SHORT COMMUNICATION

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Effect of olive leaf powder on the performance and ileal bacterial count of broilers

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Abstract

This experiment was conducted to investigate the effects of olive leaf (OL) on the performance, abdominal fat pad and some ileal bacterial population of Cobb broiler chickens. A total number of 400 day-old chicks were randomly distributed into floor pens and reared under the same condition until 14 days of age. On day 14, each pen was randomly assigned to one of the five experimental treatments with four replicates of 20 male and female chicks. The dietary treatments were consisted of a control group which fed basal diet without OL entire period of the study and groups 2 to 5 that fed diets supplemented with 0.25, 0.50, 0.75 and 1.00% OL powder, respectively. On days 21 and 42 of the experiment, ileal digesta samples were collected under the sterile condition to evaluate ileal bacterial population. The results indicated that birds fed diets containing various levels of OL, had higher body weight gain (except for 1.00% OL) and lower feed conversion ratio compared to that of the control group. Dietary inclusion of OL resulted in a higher count of *Lactobacillus sp.* compared to the control group on 42 days of age, while *Escherichia coli* count significantly was not influenced. The abdominal fat pad was lower in birds fed OL supplemented diets. In conclusion, findings of the current experiment showed that the OL had positive effects on feed conversion ratio, abdominal fat pad deposition and ileal bacterial count of broiler chickens.

Introduction

Prohibition of antibiotic growth promoters in animal and poultry nutrition has stimulated the application of bioactive secondary metabolites as alternative growth enhancers in poultry nutrition. There are many non-therapeutic alternatives such as enzymes, inorganic acids, probiotics, prebiotics, herbs, immune stimulants, and other management practices.[1]

Olive leaf (OL) from *Olea europaea* has been known for its medicinal properties since ancient times. Leaves and fruits are usable parts of olive tree. The OL contains various compounds such as secoiridoid polyphenols (oleuropein and its derivatives), hydroxytyrosol, polyphenols (verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside), triterpenes including oleanolic acid, and flavonoids (rutin and diosmin).[2] Therapeutic properties of OL extracts could be attributed to oleuropein.[2] The high concentration of oleuropein is found in fruit and leaves, although, it could also be available in oil. Different properties have been reported for OL, including hypoglycemic, hypotensive, anti-arrhythmic, anti-atherosclerotic and vasodilatory effects.[2]

Gut microflora plays a vital role in poultry health and performance. Antioxidant and antibiotic activities of herb extracts are associated with secondary metabolites such as phenolic compounds.[1] Antibiotic activity of phenolic components present in OL against viruses, bacteria, yeasts, molds, fungi, retroviruses, and other parasites have been reported.[3] Supplementation with plant extracts rich in polyphenolic compounds has different effects on gut microorganisms.[4] There is only limited data on the influence of OL on broiler chickens, therefore, this experiment was conducted to determine the effects of OL as a growth promoter on the performance, abdominal fat pad, and gut flora in the broiler chickens.

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Materials and Methods

Olive leaves were collected in October from a garden around Kazeroon, Fars Province, Iran. They were dried in shadow, ground finely and included in diets from 14 to 42 days of age. Feed consumption of each pen was determined weekly. All procedures used in the current experiment were approved by the Institution of Animal Care Committee of the University of Yasouj (5419-11.10.2012). Four hundred day-old male and female broiler chicks (Cobb 500) obtained from a local commercial hatchery. Birds were weighed in the group, randomly distributed into 20 pens and fed a basal diet until 14 days of age. On day 14, pens were randomly assigned to one of the five experimental treatments. There were no significant differences in body weights of different groups on day 14. Each treatment had four replicates of 20 chicks each. Experimental treatments included one diet without OL as the control and diets 2 to 5, which were supplemented with 0.25, 0.50, 0.75 and 1.00% OL, respectively. The mash starter and finisher diets were offered form 1-21 and 22-42 days of age, respectively. The composition of the experimental diets is shown in Table 1. Birds had free access to feed and water throughout the experiment. Birds in each pen were weighed weekly as a group. Dead birds were recorded daily and feed conversion ratio (FCR) adjusted for mortality. The mortality rate was normal (around 4.00% for all treatments) and was not influenced statistically by experimental treatments. On days 21 and 42, two birds from each replicate (eight birds per treatment) were randomly selected and slaughtered by decapitation and digestive system removed immediately and the weight of the abdominal fat pad was measured.

