

RESEARCH ARTICLE

Effect of Solid-State Fermentation on Olive Byproducts on Performance, Beneficial Microorganisms, and Expression of Digestive Tract of Broiler Chickens

Ahmed M. Abd-El-Rahman^{1*}, Mohamed E. Badawi², Doaa I. Mohamed², and Wafaa A. El-Eraky²

¹CEO Key Vet Cooperation (KVC), Zagazig, B. V. Sc., Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

²Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

*Corresponding author; Email: keyvetcooperation@gmail.com

ARTICLE INFO

Article History:

Received:

Accepted:

Published online:

Key words:

Broilers, Microbial fermentation, Performance, Gene expression.

ABSTRACT

The application of dried olive byproducts as supplementary feed sources in poultry feed remains constrained owing to their nutritional value and high contents of fiber. Solid-state fermented olive byproducts with addition of exogenous enzymes; is being investigated as an approach to improve its nutritional value. The consequences olive byproducts treated with fermentation with enzymes on growth performance; modification of genes encoding secretion of digestive enzymes, beneficial microorganisms of broiler chickens. A total of 72 one-day-old broiler chicks (Ross 308) were divided into four dietary groups, with three replicates of six birds per replicate. Dietary treatments were divided as follows: the control group while other treatments were basal diet with inclusion rate of fermented olive pulp (FOP) by three levels (5, 10, and 15%) of fermented olive byproducts for 42 days. The group fed 10 and 15% of FOB showed the highest body weight gain and the most improved feed conversion rate (FCR). Moreover, the expression of AMY2A, and PNLIP genes was significantly upregulated ($p < 0.05$) by elevating levels of FOB when compared with the control. Notably, the beneficial probiotics bacteria including lactobacilli was significantly increased and reached its peak load in FOBIII supplemented group. The findings of the current study implied that dietary incorporation of 15% FOB improved growth performance attributes, directed cecal microbes toward the beneficial one long with upregulation of digestive enzymes encoding genes.

Introduction

Among the agro-industrial byproducts that provide chicken farms with an environmentally friendly substitute feed source are olive byproducts. Simple phenolic and other useful chemicals are thought to be abundant in olive byproducts (OB), polyphenols, oleuropein, and flavonoids, which can improve animal health and performance. Olive byproducts are regarded as an affordable alternative energy source due to its In addition to their high oil content, olive byproducts are also a major source of polyunsaturated fatty acids (PUFAs) [1]. Likewise, birds can consume dried olive byproducts more efficiently in older age [2]. This is explained by the high fiber content that includes non-starch polysaccharides [3] that restricted its use in broiler diets, especially when the animals were young and their digestive tracts were still developing. [4]. Fermentation may improve the nutritional value of chicken feeds by lowering crude fiber and raising crude protein concentrations [5, 6], removing harmful substances and other anti-nutritional elements from feed ingredients [7]. Additionally, adding exogenous microbial enzymes to poultry diets during feeding facilitates the digestion of fiber and breaks down phytic phosphorus (via phytase). [8]. Also, since these enzymes function best in a pH range of 4 to 6, their activity is primarily limited to the crop, proventriculus, and gizzard. [9, 10]. Furthermore, it improves the feed palatability [11, 12], enhance growth performance, immunological resistance, and beneficial gut microbiota in chicken farms. [13]. The nature of broilers [14] may regulate intestinal enterocytes' transporter proteins and digestive enzymes to aid in the digestion and absorption of dietary nutrients [15, 16]. Therefore, the expression of genes

encoding digestive enzymes (intestine GLUT-2, lipase, and pancreatic amylase) after consuming fermented olive byproducts and cecal microbes may mimic its ability to enhance broiler development performance. Hence, this study is aimed to investigate the effect of fermented olive pulp on growth performance, modification of genes encoding secretion of digestive enzymes, and beneficial microorganisms of broiler chickens.

Materials and Methods

Preparation of olive byproducts by solid fermentation with enzymatic treatment

Bacillus licheniformis was used in this experiment to ferment the olive byproducts, *Aspergillus oryzae* (PTCC5163) and *Lactobacillus casei*, besides, commercial exogenous enzymes at the level 50 g/ton (HOSTAZYME-X), beta xylanase and beta-glucanase were introduced. After fermentation, the fermented olives by product were dried for two days at 50°C. Other feed components were combined with ground dried samples to form the diets.

Study animals

Seventy-two one day old male Ross-308 broiler chicks were weighed to be 46.00 ± 0.388 g when arrived. The chicks were raised using sawdust as litter in an open, naturally ventilated housing. The lighting, relative humidity, and room temperature were examined in compliance with the Ross-308 poultry management guidelines [15]. The Institutional Ethics Committees of the Nutrition, Clinical Nutrition, and Animal Wealth departments of the Faculty of Veterinary Medicine, Zagazig University, Egypt, approved the publication, "The Guide for the Care and Use of Laboratory Animals in Scientific

Investigations." All animal experiments were conducted in accordance with the guidelines described in the publication.

