Ann. Anim. Sci., Vol. 18, No. 3 (2018) 781–793 DOI: 10.2478/aoas-2018-0017

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THE INFLUENCE OF SELECTED FEED ADDITIVES ON MINERAL UTILISATION AND BONE CHARACTERISTICS IN LAYING HENS*

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Abstract

The trial with 240 caged ISA Brown laying hens was performed to evaluate the effect of selected feed additives on mineral utilisation as well as biomechanical (breaking strength, yielding load, stiffness) and geometrical (cortex thickness, cross-section area, weight, length) indices of tibia and femur bones. At 26 wks of age the layers were randomly assigned to 10 treatments with 12 replicates (cages) of two birds. In the study a 2 × 5 experimental scheme was used i.e. to 70 wks of age, the layers were fed isocaloric and isonitrogenous experimental diets containing reduced (3.20%) or standard (3.70%) Ca level. The diets with both Ca levels were either not supplemented, or supplemented with the studied feed additives i.e. sodium butyrate, probiotic bacteria, herbal extract blend and chitosan. There were no statistically significant effects of the experimental factors on the indices of the tibia bones. However, the diet with reduced Ca level decreased bone breaking strength, yielding load, stiffness, and mineralisation of the femur bones (P<0.05). The majority of used feed supplements, i.e. probiotic, herb extracts, and chitosan, increased biomechanical indices (breaking strength and yielding load) and mineralisation of the femur bones (P<0.05). Neither dietary Ca level nor feed additives affected dry matter, organic matter, ether extract, N-free extracts, crude fibre and ash digestibility, and P retention and excretion; however, Ca excretion and retention was lower in the hens fed the diets with reduced Ca level (P<0.05). Relative Ca retention (Ca retained as % of Ca intake) was improved by diet supplementation with probiotic, herb extracts and chitosan (P<0.05). In conclusion, this study has shown that decreased Ca dietary level (3.20%) can negatively affect bone quality in layers, while probiotic, herb extracts and chitosan addition may improve the selected biomechanical indices of the femurs, irrespective of Ca dietary concentration.

Key words: laying hens, feed additives, calcium retention, bones quality

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*This work was supported from the National Research Institute of Animal Production statutory activity (Research Project No. 05-019.1; Balice, Poland).

Skeletal health is one of the most important issues in the modern egg production industry. Osteoporosis, which is the main concern relating to bones disorders in high-performing laying hens, particularly towards the end of lay, is defined as a severe decrease in mineralised structural bone in which Ca is mobilised from the bone in order to contribute to eggshell formation (Whitehead and Fleming, 2000; Whitehead, 2004). The repercussions of osteoporosis and skeletal weakening lead not only to performance and economic losses in egg production, but mainly are detrimental to the welfare of birds, causing acute and chronic pain and distress to the animals (Webster, 2004; Lay et al., 2011). Results of the study of Wilkins et al. (2011) showed a very high frequency of bones breakage in end-of-lay laying hens housed in a variety of system designs. Jendral et al. (2008) found that hens kept in conventional cages are particularly vulnerable to osteoporosis, exhibiting reduced bones mineral density, mass, cortical bone, area and breaking strength in comparison to birds kept in furnished cages.

Optimal mineral nutrition is one of the main factors affecting bone quality of high-performing laying hens. Intensive egg formation uses extremely high amounts of Ca, which can increase Ca mobilisation from bones and negatively affect bone quality; thus a negative correlation between bone and eggshell quality in high-performing layers can be observed (Kim et al., 2012). For this reason, the supply of an adequate amount and form of dietary Ca is the most important nutritional factor for maintaining bone quality in hens (Olgun and Aygun, 2016). The results of some studies indicated that some feed additives, among others pre- and probiotics, organic acids and herb, extracts can, by their positive effect on intestinal health and physiology, improve mineral utilisation in poultry, which may in turn beneficially affect the mineralisation process in the organism, as well eggshell and bone quality (Świątkiewicz and Arczewska-Włosek, 2012; Abdelqader et al., 2013; Sobczak and Kozłowski, 2015; Świątkiewicz et al., 2015; Olgun, 2016; Li et al., 2017).

