INTRODUCTION

Macro minerals and trace elements have an essential role in different physiological functions, including bone formation. Rapid broiler growth, an important factor in poultry production, can have a negative effect on skeletal development (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007). Bone stability and breaking strength are mainly determined by the degree of mineralization of the bone matrix (Boivin and Meunier, 2002).

Calcium and phosphorus are the primary inorganic nutrients contributing to 95% of the mineral matrix in bones (Rath et al., 2000; Shim et al., 2012) and about 97% of total calcium is found in bones (Scott et al., 1982; Leeson and Summers, 2001). Within certain physiological limits higher levels of calcium and phosphorus in feed increase bone mineral content, bone density, and ash content (Ouyango et al., 2003; Rao et al., 2006; Coto et al., 2008). Deficiencies of calcium and phosphorus lead to their reduction in bone (Wilson and Duff, 1991; Hemme et al., 2005). Sodium and potassium as well as chloride are necessary to maintain a physiological acid-base balance, which is important for regular bone development (Leeson and Summers, 2001). With increased magnesium level (0.2% Mg citrate or 0.2% MgO supplementation in diet), calcium to phosphorus homeostasis was affected and tibiae from chicks showed abnormal development and reduced ash content (Lee et al., 1980).

Within the trace elements, copper is important for optimal bone formation because it is required for collagen crosslinking (Ospthal et al., 1982). With an increase in collagen crosslinking, bone strength increased (Rath et al., 1999). Deficiency of zinc in the chick diet resulted in a decrease in alkaline phosphatase activity, impaired collagen synthesis and turnover, and ultimately tibia deformities (Starcher et al., 1980). Manganese is involved in skeletal integrity (Gajula et al., 2008). Impact of thermal and organic acid treatment of feed on apparent ileal mineral absorption, tibial and liver mineral concentration, and tibia quality in broilers

A. Hafeez, A. Mader, F. Goodarzi Boroojeni, I. Ruhnke, I. Röhe, K. Männer, and J. Zentek

Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Str. 49, 14195 Berlin, Germany

ABSTRACT Minerals play an important role for growth and bone stability in broilers. Thermal treatment and inclusion of organic acids in feed may affect the mineral absorption and tibial quality in broilers. The study was conducted to investigate the effect of thermal processing of feed including pelleting (P), long-term conditioning at 85°C (L), and expanding at 130°C (E) without and with 1.5% of an acid mixture containing 64% formic and 25% propionic acid on the apparent ileal absorption (AIA) of calcium, phosphorus, magnesium, potassium, sodium, iron, copper, manganese, and zinc, their concentrations in liver and tibia, as well as various tibial quality parameters in broilers. In total, 480 one-day-old Cobb broiler chicks were assigned using a completely randomized design with a 3 × 2 factorial arrangement. The ileal digesta, liver, and tibia were collected at d 35. The AIA of calcium and sodium was improved in group E compared with L (P ≤ 0.02 and P ≤ 0.01). Group P and E showed higher AIA for potassium than L (P ≤ 0.01). Bone ash content was increased in group E compared with L (P ≤ 0.04). The BW to bone weight ratio was lower and tibial zinc content was higher in group P compared with E (P ≤ 0.05). Tibial iron content was higher in group L than E (P ≤ 0.03). Acid addition did not affect AIA, mineral content in tibia, or tibial quality parameters. Thermal and acid treatment did not affect mineral concentrations in the liver, except an inconsistent interaction effect for DM content and sodium (P ≤ 0.03 and P ≤ 0.04, respectively). In conclusion, long-term thermal treatment reduced AIA of some minerals compared with short-term thermal treatments, but had no impact on tibia composition. Acid inclusion had no effect on AIA of minerals and tibia quality. Thermal treatment and the use of organic acids can therefore be considered as safe with regard to their impact on bone development in broilers.

