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Modulation of Gut Microbiota, Intestinal Histology and Immune Function of Broilers Using Olive Cake as By-Product and Probiotic

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Abstract

The primary goal of this experiment was to detect the influences of adding various olive cake (OC) quantities as byproducts and probiotics to broiler diets, as well as their effectiveness on the gut microbiota, intestinal histology, and immune functions of broilers. Three hundred and thirty-six chick male broilers (Ross 308) one-day old were grown for 42 days during 2022. From them, seven randomly generated experimental groups were formed, four replicates per each groups (12 chicks/replicate) as follows: a control group, group received OC 4%, 8%, and 12% replaced, and for three final groups received OC 4%, 8%, and 12% with 0.4 g/kg Iraqi probiotic. The experimental design had a complete randomized design. Gut microbiota, immune function and intestinal histology of broilers were studied. The result indicated at the level inclusion 4% of OC with 0.4g/kg of probiotic supplementation showed that Lactic Acid Bacteria (LAB) increased and Total Coliform Bacteria decreased in the caecum digesta. Also, supplementing of OC including or without probiotics significantly increased the length of villi in the jejunum in the treatment groups especially replacing 4% by olive cake with 0.4 g/kg of probiotics. Moreover, 4% OC 0.4 g/kg probiotic was added to the feed of broilers, increased significantly the size of follicles. In conclusion, OC and probiotic positively affected beneficial bacteria in the caecum and lengthen of histology of Villi in jejunum in broilers.

Keywords: *Olive cake, Probiotic; Broiler, Gut Microbiota, Intestinal Histology*

1. Introduction

Considering growing, limited supply and a substantial increase in the costs of feed ingredient, Recent years have seen a lot of research into appropriate alternative sources for poultry feed. Animal nutrition has successfully adopted the use of agroindustrial by-products as an important strategy for reducing feeding costs and dealing with the need to recycle waste, which is expensive to dispose of (Brunetti et al. 2022; Vastolo et al. 2022). Feed costs may represent more than 70% of the overall expenditures associated with broilers producing (Al-Harthi 2017, Saleh et al. 2020). Any decrease in feed prices, which continues to maintain the broilers' health, is connected to have a direct adverse impact on the poultry production profitability. There has been a significant effort exerted to use of alternate and maiatina protein sources in the diets of broilers (Alagawany et al. 2019). As an alternative plant protein in this context, olive cake meal, which has a high amount of NSP (xyloglucan and xylan-xyloglucan complexes) and has a reasonable nutritional value lipids, 13-15%, and proteins, 9-10%, is recommended (Al-Harthi 2016, Al-Harthi 2018).

About 30 to 40 percent of the olive crop is remains after oil is extracted from the olives, which are mostly used to produce olive oil for use in industry and human consumption (Alagawany et al. 2019). This remnant could be utilized in animal feed (7). Stones, pulp, and residual oil are by-products of olive extraction that are high in oleic, linoleic, and linolenic fatty acids. This byproduct includes 5–9% crude protein and 30–40% fiber (Amici et al. 1991; Sadeghi et al. 2009). Large amounts of olive cake meal are typically produced during the extraction of olive oil. Many countries throughout the world produce olive cake meal, which can be used as a plant-based component of broiler feed. Olive cake (OC) has been fed to broiler chickens at a rate of 5–10% without having a negative impact on the broiler's performance, the characteristics of the carcass, or the blood components (Zangeneh and Torki 2011; Zarei et al. 2011).

The term "probiotics" refers to live microbial food additives or bacteria that have a beneficial effect on both human and animal health. Probiotics have a beneficial effect on the health of the host by restoring balance of gut microbiota, boosting antioxidant capacity, minimizing the damage led by pathogenic microorganisms, and improving integrity of epithelial cell and immunological processes (Mirza, 2015). Finding out how different amounts of by-products from OC and probiotic supplements affected the gut flora, intestinal histology, and immunity system of broilers was the main objective of the current study.

2. Materials And Methods

Ethics Approval

This study was approved by the Committees at Animal Resources Department, College of Agricultural Engineering Sciences, Salahaddin University, Erbil under Scientific Ethical process (No.: 1134 on May 31, 2021).

