Bone quality characteristics and performance in broiler chickens fed diets supplemented with organic acids

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ABSTRACT: A 6-week experiment with broilers was conducted to study the effect of diet supplementation with organic acids on performance, characteristics of the tibia and femur bones, and the calcium, phosphorus and zinc balance. A total of 320, one-day-old, Ross 308 chickens were randomly assigned to 1 of 8 treatments. A 2 × 4 factorial arrangement was used, with two dietary levels of calcium and available phosphorus (standard – 9.4/9.2 g Ca/kg and 4.3/4.0 g available P/kg, or reduced – 8.3/8.1 g Ca/kg and 3.7/3.5 g available P/kg, for the starter/finisher feeding phases, respectively), and with diets supplemented with organic acids (none; short chain fatty acids (SCFA), 4.0 g/kg; medium chain fatty acid (MCFA), 2.0 g/kg or SCFA + MCFA, 3.0 + 2.0 g/kg). Broilers fed diets supplemented with SCFA or MCFA displayed a performance similar to those fed the unsupplemented diet ($P > 0.05$). At 42 days, reducing the dietary levels of Ca and P decreased such bone parameters as tibia yielding load (256 vs. 270 N) and tibia stiffness (171 vs. 184 N/mm), as well as femur breaking strength (342 vs. 369 N), yielding load (233 vs. 250 N), stiffness (164 vs. 174 N/mm), and cortex thickness (1.47 vs. 1.56 mm). The organic acids had no effect on the parameters of the tibias; however, SCFA and SCFA+MCFA increased the yielding load and stiffness of the femurs. The SCFA diet supplementation significantly increased the relative retention of Ca (45.0 vs. 41.1%). There were no significant Ca and P level × organic acids interaction effects on performance parameters, bone quality indices or the Ca, P, and Zn balance. It was thus concluded that SCFA can improve the bone quality and Ca balance in broiler chickens when fed either diets with a standard level of Ca and P, or those with reduced levels of these macrominerals.

Keywords: broiler chickens; organic acids; calcium; phosphorus; bone quality; performance

Leg weakness, lameness and other bone abnormalities connected with various metabolic disorders are continuing problems in rapidly growing meat-type chickens, leading to considerable production losses and having a negative effect on bird welfare (Julian, 2005; Waldenstedt, 2006; Dibner et al., 2007). It has been reported that the bones in modern broiler lines are characterized by poor calcification and high porosity, which may cause an increased affinity for bone damage (Williams et al., 2000). The reduced walking ability caused by bone disorders can lead to difficulties in feed intake and a decreased body weight for chickens in production. The causes of bone abnormalities in broilers are usually complex, having a substantial genetic component and a high correlation with the growth rate. However, nutrition can also have an effect on the development of bone disorders and thus its optimization may be a strategy for decreasing the severity of leg lesions in broilers. Vitamin D_3 is one of the main nutritional factors crucial to Ca and P absorption and proper skeletal development. It is generally added to diets in the form of cholecalciferol; however, in order to carry out its physiological function, it must be hydroxylated in a two-step process: in the liver (to 25-OH-D_3) and in the kidneys (to 1.25-OH-D_3). Whitehead et al. (2004) suggested that the use of 25-OH-D_3 may be an alternative for increasing the permissible maximum of vitamin D_3 in the diet. The addition of 25-OH-D_3 to the broiler diet decreased the incidence of tibial dyschondroplasia, in the case of both the standard diet (Rennie and Whitehead, 1996; Fritts and Waldroup, 2003) and a diet with a low calcium level (Ledwaba and Roberson, 2003) and had a positive effect on the bone quality in broilers (Świątkiewicz and Koreleski, 2005; Świątkiewicz et al., 2006).

The results of several model studies with rats have shown that by lowering intestinal pH organic acids can have a positive effect on calcium absorption (Lutz and Scharrer, 1991; Mineo et al., 2001). Dietary acetic acid increased the Ca absorption and Ca content of the femur in ovariectomized rats, suggesting that this acid may reduce the bone turnover caused by ovariectomy and be helpful in preventing osteoporosis (Kishi et al., 1999). The positive effects of organic acids on mineral retention and bone ash were found in experiments performed on pigs (Radcliffe et al., 1998; Jongbloed et al., 2000; Mroz et al., 2000) and rainbow trout (Vielma and Lall, 1997). The addition of organic acids to the diet for laying hens had a beneficial effect on some indices of quality for eggshells and bones (Świątkiewicz et al., 2010a, b). In an experiment with quails, short chain fatty acid improved both the absorption of dietary phosphorus and tibia bone mineralization (Sacakli et al., 2006).

