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The effect of diet supplementation with propolis and bee pollen on the physicochemical properties and strength of tibial bones in broiler chickens

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Abstract

The objective of this study was to determine the effect of diet supplementation with propolis and bee pollen on selected physical, biomechanical and chemical properties of tibial bones in broiler chickens. The experimental materials comprised 384 Ross 308 chickens (four groups, three replications), divided into groups of males and females, and raised to 35 days of age. Control group 1 birds were fed a commercial diet throughout the experiment. Birds of three other experimental groups received for the first two weeks of rearing standard diets with a different content of propolis and/or bee pollen: group 2 – 0.025 % propolis, group 3 – 0.5 % bee pollen, group 4 – 0.5 % bee pollen and 0.025 % propolis. The following parameters of the *tibia* were determined: weight, length, mid-shaft circumference, vertical and horizontal external diameters, vertical and horizontal internal diameters. The geometric characteristics of the bones were determined based on the above measurements. Mid-shaft shear strength was measured using an Instron universal testing machine 4301 (Instron, Norwood, MA, USA). The crude ash, calcium (Ca) and phosphorus (P) content of the *tibia* was also determined. Higher values of geometric parameters were noted in the tibial bones of broilers fed a diet supplemented with a combination of propolis and bee pollen, in comparison with birds fed diets containing propolis or bee pollen administered alone. The shear strength values and physical properties of bones were similar in all groups.

Keywords: broiler chickens, propolis, bee pollen, bones, physical properties, bone strength, chemical composition

Introduction

Broiler chickens are characterized by a very fast growth rate, therefore broiler diets should contain high concentrations of available nutrients (Zimnoch *et al.* 2000). Until recently, antibiotics had been used to improve feed utilization efficiency in poultry, but after the ban imposed in the European Union they were replaced by natural feed additives, including herbs (Kwiecień & Winiarska-Mieczan 2009). Propolis seems to be an effective natural alternative to antibiotic growth promoters. Research results (Li & Zhang 2002, Roodsari *et al.* 2004, Zeng *et al.* 2004) suggest that propolis has a beneficial influence on daily gains, feed intake and conversion in different animal species, including poultry. In a study by Roodsari *et al.* (2004), chickens fed 250 mg propolis per kg feed were characterized by significantly higher body weights and lower feed intake per kg body weight gain, compared with birds receiving diets without propolis supplements. In an experiment by Zeng *et al.* (2004), a combination of flower pollen and propolis at a ratio of 2.5 : 1 used as a feed additive increased the body weights of chickens by nearly 10 % in comparison with the control group. Li & Zhang (2002) reported that supplementing diets for broiler chickens with 2.5 % propolis contributed to higher weight gains and better feed conversion, thus increasing production profitability by 9.7 %.

The adverse effect of broiler selection programs that focus on increased body weight and a faster growth rate is a higher incidence of leg problems, in particular bone deformities and abnormalities (Leterrier & Nys 1992, Horn 2000, Williams *et al.* 2004). The skeletal system plays many important functions: it keeps the body in an upright position, it is responsible for body locomotion, it protects organs and tissues, and it serves as the main storage system for mineral compounds, mostly calcium, phosphorus and magnesium (Toba *et al.* 2000). According to Stójko *et al.* (1978), an ethanol extract of propolis accelerates the healing of bone fractures. Ang *et al.* (2009) have recently demonstrated that a bioactive component of propolis, caffeic acid phenethyl ester (CAPE), has very strong properties supporting the growth and development of human bones. According to the cited authors, CAPE is a potential treatment for bone lytic diseases, and a therapeutic agent for osteoporosis. Propolis has been also found to significantly improve the availability and utilization of dietary calcium and phosphorus, which has a beneficial effect on the skeleton (Haro *et al.* 2000).

Various studies show that propolis positively affects chicken performance, yet scientific literature provides scant information on the effects of propolis on bone strength in birds. In view of the above, the objective of this study was to determine the effect of diet supplementation with propolis and bee pollen on the physical, biomechanical and chemical properties of tibial bones in broiler chickens.

