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The efficacy of using olive cake as a by-product and probiotic supplementation on growth performance and blood characteristics of broiler chickens

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ABSTRACT

Olive cake has high nutritional content, which can be used as a protein and fat source in poultry diets. The main aim of the work was to determine the impact of using different levels of Olive cake as food processing by-products and probiotics in broiler diets and their efficacy on growth performance and blood characteristics. In the feeding experiment, 336-day-old male broiler chicks (Ross 308) were raised for 42 days. They were randomly divided into seven designed groups experimentally; each included four replicates and 12 chicks. The dietary treatments were as follows; Basal diet without Olive cake (T1), Basal diet with (4%, 8% and 12%) olive cake for (T2, T3 and T4), respectively, and Basal diet with (4%, 8% and 12%) Olive cake + 0.4 g/kg Iraqi probiotic for (T5, T6 and T7), respectively. The results indicated that 4% OC with 0.4 g/kg probiotic showed the highest live body weight, body weight gain, European Production Efficacy Factor and improved Feed conversion ratio at the end of the experiment. Also, the supplementation of Olive cake with or without probiotics substantially increased lymphocyte numbers and lowered Heterophil/Lymphocyte (H/L) ratio. It decreased cholesterol levels compared to the control group at the end of the bird's life. Also, haematological and biochemical parameters significantly ($P<0.05$) were improved when 4% Olive cake 0.4 g/kg probiotic was added to the diet of broilers. In conclusion, Olive cake and probiotic supplementation positively impacted chicken performance and broilers' blood characteristics.

Keywords: Olive by-product; Probiotic; Broiler Chicken Productivity; Haematology.

1. Introduction

Approximately 70% of poultry production cost is spent on feeding. Resources for feeding animals are limited in emerging nations, and the situation got worse when grains and oilseeds were used to make biodiesel and biofuel, respectively[1]. To reduce the overall cost of poultry production, it is necessary to find a new product, non-traditional, low-cost feedstuffs for poultry diets[2-4]. As long as animals have been domesticated, agricultural waste has been given to livestock. The area used for olive trees has increased because of a rise in the global consumption of olive oil in recent years. The Mediterranean region accounts for 65% of all olive-growing territory worldwide[3]. Olive crop cultivation has dramatically grown because olive oil is used for human health benefits[5]. A cheap supply of poultry feed can be made from the leftovers from the extraction of olive oil[6]. Olive cake (OC), olive pulp and olive leaves are three major generated by-products of olive oil extraction, though olive pulp with seeds or without seeds predominates[5]. A by-product of the extraction of olive oil called olive cake is fed to livestock. However, we cannot extract all of the oil from the seeds during the oil-extracting methods. Thus, this by-product was higher in fat content, and a few compounds that may have antioxidant properties are included in this by-product[7]. Using olive cake (OC) in chicken diets is advantageous for recycling these waste materials[6].

Additionally, the fatty acid composition in meat muscle is impacted by the unsaturated fatty acids (linoleic acid, oleic acid, and linolenic acid) found in OC[3]. According to the literature, laying hens have been given up to 10% of OC without detrimental effects on their productivity, egg quality, and liver and kidney functions[8, 9].

Probiotics are also considered as live microbial food additives or bacteria that positively impact human and animal health. Probiotics positively influence the host's health by balancing the gut microbiota, increasing antioxidant activity, and reducing the harm caused by pathogenic microbes, enhancing epithelial cell integrity and immunological functions[10].

The main aim of the current study included finding out the effectiveness of using various quantities of OC by-products and

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probiotic supplementation on broiler poultry growth performance and some blood characteristics.

2. Material and Methods

2.1 Ethics Approval

The Scientific Ethical Committee of the Animal Resources Department College of Agricultural Engineering Sciences, Salahaddin University, Erbil, approved this study (No.: 1134 on 31/5/2021).

