PEATSALE OF CLA AND *CAMELINA SATIVA* SEED OIL
ON BONE PROPERTIES IN BROILER CHICKENS

IWONA PUZIO, TERESA JAŚKIEWICZ, AGNIESZKA SAGAN, MAREK BIEŃKO, AND DOROTA GRABOŚ

Department of Biochemistry and Animal Physiology,
1Department of Biological Bases of Food and Feed Technologies,
University of Life Sciences in Lublin, 20-950 Lublin, Poland
iwona.puzio@up.lublin.pl

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Abstract

The objective of this study was to evaluate the effect of dietary conjugated linoleic acid (CLA) and false flax (*Camelina sativa*) seed oil (CS) on bone quality in broiler chickens. Experiment was carried out on 96 chickens randomly divided into four groups: control group fed diet with sunflower oil (SO) and three experimental groups fed diet with addition of CLA (2.86% starter, 4.32% grower) and diet with addition of CLA (1.43% starter, 2.16% grower) and SO or CS (1.43% starter, 2.16% grower). On the 35th day of life eight birds per treatment were slaughtered and the femur, humerus, and tibia were isolated for further analysis. Using DXA method, bone mineral content (BMC) and bone mineral density (BMD) were measured. Furthermore, weight and length of bones were assessed. The weight, BMD, and BMC in chickens from CS+CLA group were significantly higher when compared with CLA group. The significant differences were noted for BMC between control and CS+CLA birds. No marked differences of bone parameters were observed between control group and CLA, and SO+CLA groups. In conclusion, the results indicate that false flax oil and CLA can replace sunflower oil in chickens feeding, and demonstrate the effectiveness of false flax oil on the enhancement of bone properties in broiler chickens.

Key words: bone, chicken, feeding, conjugated linoleic acid, *Camelina sativa*.

The causes of skeletal abnormalities in meat-type birds have been of significant interest to the poultry industry for many years. The modern-day commercial broiler chickens and turkeys have fast metabolic rates, good feed conversion ratio, fast growth ratio, and massive muscle development. These features promote an increased overload on the skeletal system and in consequence leg disorders. There is evidence that bone defects leading to eventual lameness can be induced at an early age in birds. Moreover, physiological studies have shown the importance of early nutrition on bone development in poultry.

The essential polyunsaturated fatty acids (PUFAs) from n-6 and n-3 classes are currently being studied to understand their effects on bone. PUFAs and their metabolites are involved in the regulation of a variety of biological processes including bone metabolism. As the structural components of cell membrane bilayers as well as the precursors of series of potent biologically active eicosanoids, PUFAs change the fatty acid composition of membrane phospholipids influencing cellular metabolism, specifically the biosynthesis of bone resorptive PGE₂. Therefore, the biochemical and physiological actions of n-3 PUFAs on prostaglandin metabolism and cytokine production could explain their beneficial effects on bones (23, 24, 26). Few animal studies have addressed the effect of n-3 and n-6 fatty acids on bone characteristics in broiler chickens, turkeys, and quails (1, 8, 12, 13, 22).

False flax (*Camelina sativa*) seed oil (CS) is known to have a high content of PUFAs, especially of omega-3 fatty acids such α-linolenic acid (18:3 n-3, ALA) amounting to about 36%-42% of total FAs (5). This oil is also characterised by a high content of linoleic acid (18:2 n-6, LA), 16%-24%. The FAs profile of CS appears to be very interesting and has important implications from a nutritional point of view (5, 6, 17, 20). As far as the authors know, there are no published reports describing the influence of camelina oil on bones in animals.

Recently, attention has been focused on the effects of conjugated linoleic acid isomers (CLAs) on skeletal health. However, while CLA has been shown to increase bone mass, ash, and/or mineral content in growing chicks (3) and mice (16), others have reported a lack of effect in rats (9, 28) and pigs (4, 15).

The aim of the study was to determine the effects of the consumption of CLA concentrate or its mixtures with sunflower oil, or *Camelina sativa* oil used as a replacement of sunflower oil, on bone performance of broiler chickens. The objective was also to study the influence of false flax (camelina) oil as a new source of
α-linolenic acid, used simultaneously with the conjugated dienes of linoleic acid from CLA concentrate, on the bones of broiler chickens.