Materials and methods

The experimental materials comprised 384 Ross 308 chickens (four groups, three replications), divided into groups of males and females, and raised to 35 days of age. The birds were fed *ad libitum* starter, grower and finisher diets, from day 1 to 14, from day 15 to 28 and from day 29 to 35, respectively (Table 1). Control group (1) birds were fed a commercial diet throughout the experiment. Birds of three experimental groups received, for the first two weeks of rearing, standard diets with a different content of propolis and/or bee pollen:

- group 2 - 0.025 % propolis,
- group 3 - 0.5 % bee pollen,
- group 4 - 0.5 % bee pollen and 0.025 %.

Propolis dry extract standardized to contain 5 % quercetin was purchased ay Phytopharm Klęka (Poland). Dry bee pollen was supplied from an apiary of the Department of Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Poland. Propolis and bee pollen, in powder form, were mixed with dry feed.

Table 1
Nutritive value of diets

Nutrients	Diet		
	Starter	Grower	Finisher
Energy, kcal	2 950	3 040	3 120
Total protein, %	21.4	20.2	19.8
Lysine, %	1.35	1.25	1.2
Methionine+cystine, %	0.96	0.92	0.92
Threonine, %	0.78	0.82	0.76
Clcium, %	0.80	0.80	0.82
Available phosphorus, %	0.42	0.40	0.37
Sodium, %	0.16	0.17	0.17
Vitamin A, IU	15 000	12 000	12 000
Vitamin D_3, IU	3 750	3 000	3 000
Vitamin E, mg	52	41	41
Crude fiber, %	3.0	3.1	3.4
Coccidiostat salinomycin, ppm	60	60	-

At the completion of the experiment, 12 chickens (6♀ and 6♂) randomly selected of each group were fasted for 12 h, weighed and sacrificed. The carcasses (after the removal of head and feet, and evisceration) were chilled at +4 °C for 18 h, weighed and dissected. Tibial bones were separated, removing cartilage. The weight, length and mid-shaft circumference of the *tibia* were determined.

Mid-shaft shear strength was measured using an Instron universal testing machine 4301 (Instron, Norwood, MA, USA), coupled to a computer to obtain force-deformation curves illustrating the correlation between shear force perpendicular to the long axis of the bone and deformation. Load values were recorded at a constant speed (V=10 mm/min) of a single cutting blade (Warner-Bratzler Shear, type 2830-013). Force capacities ranged from 0 to 1 000 N. The measurements were performed in 12 replications per sample (variant), at room temperature. The data were analyzed using Instron Series IX Instron Series IX Software 8.34.00 (Instron, Norwood, MA, USA)

Bone strength was determined according to the method proposed by Szeremeta *et al.* (2003), based on the following measurements: shear force F_1 (N) needed to break the bone, the maximum shear force (intersection point, F_{max}, N) needed for bone disintegration and interruption of the bone structure, and bone deformation in response to the maximum shear force (d_{max}, mm). Bone stiffness was calculated as the F_{max} to d_{max} ratio (N/mm).

The geometric parameters of the bones, determined after a mid-shaft shear test, included vertical (A) and horizontal (B) external diameters, vertical (C) and horizontal (D) internal diameters. The measurements were taken with an electronic caliper. Based on the geometric characteristics of the *tibia*, the following parameters were determined as described by Brzóska *et al.* (2005):

- Cortical thickness (mm) B – D
- Cortical index (B – D)/B × 100
- Cortical area (mm^2) $B^2 - D^2$
- Cortical area ratio $(B^2 - D^2)/B^2 \times 100$
- Cross-sectional moment of inertia (CSMI) $3.14 (A^3B - C^3D)/64$

- Cross-sectional area (CSA) (mm^2) 3.14 (BA – DC)/4
- Mean relative wall thickness (MRWT) [(A – C)/C + (B – D)/D]/2

