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The Impact of Dietary Linseed Oil Supplementation on the Performance of Broiler Chicks

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Abstract | The aim of the current research was to evaluate the effects of linseed oil (LO) nutritional supplementation on the growth performance of broiler chicks, in addition, their effects on immunological and biochemical parameters of serum, total antioxidant capacity, intestinal morphology, mortality percentage, and estimates of economic efficiency. Over the course of the five-week trial period, 150 (Ross 308) one-day-old chicks were separated into five groups, each consisting of three replicates (10 chicks/replicate). The chicks were fed five different experimental diets with varying quantities of oil (0% oil, 1 (SBO and 1% LO), 2% LO, and 3% LO, respectively). For assessment, four chicks from each replication were randomly used. The findings showed that while there was no discernible variation in feed intake across all groups, broiler chicks' body weight (Bw) increased (P<0.05) when linseed oil was added to their feeds. Supplementing linseed oil resulted in a significant (P<0.05) improvement in dressing % and carcass attributes. The breast muscle of broilers fed linseed oil had considerably (P<0.05) greater values of poly unsaturated fatty acids and n-3 polyunsaturated fatty acids than that of the control groups. Additionally, there was a significant (P<0.05) decrease in the amounts of triglycerides, cholesterol, LDL, VLDL, and TAC. Nevertheless, consuming meals containing linseed oil led to a significant (P<0.05) increase in serum levels of HDL-c, total protein (TP), globulin (GL), and GSH-Px. Additionally, giving broiler chickens linseed oil enhances their immunological response to the Newcastle vaccine. The current study showed that adding linseed oil to chick feeds increases immunological response, body composition, serum biochemical parameters, intestinal morphology and growth performance beside decreasing mortality ratio. However, it is not economically feasible when matched with control groups (0% oiland 1% SBO).

Keywords: Broiler, Linseed oil, Performance, Immunity, Gut, Economic efficiency

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INTRODUCTION

The production process of poultry meat has steadily grown over the years, and this trend is projected to continue to operate. Otherwise, modifications to genes in avian strains and greater understanding of nutrition allow chickens achieve an acceptable selling weight of 2 kg at 35 days of age, and the efficiency of turning feed into poultry products further improves (Ravindran, 2013). Also, Gous (2010) recommended that genetic modifications have been very successful in improving possible growth of broilers over the past fifty years, and it is likely that more genetic advancement is possible. So that it will be necessary for nutritionists to review the techniques in which these fast growing strains are fed. Also, Hassanein *et al*. (2023) reviewed nutritional strategies to meet fast growth of new genetic modification of Ross broiler chicks.

The supplementary use of various oils results in meat enhanced with bioactive n-3 PUFA. As a result, chicken meat is now advised for eating by anybody concerned with their health and their food choices. Also, provide the added benefit of enhancing the bird's health (Kralik *et al*., 2018). The vast majority vegetable oils include a high omega-6/omega-3 fatty acid percent. Soybean oil ranks in the middle of the omega-6 to omega-3 ratios (Doppenberg and Van der Aar, 2017).

Broilers' diets also can be supplemented with a variety of n-3 PUFA sources, including fish oil; linseed meal, marine algae, and canola oil (Coorey *et al*., 2015). ALA has a lower reported favorable effect on human health than EPA and DHA, and its metabolism efficiency to EPA and DHA in the human body is only 2-10% or less. The primary source of EPA and DHA is seafood, especially fatty fish, which may not be sustainable. ALA and SDA are synthesised in plants and represent more sustainable sources of ω-3 PUFAs Gillingham (2013). LNA is a precursor for the synthesis of EPA and DHA. N-3 PUFA and the ratio of n-3 to n-6 fatty acids in the human diet (about 2:1) are critical in lowering the occurrence of lifestyle illnesses such as hypertension, coronary artery disease, atherosclerosis, peripheral artery disease, Alzheimer's disease, arthritis, cancer, and diabetes. In addition to several autoimmune and inflammatory health problems Chiu *et al*. (2008). Omega-3 PUFAs appear to be universally acknowledged as a part of modern-day dietary habits because of their favorable effects on metabolism (Gogus and Smith, 2010).