Key words: feed decontamination, organic acid, pelleting, mineral absorption, tibia

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Corresponding author: juergen.zentek@fu-berlin.de
2011) as it is required for glucosyltransferases (Yang and Klimis-Tavantzis, 1998). Dietary manganese influenced the calcification positively and feeding diets low in manganese and calcium concentrations to young layers resulted in lower tibia strength, bone weight, and ash content (Ochrimenko et al., 1992). Iron is mainly stored in the bone marrow (Aoyagi and Baker, 1995) and supplementation of 3.22% Fe₂(SO₄)₃ in layer diet containing 0.9% Fe has been shown to impair phosphorus availability, resulting in lower bone ash (Deobald and Elvehjem, 1935).

The effects of thermal treatment on mineral absorption, storage, and tibial quality have not been widely considered up to now, although mainly positive effects of thermal treatment on microbial contaminations in feed, pellet stability, feed intake, growth, feed conversion ratio, feed efficiency, and nutrient digestibility are known (Coelho, 1994; Fancher et al., 1996; Beyer, 2000; Abdollahi et al., 2013). Nevertheless, it could be demonstrated that phosphorus availability improved when diets were pelletled, as indicated by improved bone ash (Bayley et al., 1968).

Various organic acids are used in the feed industry to improve feed hygiene by decontamination by avoiding recontamination (Martin and Maris, 2005; Ricke, 2005), lower the pH of digesta, improve digestive enzyme and phytase activity, and to beneficially affect gastrointestinal functions (Jongbloed et al., 2000; Dibner and Buttin, 2002). Additionally, some data on mineral absorption, storage, and tibial quality are available. Citric acid improved phosphorus utilization as well as the mineral concentration in tibia by increasing bone ash (Rafacz-Livingston et al., 2005), affected retention of calcium and zinc, tibial ash, as well as tibial phosphorus and calcium content in broilers (Brenes et al., 2003) and enhanced bone strength in broilers by improving total digestibility of calcium, phosphorus, and magnesium (Islam et al., 2012).

To our best knowledge, no more data on the impact of thermal and acid treatment of feed on the apparent ileal digestibility and the concentration of minerals in tibia and liver and the tibia quality in broilers were published.

Therefore, the purpose of the study was to evaluate if apparent ileal absorption, tibial and liver mineral content, and bone development can be affected by different levels of thermal processing without or with the use of organic acids and their interaction in broiler feeds.

**MATERIALS AND METHODS**

The experiment was conducted under approval of the experimental protocol by the State Office of Health and Social Affairs Berlin (LAGeSo Reg. No. 0113/11).

**Birds and Experimental Design**

The experiment lasted for 35 d. One-day-old male (Cobb) chicks were purchased from a commercial hatchery (Cobb Germany Avimex GmbH, Wiedemar-Wiesenena). Four hundred eighty chicks were randomly distributed into 48 pens with 10 birds per pen. Each pen had 1.20 m × 1.75 m space. Softwood shaving was used as bedding material for all pens. The temperature of the stable was maintained at 33°C for the first week. From the second week onward, the temperature was reduced by 3°C per week until the end of the experiment. During brooding period, all pens were provided with 24 h light for first 3 d. Light was reduced to 20 h for the next 4 d and 16 h light was provided from d 8 to 35. Feed and water were provided ad libitum throughout the experiment.

During the first 21 d of age, the chicks were provided with starter feed; grower feed was offered during the last 14 d. Diet formulation was done according to the recommendations of the German Society of Nutritional Physiology (GfE, 1999). To analyze apparent mineral absorption in the ileum, the grower diet was supplemented with TiO₂ (Sigma Aldrich, St. Louis, MO) as an indigestible marker at 2 g/kg. Table 1 shows the composition of starter and finisher diets. The concentrations of minerals in the grower feed are given in Table 2.

The study was conducted using a completely randomized design with a 2-factorial arrangement. Feed was treated with 3 types of thermal processing including steam conditioning at 70°C for 15 to 20 s followed by pelletling (P), long-term conditioning at 85°C for 3 min and subsequent pelletling (L), and expansion at 130°C for 3 to 5 s (E) without (0%) or with 1.5% supplementation of a commercial product containing 63.75% formic acid, 25.00% propionic acid, and 11.25% water (Lupro-Cid, BASF SE, Ludwigshafen, Germany). The 6 different diets, formulated using a 3 × 2 design, were then randomly assigned to chicks within 48 pens with 8 pens per diet. The procedure of feed production and its technical details were according to Goodarzi Boroojeni et al. (2014).

