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Effect of the supplementation of olive leaves and olive cake on growth performance and bone mineralisation of broiler chickens

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The by-products of olive oil production are not traditional feed ingredients in poultry nutrition. Despite their low nutritional value, they can be used not only as a source of energy and nutrients, but also as bioactive substances in nutrition of broiler chickens. The aim of the study was to determine whether the use of olive leaves and cake in feed mixtures for broilers affects their growth performance and bone mineralisation. In the feeding experiment 120 male Ross 308 broilers were used. At the age of 21 days the chickens were randomly assigned to 5 experimental groups each with two pens (replicates) of 12 animals and fed with 5 different feed mixtures without (Control) or supplemented with 5% or 10% olive leaves (OLeave5, OLeave10) or cake (OCake5, OCake10). Live weight and feed intake per pen were recorded weekly. At the end of the feeding experiment (42 days), 12 chickens per group were randomly selected and slaughtered. At the slaughter line, bone samples (femur, tibia and humerus) were taken from each chicken. The supplementation of olive leaves and cake had no significant effect on the growth performance and mineral content in femur, tibia and humerus of the broilers. The addition of 5 and 10% olive leaves to the feed resulted in higher Cu content in the humerus, but bone mineralisation did not change. In conclusion olive leaves and cake did not modify bone mineralisation but could be supplemented in the feed mixtures for broilers without negative effects on growth performance.

Keywords: broiler, olive by-product, live weight, bone mineralisation

1 Introduction

Olive oil production generates many by-products and wastes, which are usually released into the environment in large quantities and represent a major environmental problem due to their phytotoxicity and high organic content (Nunes et al., 2016). On the other hand, olive by-products, such as olive leaves and cake, could be used as (alternative) supplements in poultry diets (Salobir et al., 2013). The main problem with the use of olive by-products in animal diets is the variability of the chemical composition, which depends on the origin, age and variety of the olive tree, soil composition and climatic conditions (Molina-Alcaide and Yanez-Ruiz, 2008), as well as on the factors of olive oil production and processing. It is known that both olive leaves and olive cake have a poor nutritional value, mainly due to their high fibre content, unpleasant taste, low energy and digestible protein and a certain mineral content, but can be additional sources of Mn, Zn and Ca in animal feed (Sayehban et al., 2016). They can be added to feed mixtures in limited quantities, as animals can consume large amounts of antinutritive substances, namely lignin, tannins, Cu and polyphenols. In addition, olive leaves and cake contain high levels of structural carbohydrates that are difficult to digest, especially for non-ruminants (Al-Harthi, 2017). On the other hand, the beneficial effects of olives on health, welfare and performance are mainly attributed to polyphenols such as oleuropein, hydroxytyrosol and tyrosol, which are known to exert a variety of biological activities in animals, including antimicrobial, antiatherogenic, antioxidant, anti-inflammatory, anticoagulant, antihypertensive, hypolipidemic and anticancer activities (Ghanbari et al., 2012; Leskovec et al., 2018; Şahin and Bilgin, 2018; Salobir et al., 2013).

With the genetic selection of broilers for rapid growth, bone problems such as tibiadyshondroplasia, rickets and skeletal deformities caused by limping have increased. The main reasons for these problems are insufficient adaptation of the skeletal system to weight gain, insufficient absorption of Ca

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and P and genetic factors (Suchý et al., 2009). Skeletal deformations have a negative effect on animal welfare (Fleming, 2008) and their performance due to pain and lameness (Talaty et al., 2009). Bone growth, mineralisation and the formation of the bone collagen network are influenced by various factors such as gender, nutrition, genetics, disease, environment, rapid growth and ageing (Rath et al., 2000). Some *in vitro* and *in vivo* studies have reported positive effects of olives and their by-products on bone health by stimulating bone formation and inhibiting bone resorption, improving osteoblastogenesis and inhibiting osteoclastogenesis due to their anti-inflammatory and osteoimmunological effects (Đudarić et al., 2015; Idrus and Saim, 2019). The aim of the present study was to determine whether the dietary supplementation with olive leaves (*Olea europaea* L.) and olive cake at different levels affects growth performance and bone mineralisation of three bones (femur, tibia and humerus) in broiler chickens.

