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Original

Exploring the Relationship between Olive Cake Meal, Bacillus licheniformis, and Broiler Chicken Growth, Muscle Fatty Acid Composition, and Health Parameters

Exploración de la relación entre la harina de torta de oliva, el Bacillus licheniformis y el crecimiento, la composición de ácidos grasos musculares y los parámetros de salud de los pollos de engorde

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ABSTRACT

The research investigates how Olive Cake Meal (OCM) and Bacillus licheniformis can influence broiler chicken growth, muscle fatty acid (FA) composition, and various health parameters. A total of 1 300 newly hatched Red Broiler chickens were randomly assigned to the Control Group (n = 451) and the Experimental Group (n = 849), which were then divided into three additional groups Ex1 received a low, (4 %), Ex2 contains moderate (6 %) and Ex3 indicates high (9 %) concentration of OCM and Bacillus licheniformis. During the 45 days, Body Weight (BW), Feed Intake (FI), and Feed Conversion Ratio (FCR) were measured, for muscle FA composition and health markers. The data were analyzed statistically using one-way ANOVA and the Kruskal-Wallis test, where significant differences ($p < 0,05$) were determined for BW, FI, and FCR in the experimental groups, especially during the earlier growth stages. The experimental subgroups of chickens demonstrated better nutrient absorption, improved FA profiles, and increased muscle vitamin E content, with the superlative results perceived in the Ex3 condition. Results indicate that OCM and Bacillus licheniformis can enhance broiler chicken growth performance, improve muscle FA composition, and potentially improve overall chicken health status, which would have implications for poultry nutrition.

Keywords: Alternative Ingredients; Broilers; Lipid Peroxidation; Probiotics; Growth.

RESUMEN

La investigación analiza cómo la harina de torta de oliva (OCM) y el Bacillus licheniformis pueden influir en el crecimiento de los pollos de engorde, la composición de ácidos grasos (AG) de los músculos y diversos parámetros de salud. Se asignó aleatoriamente un total de 1300 pollos de engorde rojos recién nacidos al grupo de control (n = 451) y al grupo experimental (n = 849), que a su vez se dividieron en tres grupos adicionales: Ex1 recibió una concentración baja (4 %), Ex2 una concentración moderada (6 %) y Ex3 una concentración alta (9 %) de OCM y Bacillus licheniformis. Durante los 45 días, se midieron el peso corporal (PC), la ingesta de alimento (IA) y el índice de conversión alimenticia (ICA), así como la composición de

AG del músculo y los marcadores de salud. Los datos se analizaron estadísticamente mediante ANOVA unidireccional y la prueba de Kruskal-Wallis, donde se determinaron diferencias significativas (p < 0,05) para el PC, la IA y el ICA en los grupos experimentales, especialmente durante las primeras etapas de crecimiento. Los subgrupos experimentales de pollos mostraron una mejor absorción de nutrientes, mejores perfiles de ácidos grasos y un mayor contenido de vitamina E en los músculos, con los mejores resultados observados en la condición Ex3. Los resultados indican que el OCM y el Bacillus licheniformis pueden mejorar el rendimiento del crecimiento de los pollos de engorde, mejorar la composición de ácidos grasos en los músculos y, potencialmente, mejorar el estado general de salud de los pollos, lo que tendría implicaciones para la nutrición avícola.

Palabras clave: Ingredientes Alternativos; Pollos de Engorde; Peroxidación Lipídica; Probióticos; Crecimiento.