Experimental design and diets

Four groups of eighteen experimental chicks (Ross 308, Dakahlia company, Egypt), each consisting of three replicates and six birds, were assigned to the groups. A diet supplemented with 5%, 10%, and 15% fermented olive byproducts (FOB) or a control corn-soybean diet were given to the treatment experimental groups. The duration of the experiment was 42 days. Every chick had unrestricted access to food and water. The mash-based experimental meals were prepared in accordance with the Ross Manual's recommendations [15] as shown in Table 1. Standard techniques described by Association of Official Agricultural Chemists [16] were used to perform proximate analysis of a variety of nutrients in feed ingredients and diets, such as ether extract (EE), crude protein (CP), crude fiber (CF), and dry matter (DM).

Growth performance and nutrient digestibility:

Average body weight (BW) and total feed consumption were measured at the starter and grower - finisher stages. Then, for every phase and the entire 42-day growing period, the feed conversion ratio (FCR) and body weight BW increases were computed. The protein efficiency ratio (PER), which was computed from the total protein intake, was obtained by dividing the weight gain (g) by protein intake (g).

Sampling and analytical procedures

At day 42, the birds were chosen at random and put down by cervical dislocation. After that, birds were de-feathered, eviscerated, cecal samples were collected. Small tissue samples ($n =$

3/replicate) were collected from the pancreas and duodenum, cleansed with phosphate-buffered saline, and kept in Eppendorf cap-lock tubes at -80°C in preparation for RNA extraction.

mRNA extraction and reverse transcription polymerase chain reaction (RT-PCR) to analyze gene expression

RNA isolation was performed using the QIAamp RNeasy Mini kit (Qiagen GmbH, Hilden, Germany). Duodenal and pancreatic samples were kept in Eppendorf tubes after being snap-frozen in liquid nitrogen. The amount of RNA at an optical density of 260 nm was measured using a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

The 25- μL reaction mixtures used for SYBR Green RT-PCR amplification contained 0.25 μL of RevertAid reverse transcriptase (Thermo Fisher Scientific, Germany), 12.5 μL of 2x QuantiTect SYBR Green PCR master mix (Qiagen), 0.50 μL of each primer, 8.25 μL of RNase-free water, and 3 μL of the RNA template. Real-time PCR was amplified using the Rotor-Gene Q2 Plex (Qiagen Inc., Valencia, CA, USA). The primer sequences for the glucose transporter-2 (SLC2A2), lipase (PNLIP), sodium-dependent glucose cotransporters (SGLT-1), and pancreatic alpha-2A amylase genes (AMY2A) were provided in Table 2. The target genes' expression levels were brought back to normal by using GAPDH as an internal reference.

Bacteriological assay

At the end of the experiment; the spread plate technique was used to quantify specific bacteria in the cecal contents. Sterile saline used to serially dilute one gram of cecal material 10 times. Lactobacilli were counted using De Man, Rogosa, and Sharpe's (MRS, CM1153,

Oxoid, UK) agar medium. Violet-red bile glucose agar (VRBG, CM485, Oxoid) was utilized to count *E. coli*. On Rogosa agar (Oxoid, UK) plates, total *lactobacillus* counts were obtained after three days of anaerobic incubation at 37 °C. Log¹⁰ colony forming units (CFU)/g of the cecal contents is the average result of the duplicate data.

Statistical Analysis

Levene's test was used to validate homogeneity among experimental groups and Shapiro-Wilk's test was performed to confirm the normality, the experimental data was evaluated using SPSS's general linear model (GLM) technique (SPSS Inc., Chicago, Illinois, USA). The data deviation was expressed using the standard error of the mean (SEM), and significant differences between mean values were assessed using Tukey's test 0.05 was chosen as the predicted significance threshold. Relative fold changes in target gene expression were determined using the 2^{-ΔΔCt} method [17].

Results

Growth Performance

Growth performance parameters of broilers are presented in Tables 3 and 4. In contrast to the control diet of corn and soybeans, during the initial phase, replacing a control with 5%, 10%, and 15% fermented olive byproducts FOB harbor had no influence on body weight and body weight gain (BWG), however, replacing 15% and 25% FFB decreased BWG ($p < 0.05$) feed index FI and feed conversion rate FCR. In comparison to the control treatment, broilers fed 5%, 10%, and 15% FOB displayed higher ($p < 0.05$) BWG during the grower-finisher period. Additionally, the 10% fermented olive byproducts FOB group experienced a significant ($p < 0.05$) drop in feed intake

FI during the starter stage, but the dietary inclusion of 5%, 10%, and 15% fermented olive byproducts FOB harbor no influence on cumulative feed intake during the grower-finisher period (Table 5). During stater period and grower-finisher stage, Table 5 shows that broilers given 5% and 10% fermented olive byproducts FOB-substituted diets had a considerably higher FCR ($p < 0.05$). The group fed 10% fermented olive byproducts FOB had the lowest FCR (Table 6) and the most significant body weight gain BWG, according to the overall performance findings.

