L4 segment at 41 days between Group 6 and the other groups (Table 6).

Tibiotarsus - A significant difference (P<0.05) was registered in the L1 tibiotarsus segment between Group 6 and the other groups. Moreover, a significant difference (P<0.05) was found at the first period of growth between Groups 3 and 6, the control group and the other groups on the L2 segment. On L3, any significant difference was recovered at the different intervals, while on L4 a significant difference (P<0.05) between Group 6, the control group and the other groups was registered at the end of the first period of growth.

Table 10. Femur – craniocaudal diameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
Cr-cd A	21d	8.13	8.00	8.20	7.60	8.10	7.53	0.118
diameter (mm)	41d	10.75	12.07	11.87	10.10	10.70	11.03	0.306
	61d	13.80	12.53	13.87	14.10	13.87	12.87	0.262
Cr-cd B	21d	6.60	6.37	7.10	6.30	6.33	6.30	0.129
diameter (mm)	41d	8.65	9.80	9.83	9.43	9.70	9.97	0.197
	61d	10.87	10.63	11.90	12.03	12.37	11.50	0.279
Cr-cd C	21d	7.80	6.40	7.00	6.40	6.67	6.93	0.214
diameter (mm)	41d	8.85	9.50	9.67	9.47	9.70	9.90	0.147
	61d	10.90	10.23	11.23	11.10	11.57	11.40	0.193

(*) µg/kg of diet.

Table 11. Tibiotarsus – craniocaudal diameters.

Groups		1	2	3	4	5	6	
Added biotin (*)		Control	100	200	300	400	500	SEM
	Periods							
Cr-cd A	21d	7.60	8.03	8.30	7.97	7.90	7.53	0.116
diameter (mm)	41d	10.40	11.50	11.67	11.93	11.43	11.43	0.214
	61d	14.87	12.80	13.63	13.60	14.90	12.47	0.414
Cr-cd B	21d	5.93	5.27	5.53	5.10	5.33	5.23	0.121
diameter (mm)	41d	7.00	7.33	7.67	7.93	8.23	7.57	0.178
	61d	10.50	9.23	10.37	10.40	9.27	9.47	0.249
Cr-cd C	21d	5.87	5.40	5.57	4.97	5.57	5.37	0.122
diameter (mm)	41d	7.90	8.30	7.80	7.97	8.30	8.30	0.094
	61d	9.87	8.70	9.73	9.60	9.90	9.40	0.183

(*) µg/kg of diet.

The greatest increment in bone density was registered on segment L1 and generally the bone density tends to raise passing from the first to the last period of growth in all the segments independently from biotin integration (Table 7).

Partial bone mineral content of the L1 femoral segment is not influenced by biotin supplementation because ossification is not achieved

in the proximal epiphysis at the experimental times. This could be explained on the basis of encondral ossification in poultry which is different from the one in mammals. In poultry, long bones epiphysis are cartilaginous, as secondary centres of ossification in the epiphysis do not exist in the sampled bones. Epiphysarian secondary centres of ossification are only in the proximal and distal portion of the tibia and in the distal portion of metatarsus (fused tarsal bones). The process of ossification reaches the epiphysis from the primary centre of ossification.

Any particular trend was observed in segments L2 and L3 with increasing biotin levels in the diet even if the primary centre of ossification is active starting from 20 days of age.

An incomplete ossification of the proximal epiphysis during the three experimental ages is registered in the tibiotarsus. In fact, as already mentioned, this bone segment indudes a secondary centre of ossification in the epiphysis. In general, at the end of hatching the chicks present two more bones on the distal portion of the tibia: calcaneal bone and talus. During development these two bones increase their volume until they form one single bone, which is the distal border of the tibia. Blenau (1990a and 1990b) radiologically analysed the distal segment of the tibiotarsus, showing that calcaneal bone, a talus sinostosis, appears between 42 and 62 days of life. Comparing these physiological parameters with our data, it is possible to point out a bone mineral content increase in the L4 segment, that is the distal epiphysis of tibiotarsus, especially when 500 μg/Kg of vitamin are added. In relation to this densitometrical increase, we hypothesize greater osteogenic activity due to the fusion of tarsal bones. Once again biotin appears to be more useful in the last growing period at doses of 200, 300, 400 μg/Kg.

Diameters

Femur - No significant differences were found with regard to laterolateral and craniocaudal diameter measurements at the lines of the three surveys (Table 8 and 10).

Tibiotarsus - Any significant differences were found with regard to laterolateral and craniocaudal diameter measurements at the three intervals considered (Table 9 and 11).

Conclusions

On the basis of our results, biotin appears to improve poultry health and performance. Biotin treatment did not affect mortality rate and any clinical sign associated with treatment was observed during the trial. Biotin appeared to improve, from a clinical point of view, standing and walking ability. Moreover, biotin at doses of 200, 300 and 400 μg/Kg of feed increased growth rate during the second and third period of growth, while the feed conversion rate was improved throughout the productive cycle with the higher dose of biotin at 400 μg/Kg of feed .

Volumetrical and densitometrical data appear advantageous for productive purposes (decreased volume and increased density) at doses between 200 and 400 μg/Kg of feed. We conclude that these levels of biotin, administered during the second and third period of growth, could be the most suitable for the diet of broilers.

The authors would like to thank Mr. Luigi Faroldi for his technical collaboration.

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