2 Material and methods

2.1 Broilers and dietary treatments

One hundred and twenty male Ross 308 broilers were housed in 10 pens, each measuring 100 cm width x 130 cm length and equipped with nipple drinkers, with 12 chickens per pen reared on deep litter with sawdust. From the day of hatching until the age of 21 days, the animals were fed a commercial starter feed mixture (Table 1), which contained neither olive leaves nor cake. On day 21, chickens were individually labelled and randomly divided into 5 experimental groups corresponding to 5 dietary treatments (24 chickens per treatment) and further fed with the commercial finisher feed mixture. All feeds (starter and finisher feeds) were formulated to meet the recommendations for Ross 308 broilers (Aviagen, 2014). The experimental finisher diets were supplemented with 0% olive leaves and cake (Control), 5% olive leaves (OLeave5), 10% olive leaves (OLeave10), 5% olive cake (OCake5) and 10% olive cake (OCake10). In the Control feed mixture, dehydrated alfalfa and wheat bran were used instead of olive leaves and olive cake. The composition of the feed mixtures, the energy value and the chemical composition of the starter and finisher feed mixtures are presented in Table 1. The broilers were fed *ad libitum* during the experiment and their growth performance was measured weekly. Throughout the experiment, the lighting program, temperature and relative humidity were regulated in accordance with the recommendations for Ross 308 broilers (Aviagen, 2018). At the age of 42 days, 12 chickens per group were randomly selected and sacrificed by cervical dislocation. Whole bone samples (femur, tibia and humerus) were isolated, cleaned of muscle tissue, packed in plastic bags to prevent dehydration and stored at −20°C for further analysis.

Table 1 Formulation of feed mixtures, estimated metabolizable energy and chemical composition

Item	Starter	Control	OLeave5	OLeave10	OCake5	OCake10
Ingredient (%)						
Maize	48.32	56.71	54.80	52.90	56.30	55.87
Wheat bran	1.50	5.00	2.50	-	2.50	-
Dehydrated alfalfa	1.50	5.00	2.50	-	2.50	-
Soybean meal	40.00	26.00	27.00	28.00	26.60	27.20
Vegetable oil	4.00	3.40	4.20	5.00	3.00	2.60
Sodium chloride	0.44	0.44	0.44	0.44	0.44	0.44
Limestone	1.27	0.99	0.95	0.90	1.04	1.10
Monocalcium phosphate	1.87	1.45	1.55	1.65	1.55	1.65
l-lysine	0.15	0.17	0.19	0.20	0.20	0.22
dl-methionine	0.35	0.28	0.29	0.31	0.29	0.31
l-threonine	0.10	0.06	0.08	0.10	0.08	0.10
tryptophan	-	-	-	-	-	0.01
Olive leaves	-	-	5.00	10.00	-	-
Olive cake	-	-	-	-	5.00	10.00
Mineral-vitamin supplement*	0.50	0.50	0.50	0.50	0.50	0.50
Metabolizable energy** (MJ/kg)	12.21	12.18	12.18	12.18	12.18	12.18

Note: Table 1 continues on the next page

Proximate chemical composition (g/kg)						
Dry matter	954	896	897	901	898	899
Crude protein	229	173	176	178	176	174
Crude fat	61.1	60.3	70.2	77.0	65.9	74.3
Crude fiber	47.0	45.1	49.7	51.4	55.3	53.1
Crude ash	56.5	62.1	52.5	51.5	52.3	52.2
Nitrogen free extract	560	555	548	543	549	545
K	12.4	11.5	11.1	11.1	11.9	11.8
P	8.03	7.04	6.96	6.91	7.04	6.79
Ca	11.0	10.5	10.4	10.7	10.2	10.3
Na	2.75	4.98	2.12	2.41	2.24	2.18
Mg	1.81	2.05	1.91	1.83	1.89	1.68

*Calculated to meet the vitamin and mineral requirements for Ross 308 finisher diets (per kg of diet): Cu - 15 mg, I - 1 mg, Fe - 43 mg, Mn - 101 mg, Se - 0.3 mg, Zn - 74 mg, vitamin A - 10,000 IU, vitamin D3 - 5,000 IU, vitamin E - 50 IU, vitamin K - 3 mg, thiamine (B1) - 3 mg, riboflavin (B2) - 6 mg, niacin (B3) - 60 mg, pantothenic acid - 15 mg, pyridoxine (B6) - 4 mg, biotin - 0.15 mg, folic acid - 2 mg, vitamin B12 - 0.15 mg.