Cecal Microbes

The effect of diets containing fermented olive pulp (FOP) on broiler chickens' cecal bacteria (Log₁₀ CFU/g fresh digesta) is displayed in Table 7. The broiler fed diets containing 15% fermented olive pulp FOP showed the highest significant value ($P < 0.05$) of total microbes while, there was no significant effect ($P > 0.05$) in comparison with other groups and the control one. The broiler fed diets containing 10% and 15 % fermented olive byproducts FOB showed the lowest significant value ($P < 0.05$) of *E. coli* in comparison with the control one. On contrast, the highly significant ($P < 0.05$) increase ($P > 0.05$) in *lactobacillus* species as found in group fed on diets containing 10 and 15% FOB in comparison with control group.

Expression of digestive related genes in response to feeding on olive byproducts

Effect of diets containing fermented olive pulp FOP on broiler chicks' relative mRNA expression is displayed in Figure 1. The highest relative mRNA expression increase ($P < 0.05$) for (GLUT2/ GAPDH) gene was noted in the group fed diets containing 15% when compared to control one Figure 1A. Broilers group fed diets containing 15% followed by groups fed 5

and 10 of fermented olive pulp FOP had the highly relative mRNA expression in the genes for lipase/GAPDH and amylase/GAPDH increased significantly ($P<0.05$), as demonstrated in Figure 1B and C, respectively.

Discussion

Crude fiber is difficult or impossible to be digested and therefore has a 'poor reputation' in poultry nutrition. Treating fermented olives by product (FOB). Therefore, treating fermented olive by product FOB with microbial fermentation in conjunction with commercial exogenous enzymes can be a useful strategy for improving the nutritional value of fermented olive by product FOB. In the current study, groups given fermented olive by product FOP up to 15% were thought to have better broiler chicken development performance. These outcomes align with the conclusions of [18, 19] which demonstrated that dietary inclusion of feed treated with fermentation (Bactocell as starter inoculum) increased the broiler chickens' weight gain and feed consumption. Additionally, broiler chicken growth performance and intestinal health are positively impacted by microbial fermentation [20]. Furthermore, a high concentration of advantageous bacteria with probiotic effects on the gastrointestinal system can be obtained by fermenting an item [21]. Fermented foods can improve nutrient absorption and digestion, which will improve the birds' growth performance [22, 23]. Similarly, adding mixture of enzymes such as pectinase, glucanase, and xylanase through fermentation process decreased rapeseed cakes' NSP by 30% to 45%. Our results support the idea that *B. subtilis*-based microbial fermentation can improve the feed palatability [24], produce digestive enzymes (lipases, proteases, and amylases) to break down complex plant

carbohydrates, thereby stimulating nutrient digestion and absorption. Furthermore, it creates active complexes (bacitracin, gramicidin, nystatin, and polymyxin) that prevent the endogenous pathogens propagation [25]. The superior performance of broiler chicks in the fermented olive pulp FOP-fed groups over control one could be attributed to the functional metabolites generated during microbial fermentation. Furthermore, the microbial enzymes generated during fermentation become more active when fibro lytic foreign enzymes are added.

Additionally, decreasing PH of digestive tract inhibit the activity of pathogenic bacteria [26]. Additionally, the increased concentration of organic acids brought on by fermentation may be connected to the decreased number of enteric harmful bacteria, including *E. coli*, after feeding higher quantities of fermented olive by product FOB. This made it easier for LAB to grow and proliferate, which lowered stomach pH and stopped the growth of pathogens [27, 28]. A microbially fermented meal was shown to boost the quantity of good bacteria and prevent the growth of harmful ones. Furthermore, more favorable bacteria taxa develop in the intestine when the environment is more acidic. Lastly, by influencing the proliferation of both pathogenic and non-pathogenic bacteria in the broilers' digestive system, fermentation improves feed nutritional efficiency and accelerates growth [29].

On the other hand, fermentation can enhance the digestibility and nutritive value of unconventional feed stuff [30]. Digestive enzymes play a crucial function in breaking down the particles of feed stuff, both of which are essential for the growth and overall health of birds. Promoting expression of digestive enzymes linked genes can boost digestive enzymes activities and feed utilization in

poultry [31]. Likewise, boosting digestive enzymes secretion (lipases, amylases, and trypsin) next to feeding on fermented feeds are blamed to increasing the bird's growth rate [32]. In this case, GLUT2 was upregulated in groups given varying amounts of fermented olive pulp FOP, thus, dietary inclusion of poultry enzymes can increase intestinal GLUT2 expression and speed up the absorption of micronutrients [33]. Additionally, broilers fed cottonseed meal fermented with *B. subtilis* showed increased activity of the enzyme's amylase and protease [34]. This was due to *B. subtilis*'s contribution to the production of these enzymes. Similarly, the addition of xylanase significantly increased GLUT2 expression after eating, which may indicate improved absorption in birds [35]. In accordance, [36] discovered that feeding Ross broiler chicks microbially fermented dry brewer's

grains enhanced the production of genes for the pancreatic enzymes lipase, protease, and amylase. Additionally, after consuming fermented soybeans, birds' pancreatic enzyme activity increased [37].