Therefore, the objective of this experiment was to investigate the influence of selected feed additives i.e. sodium butyrate, probiotic bacteria, herbal extract blend and chitosan in laying hens fed with standard or decreased dietary Ca, on mineral utilisation, as well as biomechanical and geometrical indices of the tibia and femur bones in laying hens.

Material and methods

Birds and experimental diets

All experimental procedures were performed in accordance with the guidelines of the Local Krakow Ethics Committee for Experiments with Animals. A total of 240 18-wk-old ISA Brown hens, obtained from a commercial source, were placed in a poultry house, in cage on a wire-mesh floor, under controlled climate conditions. The cage dimensions were 30 cm × 120 cm × 50 cm (3600 cm^2 of total floor space). During the pre-experimental period, up to the hens' 26 wks of age, the birds were fed a standard laying-hen diet *ad libitum*, containing 170 g/kg crude protein, 11.6 MJ/kg AME_N, 37.0 g/kg calcium and 3.8 g/kg available phosphorus.

At wk 26, the hens were randomly allocated to one of 10 treatments and fed experimental diets until wk 70. Each treatment comprised 12 replicates of 2 hens (in one cage). During the experiment the hens were provided feed and water *ad libitum*, and were exposed to a 14 L:10 D lighting schedule, with a light intensity of 10 lux.

Table 1. Composition and nutrient content of experimental diets, g/kg air dry matter

Item	Reduced dietary level of Ca	Standard dietary level of Ca
Ingredient (g/kg):		
corn	417.1	423.1
wheat	240.0	210.0
soybean meal	230.0	236.0
rapeseed oil	13.0	19.0
limestone	78.0	90.0
monocalcium phosphate	12.5	12.5
NaCl	3.0	3.0
DL-Methionine	1.4	1.4
vitamin-mineral premix[1]	5.0	5.0
Nutrients composition:		
metabolizable energy (MJ/kg)[2]	11.60	11.60
crude protein	170.0	170.0
Lys	8.35	8.35
Met	4.10	4.10
Ca	32.0	37.0
total P	6.15	6.15
available P	3.90	3.90

[1]The premix provided per 1 kg of diet: vitamin A – 10,000 IU; vitamin D_3 – 3,000 IU; vitamin E – 50 IU; vitamin K_3 – 2 mg; vitamin B_1 – 1; vitamin B_2 – 4 mg; vitamin B_6 – 1.5 mg; vitamin B_{12} – 0.01 mg; Ca-pantothenate – 8 mg; niacin – 25 mg; folic acid – 0.5 mg; choline chloride – 250 mg; manganese – 100 mg; zinc – 50 mg; iron – 50 mg; copper – 8 mg; iodine – 0.8 mg; selenium – 0.2 mg, cobalt – 0.2 mg.

[2]Calculated according to European Table (Janssen, 1989) as a sum of the ME content of components.

The composition of the experimental cereal-soybean diets is given in Table 1. In the study a 2 × 5 factorial arrangement was used, so the experimental diets contained two levels of Ca (reduced – 3.20% or standard – 3.70%), and were supplemented with five experimental additives: none, sodium butyrate (700 mg/kg, GUSTOR XXI B 70, NOREL S.A., Spain), probiotic bacteria (150 mg/kg, PROTEXIN commercial preparation containing in 1 g: *Lactobacillus plantarum* – 1.26 × 10^7 cfu; *Lactobacillus bulgaricus* – 2.06 × 10^7; *Lactobacillus acidophilus* – 2.06 × 10^7; *Lactobacillus rhamnosus* – 2.06 × 10^7; *Bifidobacterium bifidum* – 2.00 × 10^7; *Streptococcus thermophilus* – 4.10 × 10^7; *Enterococcus faecium* – 5.90 × 10^7; *Aspergillus oryzae* – 5.32 × 10^6), herb extract blend (2000 mg/kg feed, 1 kg of blend provided: dry extract from *Echinacea purpurea*, 4000 mg; oleoresin *Salvia officinalis*, 27 800 mg; oleoresin *Thymus vulgaris*, 5 000 mg; oil extract from *Rosmarinus officinalis*, 2 500 mg; oil from *Allium sativum*, 1 670 mg; and oil from *Origanum vulgare*, 1 000 mg; Intermag Sp. z o.o., Poland), or chitosan (100 mg/kg used as Chimet-pasz preparation, Gumitex Poli-Farm, Poland).