Collecting and Preparation of Olive Cake

Raw olive cake was gathered in a neighborhood olive oil extraction factory in the Kurdistan area of Iraq during 2022. The materials were collected and stored for further analysis throughout the olive extraction season. The remaining olive cake OC materials, including stones and pulp, were brought to the test farm after the oil had been extracted. To allow the solar heat to dry it, the olive cake was spread out over the polyethylene bags and mixed every two hours. Around 45°C Celsius was the temperature that was recorded. For feeding, the olive cake was dried, grinded until powdered. After that, the olive cake was put into polyethylene bags for later usage. Before adding OC to the diets, in the Erbil Feed Company determined its approximate chemical composition it’s shown in Table 1.

Table (1): Olive cake chemical compositions of utilized in the diet.

Contents	Chemical composition
Dry Matter DM %	87,60
Crude Protein CP%	6.37
Crude Ash %	2,88
Crude Fat %	21,08
Crude fiber %	37.92
Metabolic Energy kcal/kg	3700 kcal

Experimental Design

This work was done on specified farm in the Kurdistan Region of Iraq's Garmian Administration during September of 2022 until the finish of the experiment. Three handed and thirty-six chick broiler males (strain Ross 308) were allocated into seven groups at random, four replicates per each, and grown for forty-two days. On a floor, broilers were kept in pens that were measuring (2 x 2) meters in size. The designed diets were formulated in accordance with Aviagen nutritional specification standards (2014) and were iso-nitrogenous, with iso-caloric olive cake OC incorporated in iso-nutritive diets for broilers. From them, seven randomly generated experimental groups were formed,

as follows: a Control group, group received OC 4%, 8%, and 12% replaced, and for three final groups received OC 4%, 8%, and 12% with 0.4 g/kg Iraqi probiotic includes *Bacillus subtilis* 10^9, *Lactobacillus acidophilus* 10^8, *Saccharomyces cerevisiae* 10^9 and *Bifidobacterium* spp. 10^8. Chicks were given free food and water ad libitum. Table 2 shows the composition of the diet used at different broiler life stages.

Table (2): Ingredients of diets that used during the experimental period

Components	Feed composition (kg/100 kg)							
	Starter diet (1-21 days)				Grower diet (22-42 days)			
	T1 Cntrol	T2 4% O.C	T3 8% O.C	T4 12% O.C	T1 Cntrol	T2 4% O.C	T3 8% O.C	T4 12% O.C
Maize.	41.80	39.20	38.20	38.20	44.20	43.10	43.00	43.40
Wheat.	10	10	9	8	12.31	10.80	9	6
Wheat bran.	5	4	2	0	4	3	1.45	0
Soy bean 48%	34.50	34.71	35.21	35.60	29.50	29.71	30.14	31
Soy bean oil	4.51	3.90	3	2	5.11	4.50	3.50	2.80
Limeston	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	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt.	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total.	100	100	100	100	100	100	100	100
	determined values							
ME (kcal/kilogram)	2941.33	2942.60	2948.43	2949.67	3051.11	3054.95	3050.17	3054.44
C.P %	22.03	22.02	22.04	22.01	20.07	20.04	20.02	20.08
Fibre	3.65	4.98	6.33	7.64	3.32	4.73	6.08	7.52
Lysine %.	2.96	2.96	2.95	2.95	2.81	2.82	2.82	2.84
Methionine %.	2.38	2.37	2.37	2.37	2.35	2.4	2.35	2.35
Calcium %.	1.21	1.24	1.27	1.28	1.04	1.07	1.08	1.14
Available .phosphate %.	0.33	0.34	0.35	0.36	0.32	0.33	0.35	0.36

Analysis of the gut microbiota

Four birds were randomly chosen from each treatment at the completion of the experiment, and their caecal digesta were completely aseptically taken to analyse the intestinal microbes (*Lactobacillus* spp. and total coliform bacteria). Consequently, serially diluted between 10^{-1} and 10^{-7}. Targeted bacteria groups were counted by plating 0.1 ml from each dilution onto sterile selective medium agar as follows: (Sigma-Aldrich, UK) MacConkey agar MRS agar for lactic acid bacteria and total coliform, respectively. The colony forming units (CFU) were then calculated from the number of microbial colonies. For fresh caecal digesta, logarithms were used to compute and express CFU/g.