To date, the amount of experimental data on the effect of organic acids on mineral utilization and bone quality in broiler chickens is limited and much of the studies carried out have focused on citric acid. Rafacz-Livingston et al. (2005b) indicated that by means of a positive effect on P utilization, citric acid, but not fumaric acid, increased the crude ash content in tibias of broilers fed a P-deficient diet. The positive influence of citric acid on tibia mineralization was also found in several other experiments (Boiling et al., 2000; Angel et al., 2001; Brenes et al., 2003; Snow et al., 2004; Rafacz-Livingston et al., 2005a; Chowdhury et al., 2009). In a study with broilers fed a P-deficient diet, the results obtained by Liem et al. (2008) as regards bone ash, P and Ca retention, and body weight gain data may suggest that, in terms of improving mineral utilization, citric acid was the most efficacious of the acids studied, followed by malic and fumaric acids. In a recent experiment, feeding broilers with a low-Ca diet negatively affected performance and tibia characteristics, whereas the addition of organic acids had a positive effect on these indices and helped the birds to overcome the problems related to a low-Ca diet (Houshmand et al., 2011).

The aim of this study was to examine the effect of short- and medium-chain fatty acids (SFCA and MCFA, respectively) on performance indices, the biomechanical and geometrical properties of the tibia and femur bones, and the calcium, phosphorus and zinc balance in broilers fed diets with different levels of Ca and available P.

MATERIAL AND METHODS

Birds and experimental diets

The Local Krakow Ethics Committee for Experiments with Animals gave its approval to all the experimental procedures relating to the use of live animals. A total of 320, one-day-old, Ross 308 chickens were used, obtained from a commercial hatchery and with an average initial weight of 40 g. The birds were housed in electrically heated, wire mesh batteries, in an environmentally controlled room in the poultry house at the Experimental Station of the National Research Institute of Animal Production in Balice, Poland. The chicks were weighed and randomly assigned to 1 of 8 treatments, each comprising 5 replicate cages, with 8 birds (4♂ and 4♀) per cage. At 21 days of age, the birds were transferred to finisher cages. During the experiment, namely from 1 to 42 days of age, all the chickens were provided with water and feed *ad libitum*.

A 2 × 4 factorial arrangement was used, with two dietary levels of Ca and P and with the diets supplemented with the additives being studied. The experimental diets (Table 1) contained either standard levels of these macroelements (9.4/9.2 g Ca/kg and 4.3/4.0 g available P/kg) or reduced (8.3/8.1 g Ca/kg and 3.7/3.5 g available P/kg, for the starter/finisher feeding phases, respectively). These diets were either unsupplemented or supplemented with organic acids (per kg of diet) as follows: 4.0 g SCFA (1.5, 1.0 and 1.5 g of formic, propionic and acetic acid, respectively), 2.0 g MCFA (1.0 g of caproic and 1.0 g of capric acid) or 3.0 g SCFA + 2.0 g MCFA. Formic, propionic, acetic, caproic, and capric acids (chemical grade) were obtained from B&K Company (Bytom, Poland) and their mixtures as SCFA and MCFA blends were specially prepared for this experiment. Used doses of organic acids were established basing of our previ-

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

Item	Starter (1–21 days)		Finisher (22–42 days)	
	control	reduced level of Ca and P	control	reduced level of Ca and P
Corn	554	560	600.5	607.0
Soybean meal	378	379	320.0	320.0
Rapeseed oil	27	25	38.0	36.0
Limestone	17	15	17.0	15.0
Monocalcium phosphate	14	11	13.5	11.0
NaCl	3	3	3.0	3.0
DL-methionine	2	2	2.0	2.0
L-lysine HCl	–	–	1.0	1.0
Vitamin-mineral premix[1]	5	5	5.0	5.0
Calculated composition				
Metabolizable energy (MJ/kg[2])	12.6	12.6	13.0	13.0
Crude protein	225	225	200.0	200.0
Lys	12	12	11.2	11.2
Met	5.4	5.4	5.1	5.1
Ca	9.4	8.3	9.2	8.1
Total P	6.9	6.4	6.5	6.1
Available P	4.3	3.7	4.0	3.5