For microbial counts, ileal digesta samples were collected under a sterile condition and put in sterile plastic tubes and immediately transferred to the refrigerator (4 ˚C). For counting *Lactobacillus sp.* and *Escherichia coli* populations, digesta samples were diluted and poured on the Man Rogosa Sharpe (MRS; Quelab, London, UK) and eosin methylene blue agar (EMB; Quelab), incubated at 37 ˚C for 24 hr in the anaerobic and aerobic conditions, respectively, and the number of bacteria was determined using an electronic colony counter (Sana SL-902; Hoor Teb, Tehran, Iran). The results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of ileal content. All data were analyzed using the ANOVA procedure of SAS (version 9.1; SAS Institute, Cary, USA). Means were compared by Duncan's multiple range test at 5.00% probability. The statistical model was as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where, Y_{ij} is the observed size of the test, μ is the population mean, T_i is the treatment effect, and ε_{ij} is the error of the experiment.

Results

The effect of experimental treatments on broiler performance is shown in Table 2. In the starter period, birds fed the diet containing 0.25% OL had a higher body weight gain (BWG) than the control group ($p < 0.05$).

Table 1. Composition of the experimental diets (g kg^{-1} diet).

Feed ingredients (g kg^{-1})	Starter					Grower-finisher				
	Control	0.25%	0.50%	0.75%	1.00%	Control	0.25%	0.50%	0.75%	1.00%
Corn	550.00	549.00	547.50	546.20	54.50	625.00	623.70	622.50	621.20	620.00
Soybean meal	361.00	360.00	358.50	357.20	35.60	302.00	300.80	299.50	298.30	297.00
Soybean oil	45.00	45.00	45.00	45.00	45.00	30.00	30.00	30.00	30.00	30.00
Limestone	14.00	14.00	14.00	14.00	14.00	13.00	13.00	13.00	13.00	13.00
Dicalcium phosphate	18.40	18.40	18.40	18.40	18.40	15.00	15.00	15.00	15.00	15.00
Common salt	3.60	3.60	3.60	3.60	3.60	3.00	3.00	3.00	3.00	3.00
Vitamin and mineral premix*	5.00	5.00	5.00	5.00	5.00	10.00	10.00	10.00	10.00	10.00
DL-Methionine	3.00	3.00	300	3.00	3.00	2.00	2.00	2.00	2.00	2.00
Olive leaf	0.00	2.50	5.00	7.50	10.00	0.00	2.50	5.00	7.50	10.00
Calculated analysis										
Metabolizable energy (Kcal kg^{-1})	3126.00	3118.00	3111.00	3104.00	3097.00	3148.00	3141.00	3133.00	3126.00	3119.00
Crude protein	223.80	223.00	222.40	221.70	221.10	187.60	187.00	186.30	185.70	184.90
Lysine	11.50	11.50	11.50	11.50	11.50	9.90	9.90	9.90	9.90	9.90
Methionine + cystine	5.90	5.90	5.90	5.90	5.90	5.40	5.40	5.40	5.40	5.40
Tryptophan	8.30	8.30	8.30	8.30	8.30	7.60	7.60	7.60	7.60	7.60
Arginine	13.40	13.40	13.40	13.40	13.40	11.90	11.90	11.90	11.90	11.90
Calcium	10.6	10.60	10.60	10.60	10.60	9.30	9.30	9.30	9.30	9.30
Available phosphorus	4.90	4.90	4.90	4.90	4.90	4.20	4.20	4.20	4.20	4.20
Sodium	1.70	1.70	1.70	1.70	1.70	1.50	1.50	1.50	1.50	1.50

Control: a basal diet without olive leaf; 0.25, 0.50, 0.75 and 1.00%: diet contains 0.25%, 0.50%, 0.75% and 1.00% olive leaf, respectively.
*Vitamin and mineral premix contained (per kg of diet): vitamin A, 9,000 IU; vitamin D3, 2,000 IU; vitamin E, 18.00 IU; vitamin K3, 2.00 mg; thiamine, 2.00 mg; riboflavin, 6.60 mg; niacin 30.00 mg; D-pantothenic acid, 10 mg; pyridoxine, 3.00 mg; folic acid, 15.00 mg; cobalamin, 0.01 mg; biotin, 100 mg; antioxidant, 500 mg, cholin, 400 mg; Fe, 50.00 mg; Mn, 100 mg; Zn, 85.00 mg; Cu, 10.00 mg; Se, 0.20 mg, and I, 100 mg.