Histology Examination of Jejunum and Bursa of Fabricius

From each groups four birds were selected randomly and killed via cervical dislocation at the end of the experiment. The jejunum part of small intestine and bursa of Fabricius were separated and fixed in 10% buffered formalin for histological examinations. The processing composed of serial dehydration, impregnation with wax and clearing. Tissue sections were done by cutting microtome into five µm thick, and were fixed on slides. A standard routine protocol was carried out using eosin and hematoxylin. A light microscope and digital camera were used to examine the stained slides under a normal light microscope with 10X-magnification pictures. The crypt depth (µm) and villus height of jejunum and area of follicles of Fabricius images were measured by Image J software (Mirza, 2015).

Statistical Analysis

Software using SPSS version 27 was applied to analyze the data using one-way ANOVA analysis (SPSS, 2022). Means and standard error were included in the results of summary statistics. The Duncan's test was applied and assisted in identifying significant differences between the various parameters at 0.05 levels (Duncan, 1955).

3. Results and Discussion

Table 3 illustrates the impact of OC by-product and a probiotic supplementation on the broiler's bacteria counting in the caecum digesta at 42 days of age. The results indicated that inclusion olive cake and combination with probiotic increased beneficial microorganism Lactic Acid Bacteria and decreased total coliform bacteria in the caecum digesta compared with control group. The highest value of LAB count in caecum was recorded at the rate inclusion 4% olive cake with 0.4 gram/kilogram probiotic. According to the results, the olive cake had positive effect on all treatment groups. Researchers found that olive cake may be fed to chickens at a rate of up to 15% without having a negative impact on feed intake (El Hachemi *et al.* 2007). The obtained results are in agreement with studies that found that adding *Bacillus subtilis* probiotics to broiler diets at levels of 0.15%, 0.30%, and 0.45% decreased the number of *clostridium spp*. and coliform counts in the caecum of broiler chicks at 35 days of age compared to the control group (Sen *et al.* 2012). The reduction in pathogenic bacteria in broilers treated with the probiotic *Bacillus amyloliquefaciens* is due to the potential of *Bacillus amyloliquefaciens* to produce lactic acid, bacteriocins, which can inhibit the effect of pathogenic bacteria (Ahmed *et al*. 2014).

Table (3): The influence of OC and probiotic supplementation on Bacteria counting (Log_{10} CFU Ml^{-1}) at 42 days of age in broiler caecum digesta

Treatment	Microbes	
	Lactic Acid Bacteria	Total Coliform Bacteria
Control Group	9.17±0.06 ^{c}	6.78±0.02 ^{a}
Olive cake 4%	9.22±0.02 ^{c}	6.67±0.04 ^{bcd}
Olive cake 8%	9.25±0.03 ^{c}	6.72±0.02 ^{ab}
Olive cake 12%	9.24±0.04 ^{c}	6.70±0.01 ^{abc}
Olive cake 4%+0.4 g/k Pro	9.78±0.03 ^{a}	6.56±0.02 ^{e}
Olive cake 8%+0.4 g/k Pro	9.53±0.03 ^{b}	6.59±0.02 ^{de}
Olive cake 12%+0.4 g/k Pro	9.65±0.03 ^{b}	6.62±0.02 ^{cde}
P. value	<0.001	<0.001

^{a,b,c,d,e} *Differences letters among the groups within the same column are statistically different P<0.05.*

Table 4 illustrates the impact of olive cake by-product and probiotics on the broiler's histology of jejunum part in the small intestine at forty-two days age. The jejunum villus height was statistically (P<0.05) higher in treatment groups and the highest villi recorded at the rate inclusion 4% olive cake with 0.4 gram/kilogram probiotic also the higher crypt depth recorded in T5 and the lower recorded in control group. Also, Figure (1) showed clearly the differences among the groups and the control group at the last stage of the study. The findings of the current study agree with previous studies on broiler chickens, which found that adding olive leaves to the diet at different levels increased the size of the small intestine's villus, villus width, and crypt depth (Shiraze *et al*. 2017). The increase in villi surface area, according to the researchers, is another characteristic that affects the absorptive level in the intestines. (Saki *et al*. 2017). These findings agree with those of researchers who found that *B.subtilis* caused the villus height in the small intestine to increase (Samanya and Yamauchi, 2002). In comparison to the dietary control, the researchers found that dietary supplements of *B. subtilis* increased villus (Sen *et al*. 2012). The Outcomes of the present study recommends that lactic acid bacteria are enhanced by the dietary olive cake by-product with or without probiotics. So, it has essential application for villus length, because long gut villi are deemed healthier.