The nutrient content of the diets (Table 1) was calculated on the basis of the chemical composition of raw feedstuffs, and the ME value was calculated based on equations from European Tables (Janssen, 1989). The chemical composition of the feed materials was determined using AOAC (2000) methods for moisture (930.15), crude protein (984.13), crude fat (920.39), fibre (978.10) and ash (942.05). Amino acids were analysed in acid hydrolysates after initial peroxidation of sulphur amino acids by colour reaction with the ninhydrin reagent (Beckman-System Gold 126 AA Automatic Analyzer; Beckman Coulter, Inc., Pasadena, CA, US; Method 982.30; AOAC 2000). The Ca content was determined by flame atomic absorption spectrophotometry (Method 968.08; AOAC, 2000) and total P content was determined by colorimetry using the molybdo-vanadate method (Method 965.17; AOAC, 2000).

Measurements

At 40 wks of age, the digestibility coefficients of nutrients were evaluated by the total collection method. The total collection of excreta was conducted over 5 d and the feed consumption for each cage was recorded. Excreta was stored in plastic bags at –20ºC for 5 wks and, after thawing, was dried in an oven at 50ºC to a constant weight, weighed, and finely ground. The contents of the nutrients in the diets and excreta were estimated using the same methods as was given for the feed materials. Apparent total tract digestibility coefficient of dry matter was calculated as dry matter intake – dry matter excretion/dry matter intake. In the same way, the digestibility of organic matter, crude fat, N-free extracts, crude fibre and ash was calculated. Calcium or P retention (mg) was calculated as: Ca or P intake – Ca or P excretion. Calcium or P relative retention (as a % of Ca or P intake) was calculated as: Ca or P intake – (Ca or P intake – Ca or P excretion)/Ca or P intake × 100.

At the end of the experiment, i.e. at 70 wks of age, all of the hens were sacrificed through cervical dislocation. The tibia and femur from both legs were collected, cleaned of soft tissues, weighed and frozen (–20ºC) until analysis. For determination of ash, the left tibias and toes were dried for 24 h at 105ºC, weighed, and dry-ashed in a muffle furnace at 600ºC. A mass of 0.2 g of bone ash was dissolved in 10 mL of 6 M hydrochloric acid.

For measurements of the biomechanical and geometrical properties of the bones, the right tibias were used. Biomechanical properties were determined by means of the 3-point bending test (Instron 5542; Instron, Norwood, MA, US). Bone breaking strength and yielding load were measured as a graphical record from post-deformation curves. Stiffness in elastic conditions was calculated as a yielding load/elastic deformation ratio. Tibia length, cortex thickness and external and internal diameters (for cross-section area calculations) were measured at the breaking location, using an electronic slide caliper. The cross-section area was calculated from the equation: $3.14\ (HB - hb)/4$, where H = external vertical diameter; B = external horizontal diameter; h = internal vertical diameter; and b = internal horizontal diameter.

Statistical analysis

The data were subjected to statistical analysis using a completely randomised design in accordance with the GLM procedure of Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA). All data were analysed using two-way ANOVA. When significant differences in treatment means were detected (ANOVA), Duncan's multiple range test was applied to separate means. Statistical significance was considered to be P≤0.05.

Results

In this study, the reduced dietary level of Ca did not decrease laying performance (egg production, feed conversion ratio), but, as it was presented in our previous paper (Świątkiewicz et al., 2018), negatively affected eggshell quality in older layers. Moreover, chitosan and herb extracts had beneficial effect on laying rate and chosen indices of eggshell quality (Świątkiewicz et al., 2018).