Table 2. Effects of experimental treatments on performance, abdominal fat pad (% of live weight), *Escherichia coli* and *Lactobacillus sp.* counts (Log CFU g^{-1}).

Parameter	Experimental Treatments					SEM	*p*-value
	Control	0.25%	0.50%	0.75%	1.00%		
Bodyweight gain (g)							
Days 1-21	635.00^{b}	708.00^{a}	672.00^{ab}	680.00^{ab}	645.00^{ab}	19.80	0.12
Days 22-42	1132.00^{b}	1308.00^{a}	1334.00^{a}	1354.00^{a}	1098.00^{b}	64.60	0.01
Days 1-42	1767.00^{b}	2016.00^{a}	2006.00^{a}	2034.00^{a}	1743.00^{b}	70.80	0.006
Feed intake (g)							
Days 1-21	785.00	795.00	754.00	787.00	727.00	28.20	0.43
Days 22-42	2134.00	2313.00	2247.00	2245.00	2009.00	92.84	0.21
Days 1-42	2919.00^{ab}	3108.00^{a}	3000.00^{ab}	3032.00^{ab}	2736.00^{b}	99.60	0.14
Feed conversion ratio							
Days 1-21	1.23^{a}	1.12^{b}	1.12^{b}	1.15^{b}	1.12^{b}	0.01	0.002
Days 22-42	1.88^{a}	1.77^{ab}	1.68^{b}	1.62^{b}	1.85^{ab}	0.09	0.07
Days 1-42	1.65^{a}	1.54^{b}	1.49^{b}	1.49^{b}	1.57^{b}	0.05	0.03
Abdominal fat pad (day 42)	3.00^{a}	2.00^{b}	2.10^{b}	2.20^{b}	1.90^{b}	0.10	0.006
E. coli							
Day 21	7.00	6.90	7.10	7.00	7.40	0.40	0.86
Day 42	10.70	10.30	10.30	10.30	9.90	0.30	0.61
Lactobacillus sp.							
Day 21	9.40	9.40	9.30	9.70	9.80	0.30	0.79
Day 42	7.80^{c}	7.60^{c}	8.40^{b}	8.40^{b}	9.90^{a}	0.10	0.0001

Control: a basal diet without olive leaf; 0.25, 0.50, 0.75 and 1.00%: diet contained 0.25%, 0.50%, 0.75% and 1.00% olive leaf, respectively.
^{abc} Means with different letters in each row, differ significantly ($p < 0.05$).

Dietary inclusion of 0.25, 0.50 and 0.75% OL resulted in significant increases in BWG during finisher period (days 22-42) and whole experimental period (days 1-42). There were no significant differences in BWG between the control and 1.00% OL groups throughout the study period.

Dietary treatments did not have a significant effect on feed intake (FI) during 1 to 21 and 22 to 42 days of age. However, birds fed diet supplemented with 1.00% OL had lesser feed intake compared to those fed 0.25% OL. From 1 to 21 days of age, supplementation with all levels of OL significantly improved FCR compared to the control group. Also, FCR was better for 0.50 and 0.75% groups compared to the control group during the finisher period. Similar to the starter phase, birds fed diets containing different levels of OL, had better FCR than the control group. In our study, the addition of OL powder to the diets significantly improved BWG (except for 1.00% OL) and FCR compared to the control group.

The effects of experimental treatments on ileal bacterial population are shown in Table 2. Dietary treatments did not have a significant effect on *E. coli* count at 21 and 42 days of age. Supplementation with OL did not have a significant effect on the *Lactobacilli* population at 21 day of age. However, on day 42, birds fed diets added with 0.50, 0.75 or 1.00% OL, had a higher number of *Lactobacilli* than the control group.