Table (4): Effect of OC and probiotic on histology of jejunum at 42 days of age (Mean ± Standard Error)

Treatment	Histology of Jejunum	
	Villi height (um)	Crypt Depth (um)
Control Group	657.17± 6.95 ^{e}	91.16±0.86 ^{e}
Olive cake 4%	706.72±6.38 ^{d}	92.18±1.50 ^{de}
Olive cake 8%	755.32±5.56 ^{c}	94.93±1.51 ^{cde}
Olive cake 12%	759.77±5.87 ^{bc}	97.52±1.72 ^{bc}
Olive cake 4%+0.4 g/k Pro	807.96±7.05 ^{a}	103.83±1.98 ^{a}
Olive cake 8%+0.4 g/k Pro	775.94±4.80 ^{b}	96.18±1.75 ^{bcd}
Olive cake 12%+0.4 g/k Pro	770.17±4.30 ^{bc}	100.30±1.68 ^{ab}
P. value	<0.001	< 0.001

^{a,b,c,d} *Differences letters among the groups within the same column are statistically different P<0.05.*

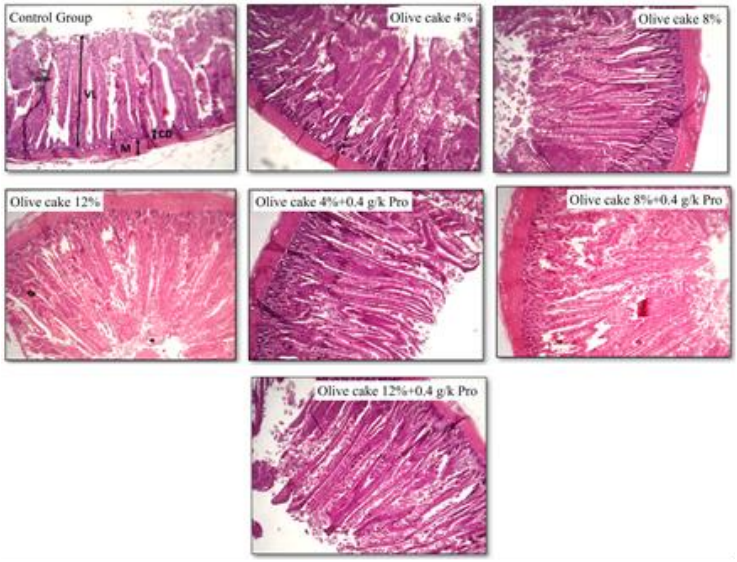

Figure (1). A haematoxylin and eosin stained section of jejunum from chicks fed diets at 42 days of age. The groups including; Control group, olive cake 4%, 8%, and 12% and three final groups olive cake 4%, 8%, and 12% with 0.4 probiotic. L is for Lumina, LP stands for Lamina propria, VL stands for Villus length, CD stands for Crypt depth, and M is stands for Muscular. (Magnification 10X).

Figure 2 shows the Bursa Histology results in birds at 42 days of age. In addition, the effects of seven treatments were detailed in microscopic pictures of tissue sections of the Bursa of Fabricius (Figures 3). When compared to the control diet all treatments increased the size of follicles of Fabricius, while only the rate 4% olive cake with 0.4 g/kg probiotic significantly (P<0.05) increased the size of follicles of Fabricius compared with control group. Additionally, the findings demonstrated that there were not significant differences between Groups 4, 5, 6, and 7 for Fabricius' follicle growth at 42 days of age. An immune system organ called the Bursa of Fabricius is in responsible for B lymphocyte maturation (Alloui *et al*. 2005). Small follicle birds did not generate any measurable antibodies against IBDV or later injected antigen. It was shown that the ability to create Ig responses was connected with the existence of bigger follicles. The entire bursal B-cell growth pathway, however, was unable to be supported by the tiny follicles (Mirza, 2015).

