Kafkas Univ Vet Fak Derg
25 (1): 61-70, 2019
DOI: 10.9775/kvfd.2018.20329

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Effects of Calcium Soaps of Animal Fats on Performance, Abdominal Fat Fatty Acid Composition, Bone Biomechanical Properties, and Tibia Mineral Concentration of Broilers

Ali ÇALIK [1,a] Sakine YALÇIN [1] Seher KÜÇÜKERSAN [1] Pınar SAÇAKLI [1] Gültekin YILDIZ [1]
Muhammad Shazaib RAMAY [1] Ozan AHLAT [2] Fatma Kübra ERBAY ELIBOL [3] Süleyman TABAN [4]

[1] Department of Animal Nutrition & Nutritional Diseases, Faculty of Veterinary Medicine, Ankara University, TR-06110 Ankara - TURKEY
[2] Department of Pathology, Faculty of Veterinary Medicine, Ankara University, TR-06110 Ankara - TURKEY
[3] Department of Micro and Nanotechnology, TOBB University of Economics and Technology, TR-06560 Ankara - TURKEY
[4] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ankara University, TR-06110 Ankara - TURKEY
[a] ORCID: 0000-0003-4550-9321

Article ID: KVFD-2018-20329 Received: 08.06.2018 Accepted: 15.10.2018 Published Online: 15.10.2018

How to Cite This Article

Çalik A, Yalçin S, Küçükersan S, Saçakli P, Yildiz G, Ramay MS, Ahlat O, Erbay Elibol FK, Taban S: Effects of calcium soaps of animal fats on performance, abdominal fat fatty acid composition, bone biomechanical properties, and tibia mineral concentration of broilers. *Kafkas Univ Vet Fak Derg,* 25 (1): 61-70, 2019. DOI: 10.9775/kvfd.2018.20329

Abstract

This study evaluated the effects of a graded concentration of dietary calcium soaps of tallow (CST) supplementation on broiler performance, carcass characteristics, abdominal fat fatty acid profile, bone biomechanical properties, and bone mineral composition. One hundred and forty 11-d-old male broiler chickens were randomly allocated to 4 experimental groups with 5 replicate pens containing 7 birds per each. The birds received corn-soybean meal based diet and CST (CST0, CST10, CST20, and CST30, respectively) was included in the grower (0, 10, 20, and 30 g/kg) and finisher (0, 15, 30, and 45 g/kg) diet at the expense of vegetable acid oil and limestone. Dietary supplementations had no significant effect on BWG and FI during the entire experimental period. However, FCR was improved in CST0 and CST10 groups in comparison to those of the CST20 group from d 11 to d 42. According to the present study result, dietary supplementation of low level of CST significantly influenced intestinal microarchitecture of the jejunum and ileum by improving villus height/crypt ratio and villus height, respectively. Femur (P=0.001) and tibia (P=0.020) stiffness increased linearly with the increasing level of dietary CST. Tibia Ca (P=0.009) and P (P=0.009) concentration of CST10 and CST30 groups were lower than the CST0 group. Increasing levels of CST in diets significantly reduced the Fe and Mn concentrations in tibia samples. In conclusion, supplementation of CST has no detrimental effect on broiler performance parameters and might be used as an alternative dietary fat source in the broiler industry.

Keywords: *Acid oil, Broiler, Calcium soap, Fat, Fatty acid composition, Tallow*

Hayvansal Yağın Yağ Asitleri Kalsiyum Tuzunun Broyler Performansı, İç Yağın Yağ Asidi Kompozisyonu, Kemiklerin Biyomekanik Özellikleri ve Tibia Mineral Düzeyi Üzerine Etkisi

Öz

Bu çalışmada rasyonlara artan düzeylerde ilave edilen hayvansal yağın yağ asiti tuzunun (CST) broylerlerde performans, karkas karakteristikleri, abdominal yağın yağ asiti kompozisyonu, kemiklerin biyomekanik özellikleri ve kemik mineral kompoziyonu üzerine olan etkisi incelenmiştir. Toplamda 140 adet 11 günlük erkek broyler 4 deneme grubuna ve her biri 7 civcivden oluşan 5 tekerrür grubuna rasgele olacak şekilde ayrılmıştır. Broylerler mısır-soyadan oluşan rasyon ile beslenmiştir. Büyütme (0, 10, 20 ve 30 g/kg) ve bitirme (0, 15, 30 ve 45 g/kg) dönemi rasyonları artan düzeylerde CST (CST0, CST10, CST20 ve CST30, sırasıyla) kapsayacak şekilde düzenlenmiştir. Karma yeme ilave edilen yağın, tüm deneme periyodu boyunca, canlı ağırlık artışı ve yem tüketimi üzerine önemli bir etkisi olmadığı görülmüştür. Ancak, araştırmanın 11-42 günleri arasında, CST0 ve CST10 gruplarının CST20 grubu ile karşılaştırıldığında daha iyi yemden yararlanma değerine sahip olduğu görülmüştür. Elde edilen araştırma sonuçlarına göre, karma yeme düşük düzeyde ilave edilen hayvansal yağın jejunum villus yüksekliği/kript derinliği oranını ve ileum villus yüksekliğini arttırmak suretiyle bağırsak histomorfolojisi üzerine olumlu bir etkisi olduğu tespit edilmiştir. Femur (P=0.001) ve tibia (P=0.020) sertliği rasyonlara artan düzeyde CST ilavesi ile birlikte doğrusal bir artış göstermiştir. CST10 ve CST30 gruplarının tibia Ca (P=0.009) ve P (P=0.009) konsantrasyonu, CST0 grubu ile karşılıştırıldığında daha düşük bulunmuştur. Karma yemde CST düzeyinin artması, tibia örneklerindeki Fe ve Mn konstrasyonunu önemli ölçüde azaltmıştır. Sonuç olarak, rasyonlara ilave edilen hayvansal yağın yağ asiti tuzlarının broyler performansı üzerine olumsuz bir etkisi olmadığı ve ucuz bir alternatif yağ kaynağı olarak kullanılabileceği görülmüştür.

Anahtar sözcükler: *Asit yağ, Broyler, Hayvansal yağ, Kalsiyum sabunu, Yağ, Yağ asidi kompozisyonu*

İletişim (Correspondence)
+90 312 3170315/4247
calik@ankara.edu.tr

INTRODUCTION

Production animals have specific energy needs and numerous nutrient requirements, such as amino acids, fatty acids, vitamins, and minerals for maintenance and growth. In the modern broiler production, diets are delicately formulated by nutritionists to meet a bird's requirements in a cost effective manner. For this purpose, fats and oils are preferred as an energy source in diets to meet high energy requirements of broilers since they contain more energy in comparison to carbohydrates and proteins [1]. There are numerous types of fats and oils, of varying quality and price, including vegetable oils (soybean oil, sunflower oil), animal fats (tallow, lard, and poultry fat), acidulated soapstocks, hydrogenated fats and oils that are available to use in diets [2]. Dietary supplementation of these products also confers beneficial effects such as improved palatability, improved absorption of fat-soluble vitamins, reduced dustiness and wastage of feed during processing, and improved texture in mash diets [2,3]. In addition, dietary fats and oils increases nutrient absorption and utilization by slowing down passage rate of digesta through the gastrointestinal tract of chickens [4-6].