[1]the premix provided per 1 kg of starter diet: vitamin A (retinol) 4.05 mg; vitamin D_3 (cholecalciferol) 0.0875 mg; vitamin E (α-tocopherol) 45 mg; vitamin K_3 (menadione) 3 mg; vitamin B_1 (thiamine) 3.25 mg; vitamin B_2 (riboflavin) 7.5 mg; vitamin B_6 (pyridoxine) 5 mg; vitamin B_{12} (cyanocobalamin) 0.0325 mg; biotin 0.15 mg; Ca-pantothenate 15 mg; niacin 45 mg; folic acid 1.5 mg; choline chloride 600 mg; manganese 100 mg; zinc 75 mg; iron 67.5 mg; copper 17.5 mg; iodine 1 mg; selenium 0.275 mg, cobalt 0.4 mg; per 1 kg of finisher diet: vitamin A (retinol) 3.6 mg; vitamin D_3 (cholecalciferol) 0.8125 mg; vitamin E (alpha-tocopherol) 40 mg; vitamin K_3 (menadione) 2.25 mg; vitamin B_1 (thiamine) 2 mg; vitamin B_2 (riboflavin) 7.25 mg; vitamin B_6 (pyridoxine) 4.25 mg; vitamin B_{12} (cyanocobalamin) 0.03 mg; biotin 0.1 mg; Ca-pantothenate 12 mg; niacin 40 mg; folic acid 1.0 mg; choline chloride 450 mg; manganese 100 mg; zinc 65 mg; iron 65 mg; copper 15 mg; iodine 0.8 mg; selenium 0.25 mg, cobalt 0.4 mg

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

ous experiment with laying hens (Świątkiewicz et al., 2010a).

The experimental diets were fed from 1 to 42 days, covering the starter (1–21 days) and finisher (22–42 days) phases. The nutrient content of the diets was calculated on the basis of the chemical composition of raw feedstuffs, and metabolizable energy value, in line with equations from the European Tables (Janssen, 1989). Samples of feed components were analyzed, using standards methods (AOAC, 1990), for moisture (method 930.15), crude protein (984.13), crude fat (920.39), and ash (942.05). The Ca content of the ingredients and diets was analyzed by flame atomic absorption spectrophotometry (AOAC, 1990; method 968.08) and the total P content by colorimetry, using the molybdo-vanadate method (AOAC, 1990; method 965.17).

Measurements

The chickens' body weight and feed intake were recorded at 21 and 42 days of age and mortality was registered. The body weight gain (BWG) and feed conversion ratio (FCR) were calculated for the starter period (1–21 days), the finisher phase (22 to

Table 2. Effects of dietary treatments on performance in the entire period of feeding (1–42 days of age)

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Body weight gain (g)	none	2278	2220	2249	9.99	NS	NS	NS
	SCFA	2241	2261	2251				
	MCFA	2285	2241	2263				
	SCFA + MCFA	2253	2240	2246				
	mean	2264	2240					
Feed intake (g)	none	3925	3933	3929	18.7	NS	NS	NS
	SCFA	3952	4037	3995				
	MCFA	3978	3912	3946				
	SCFA + MCFA	3900	3190	3910				
	mean	3939	3951					
Feed conversion ratio (g/g)	none	1.72	1.77	1.75	0.01	NS	NS	NS
	SCFA	1.76	1.78	1.77				
	MCFA	1.74	1.75	1.75				
	SCFA + MCFA	1.73	1.75	1.74				
	mean	1.74	1.76					
Production index	none	320	304	312	2.12	NS	NS	NS
	SCFA	303	307	304				
	MCFA	304	311	308				
	SCFA + MCFA	316	314	315				
	mean	311	305					

NS = $P > 0.05$

42 days), and the entire feeding period (1–42 days of age). Mortality rate was recorded on a daily basis and the weights of all mortalities were registered to correct the FCR. The production index (PI) was calculated for the entire feeding period, using the formula described by Koreleski et al. (2011).

On the 3rd week of age, Ca, P and Zn balance estimation was made. The total collection of excreta was carried out over 5 days, and feed consumption for each cage was recorded. Excreta were stored in plastic bags at –20°C for two weeks and, after thawing, they were dried in an oven at 50°C, to a constant weight, then weighted and finely ground. The total P content in the excreta was determined colourimetrically by the molybdo-vanadate method (AOAC, 1990; method 965.17) and the Ca and Zn content by flame atomic absorption spectrophotometry (AOAC, 1990; method 968.08). Calcium (phosphorus, zinc) retention (mg) was calculated as: Ca intake – Ca excretion. Calcium (phosphorus, zinc) retention as a % of Ca (P, Zn) intake was calculated as: Ca intake – (Ca intake – Ca excretion)/Ca intake × 100.