Conclusion

Microbial fermentation is a cutting-edge processing technique that can improve the nutritional content and use of unconventional feed resources, such olive by-products. According to this study, broiler chicks fed fermented olive byproducts FOB have better development performance because their digestive systems are functioning better. These results boost the chicken feed industry's recommendation of fermented olive byproducts FOB as a nutrient-dense non-traditional feed ingredient, hence eroding faith in the conventional ones.

Tables

Table 1. Ingredients of fed stuff and the chemical composition (%) of the experimental diets used in the study stages for Broiler Chickens

Ingredients	Experimental diets							
	Starter				Grower-Finisher			
	Control		Fermented olive pulp		Control		Fermented olive pulp	
	0%	5%	10%	15%	0%	5%	10%	15%
Yellow corn	52.70	50.25	45.70	40.20	57.90	52.70	47.15	45.11
Soybean meal, 46%	35.00	28.00	26.70	27.25	32.85	31.65	32.20	29.06
Fermented olive byproduct*	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Corn gluten, 60%	5.17	9.75	10.35	9.80	2.00	2.60	2.10	3.75
Soybean oil	2.35	2.20	2.50	3.00	3.00	3.90	4.50	3.00
Calcium carbonate	0.75	0.60	0.50	0.50	0.75	0.60	0.50	0.50
Calcium dibasic phosphate	2.60	2.60	2.60	2.60	2.15	2.15	2.15	2.15
Common salt	0.30	0.30	0.30	0.30	0.30	0.25	0.30	0.29
Sodium bicarbonate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.20
Premix ¹	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine HCL, 78%	0.25	0.45	0.50	0.50	0.15	0.20	0.20	0.30
DL-Methionine, 98%	0.13	0.10	0.10	0.10	0.15	0.20	0.15	0.14
Choline chloride, 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated composition								
ME, Kcal/Kg	3.05	3.05	3.01	3.00	3.10	3.10	3.05	3.05
CP, %	23.01	23.01	23.00	23.00	20.51	20.51	20.52	20.50
EE, %	4.99	5.01	5.40	5.95	5.71	6.67	7.32	6.00
CF, %	3.33	3.66	4.24	4.90	3.27	3.84	4.50	5.03
Ca, %	1.01	1.00	1.01	1.07	0.91	0.91	0.93	0.97
Available phosphorus, %	0.45	0.45	0.45	0.45	0.38	0.38	0.38	0.38
Lysine, %	1.46	1.45	1.45	1.45	1.30	1.30	1.30	1.30
Methionine, %	0.54	0.55	0.56	0.55	0.50	0.55	0.50	0.50

* premix : Each 3 kg contains the Following vitamins and minerals: Vitamin A (10,600,000 IU), Vitamin D3 (2,650,000 IU), Vitamin E (30,000 IU), Vitamin K3 (1.990 mg), Vitamin B1 (1,060 mg), Vitamin B2 (6.8 g), Vitamin B6 (1.5 g), Pantothenic acid (10.2 g), Vitamin B12 (13 mg), Niacine (30.5 g), Folic acid (1,030 mg), Biotin (50 g), Fe (40 g), Mn (70 g), Cu (8.8 g), I (1,620 mg), Co (252 mg), Se (410 mg), and Zn (51.6 g).

* Fermented olive byproduct was analyzed (Moisture, 11%, crude protein, 10 %, Ether extract, 5.20 %, crude fiber, 15%, Calcium, 1.10 %, Available phosphorus, 0.04 %, lysine, 0.10 %, methionine, 0.16 %).

Table 2. The Primers Sequences of enzymatic genes applied for quantitative real-time PCR Broiler Chickens.

Genes	Gene full name	Primer sequence (5'-3')	Accession no
AMY2A	Pancreatic alpha 2A amylase	F-CGGAGTG↓GATGTTAACGACTGG R-ATGTTCCGACACCCAGTCATTG	NM_001001473.2
PNLIP	Pancreatic lipase	F-GCATCTGGGAAG↓GAACTAGGG R- TGAACCACAAGCATAGCCCA	NM_001277382.1

GLUT2	Glucose transporter-2 (SLC2A2)	F-TGATCGTGGCACTGATGGTT R-CCACCAGGAAGAC↓GGAGATA	NM_207178.1
GAPDH	Glyceraldehyde - 3-phosphate dehydrogenase	F-GGTGGTGCTAAGCGTGTTA R-CCCTCCACAATGCCAA	NM205518

AMY2A : Pancreatic alpha-amylase, *PNLIP*: Pancreatic Lipase, *GLUT2*: glucose transporter 2, *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase

Table 3. Body weight (g) of broiler chickens fed diets containing fermented olive byproducts (means \pm SE) at end of starter and grower-finisher periods.