Almost all ingredients used in broiler diets are vegetable based and therefore the fatty acid composition of the basal diets is highly unsaturated [7]. According to previous studies, inclusion of animal fats to poultry diets, especially at low levels, resulted in increased apparent metabolisable energy (AME) values of the added fat [7-9]. Tallow is a rendering by-product of the red meat industry and commonly used as an energy source in broiler diets because of its relatively low price [10]. However, it is well documented that digestibility and metabolisable energy content of tallow are lower than vegetable oils due to the fatty acid composition, which is mostly saturated [11,12]. Previous studies suggested that the dietary fat source has no effect on performance of broilers since energy, protein, and amino acid levels are balanced, even though the tallow has a lower AME value than those of vegetable oils [13]. However, dietary inclusion level of tallow or the combined use with several vegetable oils might affect broiler performance. Tancharoenrat and Ravindran [10] indicated that broilers have had higher fat retention and ileal fat digestibility rate when fed a diet containing 4% tallow than those fed 8% tallow. Newman et al.[14] reported that dietary addition of 8% beef tallow significantly reduced broiler performance versus those fed with sunflower oil. Moreover, rather than used alone, combined use of tallow with vegetable oils was determined to have synergistic effects [12] and improved body weight (BW) and feed efficiency in broilers [3].

Calcium soaps of tallow (CST) is preferred energy sources, especially for ruminants, due to their chemical structure which makes these compounds inert in the rumen environment [15]. On the other hand, they are efficiently digested and absorbed in the small intestine. CST can be easily incorporated into diets without any specialized equipment due to their granulated-solid nature and are more resistant to oxidation than tallow [15]. Rising et al.[16] concluded that calcium soaps from animal fat were utilized efficiently by laying hens. However, as far as we know, no available data exists about the effects of CST on broiler performance.

A diverse variety of fats and oils are available but the choice of these energy products mostly depends on their price. In this manner, interest in cost-effective alternative energy sources by the modern broiler industry is growing each year. Therefore, the purpose of this experiment was to evaluate the effects of a graded concentration of CST on performance, abdominal fat fatty acid deposition and bone characteristics of broiler chickens.

MATERIAL and METHODS

Animal Care and Use

All experimental procedures were approved by The Animal Ethics Committee of the Ankara University (2015-15-166).

Birds and Management

One-day-old Ross 308 male broiler chickens were obtained from a commercial company (Beypiliç, Bolu, Turkey) and reared to 11 d of age in a broiler house under standard conditions and were fed broiler starter diets. One hundred and forty 11-day old male broiler chickens, with average BW of 365.12±4.77 g (Mean ± SD) were selected for the experiment. The chicks were randomly allocated to 4 experimental dietary groups (5 replicate floor pens, 7 birds /pen). Birds were kept under environmentally controlled room for 31 days. Temperature was adjusted to the according to the recommended conditons for Ross 308 broiler during the study [17]. The grower and finisher diets were based on maize-soybean meal and were offered to birds from 11-21, and 21-42 days of age, respectively *(Table 1)*. All diets were formulated to meet or exceed NRC [1] nutrient recommendations. Water and diets (in mash form) were provided *ad libitum* throughout the experimental period.

Calcium soap of tallow was included to grower and finisher diets as follows: Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST. Diets were mixed individually and CST (in fine powder form) was included to diets at the expense of vegetable acid oil and limestone. Full fat soybean was used in diets as unsaturated vegetable oil source and protein source. Vegetable acid oil (VAO) was added to experimental diets,

as a substitute for CST, due to its unsaturated fatty acid nature and low energy content (7.400 kcal/kg). Moreover, due to the Ca content (analysed data, 10%) in the tallow product, limestone level was reduced in diets with the increase of dietary CST level in order to provide the same amount of Ca. All experimental diets were isocaloric and isonitrogenous *(Table 1)*. Crude protein contents in diets were determined according to AOAC [18] and metabolizable energy levels were calculated according to Carpenter and Clegg [19]. Fatty acid composition of fat sources and diets are detailed in *Table 2*. The CST product was provided by KRV Oil and Bone Inc. (Kayarlar Group, Sakarya, Turkey).

Sampling Procedures

All chicks were weighed individually and feed intake (FI) was recorded at d 21, 28, 35, and 42. Body weight gain (BWG) and feed conversion ratio (FCR) were subsequently calculated based on the performance values. At the end of the study (d 42) two birds from each replicate were selected according to average BW of each replicate. Birds were slaughtered by exsanguination and the intestinal tract was removed immediately. The tissue samples for histomorphological analysis were taken from the mid part of the duodenum, jejunum, and ileum. Afterwards, the tissue samples were flushed with saline solution to remove adherent intestinal contents and fixed in 10% neutral buffered formalin solution for 24 h [20]. Then the carcasses were manually dissected and weighed individually to calculate carcass percentage. Liver and abdominal fat were also weighed and expressed as the percentage of carcass weight. Then abdominal fat from each carcass was collected in individual sample bags and stored at -20°C

Table 1. *Composition of experimental diets*[1]

Ingredients, g/kg	Grower (11 to 21 d)				Finisher (22 to 42 d)			
	CST0	CST10	CST20	CST30	CST0	CST10	CST20	CST30
Maize	495.8	498.3	500.8	501.8	510.0	514.6	520.2	524.6
Soybean meal (CP 47%)	272.0	270.0	270.0	265.5	180.1	180.0	181.3	181.5
Soybean (full fat, CP 36%)	165.0	167.0	167.0	173.0	231.0	230.0	227.1	226.0
VAO[2]	30.0	20.0	10.0	0	45.0	30.0	15.0	0
CST[3]	0	10.0	20.0	30.0	0	15.0	30.0	45.0
Limestone	10.0	7.50	5.00	2.50	11.0	7.5	3.5	0
MCP	19.0	19.0	19.0	19.0	16.0	16.0	16.0	16.0
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-methionine (98%)	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4
L-lysine-HCl (78%)	1.2	1.2	1.2	1.2	0	0	0	0
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	1000	1000	1000	1000	1000	1000	1000	1000
Analysed composition								
Crude protein, g/kg	219.9	220.1	219.8	220.2	208.4	207.6	207.9	208.3
ME, kcal/kg[6]	3.168	3.164	3.158	3.159	3.342	3.339	3.322	3.319
Calculated composition								
ME, kcal/kg	3.44	3.146	3.149	3.156	3.305	3.307	3.310	3.313
Crude Protein, g/kg	225.7	225.9	226.1	226.2	206.4	206.3	206.3	206.3
Ether Extract, g/kg	84.5	83.8	83.1	83.4	110.1	109.4	107.9	106.6
Ash, g/kg	59.2	57.8	56.4	55.0	55.5	53.6	51.2	49.3
Lysine, g/kg	14.0	14.0	14.0	14.0	11.8	11.8	11.8	11.8
Meth.+Cyst., g/kg	8.90	8.90	8.90	8.90	82.4	82.4	82.4	82.4
Threonine, g/kg	9.10	9.10	9.10	9.10	83.1	83.1	83.1	83.1
Ca, g/kg	8.60	8.70	8.70	8.70	8.40	8.60	8.50	8.60
Total P, g/kg	8.30	8.30	8.30	8.30	7.50	7.50	7.50	7.50
Available P, g/kg	5.10	5.10	5.10	5.10	4.50	4.50	4.50	4.50