At the conclusion of the experiment, and after 12 h of starvation, 5 representative males and 5 females were chosen from each group and decapitated. The mass of the cooled carcasses and edible giblets was estimated, and the carcass and breast meat yields and relative weights of abdominal fat, liver, and heart were calculated.

The tibia and femur from the right leg were collected, cleaned of adhering tissues, weighed, and frozen (–20°C) until the analysis. Measurements of the bones biomechanical properties were taken by means

Table 3. Effects of dietary treatments on biomechanical parameters of tibia bones

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Bone breaking strength (N)	none	416	385	401	6.78	NS	NS	NS
	SCFA	404	414	409				
	MCFA	434	430	432				
	SCFA + MCFA	438	410	425				
	mean	423	410					
Bone breaking strength/cross section area ratio (N/mm^2)	none	10.1	9.80	10.0	0.25	NS	NS	NS
	SCFA	10.2	9.70	10.0				
	MCFA	10.7	10.8	10.7				
	SCFA + MCFA	11.0	11.8	11.4				
	mean	10.5	10.5					
Yielding load (N)	none	258	248	253	3.03	*	NS	NS
	SCFA	265	254	260				
	MCFA	278	261	270				
	SCFA + MCFA	277	262	270				
	mean	270	256					
Stiffness (N/mm)	none	183	165	174	2.03	**	NS	NS
	SCFA	181	170	176				
	MCFA	189	173	181				
	SCFA + MCFA	184	176	180				
	mean	184	171					

NS = $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$

of the three-point bending test, using an Instron 5542 testing machine (Istron Ltd., High Wycombe, U.K.) (constant speed of crosshead – 10 mm/min and distance between supports – 50 mm). 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/elas-tic deformation ratio.

Tibia length, cortex wall thickness, external and internal diameters (for cross section area calculations) were measured at the breaking point, 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
b = internal, horizontal diameter

Statistical analysis

The data were subjected to statistical analysis using a completely randomized design, in accordance with the GLM procedure, on Statistica 5.0 (Statsoft, Inc., Tulsa, USA). All the data were analyzed using two-way ANOVA. When significant differences in treatment means were detected by the ANOVA, Duncan's Multiple Range Test was applied to the separate means. Statistical significance was considered at $P \leq 0.05$.

RESULTS

Performance

There were no statistically confirmed differences ($P > 0.05$) between the performance indices for the chickens fed a standard Ca and available P diet and those fed a diet with reduced level of these

Table 4. Effects of dietary treatments on geometrical parameters of tibia bones

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Cortex thickness (mm)	none	1.71	1.73	1.73	0.02	NS	NS	NS
	SCFA	1.69	1.82	1.75				
	MCFA	1.80	1.77	1.79				
	SCFA + MCFA	1.71	1.52	1.62				
	mean	1.73	1.72					
Cross section area (mm^2)	none	42.3	39.3	40.8	0.83	NS	NS	NS
	SCFA	39.8	43.2	41.5				
	MCFA	42.1	41.5	41.8				
	SCFA + MCFA	40.0	35.3	37.6				
	mean	41.0	39.8					
Tibia weight (g)	none	15.0	14.0	14.5	0.26	NS	NS	NS
	SCFA	14.7	15.2	15.0				
	MCFA	14.9	14.4	14.7				
	SCFA + MCFA	13.8	14.5	14.1				
	mean	14.6	14.5					
Relative tibia weight (g/100 g of body weight)	none	0.555	0.535	0.545	0.006	NS	NS	NS
	SCFA	0.555	0.557	0.556				
	MCFA	0.543	0.557	0.550				
	SCFA + MCFA	0.514	0.537	0.526				
	mean	0.542	0.546					
Tibia length (mm)	none	102	102	102	1.49	NS	NS	NS
	SCFA	104	96	98				
	MCFA	103	102	102				
	SCFA + MCFA	100	102	101				
	mean	102	99					