The content of crude ash and minerals (calcium and phosphorus) in tibial bones was determined after dry mineralization at 650 °C. Ca content was measured by spectrophotometry, and P content was estimated colorimetrically. Bone samples were mineralized in a mixture of nitric acid and perchloric acid (3:1) (Merck, Darmstadt, Germany). Mineralization was carried out in an aluminum heating block with temperature control (VELP DK 20, VELP Scientifica, Usmate, Italy). Reference samples were prepared together with test samples. The Ca content of mineralizates was determined by flame atomic absorption spectrometry (acetylene-air flame), with the use of Unicam 939 atomic absorption spectrometer (Solar, Cambridge, UK), equipped with an Optimus data station, a background correction source (deuterium lamp) and cathode lamps (Whiteside & Miner 1984). In order to determine Ca content, a 10 % aqueous solution of lanthanum chloride was added to all experimental solutions, in the amount ensuring the final La^{+3} concentration of 1 %. Phosphorus concentration was determined in mineralizates by colorimetry with ammonium molybdate and with sodium sulfate and hydroquinone. Absorbance was measured using a VIS 6000 spectrophotometer (A. KRÜSS Optronic GmbH, Hamburg, Germany), at a wavelength of λ=610 nm (Fiske & Subbarow 1925). The content of Ca and P was determined using standards at a concentration of 1 mg/cm^3, diluted with a 0.1 M solution of HNO_3 (BDH Prolabo, VWR International GmbH, Darmstadt, Germany).

The obtained results were processed statistically (means, standard deviations SD), and the significance of differences between mean values in experimental groups was estimated in males and females (two-way ANOVA) (Statistica 8, StatSoft, Inc. Tulsa, OK, USA).

Results and discussion

The weight and length of tibial bones and their percentage share of total body weight were similar in all groups of broilers. The values of the above parameters were affected by gender – the bones of males were heavier and longer than the bones of females (Table 2), which is consistent with the findings of Szeremeta *et al.* (2005).

The data in Table 3 show that group 4 chickens, fed a diet supplemented with 0.5 % bee pollen and 0.025 % propolis, were characterized by higher values of the geometric parameters of the *tibia* (cortical thickness, cortical index, cortical area ratio, mean relative wall thickness), compared with the other groups. Cortical thickness, cortical area, cross-sectional moment of inertia and cross-sectional area were significantly higher in males than in females (Table 3). According to Rath *et al.* (1999), such differences may be due to a different mode of action of male and female sex hormones.

In poultry, bone strength is affected by numerous factors, including nutritional regime, genetic factors, sex, age, management conditions and production system. Recent years have witnessed an increasing interest in the use of various feed additives and dietary supplements believed to improve skeletal integrity in poultry, such as phytase, herbs and ascorbic acid (Kocabagli 2001, Kwiecień, Winiarska-Mieczan 2009, Yildiz *et al.* 2009). An analysis of the biomechanical properties of the *tibia* in broiler chickens revealed that propolis and bee pollen did not lead to significant differences in the values of shear force, maximum shear force, bone deformation caused by the maximum shear force, and bone stiffness (Table 4). It is difficult to compare the current results with the findings of other authors since the available scientific literature provides no relevant information.

Table 2
Physical properties of the *tibia* in broiler chickens

Specification	Statistical measures	Group 1	2	3	4	Gender ♂	♀
Tibia weight, g	mean	22.71	22.56	23.29	22.15	25.67**	19.68
	SD	2.20	2.08	1.84	1.62	1.35	0.93
Tibia length, mm	mean	93.0	92.4	92.1	92.2	93.4**	91.4
	SD	2.5	3.2	1.8	1.3	2.3	1.8
Tibia percentage in the	mean	1.01	1.06	1.04	1.00	1.07**	0.98
total body weight, %	SD	0.09	0.08	0.09	0.07	0.07	0.07