[1] As-fed basis; [2] Vegetable acid oil, derived from vegetable oil refining (estimated AME: 7400 kcal/kg); [3] Calcium Soaps of Tallow (estimated AME: 7000 kcal/kg; Ca, 100 g/kg); [4] Provided per kilogram of complete diet: vitamin A, 15.000 IU; vitamin D_3, 5.000 IU; vitamin E, 100 mg; vitamin K_3, 3 mg; thiamin, 5 mg; riboflavin, 8 mg; pyridoxine, 5 mg; pantothenic acid, 16 mg; niacin, 60 mg; folic acid, 2 mg; biotin, 200 µg; vitamin B_{12}, 20 µg; [5] Provided per kilogram of complete diet: Cu, 16 mg; I, 1.5 mg, Co, 500 µg; Se, 350 µg; Fe, 60 mg; Zn, 100 mg; Mn, 120 mg; Mo, 1 mg; [6] Carpenter and Clegg [19]

Table 2. *Fatty acid composition (%) of the experimental diets*

Item	Supplemented Fats			Grower[1] (d 11 to 21)				Finisher[2] (d 22 to 42)			
	FFSB[3]	VAO[4]	CST[5]	CST0	CST10	CST20	CST30	CST0	CST10	CST20	CST30
C12:0	ND	0.36	0.24	ND[6]	ND	ND	ND	ND	ND	ND	ND
C14:0	0.21	0.38	4.22	0.09	0.27	0.28	0.52	0.11	0.27	0.14	0.54
C16:0	11.44	9.92	33.42	11.89	13.29	14.27	16.91	11.53	12.61	14.45	16.66
C16:1	0.14	ND	3.07	ND	ND	ND	ND	ND	ND	ND	ND
C18:0	3.35	3.77	21.57	4.39	5.27	6.05	7.66	4.18	5.23	6.49	7.46
C18:1 cis-9	25.76	27.90	33.12	25.48	28.49	29.77	33.31	26.39	27.74	29.62	33.77
C18:2	51.45	55.63	3.75	53.68	49.30	45.84	38.66	53.28	50.15	45.87	38.79
C20:0	0.27	0.42	0.05	3.56	3.06	3.01	2.44	3.57	3.37	2.68	2.41
C18:3	5.89	0.10	0.31	ND	ND	ND	ND	ND	ND	ND	ND
Others	0.68	1.51	0.26	0.90	0.32	0.78	0.51	0.94	0.63	0.75	0.37
Unsaturated (U)	84.05	83.63	40.25	79.16	77.79	75.61	71.97	79.67	77.89	75.49	72.56
Saturated (S)	15.27	14.85	59.50	19.93	21.89	23.61	27.53	19.39	21.48	23.76	27.07
U:S	5.50	5.63	0.68	3.97	3.55	3.20	2.61	4.11	3.63	3.18	2.68

[1] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST; [2] Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST; [3] Full fat soybean; [4] Vegetable acid oil derived from vegetable oil refining; [5] Calcium soaps of tallow; [6] ND: Not determined

for fatty acid analysis. Left femur and tibia samples were collected and stored at -20ºC to determine bone breaking strength and bone mineral level.

Fatty Acid Analysis of Feed and Abdominal Fat

Approximately 1 g of feed sample was weighed and fat contents were extracted according to Soxhlet method [18]. Obtained fats were stored at -20ºC for fatty acid analysis. On the day of analysis, extracted fats of the feed samples were mixed with 4 mL anhydrous diethyl ether (containing 100 ppm BHT), transferred to Teflon-capped tubes, and the solvent was evaporated. Abdominal fats were thawed at 4ºC and approximately 200 mg fat sample was placed in Teflon-capped tubes. Both feed and abdominal fat samples were dissolved in 5 mL of 2N NaOH-methanol in a 60°C bath for 15 min. Subsequently, 2.175 mL of BF3-methanol (10% w/w) was added and samples were placed in a 60°C bath for 30 min. Fatty acid methyl esters were extracted with 1 mL of hexane and 2 mL of saturated sodium chloride. After centrifugation, at 4.000 rpm for 5 min, the top layer was transferred to gas chromatography vials for analysis. Supernatants were analysed using gas chromatography (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) coupled with 30 m×0.25 mm i.d. column (SP™-2330, Supelco, Bellefonte, PA) and a flame ionization detector to determine the fatty acid methyl esters of the feed and abdominal fat samples. Conditions were as follows: injector: 250°C; detector: 250°C; oven: 160°C for 1 min, increased to 240°C (4°C/min), and held for 1 min. One microliter supernatant was injected automatically with a split of 1:100. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the F.A.M.E Mix C8-C24 (Supelco 18918-1AMP) methyl ester standard. Those data were presented as relative percentage of total fatty acids.

Histomorphological Measurements

Intestinal tissue samples were processed with ethanol and xylol and supsequently embedded in paraffin. Cross sections from intestinal segments, at a thickness of 5 μm, were prepared and stained with hematoxylin and eosin in order to determine small intestinal morphometry: villus height, width and crypt depth using a microscope (Olympus BX51-DP71, Tokyo, Japan) with Cellsens programs (CS-ST-V1.8) [21,22]. Subsequently, villus surface area was calculated according to the following geometric formula; $2\pi \times$ (villus width/2) $\times$ villus height [23].

Femur and Tibia Biomechanical Properties

Left femur and tibia samples were thawed at 4ºC and cleaned of all tissue. Length and width of femur and tibia samples were measured by using digital caliper. Afterwards bone samples were stored at -20ºC for further analyses. Femurs and tibias were subjected to the three-point bending tests until failure occurred, with Instron 5944 testing frame (Instron, Norwood, MA, USA). Loading rate was 5 mm/min. Spon length was 70 mm for bones. Load was applied to the midpoint of the shaft. Load versus displacement data was collected for each sample. Stiffness values were calculated from the slope of the linear region of the load displacement curves. Ultimate load (UL) and displacement at ultimate load (DUL) were determined from the load displacement curves as well. Yield load (YL) is the load which permanent deformation of the system begins.