NS = $P > 0.05$

macroelements, during either the first (1–21 days) (data not shown) or the second (22–42 days) (data not shown) phase, or for the entire feeding period (Table 2). Similarly, the addition of SCFA, MCFA or SCFA + MCFA had no significant effects on performance indices for either the starter or the finisher feeding period (Table 2). There were no statistically confirmed diet × additive interactions for performance indices. The cumulative mortality rate during the first 21 days averaged 0%; from 22 to 42 days it was 1.0%; and for the entire feeding period it made also 1.0% being random across the treatments. The results of the slaughter analysis were similar ($P > 0.05$) for broilers fed diets with different levels of Ca and available P. The carcass yield, breast meat yield, abdominal fat pad, and relative weight of the liver were not affected ($P > 0.05$) by the addition of SCFA, MCFA, or SCFA + MCFA (data not shown).

Bone quality characteristics

The dietary level of Ca and available P had a significant effect on some of the biomechanical parameters of the bones (Tables 3 and 5). In the chickens fed the

Table 5. Effects of dietary treatments on biomechanical parameters of femur bones

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Bone breaking strength (N)	none	355	329	342	5.59	*	NS	NS
	SCFA	372	358	365				
	MCFA	389	326	357				
	SCFA + MCFA	361	353	357				
	mean	369	342					
Bone breaking strength/cross section area ratio (N/mm^2)	none	8.41	8.48	8.45	0.16	NS	NS	NS
	SCFA	9.29	8.69	8.99				
	MCFA	8.97	8.82	8.90				
	SCFA + MCFA	8.92	9.28	9.10				
	mean	8.90	8.81					
Yielding load (N)	none	225	225	225^a	3.46	*	*	NS
	SCFA	258	243	250^b				
	MCFA	263	226	244^b				
	SCFA + MCFA	254	239	247^b				
	mean	250	233					
Stiffness (N/mm)	none	161	158	159^a	2.17	*	*	NS
	SCFA	182	169	175^b				
	MCFA	179	160	170^{ab}				
	SCFA + MCFA	174	169	172^b				
	mean	174	164					

^{a,b}values with different letters differ significantly ($P \le 0.05$), NS = $P > 0.05$, $^*P \le 0.05$

diet with reduced levels of dietary Ca and P, reductions of 5.2% ($P \le 0.05$) in the tibia yielding load, 7.1% ($P \le 0.05$) in tibia stiffness, 6.8% ($P \le 0.05$) in the femur yielding load, and 5.7% ($P \le 0.05$) in femur stiffness were found. The diet with a lower level of Ca and available P also had a negative effect ($P \le 0.05$) on the cortex thickness of the femurs (Table 6), but had no influence on other geometrical indices in the tibias and femurs (Tables 4 and 6).

The organic acids had no significant effect on tibia characteristics (Tables 3 and 4) and the geometrical indices of femurs (Table 6); however, the addition of SCFA, MCFA or SCFA + MCFA increased the yielding load for femurs ($P \le 0.05$), and the addition of SCFA or SCFA + MCFA increased femur stiffness ($P \le 0.05$) (Table 5). No interactions between the experimental factors were found for the results of the analysis of bones (Tables 3–6).

Balance results

The results of the balance trial showed that the chickens fed the diet with a higher Ca level excreted and retained significantly ($P \le 0.05$) more of this macroelement (Table 7). The addition of SCFA to the diet significantly ($P \le 0.05$) improved the retention of Ca (Table 7). The dietary level of Ca and P and of organic acids had no effect on the P and Zn balance results (Tables 8 and 9). No interactions between experimental factors were found for the results of balance trial (Tables 7–9).

DISCUSSION

The results obtained in our study have shown that using a diet with a reduced level of Ca and P had no negative effect in any of the feeding peri-