INTRODUCTION

The poultry business continually looks for alternate feed components and additives to maximize broiler chicken growth, boost meat quality, and improve general health metrics. OCM, a leftover from the production of olive oil, is one such substance that has attracted interest. Although rich in nutrients, OCM also includes different amounts of fiber and ant nutritional elements, which could affect the performance of broilers.[1] Probiotics like Bacillus licheniformis have been investigated for their possible advantages in reducing potential negative effects and improving performance.[2] To optimize poultry diets and advance animal welfare, it is essential to comprehend the connections between OCM, Bacillus licheniformis, broiler chicken development, muscle FA composition, and health indicators. Poultry farmers increase productivity and produce meat of the highest quality by considering the influence of these factors on broiler performance and health.[3] Suitable alternative sources for chicken feed have lately undergone extensive research due to rising demand, tight availability, and a sharp increase in the price of feed components. Feed expenses can represent more than 70 % of the broiler's manufacturing costs. Reduced feed prices without sacrificing broiler health can boost poultry farming efficiency.[4] To identify sustainable protein sources for broiler diets. After multiple hydrations, filtrations, and degumming processes, the most often used fat component in the formulation of chicken feed is soybean oil (SO). But it is expected to have a limited supply and be more expensive. A sustainable method for chicken slaughterhouses is to render chicken fats, an alternative to SO produced by processing wastes that are readily accessible and inexpensive.[5] Olives frequently generate OCM.[6] An aromatic element utilized in broiler feed is both inexpensive and accessible in many areas across the world. Due to the high amounts of FA per oxidation (Oxid), malnutrition, and poor palatability caused by the large levels of fiber and unsaturated FA present, there are potential issues with feeding OM. Up to 10 % of the total ration of chicken diets was effectively supplemented with OCM. OCM can be combined with the right microorganisms to provide advantageous effects, increasing the meal's effectiveness in the broilers' diet.[7] The main contribution of this work is the investigation of the connections between OCM, Bacillus licheniformis, broiler chicken development, FA content, and health indices.

Related Work

Author [examined the effects of several feeding techniques on developmental efficiency, gastrointestinal histomorphometry, and gene regulation in lipid metabolism and development of broiler poultry.[8] These feeding techniques included wet feed, dry feed, and wet meal soured with Bacillus licheniformis. Research how different amounts of dietary OCM affected blood pressure, cholesterol, and the levels of FA in breast muscle. For this reason, 400-day-old chicks of both sexes of the Japanese quail (Coturnix coturnix japonica) were divided into four experimental groups, each having five duplicates of 20 animals.[9]

The research suggested Broiler chicken feed additives of the effects of BL or 6-phytase on growth effectiveness, amounts of nutrients with apparent ileal digestibility coefficients Acquired immunodeficiency syndrome (AIDs), microbial activity, and populations of bacteria in the cecum assessed either singly or in combination.[10] The effectiveness of varying quantities of olive cake as probiotics and food processing by-products as measured by growth performance in broiler diets and blood parameters.[11] Research suggested the effects of Iberian sow performance; antibody levels and serum indicators, antioxidant capability, and fecal microbiota were studied after adding olive pulp.[12] Antioxidant levels and bacterial counts in the sow's feces responded favorably to the presence of olive pulp, but no other effects were observed for the other parameters tested.

The introduction of olive pulp affected Iberian sows' fecal microbiota, antioxidant capacity immunoglobulin levels and serum indicators, and performance.[13] Olive pulp addition improved the antioxidant status and the bacterial counts in the feces of sows, but no significant advantages were seen for the other measures examined. To assess how feeding broilers With or without an enzyme combination, peanut meal and linseed meal (LSM) had an impact on the animals' development, muscle amino acid profiles, nutrient digestibility, plasma metabolite profiles, and gene expression related to nutrient absorption.[14] Research examined an investigation to assess the impact of Olive leaf extract (OLE) on caecal microbiota modulation, antioxidant capability, and breast meat quality.[15] OLE, which has a 51,8 % oleuropein content, a 25,3 % polyphenol content, and a 6,5 % flavonoid content, was created to complement a baseline diet at amounts varying from 0,7 % OLE (OLE0,6) to 0,5 % OLE (OLE0,3). After the egg hatches from the egg after seven days, the entire feeding phase of 36 days lasts.

METHOD

This research involves 1300 newly hatched Red Broiler chickens to compare the effects of OCM and Bacillus licheniformis supplementation on growth, muscle FA composition, and health parameters. The research used SPSS software for statistical analysis, including one-way ANOVA and Kruskal-Wallis tests, to assess growth performance and health parameters.

Data Collection

A total of 1300 newly hatched Red Broiler chickens were randomly divided into two main groups: the Control Group consisting of 451 chickens and the Experimental Group, with 849 chickens. The Experimental Group was further divided into three subgroups.