Displacement at yield load (DYL) is the displacement at which permanent deformation begins [24].

Tibia Ash and Mineral Concentrations

After bone breaking strength analysis, tibia samples were defatted in chloroform and methanol (2:1) for 72 h. Subsequently, tibia samples were dried for 12 h at 105ºC and ashed overnight at 600ºC to determine dry matter and ash percentage, respectively. Tibia ash samples were crushed manually, weighed (approximately 300 mg) and mixed with 8 mL HCl and 2 mL nitric acid. Afterwards, digested tibia ash samples were diluted and concentrations of calcium, phosphorus, magnesium, potassium, copper, iron, manganese, and zinc were determined by ICP-OES (Perkin Elmer Optima™ DV 2100 Model, Dual View, Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA) [25].

Statistical Analysis

Data were analysed using the ANOVA procedure of the SPSS version 14.01 (SPSS Inc., Chicago, IL, USA) [26]. Significant differences among treatment groups were tested by Tukey's multiple range tests. The effect of graded levels of dietary CST on different variables was analysed using polynomial contrasts. Statistical differences were considered significant at P<0.05.

RESULTS

The effects of graded levels of CST on BWG, FI, and FCR are shown in *Table 3*. No significant differences were observed among the treatment groups in terms of BWG, FI, and FCR between d 11-21. Similarly, BWG and FI were not affected by dietary addition of CST between d 21-42 and also during the entire experimental period. However, dietary CST supplementation at 10 g/kg during grower and 15 mg/kg during finisher period (CST10) significantly improved FCR in comparison to CST20 between d 21-42 (P=0.007) and 11-42 d (P=0.001) of the study.

The effects of graded levels of CST on carcass characteristics are shown in *Table 4*. Dietary supplementation of CST had no significant effect on carcass, liver and abdominal fat weights and also their relative percentages to BW.

The effects of graded levels of CST on the fatty acid composition of abdominal fat are shown in *Table 5*. Fatty acid composition of the abdominal fat was significantly influenced (P<0.001) by the increasing level of CST and the ratio of unsaturated to saturated fatty acids (U:S) were reduced with the increase in the level of CST (grower phase; 0, 10, 20, 30 g/kg; finisher phase; 0, 15, 30, 45 g/kg).

Morphological measurements of the duodenum, jejunum, and ileum are shown in *Table 6*. Dietary CST supplementation had no effect on duodenum histomorphological measurements. CST10 and CST20 had shallow crypt as compared to CST0 (P=0.005). Villus height to crypt depth ratio of the jejunum was found to be higher (P=0.011) in CST10 birds in comparison to those birds in CST0 and CST30 groups. Similarly, ileum villus height (P=0.014) and surface area (P=0.047) were significantly increased in birds fed diet supplemented with 10 g/kg (grower phase) and 15 g/kg (finisher phase) in comparison to those fed CST0 diet.

The effects of graded levels of CST on femur and tibia biomechanical characteristics are shown in *Table 7*. Dietary fat treatment had no effect on tibia and femur characteristics in terms of length and width. Significant linear response in UL was observed with the increasing level of CST in femur samples (P=0.020). No differences were observed in DUL, YL, and DYL parameters in both femur and tibia samples. Stiffness of femur was significantly

Table 3. *Effects of dietary fat treatments on BWG, FI and FCR of broilers*[1]

Period	Item	Dietary Treatment[2]				Statistics		Contrast	
		CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	*Q*
11 to 21 d	BWG (g)	597.4	608.4	603.8	579.2	5.38	0.242	0.219	0.104
	FI (g)	848.5	842.9	862.4	836.0	4.92	0.288	0.679	0.293
	FCR	1.422	1.386	1.430	1.445	0.01	0.262	0.236	0.237
21 to 42 d	BWG (g)	2326	2373	2268	2337	16.75	0.159	0.624	0.725
	FI (g)	3814	3844	3830	3842	19.07	0.952	0.705	0.827
	FCR	1.641^{ab}	1.619^{a}	1.689^{b}	1.644^{ab}	0.01	0.007	0.160	0.331
11 to 42 d	BW (g), on d 42	3289	3343	3237	3283	17.68	0.211	0.420	0.912
	BWG (g)	2923	2981	2871	2917	17.69	0.180	0.399	0.844
	FI (g)	4662	4686	4693	4678	20.47	0.965	0.793	0.664
	FCR	1.595^{a}	1.572^{a}	1.634^{b}	1.605^{ab}	0.01	0.001	0.038	0.732

^{a,b} Means with different superscripts in the same row are significantly different (P<0.05)
[1] Data represent mean values of 5 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST

Table 4. *Effects of dietary fat treatments on carcass, liver, and abdominal fat parameters on d 42*[1]

Item	Dietary Treatment[2]				Statistics		Contrast	
	CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	*Q*
BW, g, slaughtered	3227	3211	3212	3177	12.03	0.512	0.170	0.698
Carcass, g	2355	2356	2345	2341	8.81	0.912	0.514	0.864
Carcass, %	72.97	73.39	73.01	73.69	0.19	0.502	0.299	0.742
Liver, g	61.27	61.24	61.32	60.53	0.45	0.924	0.616	0.689
Liver, %	1.90	1.91	1.91	1.91	0.15	0.995	0.844	0.853
Abdominal Fat, g	37.51	38.72	40.37	40.15	1.22	0.837	0.400	0.778
Abdominal Fat, %	1.16	1.21	1.26	1.26	0.04	0.782	0.324	0.814

[1] Data represent mean values of 10 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST

Table 5. *Effects of dietary fat treatments on fatty acid composition of abdominal fat on d 42*[1]

Item	Dietary Treatment[2]				Statistics		Contrast	
	CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	*Q*
C14:0	0.45^{c}	0.54^{b}	0.83^{a}	0.91^{a}	0.03	<0.001	<0.001	0.651
C16:0	15.69^{b}	16.87^{b}	19.81^{a}	20.11^{a}	0.34	<0.001	<0.001	0.211
C16:1	2.12^{b}	2.69^{b}	3.67^{a}	3.89^{a}	0.14	<0.001	<0.001	0.320
C18:0	4.91^{c}	6.13^{b}	6.82^{a}	7.10^{a}	0.16	<0.001	<0.001	0.009
C18:1 cis-9	30.30^{d}	32.21^{c}	36.54^{b}	38.30^{a}	0.55	<0.001	<0.001	0.852
C18:2	43.65^{a}	38.51^{b}	30.09^{c}	27.15^{d}	1.10	<0.001	<0.001	0.109
C20:0	2.60^{a}	2.62^{a}	2.09^{b}	2.37^{ab}	0.05	<0.001	<0.001	0.095
Others	0.28	0.44	0.15	0.18	0.03			
Unsaturated (U)	76.10^{a}	73.54^{b}	70.32^{c}	69.38^{c}	0.47	<0.001	<0.001	0.040
Saturated (S)	23.65^{c}	26.16^{b}	29.55^{a}	30.48^{a}	0.49	<0.001	<0.001	0.056
U:S	3.23^{a}	2.82^{b}	2.39^{c}	2.28^{c}	0.07	<0.001	<0.001	0.009

[a-d] Means with different superscripts in the same row are significantly different (P<0.05); [1] Data represent mean values of 10 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST

higher (P=0.003) in CST30 group in comparison to CST0 and CST10. Dietary inclusion of CST (20 g/kg grower and 30 g/kg finisher phase) resulted with an increase (P=0.020) of stiffness in tibia samples when compared with birds fed a diet containing 0 g/kg CST (CST0).