Table 2. Effects of dietary treatments on biomechanical parameters of tibia bones

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
Bone breaking strength (N)	None	169	172	**170**	2.25	0.421	0.488	0.661
	Sodium butyrate	173	176	**174**				
	Probiotic	170	175	**173**				
	Herb extracts	173	178	**175**				
	Chitosan	173	177	**175**				
	Mean	**172**	**176**					
Yielding load (N)	None	107	109	**108**	1.12	0.580	0.782	0.976
	Sodium butyrate	109	110	**109**				
	Probiotic	107	112	**109**				
	Herb extracts	111	114	**112**				
	Chitosan	108	112	**110**				
	Mean	**108**	**111**					
Stiffness (N/mm)	None	132	137	**134**	1.87	0.392	0.519	0.852
	Sodium butyrate	132	139	**136**				
	Probiotic	133	136	**135**				
	Herb extracts	138	143	**140**				
	Chitosan	138	143	**140**				
	Mean	**135**	**139**					

In our experiment there was no statistically significant influence of experimental factors (dietary Ca concentration, used feed additives) on the biomechanical and geometrical parameters, as well as mineralisation of the tibia bones (Tables 2 and 3). However, the femur bones in the laying hens fed the diet with reduced Ca level were characterised by significantly decreased breaking strength, yielding load, stiffness and mineralisation of femur bones (Tables 4 and 5). The majority of the used feed additives had a significant, positive influence on the biomechanical indices of the femur bones. Thus, the femurs of the laying hens fed the diet supplemented with probiotic bacteria, herb extracts, or chitosan had a significantly higher (P<0.05) breaking strength, yielding load and mineralisation than in the unsupplemented group (Tables 4 and 5). The other additive used, i.e. sodium butyrate, did not affect any of the analysed bone characteristics. Neither dietary Ca level nor diet feed additives had an effect on nutrients (dry matter, organic matter, ether extract, N-free extracts, crude fibre and crude ash) digestibility, as well as P retention and excretion (Table 6), but Ca excretion and retention were significantly lower in the hens fed the diets with reduced Ca level (Table 7). The beneficial influence of probiotic, herb extracts and chitosan on femur bone quality was assisted by their positive effect on relative Ca retention (Ca retained as % of Ca intake) (Table 7).

Table 3. Effects of dietary treatments on geometrical parameters and mineralization of tibia bones

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
Cortex thickness (mm)	None	0.961	0.970	**0.965**	0.0181	0.719	0.617	0.564
	Sodium butyrate	0.972	0.974	**0.973**				
	Probiotic	0.966	0.964	**0.965**				
	Herb extracts	0.965	0.975	**0.970**				
	Chitosan	0.964	0.972	**0.968**				
	Mean	**0.966**	**0.971**					
Cross-section area (mm^2)	None	19.4	19.8	**19.6**	0.289	0.849	0.889	0.556
	Sodium butyrate	19.8	20.0	**19.9**				
	Probiotic	19.5	19.5	**19.5**				
	Herb extracts	19.5	19.9	**19.7**				
	Chitosan	19.6	19.8	**19.7**				
	Mean	**19.6**	**19.8**					
Tibia weight (g)	None	11.5	11.5	**11.5**	0.104	0.827	0.912	0.662
	Sodium butyrate	11.5	11.9	**11.7**				
	Probiotic	11.6	11.5	**11.6**				
	Herb extracts	11.6	11.6	**11.6**				
	Chitosan	11.5	11.7	**11.6**				
	Mean	**11.5**	**11.6**					
Tibia length (mm)	None	124	124	**124**	0.432	0.886	0.734	0.754
	Sodium butyrate	123	125	**124**				
	Probiotic	122	122	**122**				
	Herb extracts	122	122	**122**				
	Chitosan	123	123	**123**				
	Mean	**122**	**123**					
Crude ash content in tibia bones (g/kg)	None	309	308	**309**	3.861	0.556	0.675	0.778
	Sodium butyrate	310	316	**313**				
	Probiotic	317	318	**317**				
	Herb extracts	313	318	**315**				
	Chitosan	315	319	**317**				
	Mean	**313**	**316**					

Table 4. Effects of dietary treatments on biomechanical parameters of femur bones

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
Bone breaking strength (N)	None	154	170	**162 a**	3.77	0.007	0.040	0.437
	Sodium butyrate	164	169	**167 ab**				
	Probiotic	166	185	**176 b**				
	Herb extracts	166	184	**175 b**				
	Chitosan	171	188	**179 b**				
	Mean	**164 a**	**174 b**					
Yielding load (N)	None	102	112	**107 a**	1.48	0.006	0.031	0.919
	Sodium butyrate	109	117	**113 ab**				
	Probiotic	110	122	**116 b**				
	Herb extracts	112	117	**115 b**				
	Chitosan	114	121	**118 b**				
	Mean	**109 a**	**117 b**					
Stiffness (N/mm)	None	130	148	**139**	2.37	0.006	0.164	0.765
	Sodium butyrate	148	154	**151**				
	Probiotic	141	162	**152**				
	Herb extracts	145	158	**151**				
	Chitosan	144	159	**152**				
	Mean	**142 a**	**156 b**					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Table 5. Effects of dietary treatments on geometrical parameters and mineralization of femur bones