Data Splitting

The Control group was supplied with a normal diet containing no Olive Cake Meal or Bacillus licheniformis supplementation. The Control group did not undergo any additional testing or treatment for use as a comparison basis for experimental groups. The chickens in this group were monitored for growth, muscle FA composition, and health parameters, yielding a baseline for assessment to determine the effects of the dietary supplements in the experimental group.

The Experimental group received different concentrations of Olive Cake Meal and Bacillus licheniformis. The Experimental group was divided into three subgroups. Ex1 received a low, (4 %), Ex2 contained moderate (6 %) and Ex3 indicated a high (9 %) concentration of OCM and Bacillus licheniformis. All chickens were measured for similar parameters to the Control group, including growth, muscle FA composition, and health markers over the time-space of 45 days and at 9-day time-spaces (Days 9, 18, 27, 36, and 45). The performance of the subgroups, under different levels of supplementation, was determined.

Statistical Analysis

This research utilized SPSS software for statistical analysis to enhance the accuracy and reliability of the findings. To assess the growth performance of broiler chickens, a one-way ANOVA (Analysis of Variance) test is performed to compare the means of the growth performance in the Control and Experimental Groups. The Kruskal-Wallis test was employed to evaluate the FA composition of breast muscle compared among dietary groups in consideration of non-normal data distribution to obtain an accurate comparison of FA levels. The research assesses health parameters like biochemical markers to understand the functional effects of different feeding techniques on broiler chickens. The statistical significance was determined as $p < 0,05$.

RESULTS

The impact of OCM and Bacillus licheniformis on broiler chicken growth, muscle FA profile, and health parameters to improve meat quality and overall poultry health. Bacillus licheniformis improves the growth performance of poultry by increasing BW, FI, And FCR. They also enhance nutrient absorption, improve gut microbiota, reinforce immunity, and increase digestion, improving growth, efficiency, and health status. The impact of substituting corn in broiler diets with OCM on breast muscle FA composition was characterized. There were increases in linoleic, and arachidonic acid levels, while palmitic acid levels reduced. Myristic acid, palmitoleic acid, and docosahexaenoic acid were unaffected. Additionally, muscle vitamin E levels significantly increased, as did liver levels of Malondialdehyde (MDA).

Growth Performance

Table 1 compares FCR, BW, and FI between a control group and three experimental groups over five time periods, with p-values indicating statistical significance. On day 9, FCR values were similar across all groups (p = 0,042), while on average BW was highest in Ex3 (182,4 ± 9,5 g), and was lowest in Ex1 (155,7 ± 13,2 g), and statistically different (p = 0,036). FI was similar between the control and Ex1 groups and also highest in Ex3 (162 g, p = 0,051). On day 18, FCR increased from 1,1 ± 0,07 for control to 1,5 ± 0,07 for Ex3 (p = 0,028). BW was more than the control at Ex3 (410,5 ± 10,7 g) versus the control (395,4 ± 28,8 g, p = 0,031). FI was also highest in Ex3 (485,4 g, p = 0,045). On day 27, FCR was significantly lower in Ex3 (1,43 ± 0,15) compared to the control (1,5 ± 0,28, p = 0,039). BW was highest in Ex3 (750,8 ± 80,2 g) and lowest in the control (650,4 ± 119,3 g, p = 0,033). FI was significantly higher in Ex3 (2 250 g) compared to control (2 141 g, p = 0,049). On day 36, FCR in experimental groups improved even more with the lowest FCR value in Ex3 (1,3 ± 0,12, p = 0,022). BW showed a similar trend with Ex3 obtaining 1 223 ± 108,2 g versus control which had 1 140,4 ± 122,9 g(p = 0,029). FI was slightly lower in experimental groups (1 632-1 634 g) when compared to control (1860 g, p = 0,041).