The effects of graded levels of CST on tibia ash percentage and mineral concentrations are shown in *Table 8*. Birds fed CST10 diet significantly lower (P=0.011) tibia ash percentage in comparison to those fed with CST0. In addition, tibia Ca (P=0.009) and P (P=0.009; linear, P=0.030) concentrations of CST10 and CST30 groups were lower than those in the CST0 group. With the increase in dietary CST, tibia Fe and Mn concentration exhibited a linear response (P<0.001). On the contrary, tibia Mg, K, Cu, and Zn levels were not influenced by the dietary fat treatment.

DISCUSSION

Improvements in genetic capacity have led to the ability for modern broilers to gain more weight by consuming less feed. This outcome resulted in precise ration formulations to meet their energy and nutrient requirements. As an essential ingredient, both animal and vegetable based fats are used in poultry diets for their high energy content, and also for their favourable effect on feed texture, nutrient digestibility, and fatty acid metabolism. However, these ingredients are more expensive than cereals or other major ingredients that are used in diets and ever-increasing prices have led the broiler producer to seek alternative cheap fat sources.

In the present study, dietary supplementation of calcium soaps of tallow had no significant effect on BWG and FI of the birds during the entire experimental period. On the contrary, overall FCR was found to be better in birds fed diets supplemented with CST0 and CST10 diet in comparison to birds fed CST20 diet, but similar to those fed with CST30. However, no differences were observed between CST0 and CST30 groups in terms of growth performance during

Table 6. *Effects of dietary fat treatments on histomorphological parameters of the duodenum, jejunum, and ileum on d 42*[1]

Intestine	Item	Dietary Treatment[2]				Statistics		Contrast	
		CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	*Q*
Doudenum	Villus height (µm)	1565	1509	1529	1551	18.80	0.753	0.912	0.324
	Crypt depth (µm)	134.8	120.7	121.2	121.5	2.37	0.096	0.059	0.122
	VH:CD ratio[3]	11.69	12.64	12.86	12.90	0.27	0.365	0.122	0.404
	Villus width (µm)	155.6	149.9	156.3	141.5	2.25	0.068	0.067	0.291
	Villus S.A.[4] (mm^2)	0.767	0.710	0.751	0.689	0.01	0.237	0.153	0.947
Jejunum	Villus height (µm)	923.6	909.0	885.5	879.7	12.72	0.600	0.187	0.865
	Crypt depth (µm)	113.8^{a}	93.4^{b}	98.7^{b}	105.5^{ab}	2.24	0.005	0.272	0.001
	VH:CD ratio	8.18^{b}	9.82^{a}	9.04^{ab}	8.44^{b}	0.20	0.011	0.999	0.003
	Villus width (µm)	144.9	153.1	150.3	144.6	2.20	0.458	0.845	0.126
	Villus S.A. (mm^2)	0.421	0.436	0.418	0.399	0.01	0.447	0.248	0.294
Ileum	Villus height (µm)	588.6^{b}	716.3^{a}	642.4^{ab}	653.6^{ab}	14.42	0.014	0.304	0.031
	Crypt depth (µm)	99.3	108.9	104.6	112.3	2.16	0.170	0.074	0.819
	VH:CD ratio[4]	5.96	6.66	6.19	5.88	0.15	0.241	0.596	0.090
	Villus width (µm)	157.7	155.0	157.3	154.6	2.24	0.951	0.739	0.995
	Villus S.A. (mm^2)	0.289^{b}	0.348^{a}	0.318^{ab}	0.316^{ab}	0.01	0.047	0.418	0.038

[a,b] Means with different superscripts in the same row are significantly different (P<0.05)
[1] Data represent mean values of 10 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST; [3] Villus height to crypt depth ratio; [4] Villus Surface Area; 2π × (villus width/2) × villus height

Table 7. *Effects of dietary fat treatments on femur and tibia parameters on d 42*[1]

Bone	Item	Dietary Treatment[2]				Statistics		Contrast	
		CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	*Q*
Femur	Length, mm	73.22	73.78	73.59	72.89	0.39	0.863	0.743	0.434
	Width, mm	10.81	10.62	10.58	10.78	0.08	0.708	0.857	0.253
	UL, N	280.9	289.0	315.4	307.6	5.13	0.056	0.018	0.415
	DUL, mm	3.90	4.07	3.82	3.61	0.09	0.374	0.189	0.307
	YL, N	217.1	199.5	235.1	232.4	5.55	0.083	0.093	0.485
	DYL, mm	2.41	2.06	2.17	2.09	0.06	0.181	0.125	0.257
	Stiffness, N/mm	90.8^{c}	91.5^{bc}	105.5^{ab}	107.2^{a}	2.16	0.003	0.001	0.898
Tibia	Length, mm	101.8	101.9	102.0	102.0	0.44	0.997	0.850	0.925
	Width, mm	10.33	10.50	10.44	10.15	0.11	0.715	0.549	0.323
	UL, N	326.7	353.8	365.2	377.3	9.21	0.252	0.051	0.684
	DUL, mm	3.02	3.09	3.02	3.09	0.05	0.912	0.746	0.959
	YL, N	189.8	180.1	191.6	194.5	4.16	0.654	0.502	0.464
	DYL, mm	1.35	1.14	1.20	1.32	0.04	0.154	0.900	0.030
	Stiffness, N/mm	146.2^{b}	164.3^{ab}	175.7^{a}	163.5^{ab}	3.47	0.020	0.031	0.021

[a-c] Means with different superscripts in the same row are significantly different (P<0.05)
[1] Data represent mean values of 10 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST; [3] UL: Ultimate Load, DUL: Displacement at Ultimate Load, YL: Yield Load, DYL: Displacement at Yield Load

the entire study. Our results are in agreement with the previous study results of Sanz et al.[13] who reported that dietary fat source (sunflower, tallow or lard) had no effect on feed intake, body weight gain, and final body weight. Similarly, Preston et al.[27] found no differences between experimental groups fed tallow or soy oil supplemented diets. It is generally known that saturated fatty acid-rich fats such as tallow, have lower AME value than vegetable oils which are rich in unsaturated fatty acids [12]. However, previous study revealed that dietary fat source had no

Table 8. *Effects of dietary fat treatments on tibia ash percentage and mineral concentration on d 42*[1]