2.2 Olive cake collection and preparation

Raw OC was collected from a local olive extraction factory in the Kurdistan region/Iraq. The material was procured throughout the olive extraction season and stored for further Analysis. The residual materials of OC, including pulp and stones after oil extraction, were transported to the experimental farm. The olive cake was spread on the plastic bag and mixed every two hours to dry the solar heat. The recorded temperature was around 45°C. The OC was dried and powdered for feeding. The powder was then placed in plastic bags until further use. The proximate chemical composition of OC was done in Erbil Feed Company before adding it to the diet (Table 1).

Table 1: The chemical composition of olive cake by-product used in the diet.

Nutrient Content of Dry Matter (DM)	chemical composition
Dry Matter (%)	87.6
Crude Protein (%)	6.37
Crude Ash (%)	2.88
Crude Fat (%)	21.08
Crude fiber (%)	37.92
Metabolic Energy (kcal/kg)	3700

2.3 Design of Experiment

This research was conducted on an experimental farm in Garmian Administration / Kurdistan Region / Iraq. A total number of 336 one-day-old male broiler chicks (Ross 308 strain) were randomly assigned into seven groups of treatments with four replicates per each treatment and reared for 42 days. Broilers were housed on a floor in pens measuring (2×2 m). The designed diets were formulated according to Aviagen nutrient specification requirements (2014) and were iso-nitrogenous, and iso-caloric of OC was included in iso-nutritive diets for the broiler. The diets were designed as follows; Basal diet (no OC) (T1), Basal diet with (4%, 8% and 12%) OC for (T2, T3 and T4) respectively, and Basal diet with (4%, 8% and 12%) OC + 0.4 g/kg Iraqi probiotic includes (*Lactobacillus acidophilus* 10^8, *Bacillus subtilis* 10^9, *Bifidobacterium* spp. 10^8 and *Saccharomyces cerevisiae* 10^9) for (T5, T6 and T7) respectively. The chicks had free to obtain diet and water *ad libitum*. The composition of diets used during the broiler's age stages is shown in (Table 2).

Table 2: The composition of broiler diets during the experimental period was used for the experiment.

Ingredients	Composition of diet (kg/ 100 kg)							
	Starter (1-21 days)				Grower (22-42 days)			
	T1 Control	T2 4% OC	T3 8% OC	T4 12% OC	T1 Control	T2 4% OC	T3 8% OC	T4 12% OC
Maize	41.8	39.2	38.2	38.2	44.2	43.1	43.0	43.4
Wheat	10	10	9	8	12.3	10.8	9	6
Wheat Bran	5	4	2	0	4	3	1.45	0
Soybean 48%	34.5	34.7	35.2	35.6	29.5	29.7	30.15	31
Soybean Oil	4.5	3.9	3	2	5.1	4.5	3.5	2.8
Limestone	1	1	1	1	0.6	0.6	0.6	0.5
Dicalcium Phosphate	0.4	0.4	0.4	0.4	1.5	1.5	1.5	1.5
Olive cake	0	4	8	12	0	4	8	12
Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100	100	100
Calculated values								
ME (kcal/kg)	2941.34	2942.63	2948.42	2949.68	3051.10	3054.98	3050.18	3054.45
CP %	22.02	22.01	22.02	22.00	20.06	20.02	20.00	20.07
Fibre	3.64	4.99	6.32	7.65	3.33	4.72	6.09	7.51
Lysine %	2.95	2.95	2.94	2.94	2.80	2.81	2.81	2.83
Methionine %	2.37	2.36	2.36	2.36	2.34	2.3	2.34	2.34
Calcium %	1.20	1.23	1.26	1.29	1.03	1.06	1.09	1.13
Available phosphate %	0.32	0.33	0.34	0.35	0.31	0.32	0.34	0.35

2.4 Growth Performance

The live birds' initial and final weights were weighed, and feed intake was recorded during all experimental periods; the feed conversion ratio (FCR) was calculated using the equation of feed intake per unit divided by weight gain. Carcass weight was also measured. The European Production Efficiency Factor (EPEF) was calculated according to the following equation[10]: **EPEF** = livability (%) × live weight (kg) × 100/ age (d) × FCR.