**Feed Production Technology**

For primary diets, feed components were mixed using a twin-shaft paddle mixer for 3 min (type 300 LTR, Din-nissen B.V., Sevenum, the Netherlands). During continuous mixing, Lupro-Cid was continuously sprayed on the diets containing acids. After mixing, the diets were subjected to the different processing methods.

**Pelleting**

During short-term steam conditioning, the temperature of the feed exiting the conditioner (Type M-Mix, Simon-Heesen B.V., Boxtel, the Netherlands) was maintained at 70°C for 15 to 20 s. The conditioned feed was pelleted using a ring die pellet press (Type Monoroll Labor, Simon-Heesen B.V.) with a die channel diameter and length of 3 and 60 mm, respective-
ly. The pressed material was cooled on a belt cooler (Fördertechnik GmbH, Mülheim/Ruhr, Germany) to ambient temperature within 15 min.

**Long-Term Conditioning and Pelleting**

Prior to pressing, diets were preconditioned in a twin-screw preconditioner (Neuhaus, Delmenhorst, Germany) using steam and subsequently subjected to long-term conditioning in a closed container with a heated jacket. The diets were heated to 85°C for 3 min. The conditioned feed was immediately introduced into the pellet press and pelleted as described above.

**Expanding**

Diets were introduced into an extruder (type OE 8, Amandus Kahl GmbH & Co. KG, Reinbek, Germany) and processed at 130°C. The feed exited the extruder through 3-mm outlet nozzles and formed strands, which were cut to pellet shape by rotating blades. The feed was cooled to ambient temperature on a belt cooler (Fördertechnik GmbH) within 15 min. The moisture content was reduced by using preheated air in 1 of the 4 segments of the cooler. After production, the pelleted and extruded feed was crumbled using a roller mill (type A2-E, MIAG, Braunschweig, Germany) with a milling gap of 3 mm to homogenize particle sizes.

**Collection of Samples**

At the end of experiment at d 35, birds were weighed, stunned, and killed by exsanguination. Carcasses were dissected immediately after slaughter. For mineral absorption analysis, ileum digesta were collected from 6 birds, randomly selected from each of the 8 pens of each diet, taking the distal two-thirds part of the ileum after dissection from Meckel’s diverticulum to the ileo-cecal junction. The digesta of birds within one pen were pooled and immediately frozen at −80°C before analysis. For analysis of mineral concentration in liver and tibia, 2 birds from 6 pens in each diet group were randomly selected. Livers were removed and cleaned from fat and gall bladder. After weighing, the liver was frozen at −80°C. The left tibiae were removed, defleshed, and the patella was removed. After cleaning, the tibiae were weighed and frozen (−20°C) until further analysis.

**Chemical Analysis**

**Apparent Ileal Absorption.** Apparent ileal absorption of minerals was calculated using the following formula:
apparent ileal absorption (%) = 100 − [(concentration of marker in feed/concentration of marker in ileum) × (concentration of nutrient in ileum/concentration of nutrient in feed) × 100].

**Tibia Quality Parameters.** Tibiae were weighed and length was measured with the help of a caliper. Thicknesses of medial and lateral walls were obtained from the mid-point of the bone. The following formulae were used to calculate robusticity index and medullary canal diameter:

robusticity index = bone length/cube root of bone weight (Riesenfeld, 1972), and
tibiotarsal index = diaphysis diameter − medullary canal diameter/diaphysis diameter × 100 (Barnett and Nordin, 1960).

Weight of bone in water was measured using an electronic semimicro balance (type 2024MP6, Sartorius GmbH, Göttingen, Germany). Bone density was calculated using the following formula:

bone density = weight of bone in air/(weight of bone in air − weight of bone in water) × water density at water temperature.

The BW to bone weight ratio was calculated by dividing BW (g) by bone weight (g).