Bone	Item	Dietary Treatment[2]				Statistics		Contrast	
		CST0	CST10	CST20	CST30	SEM	*P*-value	*L*	Q
Tibia	Ash, %	44.31[a]	41.11[b]	43.32[ab]	41.97[ab]	0.38	0.011	0.126	0.189
	Ca, g/kg	252.8[a]	234.7[b]	250.5[ab]	234.8[b]	2.60	0.009	0.073	0.795
	P, g/kg	126.1[a]	116.8[b]	123.6[ab]	115.7[b]	1.36	0.009	0.030	0.788
	Mg, g/kg	5.56	5.09	5.44	5.03	0.09	0.072	0.096	0.881
	K, g/kg	2.05	2.13	1.99	1.98	0.06	0.847	0.550	0.720
	Cu, mg/kg	0.60	0.60	0.60	0.50	0.02	0.127	0.058	0.171
	Fe, mg/kg	204.3[ab]	224.9[a]	159.5[bc]	139.9[c]	8.03	<0.001	<0.001	0.111
	Mn, mg/kg	4.86[a]	5.44[a]	3.47[b]	3.27[b]	0.19	<0.001	<0.001	0.152
	Zn, mg/kg	136.8	147.5	134.4	133.0	3.25	0.390	0.400	0.359

[a-c] Means with different superscripts in the same row are significantly different (P<0.05)
[1] Data represent mean values of 10 replicates per treatment; [2] Grower Phase (11 to 21 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 10 g/kg CST; CST20 = basal diet containing 20 g/kg CST; CST30 = basal diet containing 30 g/kg CST. Finisher Phase (22 to 42 d): CST0 = corn-soybean meal basal diet containing 0 g/kg CST; CST10 = basal diet containing 15 g/kg CST; CST20 = basal diet containing 30 g/kg CST; CST30 = basal diet containing 45 g/kg CST

effect on performance since the diets are balanced in terms of energy and protein [13]. On the contrary, it is generally known that fat utilization is affected by level of the included fat, basal diet composition, degree of saturation of the total dietary lipid fractions, and animal age [7,12]. Studies conducted with different dietary fats have suggested that the increasing level of Ca in diets tends to decrease AME value of supplemented fat by forming insoluble soap in the gut lumen [10,28,29] and depress broiler growth rate regardless of tallow supplementation level [10]. Tallow product used in this study was in the form of calcium soaps of fatty acids. However, dietary calcium concentration of the experimental diets was balanced by reducing the limestone level with the increasing level of calcium soaps of tallow in diets. Rising et al.[16] suggests that, rather than the added calcium, preformed calcium soaps of animal fats were utilized well by laying hens. However, as far as we know, no available data exists for the use of calcium soaps of tallow in broiler diets. According to our results, dietary supplementation of calcium soap of tallow up to 30 g/kg in grower and 45 g/kg in finisher diets of broiler chicken was well tolerated and had no negative effects on broiler growth performance in terms of BWG. Moreover, due to aforementioned synergistic effects of blending saturated and unsaturated fatty acids on broiler performance [3,12], combined use of CST with vegetable oils might have positive effects under commercial conditions. However, more research needs to be conducted to determine AME value of this product for precise diet formulation.

In modern broilers, the most significant amount of fat is deposited as abdominal fat. However, this discrete fat depot has no contribution to carcass quality and is considered a waste product for producers and consumers [30-32]. According to our results, carcass yield, liver weight, and abdominal fat weight were not influenced by dietary fat treatments. The results obtained in the present study are consistent with those of earlier studies which found no differences in abdominal fat deposition in broilers fed animal or vegetable fat supplemented diets [33-35]. However, several studies reported that broilers receiving saturated fat in their diets had higher abdominal fat weight than those fed diets containing unsaturated fat [3,13]. The lack of consistency between the studies might be related to the dietary fat level or fat type. As an important finding of the present study, dietary supplementation of CST significantly influenced the fatty acid composition of the abdominal fat. It is known that tissue fatty acid composition is typically modified by dietary fat source in pigs and poultry due to the direct absorption and deposition of fatty acids [35,36]. Crespo and Esteve-Garcia [37] reported more saturated fatty acids in the abdominal fat pad, thigh muscle, and breast muscle of birds fed a diet containing tallow in comparison to those fed with vegetable oil. From this point of view, increasing the saturated fatty acid content of the tissue might have an important impact on carcass quality by producing more stable fat against oxidation. Moreover, due to the direct impact of dietary fat treatments on abdominal fatty acid composition, it can be assumed that dietary addition of CST is efficiently utilized by broilers and deposited in the fat tissue.

Morphological changes in the small intestine, such as increased villus height, villus width, and villus height to crypt depth ratio (VH:CD), are important parameters that affect broiler performance by improving nutrient digestion and absorption [38]. Earlier studies with rats suggested that source and concentration of supplemented dietary fat influenced intestinal morphology [39]. In poultry, fat digestion and absorption mainly occurs in small intestines, especially in the duodenum and jejunum. According to our results, dietary supplementation of increasing level of CST had no detrimental effect on duodenum and jejunum villus height. Significant quadratic response in jejunum crypt depth and VH:CD ratio were observed with the increasing level of CST on d 42. Moreover, ileum villus height and

villus surface area increased in birds fed CST10 diet in comparison to those fed CST0 diet. As an important finding of the present study, feeding the fat blend that contained a low level of CST improved intestinal microarchitecture, which may be accompanied by a better FCR of these birds. These results corroborate with the results from Li et al.[40] who suggested that, supplementation of pig diets with soybean oil and coconut oil (1:1) increased villus height in comparison to soybean oil or coconut oil alone. Khatun et al.[41] showed that birds fed a diet containing 2% palm oil and 4% soybean oil diet had significantly higher villi in all small intestinal segments than those birds fed 6% palm oil alone. It can be assumed that the improvements in the small intestine morphology might be related to the synergistic effects of saturated and unsaturated fatty acids.