Table 1. Impact of Broiler Growth and Performance

Period	Item	Group				p-Value
		Control Group	Experimental Group			
			Ex1	Ex2	Ex3	
9th day	FCR, g/g	0,8 ± 0,9^{a}	0,8 ± 0,08^{a}	0,8 ± 0,06^{a,b}	0,8 ± 0,03^{a,b}	0,042
	BW, g	172,4 ± 18,7^{a}	155,7 ± 13,2^{a}	158,7 ± 7,9^{a,b}	182,4 ± 9,5^{b}	0,036
	FI, g	142± 0,8	142,1 ± 0,3	141 ± 0,8	162 ± 2,2	0,051
18th day	FCR, g/g	1,1 ± 0,07^{a}	1,1 ± 0,0^{a,b}	1,3± 0,2^{b}	1,5 ± 0,07^{b}	0,028
	BW, g	395,4 ± 28,8^{a}	382,3 ± 38,3 $^{a,\ b}$	405,5 ± 36,2^{b,c}	410,5 ± 10,7 ^{b,c}	0,031
	FI, g	460 ± 1,8	462,4 ± 1,7	463,5 ± 1,8	485,4 ± 3,5	0,045
27th day	FCR, g/g	1,5 ± 0,28^{a}	1,5 ± 0,20 ^{a,b}	1,5± 0,17^{a,b}	1,43± 0,15^{b}	0,039
	BW, g	650,4 ± 119,3^{a}	680,2± 88,1 ^{a,b}	697 ± 84,3$^{a,\ b}$	750,8 ± 80,2^{b}	0,033
	FI, g	2,141± 4,3	2,140 ± 6,5	2,150 ± 2,3	2,250 ± 7,9	0,049
36th day	FCR, g/g	1,6 ± 0,18^{a}	1,4 ± 0,14^{b}	1,4 ± 0,13^{b}	1,3 ± 0,12^{b}	0,022
	BW, g	1,140,4 ± 122,9^{a}	1,175,2 ± 112,7^{a,b}	1,201,8 ± 109,5^{a,b}	1,223 ± 108,2^{b}	0,029
	FI, g	1,860 ± 21,7	1,632 ± 13,6	1,634 ± 14,8	1,634 ± 15,2	0,041
45th day	FCR, g/g	1,8 ± 0,19	1,7 ± 0,17	1,7 ± 0,16	1,7 ± 0,15	0,057
	BW, g	1,657,7 ± 186	1,697,3 ± 165,9	1,724,4 ± 157,8	1,746,4 ± 150,7	0,064
	FI, g	1,800± 28,6	1,8205± 26,4	1,964 ± 18,6	2,520 ± 23,9	0,072

By day 45, the FCR had remained similar across all groups (p = 0,057) and BW was the highest in Ex3 (1 746,4 g) but was not significantly different from the control group (1 657,7 g, p = 0,064). FI was highest in Ex3 (2 520 g) and lowest in the control group (1 800 g, p = 0,072), but while both were higher, differences were not statistically significant. Overall significant and notable differences in FCR, BW, and FI were observed in earlier stages (9th-36th day), but by the 45th day, more similar values were being noted. Over time the Ex3 groups continued to show. Furthermore, while Ex3 values for BW and FI were consistently higher, FCR scores improved over time across all groups, especially in the experimental groups. Significant differences in FCR, BW, and FI were observed on the 9th-36th day, but diminished by the 45th day. Ex3 consistently performed better in BW and FI, and FCR values generally improved over time.

Findings of fatty acid (FA)

The differences in the FA profile, liver MDA, and muscle vitamin E concentration values for the control and the three experimental diets (Ex1, Ex 2, Ex 3) with p-values to indicate significance are represented in table 2. Palmitic acid (C16:0), the control had the greatest value (25,09 mg/100 g fat) and Ex 3 had the least (20,15 mg/100 g fat) which exhibited a significant downward trend (p = 0,032). Arachidonic acid (AA, C20:4 n - 6) showed a significant increase across the experimental groups from the control (2,56 mg/100 g fat) to Ex 3 (3,04 mg/100 g fat) (p = 0,041). Myristic acid (C14:0) was relatively stable across groups (1,59–1,44 mg/100 g fat) and was not statistically significant (p = 0,089). Palmitoleic acid (C16:1) also showed a reduction from the control (6,41 mg/100 g fat) to Ex 3 (5,55 mg/100 g fat) but was not statistically significant (p = 0,064). Linoleic acid (C18:2 n − 6) significantly increased from 7,52 mg/100 g fat in the control to 9,21 mg/100 g fat in Ex 3 (p = 0,027).