**Material and Methods**

**Animals.** All procedures used throughout this study were approved by the Local Animal Welfare Committee at the University of Life Sciences in Lublin, Poland.

A total of 96 one-day-old Ross 308 broiler chickens of both sexes were purchased from a local hatchery. Chickens were housed in cages, each containing three males and three females. The temperature during experiment was controlled and continuous lighting was applied throughout the entire experimental period. Feed and water were provided *ad libitum.*

**Procedure.** Commercially-sourced pre-starter feed was provided to the 10th d of life of the birds. The experimental trial started on the 11th and lasted until the 35th d. The chickens were assigned randomly to each of the four dietary treatments, four replicates of six birds each. From the 11th to the 21st d of life, the chickens were fed control and experimental starter diets, and in the next period grower diets were given to the birds. In the experimental diet sunflower oil (SO) was replaced by purified conjugated linoleic acid (CLA) or *Camelina sativa* oil (CS). The diets were formulated to be isocaloric and to meet the nutrient requirements of broilers (starter: ME 13.2 MJ/kg, protein crude 18.7%, ether extract 6.5%, crude fiber 3.15%, ash 4.63%, calcium 8.5 g/kg, available phosphorus 4.2 g/kg). The following dietary treatments were applied: 1) control diet, SO (SO – 2.86% starter, 4.33% grower) 2) CLA (CLA replacing SO), 3) SO+CLA (SO – 1.43% starter, 2.165% grower; CLA - 1.43% starter, 2.165% grower), and 4) CS+CLA (CS – 1.43% starter, 2.165% grower; CLA - 1.43% starter, 2.165% grower). The CLA used in this study was obtained from a commercial company (BASF, the Chemical Company, Poland). The content of both isomers 9cis,11trans, and 10trans,12cis in purified CLA was at the same level.

At the end of experiment eight birds (four males and four females) per treatment were randomly selected, weighed, and euthanised with an i.v. injection of pentobarbital sodium at the dosage of 15 mg/kg b.w. (Morbital, Biowet Pulawy, Poland).

**Bone analyses.** After euthanasia, the humerus, tibia, and femur were isolated, and their wet weight (g) and length (mm) were measured. The bone samples were frozen at -25°C and stored until further analysis. The measurements of bones were performed on Norland Excell Plus X-ray densitometer (Fort Atkinson, WI, USA) including special software, Small Subjects Scan version 3.9.6. The Small Subject Scan consists of a measurement scan over an area defined by the operator. An optional scout scan was available to assist the operator in defining the scan region. Analysis was performed on the scan data using operator-defined region of interest and numeric results were calculated and displayed. The measurements of the whole single bone mineral content (BMC), and area bone mineral density (BMD) were performed using the following parameters: scout scan speed 260 mm/s, resolution 3.0x3.0 mm; measurement scan speed 30 mm/s, resolution 1.0x1.0 mm.

**Statistical analyses.** The results were presented as mean ± SEM and data were statistically analysed by one-way analysis of variance (ANOVA) with the aid of Statistica 5.0 software. Differences among each treatment group were tested by Tukey’s multiple comparison test, and were considered significant at P≤0.05.

### Table 1
Effect of dietary treatment on mean bone parameters - weight, length, BMD, and BMC of the femur, humerus, and tibia (n=8)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SO (g)</th>
<th>CLA (g)</th>
<th>SO+CLA (g)</th>
<th>CS+CLA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Femur</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>10.6±1.20</td>
<td>10.4±0.50 a</td>
<td>10.7±1.17</td>
<td>11.7±0.67 b</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>76±2.17</td>
<td>76±1.8</td>
<td>75±0.86</td>
<td>78±2.6</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>1.825±0.071 a</td>
<td>1.815±0.077 a</td>
<td>1.861±0.175</td>
<td>1.963±0.071 b</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.164±0.007</td>
<td>0.162±0.013</td>
<td>0.164±0.012</td>
<td>0.169±0.009</td>
</tr>
<tr>
<td><strong>Humerus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>7.6±1.00</td>
<td>7.2±0.48 a</td>
<td>7.8±0.79</td>
<td>8.5±0.83 b</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>69±2.3</td>
<td>69±1.4</td>
<td>69±0.66</td>
<td>74±2.8</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>1.381±0.115 ab</td>
<td>1.369±0.082 a</td>
<td>1.529±0.109 bc</td>
<td>1.548±0.098 c</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.142±0.009</td>
<td>0.134±0.014</td>
<td>0.146±0.008</td>
<td>0.146±0.009</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>15.5±2.33</td>
<td>14.7±1.83 a</td>
<td>14.7±2.34</td>
<td>16.4±1.80 b</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>117±16.7</td>
<td>114±13.8</td>
<td>112±10.0</td>
<td>108±20.4</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>2.789±0.085 a</td>
<td>2.688±0.103 a</td>
<td>2.844±0.156</td>
<td>2.996±0.111 b</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.173±0.015</td>
<td>0.165±0.008 a</td>
<td>0.174±0.005</td>
<td>0.180±0.007 b</td>
</tr>
</tbody>
</table>