**Analysis of Mineral Concentrations**

Tibiae were crushed manually into small particles, freeze-dried, and defatted. To determine the ash content, tibiae were ashed in a muffle furnace at 600°C for 6 h. The percent ash was determined with relation to fat-free DM in tibia. Concentrations of calcium, magnesium, potassium, sodium, iron, copper, manganese, and zinc were determined by atomic absorption spectrometry in an AAS Vario 6 spectrometer (Analytik Jena, Jena, Germany). The ammonium vanadate/molybdate method was adopted to measure the concentration of phosphorus as described by Gericke and Kurnies (1952). Concentrations of titanium dioxide in feed and ileum digesta were determined according to the method described by Short et al. (1996). Feed, ileum digesta, and liver samples were freeze-dried using the same procedure as for the tibia. Dried liver was cut into small pieces using a plastic knife. Dried feed, digesta, and liver were ashed in muffle furnace at 600°C for 6 h and ash content was calculated in relation to the amount of DM in feed, ileum, and liver, respectively. Analysis for mineral concentrations in feed, digesta, and liver were performed in the same way as in tibia.
**Statistical Analysis**

Data were arranged as a 3 × 2 factorial arrangement including 3 types of thermal processing (P, L, and E) and 2 levels of acid (0 and 1.5%). Normal distribution was tested using Shapiro-Wilk. On the basis of the results, normally distributed variables were subjected to ANOVA using the GLM procedure and not normally distributed parameters were analyzed by Kruskal-Wallis test of SPSS 20.0 (SPSS Inc., Chicago, IL). To achieve normality, potassium (liver) was log-transformed before analysis. Pearson correlation method was used for normally distributed parameters. Analyses were performed for main treatment effects and their interactions. Tukey’s b test was used as a post-hoc test at \( P \leq 0.05 \) for grouping of treatment means. Apparent ileal absorption variables were measured on the basis of pen as experimental unit. Bird was used as experimental unit to measure BW, liver, and tibia variables.

**RESULTS**

Thermal treatment had a significant effect \( (P \leq 0.05) \) on the apparent ileal absorption of calcium, potassium, and sodium (Table 3). The absorption of calcium and sodium was higher in group E compared with group L \( (P \leq 0.02 \text{ and } P \leq 0.01, \text{ respectively}) \), whereas for the group P, no differences could be found \( (P > 0.05) \). Enhanced apparent potassium absorption was determined in groups E and P compared with the group L \( (P \leq 0.01) \). No differences were observed among various thermal treatment groups for phosphorus, magnesium, iron, copper, manganese, and zinc \( (P > 0.05) \).

Acid supplementation and interaction between thermal treatment and acid had no effect \( (P > 0.05) \) on the apparent ileal absorption of macro and trace elements. Table 3 demonstrates various quality parameters and minerals concentrations in the tibia of broilers at 35 d of age. The ratio between BW and bone weight was reduced in the group P compared with the group E \( (P \leq 0.04) \); however, group L did not show significant differences. The amount of ash in fat-free DM was 55.0, 53.7, and 53.3% in groups E, P, and L, respectively \( (P \leq 0.4) \). Group L displayed higher iron concentrations compared with group E \( (P \leq 0.03) \). Pelleting resulted in higher tibia zinc concentration compared with group E \( (P \leq 0.01) \) and no effect could be found for group L. No differences \( (P > 0.05) \) were observed among the various treatments groups in bone length, bone weight, tibiotarsal index, robusticity index, and bone density. Dry matter percent and fat-free DM percent were comparable between the treatment groups. The concentrations of calcium, phosphorus, magnesium, potassium, sodium, copper, and manganese were at similar levels. Acid inclusion had no effect on any of the investigated variables, and there was no interaction effect between thermal and acid treatment for any tibia quality parameter or tibia mineral concentration \( (P > 0.05) \). The concentrations of minerals in the liver were not affected by thermal and acid treatment (Table 5). The interaction of thermal treatments and acid affected DM percent in liver \( (P \leq 0.03) \). The retention of sodium in the liver was significantly affected by interaction of thermal treatments and acid \( (P \leq 0.05) \), which was lower in group E+0% \( (2.02 \pm 0.07 \text{ g/kg of DM}) \) compared with groups P+0%, L+1.5%, and E+1.5% \( (2.39 \pm 0.12, 2.33 \pm 0.07, \text{ and } 2.29 \pm 0.09 \text{ g/kg of DM, respectively; } P \leq 0.04) \).