Discussion

There are inconsistent results in the literature on the growth-promoting effects of olive products in broiler chickens. Replacement of 15.00 and 30.00 g kg^{-1} wheat bran with OL in starter and finisher diets did not have a significant effect on performance and carcass characteristics of broilers, however, replacement at the level of 50.00 g kg^{-1}, reduced live body weight and carcass weight of broiler chickens.[5] Dietary inclusion of OL powder at the levels of 0.00, 5.00, 10.00 and 15.00 g kg^{-1} of diet did not affect growth parameters.[6] In contrast to those reports, beneficial effects of OL extract on the broilers performance have been shown in some studies. Findings of a recent study have shown that dietary inclusion of 15.00 mL of OL extract had beneficial effects on the performance of broilers reared under a hot humid tropical climate.[7] In another study, broilers fed diets supplemented with 100 and 200 mg kg^{-1} OL extract had higher body weight gain and better feed conversion ratio than the control group.[8]

It has been reported that antioxidant properties of active compounds in OL by reducing protein oxidation can improve nitrogen retention and, therefore, bird growth.[9] As mentioned earlier, OL contains isoflavonoids which can decrease lipid peroxidation, thereby, improving anti-oxidant status and growth performance in male broilers.[5]

The OL is rich in oleuropein (60.00 – 90.00 mg g^{-1} dry OL),[3] a compound which can increase the activity of the pancreas and small intestine digestive enzymes, growth of potentially beneficial gut bacteria as well as nutrients digestion and absorption.[8] One of such beneficial effects was observed in the current experiment. Dietary supplementation with different levels of OL (except for 0.25%) resulted in significant increases in the population of beneficial bacteria. Therefore, this effect could be considered as one of the possible reasons for the improved performance of birds in the supplemental groups.

Antibacterial activity of oleuropein against wide broad of bacteria (by cell wall degeneration) has been reported.[10] Antibacterial activity of OL could be related to the inactivation of necessary enzymes for bacterial replication or by a direct attack on the cell membrane.[11] Due to the high levels of phenols, OL extract probably has prebiotic activity.[12] Since it can act as a prebiotic, it stimulates the growth of probiotic bacteria.[10]

Plant extracts have been reported to reduce ileal pH value and increase the number of lactic acid bacteria in the ileum and caecal contents of broiler chickens.[13] Stimulation of beneficial bacteria such as *Lactobacilli* and *Bifido-bacteria* could be contributed to a balanced gut microflora and may provide an optimal condition for adequate protection against pathogenic microorganisms and intact immune system.[14] It was suggested that terpenoids and phenylpropanoids can penetrate into the membrane of the bacteria and the inner part of the cell because of their lipophilicity property.[14] It has also been proposed that structural properties such as the presence of the functional groups[15] and aromaticity[1] are responsible for the antibacterial activity. Active components in plant extract penetrate into the cell wall of pathogenic bacteria and then alter H^+ and K^+ ions transferring and interference with enzymatic reactions related to ATP production, and as a result, cell death will happen.[16]

Addition of OL, regardless of its level, significantly decreased abdominal fat pad compared to the control group. These findings were in agreement with the results reported in a previous work.[5] They reported that volatile plant oil decreased the abdominal fat content. Plant antioxidant components can increase lipase and bile acids secretions and due to these changes, the abdominal fat deposition will decrease.[5] Dietary flavonoids inhibit phosphodiesterase activity, thereby decrease tissues fat production.[17]

Furthermore, it has been shown that flavonoids reduce the availability of lipid substrates and consequently reduce VLDL production in the liver.[18] Hypolipidemic effects of olive derivates have been reported. Oleuropein in OL has inhibitory activity on the activity and/or expression of some enzymes such as hydroxymethylglutaryl-CoA synthase and hydroxyl-methylglutaryl-CoA reductase which play an important role in lipid synthesis in the liver.[8] Taking together, it is not surprising that the abdominal fat pad was reduced in OL-supplemental groups.

In the current experiment, dietary OL addition of resulted in the less abdominal fat pad, beneficial effect on the population of ileal bacteria and better performance. These findings probably indicated that nutrients utilization might be improved by supplementation with OL. Thus, it seems that OL could be included in broilers diets as a new growth promoter.

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Conflict of interest

The authors declare that there is no conflicts of interest.

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