a,b - values within a row with different superscripts are significantly different (P≤0.05).

± SEM
Results

Analysis of bone parameters indicated the highest values of weight, length, BMC, and BMD in CS+CLA chickens, and the lowest in CLA chickens (Table 1). However, no significant differences were noted for bone length between the control and experimental birds. CS+CLA feeding significantly increased bone weight as compared to CLA group, which is in accordance to the differences in body weight (2.15±0.25 vs 2.035±0.30 kg). The highest differences in bone weight (18.1%) were noted for the femur. For the femur and tibia they were 12.5% and 11.6%, respectively.

The femur and tibia of the birds fed diet with addition of SO+CLA and CS+CLA were characterised by higher values of BMC than in other birds. Statistical analysis showed significant differences in BMC of the femur and tibia between CS+CLA and SO (7.6% for femur and 7.4% for tibia), CS+CLA and CLA (8.1% for femur and 11.5% for tibia) birds. The BMC of the femur and tibia of broilers fed SO or CLA diets was similar. Significant differences in values of this parameter were consistent between CS+CLA group and SO, and CLA (13.1%) groups. Moreover, birds fed SO+CLA diet had a significantly higher BMC than CLA group. Values of BMC of the femur and humerus in different groups indicated that there was no marked variation. The highest BMC of the tibia was in CS+CLA birds, and it showed significant differences versus CLA group (9%).

Discussion

The purpose of this study was to investigate the effects of CLA mixture and false flax oil in the diets of broiler chickens on bone characteristic. Currently, there are no data concerning the effects of camellina by-products, source of ALA and LA, and combined CLA and CS feeding on bone characteristics, both in humans and animals. Thus, the present study is probably the first to estimate the influence of false flax oil on bone characteristic in growing animals. Recently, many researchers have reported that FAs intake indicated different effects on BMD and BMC. No effect of n-3 and n-6 on BMD and BMC was observed by Johnston et al. (8) in turkey breeder hens, and by Baird et al. (1) in mature laying chickens. The study of Mazzuco et al. (14) demonstrated similar findings in case of BMD in chickens. However, other animal studies indicate positive effects of n-3 on BMD and BMC. Liu et al. (12) found a significantly higher BMC in quails fed a fish oil-supplemented diet (high in n-3) compared with the birds receiving a soybean oil diet group (high in n-6). The positive effects of n-3 on BMD were also reported by Sun et al. (21).

On the other hand, the effects of CLA on bone are contradictory. CLA has been shown to either increase or decrease bone formation rates and to have variable effects on markers of bone formation and resorption. Banu et al. (2) reported that CLA increased bone weight in mice. Mice fed 5 g/kg dietary CLA had higher levels of whole body ash (16), and chickens had higher tibial bone ash (3). Furthermore, a diet containing 5-5.2 g/kg of CLA increased the bone formation rate in rats and chickens (22, 25). However, the studies also showed a null or negative effect of dietary CLA on bone physiology after providing CLA at 10 g/kg of the diet (9, 11, 27).