**DISCUSSION**

The various thermal treatments used in present study are combinations of different defined levels of temperature and duration. Each of the various factors of thermal treatment may have an influence on the investigated parameters. Nevertheless, the different factors of thermal treatment cannot be tested one by one because the study was designed to compare common thermal treatments used by the industry and the various parameters of common thermal treatments affect each other. The thermal treatments used can be differentiated into 2 factors: the maximum temperature (°C) and the duration of the feed at the maximum temperature (time). Therefore, both factors have to be taken into account when interpreting the results.
In general, the percentages of apparent ileal mineral absorption for the short-term treated diets were similar to previous data published; however, those for the long-term treated diet were partly below previously published ranges of total availability of calcium (68–71%), phosphorus (44–77%), magnesium (51–62%), copper (69–87%), manganese (48–60%), and zinc (44–58%; Nwokolo and Bragg, 1980; Awyong et al., 1983). Thermal treatment increased apparent ileal absorption of calcium, potassium, and sodium when the feed was expanded at 130°C for 3 to 5 s compared with pelleting at 80°C for 5 s and long-term treatment at 85°C (P ≤ 0.05). Additionally, apparent ileal absorption values of phosphorus reflect a similar impact, although there was no significant change. A previous study on broilers indicated that oven drying of fresh maize grains for 24 h resulted in an increased absorption of calcium at 85 and 95°C (P ≤ 0.05) compared with fresh maize. Similarly, phosphorus absorption was improved at 85°C (P ≤ 0.05). However, for both minerals the absorption decreased with a further increase in temperature up to 105°C (Iji et al., 2003). Some other studies revealed that pelleting reduced phytate phosphorus retention in coarse and fine particle size maize diets compared with nonpelleted diets (Kilburn and Edwards, 2001) and that maize had 19% and soybean meal had 44% phytate phosphorus disappearance at the ileum (Rutherfurd et al., 2002). To our knowledge, no further literature on thermal feed treatments is present.

Inclusion of 1.5% organic acids did not affect the apparent ileal absorption of minerals and trace elements, except for a trend for lower absorption of phosphorus in the acid inclusion group (P ≤ 0.08). A recent study using citric acid in broiler diets showed that the apparent absorption of calcium and phosphorus was higher by

### Table 4. Effect of thermal and acid treatment of feed on tibia quality and tibial mineral content in broilers at d 351,2

<table>
<thead>
<tr>
<th>Item</th>
<th>Thermal</th>
<th>Acid (%)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW d 35 (g)</td>
<td>Pellet</td>
<td>1.985</td>
<td>2.041</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Long-term</td>
<td>2.040</td>
<td></td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Expand</td>
<td>2.039</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Tibia length (cm)</td>
<td>8.48</td>
<td>8.38</td>
<td>8.44</td>
<td>0.43</td>
</tr>
<tr>
<td>Tibia weight (g)</td>
<td>8.50</td>
<td>8.14</td>
<td>7.76</td>
<td>0.34</td>
</tr>
<tr>
<td>BW:bone weight</td>
<td>252b</td>
<td>253ab</td>
<td>260a</td>
<td>0.24</td>
</tr>
<tr>
<td>Tibiotarsal index</td>
<td>42.4</td>
<td>42.0</td>
<td>40.9</td>
<td>0.54</td>
</tr>
<tr>
<td>Robusticity index</td>
<td>4.14</td>
<td>4.17</td>
<td>4.21</td>
<td>0.01</td>
</tr>
<tr>
<td>Bone density (g/cm³)</td>
<td>1.17</td>
<td>1.15</td>
<td>1.16</td>
<td>0.11</td>
</tr>
<tr>
<td>DM (%)</td>
<td>55.4</td>
<td>54.4</td>
<td>54.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Fat-free DM (%)</td>
<td>52.5</td>
<td>51.2</td>
<td>51.2</td>
<td>0.38</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>53.1ab</td>
<td>53.3ab</td>
<td>55.6a</td>
<td>0.30</td>
</tr>
<tr>
<td>Calcium (g/kg)</td>
<td>170</td>
<td>173</td>
<td>182</td>
<td>2.46</td>
</tr>
<tr>
<td>Phosphorus (g/kg)</td>
<td>94.6</td>
<td>93.5</td>
<td>93.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Magnesium (g/kg)</td>
<td>4.01</td>
<td>4.01</td>
<td>4.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Potassium (g/kg)</td>
<td>2.09</td>
<td>2.19</td>
<td>2.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Sodium (g/kg)</td>
<td>4.66</td>
<td>4.71</td>
<td>4.68</td>
<td>0.07</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>128ab</td>
<td>147a</td>
<td>121b</td>
<td>0.03</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>0.85</td>
<td>0.89</td>
<td>0.67</td>
<td>0.04</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>4.10</td>
<td>4.35</td>
<td>4.54</td>
<td>0.11</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>174a</td>
<td>165ab</td>
<td>160b</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**a,b**Means with different superscripts in a row differ significantly (P ≤ 0.05).