2.5 Blood parameters

Four birds were randomly selected on day 42 from every treatment. The samples of blood were taken from the wing vein and stored in sterilized tubes containing anticoagulant Di-Potassium ethylene diamine tetra acetic acid (K_2EDTA) for haematological [White Blood Cells (WBC), Red Blood Cells (RBC), Lymphocyte (LYM), Heterophil (HETR), Heterophil / Lymphocyte ratio, and Hemoglobin (HGB)] were tested using Hematology Analyzer (Full Auto) (MCL 3800, China) and other for serum biochemical parameters, lipid profiles (Cholesterol, LDL HDL, and Triglyceride) using (Roche Cobas® 6000 analyzer series, Switzerland).

2.6 Statistical Analysis

The results were analyzed using a one-way ANOVA analysis by SPSS version 26 software[12]. Results of summary statistics included means and standard error. Duncan's test utilized and aided in calculating significant differences at 0.05 levels among the various parameters[13].

3. Results and Discussion

Table 3 shows the influence of OC and probiotics on the broiler's growth performance at 42 days. The results indicated that the chicks receiving 4% of OC with 0.4 g/kg probiotic T5 had the highest body weight compared to other feeding groups, followed by T2. OC and probiotics were recorded as the highest average body weight in all treatment groups compared to the control group. While there were no significant (P>0.05) differences were observed among all treatments on the weight gain, live body weight, and FCR of the broiler at the end of the experimental period. Also, figure 1 showed no significant (P>0.05) differences among treatments on the EPEF, but all treatments recorded a high value of EPEF compared to the control group.

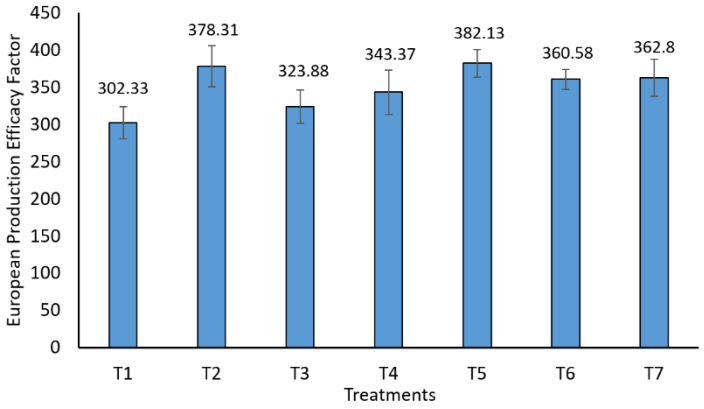

Figure 1: Effect of olive cake by product and probiotic on European Production Efficiency Factor of broiler chickens (Mean ± standard error). Treatments: (T1), Basal diet, Basal diet with (4%, 8% and 12%) OC for (T2, T3 and T4) respectively, and Basal diet with (4%, 8% and 12%) OC + 0.4 g/kg Iraqi probiotic for (T5, T6 and T7) respectively.

Table 3: Influence of olive cake by product and probiotic on growth performance of broilers at 42 days of age (Mean ± Standard error).

Treatment	Growth Performance				
	Initial weight (g)	Final weight (g)	Weight gain (g/bird)	Feed intake (g/bird)	Feed Conversion Ratio
T1	40.89±0.10	2423.43±88.29	2381.41±88.46	4181±24.12 ^{b}	1.76±0.06
T2	40.82±0.14	2664.79±55.22	2623.11±55.31	4145±12.75 ^{bc}	1.58±0.03
T3	40.90±0.02	2493.89±81.98	2452.99±81.98	4138±10.25 ^{c}	1.69±0.03
T4	40.67±0.09	2496.25±77.59	2455.57±77.63	4012±4.02 ^{d}	1.63±0.05
T5	40.86±0.08	2688.12±37.60	2647.26±37.65	4257±6.96 ^{a}	1.61±0.02
T6	40.59±0.21	2619.68±19.72	2578.59±19.70	4279±10.32 ^{a}	1.65±0.01
T7	40.70±0.16	2616.56±54.86	2575.85±54.88	4259±11.76 ^{a}	1.65±0.03
P. value	0.541	0.057	0.057	<0.001	0.132

^{a,b,c,d} Means within the same column with different superscripts differ (P<0.05).