The values of docosahexaenoic acid (DHA; C22:6 n − 3) were relatively similar across all groups ranging from 0,841 to 0,895 mg/100 g fat without significant differences (p = 0,112). The liver levels of MDA (nmol/g) significantly decreased across experimental groups. From 20 nmol/g in the control to 9 nmol/g in Ex 3 (p = 0,015), indicating a decreased level of oxidative stress. Finally, muscle vitamin E (g/100g muscle) significantly increased from 0,30 g/100 g in the control to 0,50 g/100 g in Ex 3 (p = 0,008) suggesting improved levels of antioxidants. The difference in experimental diets led to a decrease in palmitic acid and liver MDA, in addition to an increase in arachidonic and linoleic acid. The muscle vitamin E also increased in the higher percentage of experimental diets.

Table 2. The Relevance of Feeding Strategies on FA Content					
Element (mg/100 g fat)	**Groups**				**p-Value**
	Control	**Experimental Group**			
		Ex 1	**Ex 2**	**Ex 3**	
Palmitic acid (C16:0)	25,09 [a]	23,52 [a, b]	22,20 [b]	20,15 [b]	0,032
Arachidonic acid (AA, C20:4 n − 6)	2,56 [b]	2,62 [a, b]	2,79 [a]	3,04 [a]	0,041
Myristic acid (C14:0)	1,59	1,46	1,46	1,44	0,089
Palmitoleic acid (C16:1)	6,41	6,40	6,33	5,55	0,064
Linoleic acid (C18:2 n − 6)	7,52 [b]	8,21 [a, b]	8,52 [a]	9,21 [a]	0,027
Docosahexaenoic acid (DHA, C22:6 n − 3)	0,871	0,846	0,841	0,895	0,112
Liver MDA (nmol/g)	20 [a]	15 [b]	12 [c]	9 [c]	0,015
Muscle vitamin E (g/100 g muscle)	0,30 [c]	0,32 [b]	0,41 [a]	0,50 [a]	0,008

Immune Cell Distribution of Health Parameters

Figure 1 has three boxplots that compare the percentage of segmented neutrophils, monocytes, and lymphocytes in the control group and the experimental groups (Ex 1, Ex 2, and Ex 3). Each boxplot also contains a p-value to indicate statistical significance. Segmented neutrophils (%) reflect the percentage of segmented neutrophils of the different Groups, with the control group having the highest median also showing a wider IQR compared to the other experimental groups (Ex 1, Ex 2, and Ex 3), displaying an overall trend of decreasing segmented neutrophils. Outliers are prevalent in the control group. The p-value = 0,0866 indicates there is not a significant difference among the groups. Monocytes (%) depicts monocyte percentages for both the control and experimental groups. Control and Ex 1 groups have drastically higher median monocyte levels than Ex 2 and Ex 3 groups, which are noticeably decreased. The statistical analysis demonstrates a p-value = 0,0002 indicating this is a statistically significant difference between the groups; especially concerning lower values with increasing experimental conditions. Lymphocytes (%) refers to the lymphocyte percentages, being that both groups, control, and experimental groups, have similar, relatively high median values. Ex 1 has a much wider range and Ex 2 and Ex 3 are more stable in their distributions. The p-value = 0,0867 states that there was no statistically significant difference, although there still are some small differences among the groups.