**The apparent metabolizable energy values in feed was calculated considering correction for nitrogen retention, using the value of 32.42 kJ/g retained nitrogen (Hill and Anderson, 1958).

Control - control diet; OLeave5 and OLeave10 - 5% and 10% olive leaves per kg of feed mixture, respectively; OCake5 and OCake10 - 5% and 10% olive cake per kg of feed mixture, respectively.

The olive leaves (*Olea europaea* L.) used in the study were provided by the Institute of Oliveculture (Koper, Slovenia) from olive plantations in the Slovenian Istra region. The olive leaves were dried and ground with a hammer mill to a maximum particle size of 2 mm. The olive cake was obtained from a local Slovenian olive oil producer in Koper. The olive cake, which is a by-product of fruit pressing, did not contain any seeds, as the olives were deboned before grinding. Like the olive leaves, the olive cake was air-dried and ground before being mixed into the experimental feed mixtures. Before including the olive leaves and the olive cake in the experimental feed mixtures, their chemical composition and mineral content were determined (Naumann and Bassler, 1997) and their gross energy value was assessed with a calorimeter IKA C 200 (IKA, Germany) (Table 2).

Table 2 Chemical composition, estimated gross energy and mineral content of dried olive leaves and olive cake

Item	Olive leaves	Olive cake
Chemical composition (g/kg)		
Dry matter	848.4	976.1
Crude protein	72.4	91.9
Crude fat	20.2	226.0
Crude fibre	247.7	239.4
Crude ash	47.5	59.3
Nitrogen free extract	460.8	359.5
Gross energy (MJ/kg)	3.2	8.5
Mineral content		
K (g/kg)	6.78	26.7
P (g/kg)	0.89	2.18
Ca (g/kg)	13.43	2.70
Na (g/kg)	0.32	0.20
Mg (g/kg)	1.09	0.77
Fe (mg/kg)	99.2	155.8
Zn (mg/kg)	107.7	117.2

2.2 Chemical analyses

Prior to the analyses of crude ash and mineral content, all bones were defrosted and cut across the middle of the diaphysis and transversely from the proximal epiphysis to the end of the distal epiphysis of the bone for better homogenization. The content of dry matter, crude protein, crude fat, crude ash and minerals in the experimental feeds and bone samples were determined by standard methods (Naumann and Bassler, 1997). The P was determined spectrophotometrically (Varian Cary 50, Probe UV-Visible Spectrophotometer) in hydrochloric acid extract at an absorbance of 430 nm, while Ca, Na, Mg, Cu and Zn were determined by flame atomic absorption spectrometry (Perkin Elmer, Analyst 200).

2.3 Statistical analyses

Data were analysed using the GLM procedure of the SAS software v. 9.4 (SAS Institute Inc. Cary, NC, USA), with experimental diet group as fixed effect (5 levels: Control, Oleave5, Oleave10, OCake5, Ocake10). Multiple comparison of least squares means of the experimental groups for each variable was conducted through Tukey-Kramer *post hoc* test and statistical significance was set at $P<0.05$.