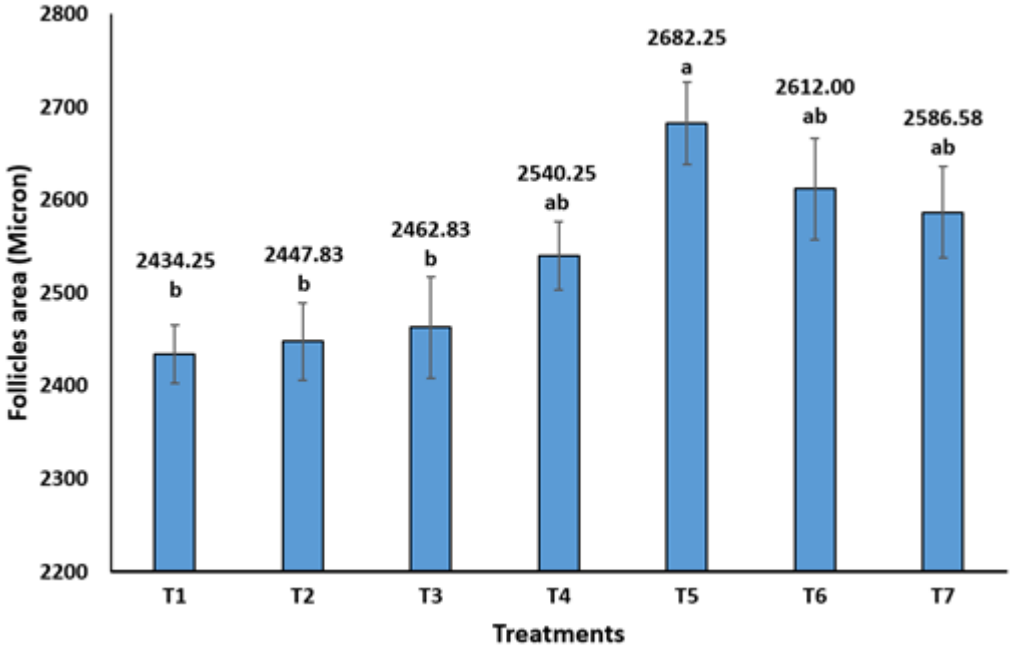

Figure (2). shows the effect of probiotics and olive cake on the follicles area (Micron) of the Bursa of Fabricius after 42 days after the end of the study. The groups including; Control group, olive cake 4%, 8%, and 12% and three final groups olive cake 4%, 8%, and 12% with 0.4 probiotic.

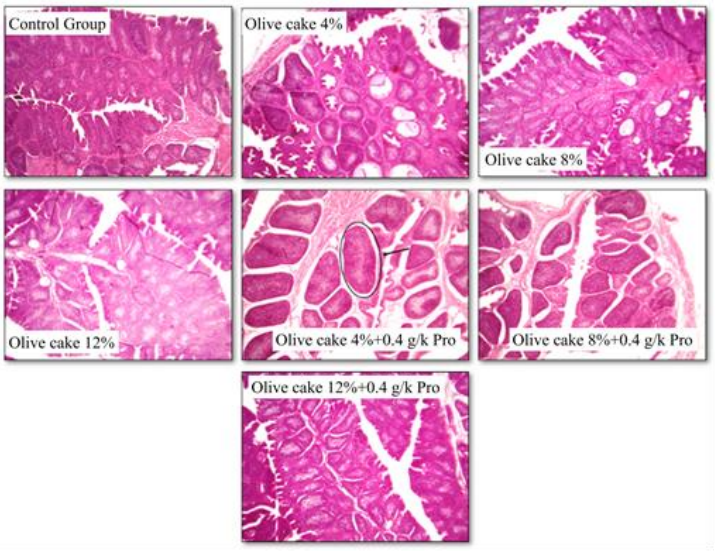

Figure (3). Follicles Bursa of Fabricius section stained with haematoxylin and eosin at the age of 42 days for chicks fed diets including olive cake with 0.4 probiotic supplementation. The groups including; Control group, olive cake 4%, 8%, and 12% and three final groups olive cake 4%, 8%, and 12% with 0.4 probiotic (10X Magnification).

4. Conclusion

The results show that feeding olive cake as a byproduct improved beneficial bacteria and decreased harmful total coliform bacteria in the small intestine, particularly at the rate of 4% olive cake combined with 0.4 gram/killogram probiotic. Also, these findings that increased the high of the villi and crypt depth in the Jejunum part of the small intestine which can lead to increase surface area of absorption of foods in the small intestine also increased the size of follicles of Fabricius.

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