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
1	2	3	4	5	6	7	8	9
Cortex thickness (mm)	None	1.008	1.005	**1.007**	0.0162	0.791	0.679	0.879
	Sodium butyrate	1.012	1.022	**1.017**				
	Probiotic	1.005	1.008	**1.007**				
	Herb extracts	1.004	1.022	**1.013**				
	Chitosan	1.018	1.020	**1.019**				
	Mean	**1.009**	**1.015**					
Cross-section area (mm^2)	None	22.1	21.9	**22.0**	0.345	0.842	0.743	0.960
	Sodium butyrate	22.4	22.7	**22.6**				
	Probiotic	21.8	22.1	**22.0**				
	Herb extracts	21.7	22.6	**22.2**				
	Chitosan	22.5	22.6	**22.5**				
	Mean	**22.1**	**22.4**					
Femur weight (g)	None	9.33	9.31	**9.32**	0.107	0.848	0.977	0.491
	Sodium butyrate	9.34	9.38	**9.36**				
	Probiotic	9.40	9.51	**9.46**				
	Herb extracts	9.39	9.48	**9.44**				
	Chitosan	9.39	9.43	**9.41**				
	Mean	**9.37**	**9.42**					

Table 5 – contd.

1	2	3	4	5	6	7	8	9
Femur length (mm)	None	87.0	86.6	**86.8**	0.290	0.419	0.953	0.656
	Sodium butyrate	85.2	87.4	**86.3**				
	Probiotic	85.9	86.7	**86.3**				
	Herb extracts	85.9	86.2	**86.1**				
	Chitosan	86.7	86.3	**86.5**				
	Mean	**86.2**	**86.6**					
Crude ash content in femur bones (g/kg)	None	303.8	310.2	**307.0 a**	2.412	0.037	0.036	0.669
	Sodium butyrate	315.9	320.1	**318.0 ab**				
	Probiotic	318.8	331.0	**324.9 b**				
	Herb extracts	319.2	331.1	**325.1 b**				
	Chitosan	320.2	328.1	**324.1 b**				
	Mean	**315.6 a**	**324.1 b**					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Table 6. Effects of dietary treatments on digestibility of nutrients (%)

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
1	2	3	4	5	6	7	8	9
Dry matter	None	75.2	74.2	**74.7**	0.151	0.295	0.095	0.755
	Sodium butyrate	76.0	75.5	**75.7**				
	Probiotic	75.6	75.9	**75.8**				
	Herb extracts	75.9	75.7	**75.8**				
	Chitosan	75.9	75.7	**75.8**				
	Mean	**75.7**	**75.4**					
Organic matter	None	76.4	75.4	**75.9**	0.159	0.399	0.093	0.725
	Sodium butyrate	77.1	76.8	**77.0**				
	Probiotic	77.0	77.2	**77.1**				
	Herb extracts	77.0	77.2	**77.1**				
	Chitosan	77.2	76.9	**77.1**				
	Mean	**76.9**	**76.7**					
Crude fat	None	56.6	56.4	**56.6**	0.423	0.692	0.951	0.963
	Sodium butyrate	57.3	57.0	**57.2**				
	Probiotic	57.9	56.6	**57.3**				
	Herb extracts	57.2	57.7	**57.5**				
	Chitosan	57.0	56.5	**56.8**				
	Mean	**57.2**	**56.9**					
N-free extracts	None	89.8	87.5	**88.6**	0.190	0.130	0.680	0.076
	Sodium butyrate	89.3	89.3	**89.3**				
	Probiotic	89.2	89.2	**89.2**				
	Herb extracts	89.1	89.3	**89.2**				
	Chitosan	89.2	89.0	**89.1**				
	Mean	**89.3**	**88.9**					

Table 6 – contd.