Leg abnormalities are highly important problems in fast-growing broilers, causing economic losses and reduced welfare [42]. A growing body of evidence indicates that dietary supplementation of saturated fats has an adverse effect on bone mineralization [43]. Our results showed that tibia ash content was reduced in birds fed CST10 diet in comparison to birds fed CST0. Moreover, increasing level of dietary CST had a negative effect on tibia Ca, P, Fe, and Mn concentrations. Results of the present study are consistent with Atteh and Leeson [29], who reported that dietary supplementation of palmitic acid significantly reduced bone ash and bone calcium content in broilers compared to oleic acid due to the increased excreted soap formation. More recently, Zhong et al.[44] suggested that supplementation of broiler diets with lard or palm oil reduced tibia calcium concentration in comparison to linseed oil. Contrary to our results, they reported that the combined use of palm oil and linseed oil, at the ratio of 60:40 or 40:60 (w:w), alleviated the adverse effect of saturated fatty acids on tibia calcium level [44]. As suggested by previous studies, structural stiffness of the bone improved with the increasing level of bone mineral content. However, at the same time, this increase negatively affects bone by making it more brittle [45,46]. Even though poor mineralization results in tibia samples were obtained in this study, it is not enough to evaluate bone strength, because bone mineral concentration needs to be evaluated along with the intrinsic and extrinsic biomechanical properties to determine bone strength and health [47]. Our results showed that femur and tibia stiffness improved with the increasing level of dietary CST concentration and it can be assumed that increased bone stiffness might help to protect against fractures [48] and improve the efficiency of leg movements in older chickens [49]. The reason for the improvement in stiffness is unclear, but it could be related to the collagen crosslinks, which are the major organic constituent of bone [50]. Rath et al.[51] revealed that crosslink content had a stronger correlation with bone strength than bone ash and density. As a fat source, the effect of dietary CST on broiler bone strength and mineral concentration has not previously been reported. Therefore, future studies should be performed to investigate the influence of saturated fatty acids on bone strength, taking into account the contribution of other determinants affecting bone strength and excreted soap formation and mineral retentions, especially Ca, P, Mg, and Zn, to determine bone quality and health.

Our results showed that dietary supplementation of CST had no detrimental effect on broiler performance parameters and might be used in broiler diets as an alternative fat source, like vegetable acid oil. However, more research needs to be conducted to determine apparent metabolisable energy value of calcium soap of tallow and to find out how this product affects fat metabolism, meat quality, and mineral retention.

Acknowledgements

The authors thank KRV Oil and Bone Inc. and Beypiliç Inc., for providing materials.

REFERENCES

1. NRC: Nutrient Requirements of Poultry. Washington, DC: National Academies Press. 1994.

2. Ravindran V, Tancharoenrat P, Zaefarian F, Ravindran G: Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. *Anim Feed Sci Technol*, 213, 1-21, 2016. DOI: 10.1016/j.anifeedsci.2016.01.012

3. Poorghasemi M, Seidavi A, Qotbi AAA, Laudadio V, Tufarelli V: Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. *Asian-Australas J Anim Sci*, 26 (5): 705-710, 2013. DOI: 10.5713/ajas.2012.12633

4. Mateos GG, Sell JL: Nature of the extrametabolic effect of supplemental fat used in semipurified diets for laying hens. *Poult Sci*, 60 (8): 1925-1930, 1981. DOI: 10.3382/ps.0601925

5. Mateos GG, Sell JL, Eastwood JA: Rate of food passage (transit time) as influenced by level of supplemental fat. *Poult Sci*, 61 (1): 94-100, 1982. DOI: 10.3382/ps.0610094

6. Kim JH, Seo S, Kim CH, Kim JW, Lee BB, Lee GI, Shin HS, Kim MC, Kil DY: Effect of dietary supplementation of crude glycerol or tallow on intestinal transit time and utilization of energy and nutrients in diets fed to broiler chickens. *Livest Sci*, 154 (1-3): 165-168, 2013. DOI: 10.1016/j.livsci.2013.03.005

7. Ketels E, De Groote G: Effect of ratio of unsaturated to saturated fatty acids of the dietary lipid fraction on utilization and metabolizable energy of added fats in young chicks. *Poult Sci*, 68 (11): 1506-1512, 1989. DOI: 10.3382/ps.0681506

8. Sibbald IR: The true metabolizable energy values of mixtures of tallow with either soybean oil or lard. *Poult Sci*, 57 (2): 473-477, 1978. DOI: 10.3382/ps.0570473

9. Sibbald IR, Kramer JKG: The effect of the basal diet on the true metabolizable energy value of fat. *Poult Sci*, 57 (3): 685-691, 1978. DOI: 10.3382/ps.0570685

10. Tancharoenrat P, Ravindran V: Influence of tallow and calcium concentrations on the performance and energy and nutrient utilization in broiler starters. *Poult Sci*, 93 (6): 1453-1462, 2014. DOI: 10.3382/ps.2013-03817

11. Blanch A, Barroeta AC, Baucells MD, Puchal F: The nutritive value of dietary fats in relation to their chemical composition. Apparent fat availability and metabolizable energy in two-week-old chicks. *Poult Sci*, 74 (8): 1335-1340, 1995. DOI: 10.3382/ps.0741335

12. Tancharoenrat P, Ravindran V, Zaefarian F, Ravindran G: Influence of age on the apparent metabolisable energy and total tract apparent fat digestibility of different fat sources for broiler chickens. *Anim Feed Sci*

Technol, 186 (3-4): 186-192, 2013. DOI: 10.1016/j.anifeedsci.2013.10.013

13. Sanz M, Flores A, Lopez-Bote CJ: The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *Br Poult Sci*, 41 (1): 61-68, 2000. DOI: 10.1080/00071660086411

14. Newman RE, Bryden WL, Fleck E, Ashes JR, Storlien LH, Downing JA: Dietary n-3 and n-6 fatty acids alter avian metabolism: molecular-species composition of breast-muscle phospholipids. *Br J Nutr*, 88 (1): 19-28, 2002. DOI: 10.1079/BJN2002581

15. Salinas J, Ramírez RG, Domínguez MM, Reyes-Bernal N, Trinidad-Lárraga N, Montaño MF: Effect of calcium soaps of tallow on growth performance and carcass characteristics of Pelibuey lambs. *Small Rumin Res*, 66 (1-3): 135-139, 2006. DOI: 10.1016/j.smallrumres.2005.07.058

16. Rising R, Maiorino PM, Mitchell R, Reid BL: The utilization of calcium soaps from animal fat by laying hens. *Poult Sci*, 69 (5): 768-773, 1990. DOI: 10.3382/ps.0690768

17. Aviagen: Ross Broiler Management Handbook. Huntsville, AL, Aviagen Group, 2014.

18. AOAC: Official Methods of Analysis of AOAC International. Gaithersburg, MD. 2000.

19. Carpenter KJ, Clegg KM: The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *J Sci Food Agr*, 7 (1): 45-51, 1956. DOI: 10.1002/jsfa.2740070109

20. Calik A, Ekim B, Bayraktaroğlu AG, Ergün A, Saçaklı P: Effects of dietary probiotic and synbiotic supplementation on broiler growth performance and intestinal histomorphology. *Ankara Univ Vet Fak Derg*, 64, 183-189, 2017. DOI: 10.1501/Vetfak_0000002797

21. Luna LG: Manual of histologic staining methods of the Armed Forces Institute of Pathology. Newyork: McGraw-Hill Book Co., 1968.

22. Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ: Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult Sci*, 82 (6): 1030-1036, 2003. DOI: 10.1093/ps/82.6.1030

23. Sakamoto K, Hirose H, Onizuka A, Hayashi M, Futamura N, Kawamura Y, Ezaki T: Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. *J Surg Res*, 94 (2): 99-106, 2000. DOI: 10.1006/jsre.2000.5937

24. Gürgül S, Erdal N, Yılmaz ŞN, Yıldız A, Ankaralı H: Deterioration of bone quality by long-term magnetic field with extremely low frequency in rats. *Bone*, 42 (1): 74-80, 2008. DOI: 10.1016/j.bone.2007.08.040

25. Boss CB, Fredeen KJ: Concepts, instrumentation and techniques in inductively coupled plasma optical emission spectrometry. 710 Bridgep Ort. Avenue Shelton, CT 06484–04794 USA: PerkinElmer Life and Analytical Sciences. 2004.

26. SPSSInc: SPSS 14.0.1 for Windows, User's Guide: SPSS Inc. Chicago, IL. 2004.

27. Preston CM, McCracken KJ, Bedford MR: Effect of wheat content, fat source and enzyme supplementation on diet metabolisability and broiler performance. *Br Poult Sci*, 42 (5): 625-632, 2001. DOI: 10.1080/00071660120088443

28. Atteh JO, Leeson S: Effects of dietary fatty acids and calcium levels on performance and mineral metabolism of broiler chickens. *Poult Sci*, 62 (12): 2412-2419, 1983. DOI: 10.3382/ps.0622412

29. Atteh JO, Leeson S: Effects of dietary saturated or unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. *Poult Sci*, 63 (11): 2252-2260, 1984. DOI: 10.3382/ps.0632252

30. Griffiths L, Leeson S, Summers JD: Fat deposition in broilers: Effect of dietary energy to protein balance, and early life caloric restriction on productive performance and abdominal fat pad size. *Poult Sci*, 56 (2): 638-646, 1977. DOI: 10.3382/ps.0560638

31. Sanz M, Flores A, Perez De Ayala P, Lopez-Bote CJ: Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *Br Poult Sci*, 40 (1): 95-101, 1999. DOI: 10.1080/00071669987908

32. Leeson S, Summers JD: Production and carcass characteristics of the broiler chicken. *Poult Sci*, 59 (4): 786-798, 1980. DOI: 10.3382/ps.0590786

33. Wongsuthavas S, Yuangklang C, Vasupen K, Mitchaothai J, Srenanual P, Wittayakun S, Beynen AC: Assessment of de-novo fatty acid synthesis in broiler chickens fed diets containing different mixtures of beef tallow and soybean oil. *Int J Poult Sci*, 6 (11): 800-806, 2007. DOI: 10.3923/ijps.2007.800.806

34. Potença A, Murakami AE, Fernandes JIM, Matsushita M, Nakagawa EL: Performance, abdominal fat deposition and bone characteristics of broilers fed diets containing different lipid sources. *Rev Bras Cienc Avic*, 10 (4): 239-244, 2008. DOI: 10.1590/S1516-635X2008000400008

35. Scaife JR, Moyo J, Galbraith H, Michie W, Campbell V: Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. *Br Poult Sci*, 35 (1): 107-118, 1994. DOI: 10.1080/00071669408417675

36. Zollitsch W, Knaus W, Aichinger F, Lettner F: Effects of different dietary fat sources on performance and carcass characteristics of broilers. *Anim Feed Sci Technol*, 66 (1-4): 63-73, 1997. DOI: 10.1016/S0377-8401(96)01126-1

37. Crespo N, Esteve-Garcia E: Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult Sci*, 80 (1): 71-78, 2001. DOI: 10.1093/ps/80.1.71

38. Calik A, Ergun A: Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. *Poult Sci*, 94 (9): 2173-2182, 2015. DOI: 10.3382/ps/pev182

39. Sagher FA, Dodge JA, Johnston CF, Shaw C, Buchanan KD, Carr KE: Rat small intestinal morphology and tissue regulatory peptides: Effects of high dietary fat. *Br J Nutr*, 65 (1): 21-28, 1991. DOI: 10.1079/BJN19910062

40. Li DF, Thaler RC, Nelssen JL, Harmon DL, Allee GL, Weeden TL: Effect of fat sources and combinations on starter pig performance, nutrient digestibility and intestinal morphology. *J Anim Sci*, 68 (11): 3694-3704, 1990. DOI: 10.2527/1990.68113694x

41. Khatun J, Loh TC, Akit H, Foo HL, Mohamad R: Influence of different sources of oil on performance, meat quality, gut morphology, ileal digestibility and serum lipid profile in broilers. *J Appl Anim Res*, 46 (1): 479-485, 2018. DOI: 10.1080/09712119.2017.1337580

42. Shim MY, Karnuah AB, Mitchell AD, Anthony NB, Pesti GM, Aggrey SE: The effects of growth rate on leg morphology and tibia breaking strength, mineral density, mineral content, and bone ash in broilers. *Poult Sci*, 91 (8): 1790-1795, 2012. DOI: 10.3382/ps.2011-01968

43. Wohl GR, Loehrke L, Watkins BA, Zernicke RF: Effects of high-fat diet on mature bone mineral content, structure, and mechanical properties. *Calcif Tissue Int*, 63 (1): 74-79, 1998. DOI: 10.1007/s002239900492

44. Zhong X, Gao S, Wang JJ, Dong L, Huang J, Zhang LL, Wang T: Effects of linseed oil and palm oil on growth performance, tibia fatty acid and biomarkers of bone metabolism in broilers. *Br Poult Sci*, 55 (3): 335-342, 2014. DOI: 10.1080/00071668.2014.891097

45. Currey JD: Physical characteristics affecting the tensile failure properties of compact bone. *J Biomech*, 23 (8): 837-844, 1990. DOI: 10.1016/0021-9290(90)90030-7

46. Turner CH: Biomechanics of bone: Determinants of skeletal fragility and bone quality. *Osteoporos Int*, 13 (2): 97-104, 2002. DOI: 10.1007/s001980200000

47. Forestier-Zhang L, Bishop N: Bone strength in children: Understanding basic bone biomechanics. *Arch Dis Child Educ Pract Ed*, 101 (1): 2-7, 2016. DOI: 10.1136/archdischild-2015-308597

48. Lories RJU, Peeters J, Bakker A, Tylzanowski P, Derese I, Schrooten J, Thomas JT, Luyten FP: Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. *Arthritis Rheum*, 56 (12): 4095-4103, 2007. DOI: 10.1002/art.23137

49. Kim WK, Bloomfield SA, Ricke SC: Effects of age, vitamin D3, and fructooligosaccharides on bone growth and skeletal integrity of broiler chicks. *Poult Sci*, 90 (11): 2425-2432, 2011. DOI: 10.3382/ps.2011-01475

50. Rath NC, Huff GR, Huff WE, Balog JM: Factors regulating bone maturity and strength in poultry. *Poult Sci*, 79 (7): 1024-1032, 2000. DOI: 10.1093/ps/79.7.1024

51. Rath NC, Balog JM, Huff WE, Huff GR, Kulkarni GB, Tierce JF: Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. *Poult Sci*, 78 (8): 1232-1239, 1999. DOI: 10.1093/ps/78.8.1232