The studies on animals suggest that intakes of CLA and different PUFAs can affect bone directly, by modulating prostaglandin (PGs) biosynthesis, or indirectly via IGF. The PGs are locally synthesised in bone from 20-carbon essential fatty acid precursors (AA 20:4 n-6 and EPA 20:5 n-3) and they are considered a potent stimulator of bone resorption and formation. The consumption of n-6 and n-3 fatty acids has been shown to have different effects on the synthesis of prostaglandins. PGs derived from n-6 family fatty acids, in particular PGE_2_, have been shown to have some inhibitory effects on bone development, while PGs derived from the n-3 series can have more beneficial effects of stimulating osteoblast function and bone formation, as demonstrated in cell culture studies (11, 22, 23). The anabolic effects of PGE_2_ may occur through stimulation of IGF-1 production by osteoblasts or by increasing bone cell responsiveness to IGF-1. Li et al. (11) and Watkins et al. (22) reported that dietary n-3 and CLA modulated the production of PGE_2_, altered the concentration of IGF-1 in bone, and led to increased or decreased bone formation rates in growing chickens and rats. The higher rate of bone formation in chicks was associated with reduced arachidonic acid level (precursor of PGE_3_). Because CLA is incorporated into membrane phospholipids, it may compete with other PUFAs in the formation of arachidonic acid to inhibit PGE_2_ biosynthesis (10). As already mentioned, PGE_2_ is also an important regulatory factor in the rate of bone formation (19). The reduced production of PGE_2_ in chicks fed a diet high in n-3 PUFA was associated with an increased rate of bone formation (22, 29).

The presented findings suggest that different factors alter action of unsaturated fatty acids. A probable explanation for the differences in animal studies concerning the effects of n-3 and n-6 FAs on bone could be the age of animals and varying ratio of n-6 to n-3 in the diet. Moreover, the dietary source of FAs may be a significant factor because it influences bioconversion of 18:2 n-6 and 18:3 n-3 PUFAs. The competitive interaction between LA and ALA exists, such that n-3 PUFA suppress the metabolism of n-6 PUFA, and n-6 PUFA suppress the metabolism of n-3 PUFA, although less strongly. Poureslami et al. (18) reported that in chickens bioconversion rate of 18:3 n-3 to 18:4 n-3 was 1.8-fold higher than the conversion rate of 18:2 n-6 to 18:3 n-6, indicating a higher affinity of D-6 desaturase for 18:3 n-3 than for 18:2 n-6. Bioconversion of 18:2 n-6 and 18:3 n-3 depended on supplemented oil. Feeding linseed oil decreased β-oxidation of 18:2 n-6, whereas fish diet increased β-oxidation of 18:3 n-3. The fish oil suppressed elongase and desaturase activity, whereas a
higher dietary supply of 18:3 n-3 and 18:2 n-6 enhanced elongation and desaturation activity on the PUFA involved in the n-3 and n-6 pathway, respectively (18).

In our studies, the main FAs components in SO were LA – 62.23% and oleic acid (18:1n-9) – 25.30%, whereas in Camelina sativa oil ALA - 36.08% and LA - 16.70%. Ratio of n-6/n-3 in CS was 0.47:1 (7). The results of the study revealed that simultaneously supplementing the diet of broilers with CS and CLA resulted in higher bone parameters - weight and BMC. Moreover, it was observed that dietary CLA supplementation resulted in lack of influence on bone when fed alone. These findings are in agreement with the data from other authors, who described no effect of CLA on bone mineral content in animal studies (15, 27). However, in the present study, the positive effect was observed when birds were fed a combined CS+CLA diet, but no significant differences were noted vs SO+CLA. These data indicate that when chicken were fed diet with CLA and SO, rich in LA, probably a competition for metabolic pathways between CLA and LA existed. However, during feeding with CLA and CS, which contain ALA as dominant component, CLA can be converted. The observed effect is probably the result of simultaneous activity of CLA and ALA. In the study of Baird et al. (1), in which no effect of n-3 FAs on bone was observed, linseed oil was the primary source of ALA, which has a low conversion rate to DHA and EPA. Furthermore, the epidemiological study of Weiss et al. (28) showed that reduced LA/ALA ratios were associated with increased BMD.

In young animals, not only total amount of both n-3 and n-6 FA, but the composition of dietary PUFAs appears to be required for bone growth. Thus, the influence of differences in specific n-3 sources on bone has yet to be clarified. According to the results of the present study, modifying the diet with the false flax oil and CLA mixture affects the bone weight and bone mineral content. These findings provide evidence that combined dietary supplementation with CLA and camelina oil can improve bone mineral content. However, this is a pilot study aiming at determination whether it is justifiable to conduct further work investigating the possible influence of addition of camelina oil, as a potential feed ingredient in chicken diets, on bone. The scope of future research on dietary false flax oil should include specifying the amount of supplementation. It should also focus on identifying the biomarkers of bone metabolism.

References