3 Results and discussion

In the present study the final live weight of broilers did not differ among the experimental groups, in agreement with results reported in similar studies (Herrero-Encinas et al., 2020; Leskovec et al., 2018; Varmaghany et al., 2013). On the other hand, Branciari et al. (2017) observed significantly higher live weights in broilers fed with diets supplemented with olive cake. The broilers in our study exhibited comparable feed intake and live weight in all groups throughout the trial period. Only during the first week of trial the feed intake was significantly affected by the dietary treatment resulting higher in the OLeave10 group compared to OLeave5 group ($P=0.05$). Moreover, no health problems were detected during the experimental period. In the whole trial period, the Control group showed feed intake and feed conversion ratio (FCR) of 145 g/day and 1.8 kg/kg, respectively. The group OLeave10 showed feed intake of 165 g/day, and the highest FCR (2.22 kg/kg). Similarly, the OCake10 group exhibited FCR higher than 2 (Table 3). The differences detected for feed intake and FCR among groups were not statistically significant, however, in the present trial, the number of birds (24) and experimental units (2) per dietary group were rather low and the results on growth performance need to be confirmed in further trials. Indeed, Herrero-Encinas et al. (2020) reported that broilers fed with supplement of olive pomace (750 ppm) had higher average daily gains and FCR compared to the control group, whereas no difference on the average daily feed intake was observed between treatments. On the other hand, Branciari et al. (2017) reported a reduced FCR in broilers fed with diets containing different percentages (8.3 and 16.5%) of olive cake. The same trend was observed for supplementation with different concentrations of olive leaves, where a higher concentration linearly reduced the FCR (Varmaghany et al., 2013).

Healthy bones play an important role in both animal welfare and production performance. The most important factor in maintaining healthy bones is a diet rich in vitamins and minerals, including vitamin D, vitamin B complex, vitamins A, E, K, and D, folic acid, Ca, P and Cu (Suchý et al., 2009). In a study by Venäläinen et al. (2006), an increased concentration of metabolizable P in the feed increased the crude ash content and the Ca and P content in the tibia of broilers. However, other components may also contribute to bone health, such as n-3 fatty acids (Liu et al., 2003) and polyphenols, which are present in *Olea europaea L.* by stimulating bone formation and inhibiting bone resorption, as they are thought to promote osteoblast differentiation and proliferation (Idrus and Saim, 2019). Several studies have shown that olives and their compounds can affect bone formation, maintain healthy bones and can be used as an effective dietary supplement in the treatment of osteoporosis, osteoarthritis, osteopenia and cartilage injuries (Đudarić et al., 2015; Hagiwara et al., 2011). Based on the results of measurements on femur, tibia and humerus (Table 4), olive leaves and olive cake until 10% had no effect on bone mineralisation. Significant differences among groups were only observed in the Cu content of the humerus. The group receiving 5% and 10% olive leaves in the feed had significantly higher Cu content in the humerus compared to the groups Control and OCake10.

The results of the present study agree with those of Leskovec et al. (2018), who reported that dietary supplementation with olive leaf extract (1%) did not affect the physical properties (weight, length, maximum force and maximum bending) and crude ash content of the tibia of broilers. On the other hand, other studies have observed that polyphenols have an inhibitory effect on the secretion of digestive enzymes (α-amylase, protease and lipase), which may reduce the digestibility of nutrients (Surai, 2014).

Table 3 Effect of the dietary treatment on live weight, feed intake and feed conversion ratio of broilers fed different amounts of olive leaves and cake from day 21 to 42 of age

Trait	Control	OLeave5	OLeave10	OCake5	OCake10	SEM	P-value
Live weight (g) at:							
21 days	906	786	843	845	918	34	0.134
28 days	1,453	1,273	1,371	1,390	1,388	49	0.150
35 days	2,102	2,071	2,048	2,034	1,918	66	0.360
42 days	2,772	2,532	2,638	2,659	2,623	82	0.362
Feed intake (g/day) from:							
21 to 28 days	133.5^{ab}	121.1^{b}	142.7^{a}	131.1^{ab}	132.2^{ab}	3.4	0.050
28 to 35 days	162.8	162.4	186.1	163.85	166.1	7.5	0.264
35 to 42 days	140.6	140.0	165.7	139.2	143.2	6.6	0.136
21 to 42 days*	145.3	141.2	164.8	146.5	148.9	5.8	0.175
Feed conversion ratio	1.83	1.94	2.22	1.82	2.08	0.12	0.214

Pen data.