1Data are means of 12 replicates per group; ash, macrominerals, and trace elements are related to fat-free DM.

2Data within one group were normally distributed.

### Table 5. Effect of thermal and acid treatment of feed on the liver mineral concentrations in broilers at d 351,2

<table>
<thead>
<tr>
<th>Item</th>
<th>Thermal</th>
<th>Acid (%)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>45.2</td>
<td>43.3</td>
<td>43.9</td>
<td>0.70</td>
</tr>
<tr>
<td>DM (%)</td>
<td>30.1</td>
<td>31.2</td>
<td>30.5</td>
<td>0.21</td>
</tr>
<tr>
<td>Ash (% of DM)</td>
<td>4.33</td>
<td>4.26</td>
<td>4.34</td>
<td>0.04</td>
</tr>
<tr>
<td>Calcium (g/kg of DM)</td>
<td>0.30</td>
<td>0.29</td>
<td>0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>Phosphorus (g/kg of DM)</td>
<td>8.93</td>
<td>8.83</td>
<td>9.16</td>
<td>0.10</td>
</tr>
<tr>
<td>Magnesium (g/kg of DM)</td>
<td>0.56</td>
<td>0.56</td>
<td>0.55</td>
<td>0.01</td>
</tr>
<tr>
<td>Potassium (g/kg of DM)</td>
<td>15.7</td>
<td>16.2</td>
<td>16.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Sodium (g/kg of DM)</td>
<td>2.27</td>
<td>2.28</td>
<td>2.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Iron (mg/kg of DM)</td>
<td>404</td>
<td>449</td>
<td>472</td>
<td>0.23</td>
</tr>
<tr>
<td>Copper (mg/kg of DM)</td>
<td>8.93</td>
<td>9.05</td>
<td>8.47</td>
<td>0.35</td>
</tr>
<tr>
<td>Manganese (mg/kg of DM)</td>
<td>7.36</td>
<td>7.76</td>
<td>7.94</td>
<td>0.25</td>
</tr>
<tr>
<td>Zinc (mg/kg of DM)</td>
<td>55.1</td>
<td>53.6</td>
<td>51.8</td>
<td>0.52</td>
</tr>
</tbody>
</table>

**a,b**Means with different superscripts in a row differ significantly (P ≤ 0.05).

1Data are means of 12 replicates per group.

2Data within one group were normally distributed except for potassium.
the addition of 0.75% acid, but further addition of acid resulted in a decline of absorption (Islam et al., 2012), the addition of 2% acid increased calcium, phosphorus, and zinc retention (Brenes et al., 2003) and 4 to 6% acid increased the phosphorus utilization without having any effects on the calcium utilization (Boling-Frankenbach et al., 2001). Addition of 5.4% sulfuric acid in poultry and ducks feed increased phosphorus utilization and tibia ash (Capdevielle et al., 1998). Liem et al. (2008) analyzed the effect of several organic acids including citric acid, malic acid, fumaric acid, and EDTA on phytate phosphorus hydrolysis and concluded that the reason why some organic acids are effective, whereas others are not is not apparent. The effects of organic acids are inconsistent and depending on dosage, buffering capacity of dietary ingredients, and cleanness of buffering environment (Dibner and Buttin, 2002), which may explain the contrast in our finding in comparison with some studies mentioned above.