Age (week)	Experimental diets			
	Fermented olive byproducts			
	Control	5%	10%	15%
Starter (0-21 day)	1106 \pm 22.10 ^a	1067 \pm 3.76 ^a	1056 \pm 22.10 ^{ab}	992 \pm 13.3 ^b
Grower-finisher (22-42 day)	2506 \pm 13.61 ^{ab}	2442 \pm 15.85 ^b	2270 \pm 25.36 ^a	2253 \pm 20.67 ^{ab}

^{a-b} Mean significances with altered letters in the similar column vary statically at $P < 0.05$.

Table 4. Body weight gain (g) of broiler chickens fed diets containing fermented olive pulp (means \pm SE) during starter and grower-finisher periods

Age (week)	Experimental diets			
	Fermented olive byproducts			
	Control	5 %	10 %	15 %
Starter (0-21 day)	878 \pm 11.53	993 \pm 22.26	954 \pm 9.55	900 \pm 5.77
Grower-finisher (21-42 day)	1183 \pm 61.58 ^c	1400 \pm 25.46 ^a	1374 \pm 19.78 ^{ab}	1215 \pm 83.21 ^b

^{a-c} Mean significances with altered letters in the similar column vary statically at $P < 0.05$.

Table 5. Cumulative feed consumption (g/bird) of broiler chickens fed diets containing varying levels of fermented olive byproducts (means \pm SE) during starter and grower-finisher periods.

Experimental diets				
Fermented olive byproducts				
Age (week)	Control	5 %	10 %	15 %
Starter (0-21 day)	1348 \pm 5.54 ^a	1338 \pm 2.51 ^a	1279 \pm 2.08 ^b	1315 \pm 4.58 ^a
Grower-finisher (21-42 day)	2841 \pm 42.77 ^a	2854 \pm 34.46 ^a	2860 \pm 27.20 ^a	2766 \pm 24.58 ^{ab}

^{a-b} Mean significances with altered letters in the similar column vary statically at $P < 0.05$.

Table 6. Feed conversion ratio (FCR) of broiler chickens fed diets containing varying levels of fermented olive byproducts (means \pm SE) during starter and grower-finisher periods.

Experimental diets				
Fermented olive byproducts				
Age (week)	Control	5 %	10 %	15 %
Starter (0-21 day)	1.53 \pm 0.02 ^a	1.35 \pm 0.03 ^b	1.34 \pm 0.01 ^b	1.46 \pm 0.02 ^a
Grower-finisher (21-42 day)	2.50 \pm 0.10 ^a	1.97 \pm 0.06 ^c	2.02 \pm 0.04 ^c	2.22 \pm 0.02 ^b

^{a-c} Mean significances with altered letters in the similar column vary statically at $P < 0.05$.

Table 7. Cecal microorganisms (Log¹⁰ cfu/g fresh digesta) of broiler chickens fed diets containing varying levels of fermented olive byproducts (means \pm SE).

Experimental diets				
Fermented olive byproducts				
	Control	5 %	10 %	15 %
Total microbes	6.13 \pm 0.09 ^b	6.25 \pm 0.06 ^b	7.15 \pm 0.03 ^b	7.35 \pm 0.03 ^{ab}
<i>Escherichia coli</i>	5.27 \pm 0.16 ^a	5.23 \pm 0.09 ^a	4.97 \pm 0.03 ^b	4.33 \pm 0.07 ^b
<i>Lactobacillus</i> spp.	6.20 \pm 0.26 ^c	6.23 \pm 0.27 ^c	6.50 \pm 0.08 ^b	6.67 \pm 0.06 ^b

^{a-c}Mean values with different letters in the same column differ significantly at $P < 0.05$.