Control - control diet; OLeave5 and OLeave10 - 5% and 10% olive leaves per kg of feed mixture, respectively; OCake5 and OCake10 - 5% and 10% olive cake per kg of feed mixture, respectively; SEM – standard error of the mean.

^{a,b}Means with different superscript letters within a row differ significantly (P<0.05).

Table 4 Effect of the dietary treatment on contents of crude ash and minerals in bones of broilers

Trait	Control	OLeave5	OLeave10	OCake5	OCake10	SEM	P-value
Femur							
Crude ash (g/kg)	154.67	154.67	160.09	162.02	158.65	3.41	0.477
P (g/kg)	27.12	27.62	28.26	27.12	27.82	0.55	0.592
Ca (g/kg)	53.67	54.46	55.95	52.44	53.94	1.37	0.487
Mg (g/kg)	1.38	1.40	1.43	1.39	1.36	0.03	0.589
Cu (mg/kg)	3.50	4.15	3.71	3.34	3.14	0.27	0.102
Zn (mg/kg)	86.68	89.33	90.65	88.98	85.72	4.39	0.931
Tibia							
Crude ash (g/kg)	172.54	175.84	174.65	182.93	182.10	5.34	0.565
P (g/kg)	30.57	31.94	30.74	31.25	32.07	0.93	0.715
Ca (g/kg)	59.91	61.33	61.39	60.33	62.18	2.33	0.962
Mg (g/kg)	1.52	1.55	1.50	1.58	1.53	0.05	0.829
Cu (mg/kg)	3.60	4.37	3.97	3.94	3.26	0.28	0.084
Zn (mg/kg)	84.50	92.59	105.24	97.44	100.28	5.14	0.082
Humerus							
Crude ash (g/kg)	182.63	193.30	181.53	181.29	190.77	5.25	0.323
P (g/kg)	31.88	34.05	32.15	31.59	33.75	1.02	0.318
Ca (g/kg)	63.35	65.99	62.89	59.80	65.59	2.17	0.300
Mg (g/kg)	1.59	1.69	1.60	1.60	1.60	0.04	0.337
Cu (mg/kg)	3.57^{a}	4.44^{b}	4.12^{b}	3.82^{ab}	3.21^{a}	0.26	0.020
Zn (mg/kg)	98.22	100.55	99.19	97.87	100.66	3.78	0.976

Control - control diet; OLeave5 and OLeave10 - 5% and 10% olive leaves per kg of feed mixture, respectively; OCake5 and OCake10 - 5% and 10% olive cake per kg of feed mixture, respectively; SEM – standard error of the mean.

^{a,b}Means with different superscript letters within a row differ significantly (P<0.05).

In addition, polyphenolic compounds can bind with minerals (e.g. Fe, Cu, Zn) and form indigestible complexes that reduce the absorption and thus the utilization of minerals (Yang and Landau, 2000; Sandberg, 2002; Surai, 2014). However, according to the results of numerous *in vitro* studies on human models investigating the effects of olive polyphenols on osteoblast differentiation and

osteoporosis prevention, olive polyphenols can have a beneficial effect especially when bone homeostasis is disturbed, because they increase bone formation and inhibit its resorption (García-Martínez et al., 2014; Idrus and Saim, 2019; Melguizo-Rodríguez et al., 2018; Santiago-Mora et al., 2011).

4 Conclusions

In the present study broiler chickens showed comparable growth and feed intake in all groups during the whole trial period (from 21 to 42 days of age). Olive leaf or cake addition did not affect broiler performance; however, these results need to be confirmed in further trials with a higher number of animals and replicates per experimental treatment. Furthermore, the addition of olive leaves and cake in broiler feed did not affect bone mineralisation and maintenance of bone homeostasis of healthy bones, as there were no differences in the mineral structure of bones between different dietary treatments. Therefore, we can assume that the dietary addition of olive leaves and cake had no inhibitory effect on the absorption and utilization of minerals. In conclusion, based on our results, olive leaves and cake can be included in the diets for broiler chickens at levels from 5 to 10% without impairing growth performance and bone mineralisation; again, further research on their use is required to support results of the present study.

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