1	2	3	4	5	6	7	8	9
Crude fibre	None	5.01	5.64	**5.33**	0.241	0.548	0.631	0.990
	Sodium butyrate	6.50	6.35	**6.43**				
	Probiotic	5.97	6.89	**6.43**				
	Herb extracts	6.57	6.94	**6.76**				
	Chitosan	6.82	7.22	**7.02**				
	Mean	**6.18**	**6.61**					
Crude ash	None	68.0	68.7	**68.4**	0.200	0.317	0.381	0.698
	Sodium butyrate	69.6	69.4	**69.5**				
	Probiotic	68.9	69.9	**69.5**				
	Herb extracts	69.7	69.1	**69.4**				
	Chitosan	68.7	69.8	**69.3**				
	Mean	**69.0**	**69.4**					

Table 7. Effects of dietary treatments on balance of calcium and phosphorus (%)

Item	Feed additives	Dietary Ca level			SEM	Effect of:		
		Reduced	Standard	Mean		Ca level	Additives	Interaction
1	2	3	4	5	6	7	8	9
Ca excretion (mg/hen per day)	None	1309	1589	**1490 b**	15.1	0.001	0.046	0.588
	Sodium butyrate	1364	1467	**1416 a**				
	Probiotic	1361	1508	**1435 ab**				
	Herb extracts	1311	1491	**1401 a**				
	Chitosan	1337	1528	**1433 ab**				
	Mean	**1353 a**	**1517 b**					
Ca retention (mg/hen per day)	None	2809	3395	**3102**	26.4	0.001	0.250	0.322
	Sodium butyrate	2955	3278	**3116**				
	Probiotic	3053	3415	**3233**				
	Herb extracts	2994	3433	**3213**				
	Chitosan	2923	3436	**3180**				
	Mean	**2947 a**	**3391 b**					
Ca retained (% of Ca intake)	None	66.9	68.0	**67.5 a**	0.265	0.237	0.038	0.975
	Sodium butyrate	68.4	69.1	**68.7 ab**				
	Probiotic	69.1	69.3	**69.2 b**				
	Herb extracts	69.5	69.7	**69.6 b**				
	Chitosan	69.4	69.8	**69.6 b**				
	Mean	**68.7**	**69.2**					
P excretion (mg/hen per day)	None	653	670	**662**	3.55	0.084	0.313	0.061
	Sodium butyrate	662	619	**640**				
	Probiotic	674	638	**656**				
	Herb extracts	649	652	**650**				
	Chitosan	654	656	**655**				
	Mean	**658**	**647**					

Table 7 – cotd.

1	2	3	4	5	6	7	8	9
P retention (mg/hen per day)	None	199	204	**201**	3.68	0.976	0.420	0.931
	Sodium butyrate	214	214	**214**				
	Probiotic	221	225	**223**				
	Herb extracts	224	212	**218**				
	Chitosan	210	215	**212**				
	Mean	**214**	**214**					
P retained (% P intake)	None	23.3	23.4	**23.3**	0.342	0.579	0.407	0.817
	Sodium butyrate	24.4	25.6	**25.0**				
	Probiotic	24.6	26.0	**25.3**				
	Herb extracts	25.5	24.5	**25.0**				
	Chitosan	24.3	24.7	**24.5**				
	Mean	**24.4**	**24.8**					

a, b – the values in the rows with different letters differ significantly (P≤0.05).

Discussion

To date, the amount of published data from poultry studies on the effects of feed additives on bone quality is rather limited. Similarly to results of this experiment, Świątkiewicz et al. (2014 a) observed a positive effect of probiotic bacteria (*L. salivarius*) on some bone quality indices in layers fed a diet with a high level of DDGS. Abdelqader et al. (2013) found increased tibia weight, density and ash concentration in aged laying hens (64–74 wks of age) fed a diet supplemented with *Bacillus subtilis* probiotic. Corresponding results were reported by Mutus et al. (2006) in broilers fed diets supplemented with *Bacillus* probiotic bacteria. They observed the positive influence of probiotic bacteria on several indices of tibia bones i.e. the thickness of the medial and lateral walls, the tibiotarsal index, percentage of ash and P content. Angel et al. (2005) found enhanced bones mineralisation and breaking strength along with increased retention of Ca and P in chickens fed a diet supplemented with *Lactobacillus* probiotic.