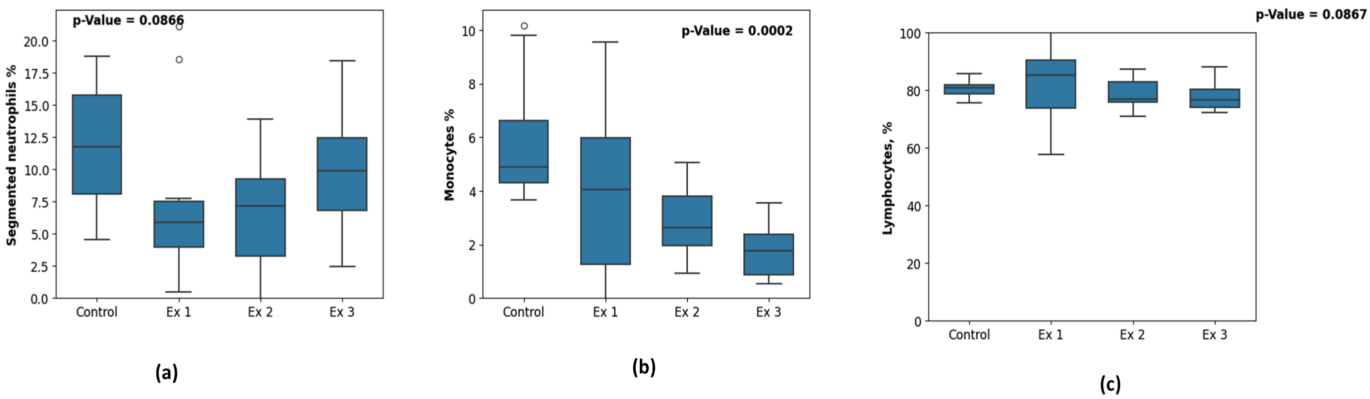

Figure 1. Effects of OCM and *Bacillus licheniformis* on broiler immune cells

The results demonstrate that experimental diets alter white blood cell composition, resulting in a statistically significant decrease in monocyte proportions in all experimental groups. The amounts of segmented neutrophils and lymphocytes show generally similar patterns of decreasing and stabilizing although these changes do not reach significance.

DISCUSSION

The positive impacts of OCM and Bacillus licheniformis inclusion on broiler growth, FA profile in muscle, and health-related parameters. The experimental groups, especially the Ex3 concentration level, demonstrated significantly increased BW, FI, and FCR compared with the control. This indicated that Bacillus licheniformis supplementation can increase nutrient absorption, promote gut microbiota balance, and enhance immunity function for overall growth performance. The inclusion of OCM in dietary groups modified FA profiles in muscle. Significantly increased levels of linoleic and arachidonic acid were noted, while palmitic acid increased. It has potential implications for improved substance quality and nutritional enhancement, thereby contributing to healthier broiler meat for consumers. This assessment would also be supported by the increase in muscle vitamin E and liver MDA levels, which can indicate antioxidant effects, therefore contributing to overall meat stability and balance in oxidative status. There was a statistically significant improvement in FCR over time for all diet combinations, and Ex3 had the lowest FCR at multiple time points ($p < 0,05$). There were significant differences in BW at various time points, with Ex3 ranked higher than the control and with some of the greatest differences occurring between days 9 and 36 ($p < 0,05$). FI was also greatest for Ex3 at several of the time points and demonstrated that the experimental diet combinations were having a positive effect on overall growth. However, by day 45, the differences in FCR, BW, and FI between groups began to diminish indicating that the majority of the effects were during the earlier stages of growth. The experimental diets significantly impacted the lipid profiles based on FA composition. Palmitic acid (C16:0) has been decreased in Ex9 compared to Control ($p = 0,032$). An apparent increase in both arachidonic acid (C20:4 n − 6) and linoleic acid (C18:2 n − 6) in the experimental groups, and more so in Ex9 ($p < 0,05$). The changes in FA composition and growth performance observed in this research showed the importance of improved diet formulations for maximizing broiler productivity and meat quality. It suggests that OCM and Bacillus licheniformis in broiler diets could be used as tools for improving growth efficiency, feed utilization, and meat composition to improve sustainable, high-quality poultry production.

CONCLUSION

The research assesses dietary supplementation of olive cake meal and Bacillus licheniformis on growth performance, muscle FA profile, and health parameters in broiler chickens to establish their usefulness in meat quality enhancement and overall poultry health. The research compared the growth performance and FA profile of the experimental diets coordinated against a control. The experimental groups, particularly Ex3, consistently demonstrated improved growth performance throughout the research. The results suggested that FA profiles can be altered with diet, and also enhanced an improvement in the potential nutritional value of the finished product. In addition, liver MDA levels decreased significantly in the experimental groups, especially Ex9 ($p = 0,015$), improving oxidative stability. The results also provide important information regarding dietary interventions aimed at improvement of growth efficiency and nutritional value, and potential candidates for future use in animal nutrition and production systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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