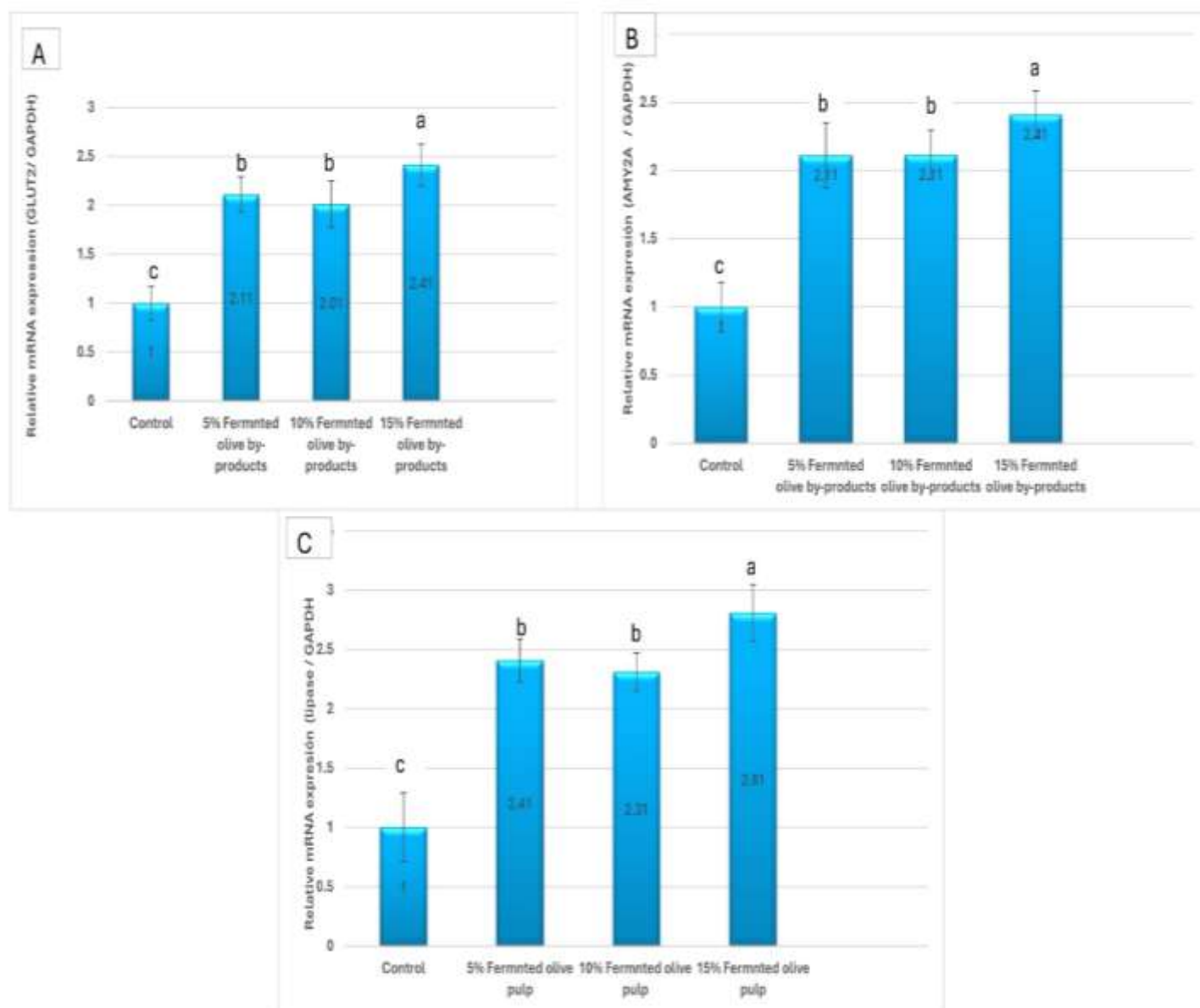


Figure 1. Influence of replacement of control diet with fermented olive byproducts on the mRNA expression of *GLUT-2*, **A** and pancreatic alpha 2A amylase (**B**, *AMY2A*), and lipase (**C**, *PNLIP*) genes in duodenum. 5% fermented olive pulp: birds fed microbially fermented olive pulp at the level of 5%, 10% fermented olive pulp: birds fed microbially fermented olive pulp at the level of 10%, 15% fermented olive pulp: birds fed microbially fermented olive pulp at the level of 15%.

References:

- [1] Donohue, M. and Cunningham, D. (2009): Effects of grain and oilseed prices on the costs of US poultry production. *J. Appl. Poult. Res.* 18, 325–337.
- [2] Molina-Alcaide, E.; Yáñez-Ruiz, D.R.; (2008): Potential use of olive by-products in ruminant feeding: A review. *Anim. Feed Sci. Technol.* 147, 247–264.
- [3] Papadomichelakis, G.; Pappas, A.; Tsiplakou, E.; Symeon, G.; Sotirakoglou, K.; Mpekis, V.; Fegeros, K. and Zervas, G. (2019): Effects of dietary dried olive pulp inclusion on growth performance and meat quality of broiler chickens. *Livest. Sci.* 221, 115–122.
- [4] Sayehban, P.; Seidavi, A.; Dadashbeiki, M.; Ghorbani, A.; Araújo, W. and Albino, L. (2016): Effects of different levels of two types of olive pulp with or without exogenous enzyme supplementation on broiler performance and economic parameters. *Braz. J. Poult. Sci.* 18, 489–500.
- [5] Noy, Y. and Uni, Z. (2010): Early nutritional strategies. *Worlds Poult. Sci. J.* 66, 639–646.
- [6] Khempaka, S.; Thongkratok, R.; Okrathok, S. and Molee, W. (2013): An evaluation of cassava pulp feedstuff fermented with *A. oryzae*, on growth performance, nutrient digestibility and carcass quality of broilers. *Poult. Sci.* 0130022.
- [7] Chiang, G.; Lu, W.; Piao, X.; Hu, J.; Gong, L. and Thacker, P. (2009): Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. *Asian-Australas J Anim Sci.* 23, 263–271.
- [8] Choct, M. (2006): Enzymes for the feed industry: past, present and future. *Worlds Poult. Sci. J.* 62, 5–16.
- [9] Svihus, B. (2011): The gizzard: function, influence of diet structure and effects on nutrient availability. *Worlds Poult. Sci. J.* 67, 207–224.
- [10] Wang, Y. and Gu, Q. (2010): Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. *Res. Vet. Sci.* 89, 163–167.
- [11] Sugiharto, S.; Yudiarti, T. and Isroli, I. (2016): Performances and haematological profile of broilers fed fermented dried cassava (*Manihot esculenta* Crantz). *Trop. Anim. Health Prod.* 48, 1337–1341.
- [12] Kheravii, S.; Morgan, N.; Swick, R.A.; Choct, M. and Wu, S.B. (2018a): Roles of dietary fibre and ingredient particle size in broiler nutrition. *Worlds Poult. Sci. J.* 74, 301–316.
- [13] Gilbert, E.; Li, H.; Emmerson, D.; Webb J.K. and Wong; E. (2007): Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. *Poult. Sci.* 86, 1739–1753.
- [14] Aviagen, W. (2018). Ross 308: broiler's management and nutrition specification.
- [15] AOAC (2006): Official Methods of Analysis. 18th ed AOAC Int, Gaithersburg, MD.
- [16] Livak, K.J. and Schmittgen, T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ ΔΔCT method. *Methods.* 25, 402–408.
- [17] Gilani, S.; Howarth, G.; Natrass, G.; Kitessa, S.; Barekatin, R.; Forder, R.; Tran, C. and Hughes, R. (2018): Gene expression and morphological changes in the intestinal mucosa associated with increased permeability induced by short-term fasting in chickens. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 102, e653–e661.
- [18] Kheravii, S.; Swick, R.A.; Choct, M. and Wu, S.B. (2018b): Upregulation of genes encoding digestive enzymes and nutrient transporters in the digestive system of broiler chickens by dietary supplementation of fiber and inclusion of coarse particle size corn. *BMC Genomics.* 19, 208.
- [19] Uchewa, E. and Onu, P. (2012): The effect of feed wetting and fermentation on the performance of broiler chick. *J Anim Sci. Biotechnol.* 28, 433–439.
- [20] Alshelmani, M.I.; Loh, T.C.; Foo, H.L.; Sazili, A.Q. and Lau, W.H. (2017): Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of palm kernel cake in broiler chickens. *Indian J. Anim. Sci.* 87, 1135–1140.
- [21] Ibrahim, D.; Abdelfattah-Hassan, A.; Arisha, A.H.; Abd El-Aziz, R.M.; Sherief, W.R.; Adil, S.H.; El Sayed, R. and Metwally, A.E. (2020): Impact of feeding anaerobically fermented feed supplemented with acidifiers on its quality and growth performance, intestinal villi and enteric pathogens of mulard ducks. *Livest. Sci.* 242, 104299.
- [22] Chiang, G.; Lu, W.; Piao, X.; Hu, J.; Gong, L. and Thacker, P. (2009): Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. *Asian-Australas J Anim Sci.* 23, 263–271.
- [23] Missotten, J.; Michiels, J.; Dierick, N.; Oryn, A.; Akbarian, A. and De Smet, S. (2013): Effect of fermented moist feed on performance, gut