Table 6. Effects of dietary treatments on geometrical parameters of femur bones

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Cortex thickness (mm)	none	1.56	1.46	1.51	0.02	*	NS	NS
	SCFA	1.48	1.52	1.50				
	MCFA	1.63	1.42	1.52				
	SCFA + MCFA	1.56	1.47	1.52				
	mean	1.56	1.47					
Cross section area (mm^2)	none	42.9	39.8	41.4	0.85	NS	NS	NS
	SCFA	40.5	41.5	41.0				
	MCFA	43.8	37.1	40.5				
	SCFA + MCFA	41.3	39.5	40.4				
	mean	42.1	39.5					
Femur weight (g)	none	10.9	10.3	10.6	0.21	NS	NS	NS
	SCFA	10.9	11.0	11.0				
	MCFA	10.8	10.3	10.5				
	SCFA + MCFA	10.1	10.5	10.3				
	mean	10.7	10.5					
Relative femur weight (g/100 g of body weight)	none	0.400	0.391	0.396	0.005	NS	NS	NS
	SCFA	0.401	0.402	0.406				
	MCFA	0.391	0.396	0.393				
	SCFA + MCFA	0.384	0.389	0.386				
	mean	0.396	0.395					
Femur length (mm)	none	75.6	74.5	75.0	0.35	NS	NS	NS
	SCFA	75.4	76.0	75.7				
	MCFA	74.8	74.9	74.9				
	SCFA + MCFA	75.2	74.4	74.8				
	mean	75.2	74.9					

NS = $P > 0.05$, *$P \leq 0.05$

ods, on weight gain, feed intake and feed conversion ratio and gave the same performance indices as the standard Ca and available P diet. Similarly, performance parameters and carcass traits were not affected by the addition of organic acid. The lack of any positive effect of organic acids on broiler performance has also been observed by others, including Hernandez et al. (2006) and Isabel and Santos (2009), who indicated that the lack of a growth-promoting action in formic acids observed in their study was probably caused by the fact that the trial was carried out in ideal conditions (Hernandez et al., 2006). As with our experiment, Skřivan et al. (2010) reported that MCFA (caprylic acid, 2.5 g/kg of diet) had no influence on broiler performance; however they observed a decline in body weight gain when a high additional dose of MCFA (5.0 g/kg) was used. Other authors have found that organic acids have a beneficial effect on FCR (Runho et al., 1997; Garcia et al., 2007), or on both BWG and FCR (Senkoylu et al., 2007; Bozkurt et al., 2009). This can be probably attributed to a better utilization of nutrients when acidifiers were added to the diet. The lack of statistically confirmed diet

Table 7. Effects of dietary treatments on balance of calcium

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Ca excretion (mg/bird per day)	none	370	314	342	4.69	**	NS	NS
	SCFA	333	335	334				
	MCFA	336	348	342				
	SCFA + MCFA	378	330	353				
	mean	354	334					
Ca retention (mg/bird per day)	none	242	233	237[a]	4.82	**	*	NS
	SCFA	287	261	274[b]				
	MCFA	284	227	256[ab]				
	SCFA + MCFA	260	253	257[ab]				
	mean	268	244					
Ca retained (% of Ca intake)	none	39.6	42.6	41.1[a]	0.59	NS	*	NS
	SCFA	46.2	43.7	45.0[b]				
	MCFA	45.8	39.3	42.6[ab]				
	SCFA + MCFA	40.7	43.5	42.1[ab]				
	mean	43.1	42.3					

[a,b]values with different letters differ significantly ($P \leq 0.05$), NS = $P > 0.05$, *$P \leq 0.05$, **$P \leq 0.01$

Table 8. Effects of dietary treatments on balance of phosphorus

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
P excretion (mg/bird per day)	none	240	216	228	2.30	NS	NS	NS
	SCFA	212	219	216				
	MCFA	213	214	214				
	SCFA + MCFA	221	213	217				
	mean	221	216					
P retention (mg/bird per day)	none	234	223	229	3.02	NS	NS	NS
	SCFA	240	226	233				
	MCFA	239	216	227				
	SCFA + MCFA	239	239	239				
	mean	238	226					
P retained (% of P intake)	none	49.5	50.8	50.2	0.41	NS	NS	NS
	SCFA	53.0	50.8	51.9				
	MCFA	52.8	50.1	51.4				
	SCFA + MCFA	52.0	52.9	52.5				
	mean	51.8	51.2					

NS = $P > 0.05$

Table 9. Effects of dietary treatments on balance of zinc

Item	Used additives	Dietary level of Ca and P			SEM	Effect		
		normal	reduced	mean		level of Ca and P	acids	interaction
Zn excretion (mg/bird per day)	none	6.09	5.90	6.00	0.07	NS	NS	NS
	SCFA	5.81	6.01	5.91				
	MCFA	5.73	5.91	5.82				
	SCFA + MCFA	5.73	6.28	6.00				
	mean	5.84	6.03					
Zn retention (mg/bird per day)	none	0.029	0.160	0.094	0.04	NS	NS	NS
	SCFA	0.024	0.221	0.123				
	MCFA	0.100	0.107	0.103				
	SCFA + MCFA	0.129	0.117	0.123				
	mean	0.071	0.151					
Zn retained (% of Zn intake)	none	0.42	2.62	1.52	0.62	NS	NS	NS
	SCFA	0.73	3.57	2.15				
	MCFA	1.84	1.82	1.83				
	SCFA + MCFA	2.02	1.81	1.92				
	mean	1.25	2.45					

NS = $P > 0.05$

× additive interactions for the performance indices in our study indicates that for broilers the efficacy of the organic acids used is not connected with the dietary level of Ca and P.