No interaction effect was observed between acid inclusion and thermal processing. This indicates that the investigated thermal and acid treatment combinations has no effect on mineral absorption in the ileum and therefore can be used safely for decontamination and to avoid recontamination during the cooling process of broiler feed formulation to enhance feed hygiene.

The present study showed some interesting results about the effect of thermal treatment on bone quality and tibial mineral contents. We used various parameters to indicate the bone status, which have been reported in previous studies including bone breaking strength (Merkley, 1981; Ruff and Hughes, 1985; Park et al., 2003; Kim et al., 2006), bone density (Watkins and Southern, 1992; Onyango et al., 2003; Kim et al., 2006), bone mineral content (Akpe et al., 1987; Onyango et al., 2003; Kim et al., 2006), and bone ash (Garlisch et al., 1982; Cheng and Coon, 1990; Park et al., 2003; Shim et al., 2008). In group P, BW to bone weight ratio was lower ($P \leq 0.04$), which suggests that higher amount of bone content was available in relation to BW in group P compared with group E. Due to no observed difference in bone density ($P > 0.05$), it was assumed that difference in this ratio was by chance and has no known biological reason. Our data indicate that in group E, ash content increased ($P \leq 0.05$) when mineral content was enhanced due to a trend for higher calcium ($P \leq 0.1$). With the increase of ash, bone hardness or strength is expected to increase (Bonser and Casinos, 2003). The organic component of bone is important in providing tensile strength and flexibility (Velleman, 2000). Higher zinc content was found in group P ($P \leq 0.01$). The literature suggests that zinc is involved in collagen synthesis and turnover (Starcher et al., 1980), bone development (Ovesen et al., 2001), and integrity of bones (Gajula et al., 2011), whereas copper plays a role in collagen crosslinking (Ospal et al., 1982). Interestingly, in group L, iron concentration was higher and ash content was lower in comparison with group E, whereas copper concentrations showed a similar trend ($P \leq 0.03$, $P \leq 0.04$, and $P \leq 0.1$, respectively). In general, iron had a negative correlation with ash percent ($-0.80$) and calcium ($-0.33$; $P \leq 0.05$). This indicates that the higher concentration of iron might have reduced mineralization. Our results are in line with the literature that suggest that the addition of 3.22% $\text{Fe}_2(\text{SO}_4)_3$ having 0.9% iron compared with 1.61% $\text{Fe}_2(\text{SO}_4)_3$ with 0.45% iron in the layer diet reduced the bone ash from 29.5 to 25.4% on a fat-free DM basis. This amount of iron was sufficient to bind one-half of the total phosphorus in the ration as $\text{FePO}_4$, thereby causing phosphorus deficiency in blood and ultimately severe rickets (Deobald and Elvehjem, 1935).

However, no differences were found for calcium and phosphorus concentrations within this study, which is in line with the findings of Edwards et al. (1999) who reported that pelleting did not improve the total digestibility of phytate phosphorus. Phosphorus availability was improved by pelleting when corn-soy-based mash diets containing no added inorganic phosphate, as indicated by improved bone ash (Bayley et al., 1968). Cereals contain 60 to 70% of phosphorus in the form of phytic acid, which is poorly available (0–50%). In young birds total phosphorus availability from mono-calcium phosphate may be as high as 98% (Leeson and Summers, 2001). In the present study, diets contained both phytic acid from the soy and maize and mono-calcium phosphate. Therefore, the lack of treatment effects on apparent phosphorus absorption could be explained by the presence of both phytic acid and inorganic phosphorus. Bone density, which can indirectly be used to determine bone strength (Shim et al., 2012), did not differ in present study. Bone length, BW, robusticity index, tibiotarsal index, DM percent, fat-free DM percent, magnesium, potassium, sodium, and manganese did not show any differences. To our knowledge, no further previous results are available regarding the effect of feed processing on tibia quality and mineral concentrations in the tibia.