- bacteria and gut histo-morphology in broilers. *Br. Poult. Sci.* 54, 627–634.
- [24] Gao, Z.; Wu, H.; Shi, L.; Zhang, X.; Sheng, R.; Yin, F. and Gooneratne, R. (2017): Study of *Bacillus subtilis* on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d broiler chickens. *Animal Nutrition.* 3, 109–113.
- [25] Li, W.; Bai, J.; Li, Y.; Qin, Y.; Yu, D.; (2014): Effects of *Bacillus subtilis* on meat quality, nutrient digestibility and serum biochemical parameters of broilers. *Chin J Vet Sci.* 34, 1682–1685.
- [26] Mathivanan, R.; Selvaraj, P. and Nanjappan, K.; (2006): Feeding of fermented soybean meal on broiler performance. *International Journal of Poultry Science.* 5, 868–872.
- [27] Jazi, V.; Boldaji, F.; Dastar, B.; Hashemi, S. and Ashayerizadeh, A. (2017): Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. *Br. Poult. Sci.* 58, 402–408.
- [28] Yin, Q.Q.; Fan, G.G.; Chang, J.; Zuo, R.Y.; Zheng, Q.H.; (2012): Effect of the combined probiotics on inhibiting pathogenic *Escherichia coli* proliferation. *Advanced Materials Research. Trans Tech Publ*, pp. 802–808.
- [29] Pacheco, W.; Stark, C.; Ferket, P. and Brake, J. (2014): Effects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreas. *Poult. Sci.* 93, 2245–2252.
- [30] Canibe, N. and Jensen, B.B. (2012): Fermented liquid feed-Microbial and nutritional aspects and impact on enteric diseases in pigs. *Anim. Feed Sci. Technol.* 173, 17–40.
- [31] Ibrahim, D.; Abdelfattah-Hassan, A.; Badawi, M.; Ismail, T.A.; Bendary, M.M.; Abdelaziz, A.M.; Mosbah, R.A.; Mohamed, D.I.; Arisha, A.H. and El-Hamid, M.I.A. (2021): Thymol nanoemulsion promoted broiler chicken's growth, gastrointestinal barrier and bacterial community and conferred protection against *Salmonella Typhimurium*. *Sci. Rep.* 11, 7742.
- [32] Feng, J.; Liu, X.; Xu, Z.; Wang, Y. and Liu, J. (2007): Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. *Poult. Sci.* 86, 1149–1154.
- [33] Saleh, A.A.; Ali, H.; Abdel-Latif, M.A.; Emam, M.A.; Ghanem, R. and El-Hamid, H.S.A. (2018): Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PLoS One.* 13.
- [34] Sun, H.; Tang, J.-w.; Yao, X.-h.; Wu, Y.-f.; Wang, X. and Feng, J. (2013): Effects of dietary inclusion of fermented cottonseed meal on growth, cecal microbial population, small intestinal morphology, and digestive enzyme activity of broilers. *Trop. Anim. Health Prod.* 45, 987–993.
- [35] Lee, S.A.; Wiseman, J.; Masey O'Neill, H.V.; Scholey, D.V.; Burton, E.J. and Hill, S.E. (2017): Understanding the direct and indirect mechanisms of xylanase action on starch digestion in broilers. *Journal of World's Poultry Research.* 7, 35–47.
- [36] Al-Khalaifah, H.S.; Shahin, S.E., Omar, A.E.; Mohammed, H.A.; Mahmoud, H.I. and Ibrahim, D. (2020): Effects of graded levels of microbial fermented or enzymatically treated dried brewer's grains on growth, digestive and nutrient transporter genes expression and cost effectiveness in broiler chickens. *BMC Vet Res.* 16, 115.
- [37] Soumeh, E.; Mohebodini, H.; Toghyani, M.; Shabani, A.; Ashayerizadeh, A. and Jazi, V. (2019): Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. *Poult. Sci.* 98, 6797–6807.

الملخص العربي

تأثير مخلفات الزيتون المخمرة والبكتريا النافعة على الأداء والتعبير الجيني للقناة الهضمية في بدارى التسمين

أحمد محمد عبد الرحمن¹, محمد السيد بدوي², دعاء ابراهيم محمد², وفاء العراقي²

¹المدير التنفيذي لشركة كي فيت كوبريشن، الزقازيق، بكالوريوس العلوم الطبية البيطرية، جامعة الزقازيق، 44511، مصر

²قسم التغذية والتغذية الاكلينيكية، كلية الطب البيطري، جامعة الزقازيق، 44511، مصر

لا يزال استخدام منتجات تفلّة الزيتون المخمرة كمصدر تغذية تكميلي في علف الدواجن مقيّدًا بسبب قيمتها الغذائية ومحتواها العالي من الألياف حيث يتم التحقق من مدى الاستفادة من منتجات تفلّة الزيتون المخمرة بعد تجفيفها جيدًا مع إضافة إنزيمات خارجية، كنهج لتحسين قيمتها الغذائية ومدى تأثيرها على نمو الطائر، وتعديل الجينات المسؤولة عن إفراز الإنزيمات الهضمية، والكائنات الحية الدقيقة المفيدة لدجاج التسمين. تم تقسيم 72 طائرا من دجاج التسمين بعمر يوم واحد (روص 308) إلى أربع مجموعات غذائية، مع ثلاث مكررات من ستة طيور لكل تكرار. وقسمت المجموعات الغذائية على النحو التالي: مجموعة ضابطة، بينما تم تقسيم الثلاث مجموعات الأخرى بثلاثة مستويات (5 و10 و15%) من منتجات تفلّة الزيتون المخمرة لمدة 42 يوما لمتابعة أعلى زيادة في وزن الجسم وأعلى معدل نمو في المجاميع المعالجة. أظهرت المجموعة التي تغذت على 10 و 15% من تفلّة الزيتون المخمرة أعلى زيادة في وزن الجسم وأعلى زيادة في معدل التحويل. علاوة على ذلك، تم رفع مستوى التعبير عن الجينات AMY2A و PNLIP بشكل ملحوظ عن طريق رفع مستويات المقارنة مع المجموعة الضابطة. والجدير بالذكر أن البكتيريا الحيوية المفيدة بما في ذلك اللاكتوباسيليس زادت بشكل ملحوظ ووصلت المجموعة التي تغذت على 15% تفلّة زيتون مخمرة الى زروتها في النظام الغذائي. وتشير الدراسة الحالية أن أعلى معدل نمو وأعلى زيادة في معدل التحويل كانت في المجموعة التي تغذت على 15 % تفلّة زيتون مخمرة.