In the experiment by Houshmand et al. (2011), the bones indices of broilers were negatively affected by a low Ca diet; however, the addition of probiotic bacteria had a beneficial influence on these parameters and helped to overcome the problems related to a low Ca dietary level. Such a positive effect of dietary probiotic on breaking strength and mineralisation of bones was attributed by the authors to the increased retention of Ca in the bones (Panda et al., 2008). As it was discussed by Scholz-Ahrens et al. (2007), the mechanism of the beneficial influence of probiotic bacteria in terms of bones indices could probably be linked to their positive effect on mineral utilisation, which can be attributed in turn to increased solubility of minerals due to the bacteria's increased production of short-chain fatty acids, alteration of intestinal mucosa and increase of the absorption surface through the beneficial effect of bacterial fermentation products on the proliferation of enterocytes, increased expression of Ca-binding proteins, release of bone modulating factors, degradation of phytates by probiotic bacteria enzymes and overall improvement of gut health.

Similarly to the beneficial effect of herb extracts on selected femur characteristics found in this study, Zhou et al. (2009) reported improved breaking strength, weight and radiographic densities of the humerus, tibia and femur in older laying hens fed a diet supplemented with traditional Chinese herbs mixture (*Epimedii, Rhizoma Drynariae, Rhizoma Atractylodis* and *Radix Astragali*). The authors speculated that the mechanism of such a positive effect of herbs dietary supplementation was probably related to their action minimising structural bone loss and stimulating bone mineral absorption in osteoporotic laying hens (Zhou et al., 2009). The positive effect of herb extracts on bone quality can also be due to their beneficial influence on Ca utilisation, as was observed in this experiment and in the study performed on breeder quails (Olgun and Yildiz, 2014). More recently, Olgun (2016) demonstrated the positive effect of herb extract blend used at a low supplementation level (25 or 50 mg/kg) on the biomechanical indices of bones in laying hens, which was assisted with increased Ca content; however, the shear force and shear stress of bones was reduced for layers fed the diet with a high level (600 mg/kg) of extracts. Correspondingly, Mühlbauer et al. (2003) found in a model rats study that essential oils and monoterpenes of selected herbs (sage, rosemary and thyme) are efficient inhibitors of bone resorption. The above mentioned effects of herb extracts and essential oils can be probably related to their beneficial effects on gut health, for instance on intestinal microbiota and morphology, as it was recently reported in broilers fed the diet supplemented with herb products (Giannenas et al., 2016; Kiczorowska et al., 2016). Recently, however, Leskovec et al. (2018) did not find any positive effect of plant extracts (marigold, olive leave extracts) on mineral utilization and bone characteristics in broiler chickens.

The positive effect of chitosan on some tibia bones indices, as observed in our study, could probably be explained by improved Ca digestibility. To date, the experimental data on the effect of dietary chitosan on bone quality in laying hens are very limited. Corresponding results were found in broiler chickens by Huang et al. (2005) and Świątkiewicz et al. (2014b), who observed that diet supplementation with chitosan (0.015%) increased the digestibility of dry matter, N, Ca, and P and, as a result of improved nutrients digestibility, growth performance indices; however, these effects were not reflected in the bones characteristics. The mechanism of the beneficial effect of chitosan addition and, in this way, on tibia characteristics as observed in our study, can probably be attributed to its positive influence on intestinal morphology (Khambualai et al., 2008; Khambualai et al., 2009). Such effect was recently confirmed in *Leiothrix lutea* birds, where diet supplementation with chitosan (0.5%) increased the apparent digestibility of most nutrients, improved intestinal histology, i.e. increased the villous height of the duodenum and ileum, as well as enhanced activity of intestinal enzymes (Le et al., 2015).

Conclusions

In conclusion, the findings of this study show that reducing Ca dietary level below 3.70% can negatively affect bone quality in high-producing laying hens, while probiotic, herb extracts, or chitosan addition may improve the selected biomechanical indices of bone quality in aged, high-producing laying hens, irrespective of Ca dietary level.

Competing interests

The authors confirm that this work has no conflict of interest.

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Received: 12 III 2018
Accepted: 21 III 2018