In our experiment, the use of a diet with a reduced level of Ca and available P negatively affected some bone quality indicators. The results obtained suggest that the supplies of 8.4/8.2 g Ca/kg and 3.7/3.5 g available P/kg (for the first and the second feeding phases, respectively) may be insufficient for maximal bone mineralization and that, for proper bone development, broiler demands for these macroelements are higher than they are for achieving maximum growth rate. Corresponding observations were made by Rama Rao et al. (2003), who reported that the Ca requirement of broiler chickens (1–39 days of age) for tibia ash content was 9.83 g/kg and was much higher than that for the maximum growth rate (7.56 g/kg). Similarly, Jamroz et al. (2007) found that a decrease of Ca and P content in the broiler diet (from 11.0 to 9.1 g/kg and 7.0 to 6.0 g/kg, respectively) had a negative effect on the biomechanical properties of bones. Venalainen et al. (2006) reported that tibia ash, Ca and P increased curvilinearly with the increasing level of dietary available P (4.0–5.5 g/kg of diet, 3.5–5.0 g/kg in the starter and finisher period, respectively), but had no effect on tibia breaking strength. However, Huyghebaert's study (1996) demonstrated that both tibia ash and tibia breaking strength diminished when the level of available P was decreased (from 4.5 to 3.0 g/kg of diet).

In our study, the addition of SCFA and the simultaneous addition of SCFA + MCFA had a positive effect on the chosen biomechanical parameters of the femur bones. Corresponding results were obtained by Liem et al. (2008), who showed that the addition of citric, malic or fumaric acid increased the tibia ash in broiler chickens fed a diet deficient in P; however, it was only the effect of citric acid that was statistically significant. They found also that citric and malic acid decreased incidency of P-deficiency rickets. Orban et al. (1993) found that the addition of ascorbic acid to the broiler's diet increased the femur breaking strength. The beneficial effect of citric acid on bone (tibia) mineralization has also been observed in laying hens (Nezhad et al., 2008). In our previous experiment, layers fed a diet supplemented with MCFA, SCFA + MCFA or inulin + SCFA displayed a significantly higher bone breaking strength and yielding load in the tibia bone than that of the control group (Świątkiewicz et al., 2010b). The increase of tibia ash as an effect of the supplementation of low-P diet with organic acids (formic + lactic, 2.5 g/kg of diet) has also been observed in quails (Sacakli et al., 2006). This positive influence of organic acids on bone properties can probably be attributed to an increased availability of Ca and P, by virtue of a decrease in pH in the upper part of the intestine and the stimulating effect of organic acids on villus height and, thus, on intestinal surface area, which was observed in broilers by Hernandez et al. (2006), Garcia et al. (2007), and Senkoylu et al. (2007). It has also been proposed that organic acids (citric acid) improve Ca availability by competitive chelating of Ca and a reduction in the formation of insoluble Ca-phytate-complexes (Boling et al., 2000).

In the present study, diet supplementation with SCFA had a positive effect on Ca retention, which was a likely reason for the improved femur quality observed in this experiment. Higher retention of Ca as an effect of the addition of SCFA to the diet was also observed in our previous experiment with laying hens (Świątkiewicz et al., 2010c). Liem et al. (2008) found that supplementation of a P-deficient broiler diet with citric, malic and especially fumaric acid numerically increased Ca and P retention, but this effect was only confirmed statistically for P. Observations corresponding to our results were made by Abdel-Fattah et al. (2008), who reported that chicks fed a diet supplemented with organic acids had a significantly higher Ca and P blood concentration. The authors attributed this to the lowering of gut pH and the increase in the absorption of these macroelements.

In conclusion, the results obtained in this study indicate that when diets with either a standard level of Ca and P or with reduced levels of these macrominerals are fed, SCFA can improve the bone quality and Ca balance in broiler chickens, without having an effect on performance indices.

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