Inclusion of organic acids did not affect of the measured tibial quality parameters and mineral concentrations. However, a trend was observed toward a higher tibiotarsal index and lower sodium concentration in 1.5% acid group. The literature suggests that bone breaking strength, bone density, and DM contents were increased by up to 0.75% dietary citric acid and decreased with increasing acid levels (Islam et al., 2012). Further, tibial ash, calcium, and magnesium decreased numerically in birds consuming a diet with 1.5% compared with 0.75% citric acid (Islam et al., 2012). With an increase of citric acid in the diet from 0 to 4%, tibia ash increased linearly (Rafacz-Livingston et al., 2005). Inclusion of citric acid at 3 to 4% in broiler feed led to improved phosphorus utilization and mineral concentration in the tibia (Rafacz-Livingston et al., 2005). Citric acid at 0.5% significantly increased tibia ash percentage (Chowdhury et al., 2009). Additionally, the inclusion of citric acid affected calcium and zinc retention, tibia ash, phosphorus, and calcium content in
tibia (Brenes et al., 2003). Our results could be interpreted in the context that the effects of organic acids are inconsistent depending on dosage, buffering capacity of dietary ingredients, heterogeneity of gut microbiota, and the presence of other antimicrobial compounds (Dibner and Buttin, 2002).

Thermal and acid treatment combinations failed to show interaction effects for any of tibial parameters or mineral concentrations ($P > 0.05$). Therefore, organic acids at 1.5% may safely be used along with various thermal treatments for sanitation of broiler feed.

Our data show that thermal treatment and acid supplementation had no effect on fresh liver weight, ash percentage, and retention of any mineral and trace element in hepatic tissue ($P > 0.05$). However, there was an inconsistent interaction between thermal processing and acid treatment for DM and sodium ($P \leq 0.05$).

In the present study, the observed mineral levels in hepatic tissue are in agreement with the literature, which revealed a wide range for some of the minerals. The concentrations reported in different studies are for magnesium (0.64–0.652 g/kg of DM), phosphorus (10.7–10.9 g/kg of DM), calcium (0.107–0.115 g/kg of DM), copper (3.0–15.1 mg/kg of DM), zinc (28.0–115 g/kg of DM), iron (75.0–634 g/kg of DM), and manganese (4.3–11.6 g/kg of DM; Thompson and Weber, 1981; Black et al., 1984; Henry et al., 1987; Skrivan et al., 2005; Bao et al., 2007). However, not only the trace elements were used at various levels in experimental diets for most of these studies but also different methods were used for preparation, digestion, and analysis of liver samples (Thompson and Weber, 1981; Korsrud et al., 1985; Henry et al., 1987; Falandysz, 1991; Skrivan et al., 2005; Bao et al., 2007), which might be the reasons for inconsistency in observed mineral values in literature. According to our knowledge, the effect of thermal and acid treatment of feed and their interaction effects on mineral concentrations in liver were not reported in previous studies.

The lack of significant differences in the mineral concentrations of various thermal processing and acid treatment groups in liver might be due to the fact that the impact on apparent ileal absorption was too low and liver is not enough sensitive for indicating mineral retention under these conditions. Previous studies reveal that the tibia is a better indicator for some specific aspects of mineral retention. For example, manganese and zinc accumulate in the tibia rather than in the liver (Gajula et al., 2011), independent of the organic or inorganic form of manganese supplemented in the diet (Berta et al., 2004).

In conclusion, long-term thermal treatment impaired apparent ileal absorption of calcium, potassium, and sodium and resulted in reduced tibial ash content compared with short-term thermal treatment. Inclusion of organic acid had no negative effect on apparent ileal absorption and tibial and liver concentrations of minerals and tibia quality. Long- and short-term thermal treatment, organic acids, and their combination seem to have no negative impact on bone development in broilers and may safely be used for sanitation of broiler feed.

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