Treatments: (T1), Basal diet, Basal diet with (4%, 8% and 12%) OC for (T2, T3 and T4) respectively, and Basal diet with (4%, 8% and 12%) OC + 0.4 g/kg Iraqi probiotic for (T5, T6 and T7) respectively.

According to the findings, the olive cake had no negative impact on broilers' growth performance, feed consumption, and FCR. The use of isocaloric and isonitrogenous diets may have contributed to this result. These findings align with previous research [14], which demonstrated that broiler chickens could be fed OC up to 10% without suffering negative impacts on growth, feed intake, or FCR. According to the researchers' findings, olive cake can be fed to broilers and hens up to 15% without impacting feed consumption[15]. However, the study's conclusion revealed that olive cake in broiler diets greatly improved FCR. However, olive cake is characterized by its high percentage of fibre, and beneficial bacteria use this fibre to improve digestion rates[16,17].

Table 4 shows the influence of OC by product and probiotic on the haematological parameters of 42 days old broilers. It was found that WBC, RBC count, LYM, HETR, H/L ratio and HGB

were significantly affected by the inclusion of different levels of OC and probiotic supplementation. The lymphocyte ratio increased at the inclusion of 4% of OC with a probiotic (T5) compared to all other treatments. In contrast heterophil ratio decreased in the treatment groups compared to the control. In this study, the treatments' heterophil/ lymphocyte H/L ratio decreased compared to the control group. Moreover, the highest haemoglobin (g/L) was recorded at the level inclusion of 4% of olive cake with probiotic (T5) and the lowest value was recorded at the inclusion of 12% olive cake without probiotic (T4).

Table 4: Influence of olive cake by product and probiotic on haematological parameters at the end of the experiment 42 days of age (Mean ± Standard error).

Treatment	Haematological parameters					
	WBC (No.×10^9/L)	RBC (No.×10^9/L)	LYM (%)	HETR (%)	H/L ratio	HGB (g/L)
T1	91.85± 5.11 ^{a}	27.30± 3.84 ^{ab}	70.12± 1.28 ^{b}	23.42± 0.93 ^{a}	0.33± 0.18 ^{a}	123.32±10.18 ^{ab}
T2	92.17± 2.78 ^{a}	21.49± 2.32 ^{b}	72.02± 0.95 ^{b}	20.77± 1.02 ^{ab}	0.29± 0.18 ^{ab}	112.67± 6.14 ^{b}
T3	57.07± 3.09 ^{b}	22.10± 2.26 ^{b}	72.50± 0.56 ^{b}	20.22± 1.05 ^{b}	0.28± 0.15 ^{b}	83.70± 3.80 ^{cd}
T4	42.52± 4.57 ^{c}	19.54± 6.59 ^{b}	70.55 ± 1.40 ^{b}	20.52± 1.08 ^{b}	0.29± 0.21 ^{ab}	61.75± 12.53 ^{d}
T5	40.30± 2.49 ^{c}	34.94± 2.53 ^{a}	76.35 ± 0.61 ^{a}	18.27± 0.89 ^{b}	0.24± 0.13 ^{b}	143.72± 8.60 ^{a}
T6	51.25± 2.56 ^{bc}	35.13± 0.80 ^{a}	71.15± 1.29 ^{b}	19.30± 0.69 ^{b}	0.27± 0.14 ^{b}	80.72± 5.43 ^{cd}
T7	89.07± 3.57 ^{a}	29.25± 3.76 ^{ab}	72.97± 0.42 ^{b}	19.32± 0.43 ^{b}	0.26± 0.06 ^{b}	100.10± 2.29 ^{bc}
P. value	<0.001	0.023	0.006	0.019	0.022	<0.001

^{a,b,c,d} Means within the same column with different superscripts differ (P<0.05).