**$P \le 0.01$

Table 3
Geometric characteristics of the *tibia* in broiler chickens

Specification	Statistical measures	Group 1	2	3	4	Gender ♂	♀
Cortical thickness, mm	mean	3.26^{ABab}	2.86^{Bb}	3.05^{ABb}	3.56^{Aa}	3.50**	2.87
	SD	0.90	0.47	0.58	0.63	0.64	0.61
Cortical index	mean	39.75^{AB}	36.17^{B}	36.37^{B}	44.55^{A}	39.97	38.45
	SD	8.17	5.70	5.12	6.91	6.16	8.23
Cortical area, mm^2	mean	42.76	37.26	41.94	44.45	48.84**	34.36
	SD	13.72	7.75	10.68	10.06	9.12	6.63
Cortical area ratio	mean	63.08^{AB}	58.96^{B}	59.27^{B}	68.81^{A}	63.60	61.47
	SD	10.15	7.31	6.65	7.61	7.05	10.23
Cross-sectional moment	mean	178.07	180.93	219.98	202.00	247.18**	143.31
of inertia CSMI	SD	101.31	60.38	106.45	83.69	95.14	36.67
Cross-sectional area	mean	32.59	31.22	34.16	36.18	38.71**	28.37
CSA, mm^2	SD	9.08	5.91	9.26	7.59	7.29	4.78
Mean relative wall	mean	0.71^{ABa}	0.63^{Aa}	0.63^{Aa}	0.86^{Bb}	0.74	0.68
thickness MRWT	SD	0.19	0.09	0.13	0.14	0.16	0.17

**$P \le 0.01$, $^{AB}P \le 0.01$, $^{ab}P \le 0.05$

Table 4
Strength parameters of the *tibia* in broiler chickens

Specification	Statistical measures	Group 1	2	3	4	Gender ♂	♀
Shear force	mean	413.88	378.38	379.90	375.88	420.93**	353.09
F_1, N	SD	101.34	99.52	78.04	87.27	83.82	85.34
Maximum shear force	mean	445.48	395.37	386.48	386.97	436.54*	370.61
F_{max} ,N	SD	115.69	121.88	74.79	75.97	104.01	84.27
Deformation d_{max} in	mean	3.70	2.64	3.35	3.23	2.77	3.69
response to the maximum shear force, mm	SD	1.89	1.46	1.97	2.17	1.35	2.21
Stiffness F_{max}/d_{max}, N/mm	mean	2.12	2.21	2.11	2.14	2.23*	2.06
	SD	11.76	9.05	11.06	13.87	8.77	12.64

**$P \le 0.01$, *$P \le 0.05$

Bone strength values, including shear force needed to break the bone ($F_{1♂}$=420.93 N, $F_{1♀}$=353.09 N) and the maximum shear force ($F_{max♂}$=436.54 N, $F_{max♀}$=370.61 N), were higher in males than in females (Table 4). As reported by Frost (1997), bone strength is directly proportional to bone mass. This correlation was also observed in the present study - the tibial bones of males were both heavier and stronger than the tibias of females.

The highest content of dry matter was noted in the tibial bones of broilers fed a diet supplemented with propolis and bee pollen (group 4). Phosphorus concentrations in the tibial bones of chickens were comparable in all groups. Concentrations of calcium in ash was highest in the tibial bones of broilers in the control group and group 4, fed a diet supplemented with 0.5 % bee pollen and 0.025 % propolis. In the other groups the level of calcium was significantly lower. The chemical composition of the *tibia* was similar in males and females (Table 5).

Table 5
Mineral composition of the *tibia* in broiler chickens

Percentage share	Statistical measures	Group 1	Group 2	Group 3	Group 4	Gender ♂	Gender ♀
Tibia dry matter	mean	94.96[ab]	94.46[b]	94.69[ab]	95.34[a]	94.72	95.01
	SD	0.62	0.81	0.99	0.72	0.79	0.86
Ash in *tibia* dry matter	mean	54.38	55.16	54.57	54.07	54.37	54.72
	SD	1.69	2.53	1.92	2.57	1.90	2.45
Phosphorus in ash	mean	17.02	17.00	17.18	17.02	16.99	17.12
	SD	0.30	0.67	0.70	0.43	0.46	0.61
Calcium in ash	mean	38.86[a]	37.86[b]	37.99[b]	38.18[ab]	38.46	37.98
	SD	1.05	0.79	1.22	0.40	0.90	1.00

Means followed by small letters are significantly different at $P \leq 0.05$

In conclusion, higher values of geometric parameters were noted in the tibial bones of broilers fed a diet supplemented with a combination of propolis and bee pollen, in comparison with birds fed diets containing propolis or bee pollen administered alone. The shear strength values and physical properties of bones were similar in all groups. In view of the positive effect of the analyzed dietary supplements on the geometric parameters and chemical properties of the *tibia* in broiler chickens, further research is needed to determine the optimal dosage of propolis and bee pollen fed to poultry.

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