Treatments: (T1), Basal diet, Basal diet with (4%, 8% and 12%) OC for (T2, T3 and T4) respectively, and Basal diet with (4%, 8% and 12%) OC + 0.4 g/kg Iraqi probiotic for (T5, T6 and T7) respectively.

The Heterophil/Lymphocyte (H/L) ratio is used as a parameter for the animal's well-being, and any increase in the H/L ratio may indicate high stress on the birds[18]. In this study, the H/L ratio was decreased at the end of the experiment in OC with or without probiotics compared to the control. A low H/L ratio during treatment may be related to the use of olive cake, and probiotic supplements may increase the beneficial bacteria in the body and reduce nutritional stress on the birds[19].

Table 5 shows the influence of olive cake by product and probiotic on biochemical factors of 42 days old broilers. In this experiment, the impact of the inclusion level of OC with 0.4g/kg probiotic on cholesterol, TG and LDL levels decreased significantly (P>0.05) compared to the control. At the same time, no significant differences (P<0.05) were noticed in the HDL level among the treatments. The results agreed with the research and indicated that adding OC at 10% in broiler diets has a positive effect due to decreased blood cholesterol and triglyceride levels and increased plasma HDL levels[20]. Also, the researcher discovered that the inclusion of the probiotics in the diet (*Lactobacillus acidophilus* at 10^6 and 10^7 cfu/g) significantly (P<0.05) reduced the cholesterol in comparison to the control[21].

Table 5: Influence of olive cake OC by product and probiotic on biochemical parameters of broilers at 42 days of age (Mean ± Standard error).

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
T1	115.72±3.50 ^{a}	74.05±4.50 ^{a}	44.75±2.95 ^{a}	54.20±4.33
T2	123.30±1.74 ^{a}	72.60±2.23 ^{a}	44.50±4.55 ^{a}	56.57±2.87
T3	113.47±2.79 ^{ab}	75.22±3.53 ^{a}	38.00±5.70 ^{ab}	54.85±3.95
T4	114.67±2.76 ^{a}	53.97±3.38 ^{bc}	39.17±6.62 ^{ab}	56.42±6.28
T5	95.27±3.20 ^{c}	50.27±2.81 ^{c}	27.00±1.29 ^{b}	57.15±2.52
T6	99.35±3.64 ^{c}	61.22±2.73 ^{b}	30.50±2.50 ^{b}	56.70±2.45
T7	104.35±4.73 ^{bc}	49.22±1.80 ^{c}	31.25±1.03 ^{b}	63.07±5.12
P. value	<0.001	<0.001	0.028	0.814

^{a,b,c} Means within the same column with different superscripts differ (P<0.05).

Treatments: (T1), Basal diet, Basal diet with (4%, 8% and 12%) OC for (T2, T3 and T4) respectively, and Basal diet with (4%, 8% and 12%) OC + 0.4 g/kg Iraqi probiotic for (T5, T6 and T7) respectively.

According to the researchers, studies showed that cholesterol, LDL, and triglycerides were significantly (P<0.05) decreased in probiotic supplementation that contains *Lactobacillus* spp. Compared to the control group[22]. Also, it has been found that broilers fed a diet that contains *Aspergillus oryzae* as a probiotic led to a decrease in blood cholesterol[23]. According to the previous researcher, a significant (P<0.05) difference was observed using the interaction between OC and probiotics on total lipids, TG, cholesterol, LDL, HDL/LDL ratio and VLDL[24]. Research using OC on broiler's biochemical blood parameters is rare. However, they need more research to find more of the positive influences of OC on decreasing total lipids and raising the LDL ratio due to OC unsaturated fatty acids[25]. Furthermore, the use of agricultural byproducts is also supported by these results due to their ability to lower the cost of feeding, boost economic returns, and lessen environmental pollution.

Conclusion

It can be concluded that feeding OC as a by-product with or without probiotic supplementation improved growth performance, promoted the best plasma lipid profiles, reduced stress on the birds, and subsequently improved the health status of broilers.

Conflict of interests

There is no conflict of interest.

Author contribution

Author has written, drafted, analyzed data and finalized the manuscript.

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