The study piloted by Konieczka *et al*. (2018) recognized that broilers were given varying amounts of fish/linseed and maize oil to produce distinct n-6:n-3 ratios. A great deal of study has looked at the consequences of linseed oil intake on broiler growth performance. For example, Smith *et al*. (2018) observed an important boost in body weight growth and feed conversion ratio (FCR), as well as raised levels of immunoglobulins, such as IgG and IgM, in broilers given a diet enriched with linseed oil corresponding to the control group. More importantly, Jones *et al*. (2018) found that broilers given a diet high in linseed oil had much better growth performance than the control group. The identical results were presented by Patel *et al*. (2021), who found that broilers that get linseed oil supplementation had an enormous boost in FCR relative to the control group, as well as greater dressed carcass weight and breast meat output. Aziza *et al*. (2014) found that the kind of fat and quantity of feed ingested both had a positive impact on the GI tract's morphology.

As a result, the present investigation was taken to investigate the effects of linseed oil supplementation on the growth performance, carcass traits, biochemical and histopathological parameters on Ross chicks. Also, because linseed oil is an available and cheap source of PUSFA in Egypt. Additional the high content of PUFA Ibrahim *et al*. (2018).

MATERIALS AND METHODS

The research project was conducted at the Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Zagazig University in Egypt.

Experimental birds, accommodation and management

A hatchery provided a total of 308 unsexed one-day-old broiler chicks (Ross). Whenever the chicks arrived, they were each individual weighed (the first mean body weight was identical), and then on random basis assigned to five identical groups, each with three replicates of ten birds. The feeding study extended 5 weeks. The entire chicks received a crumble meal throughout the starter and grower stages, followed by a pellet diet until the end of the study period, when they were fed *ad libitum*. The chicks were immunized according to vaccination schedules. Five exploratory (isocaloric and isonitrogenous) meals separated into three stages were created to contain 0 oil, 1% SBO, 1% LO, 2% LO, and 3% LO to match the nutritional needs for broiler chicks as specified by Aviagen (2019) and to have a varied ratio of n-3 to n-6 fatty acids, as indicated in Table 1. The typical sample of all feed components was tested using SupNIR-2700. Metabolic energy was computed in accordance with Janssen (1989). In order to avoid oxidative rancidity, the diets were stored in a cool, dry environment.

Growth performance and carcass traits

Growth performance was determined by assessing daily feed consumption and body weight inside each cage after a 5-week duration. At the end of the feeding trials, the overall feed intake, weight gain, and feed-to-gain ratios were determined. Added to that, four 5-week-old chicks from each duplicate were weighed, slaughtered, subsequently eviscerated without any feed withdrawal period, as suggested by Brake *et al*. (1997). The eviscerated carcass, liver, heart, gizzard, spleen, bursa, thymus gland, breast, thigh, abdominal fat yields, and complete evacuated intestinal tract were all weighed to determine the dressing ratio.

Table 1: Ingredient and nutrient composition of the experimental diets.

Ingredients	Starter					Grower					Finisher				
Feeding period (days)	0-10 days					11-24 days					25-35 days				
Groups	G 1	G 2	G 3	G 4	G 5	G 1	G 2	G 3	G 4	G 5	G 1	G 2	G 3	G 4	G 5
Yellow corn	59.71	57.10	57.00	55.10	52.70	65.00	62.74	62.71	60.48	58.17	71.42	69.11	69.07	66.84	64.60
Corn gluten meal 60% cp	12.20	9.66	9.66	6.58	3.89	15.55	12.73	12.73	9.91	7.22	17.85	15.10	15.10	12.30	9.58
Soybeenmeal 48% cp	23.32	27.60	27.70	31.79	35.96	14.93	19.12	19.15	23.32	27.40	6.45	10.62	10.6	14.80	18.85
Linseed oil	0.00	0.00	1.00	2.00	3.00	0.00	0.00	1.00	2.00	3.00	0.00	0.00	1.00	2.00	3.00
Soybean oil	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Grouned limestone	1.87	1.85	1.85	1.86	1.82	1.70	1.65	1.65	1.60	1.64	1.59	1.59	1.59	1.57	1.55
mono-calcium phosphate	1.29	1.25	1.25	1.25	1.21	1.25	1.25	1.25	1.10	1.19	1.10	1.05	1.05	1.03	1.02
L-lysine	0.41	0.33	0.33	0.25	0.17	0.46	0.38	0.38	0.30	0.22	0.53	0.44	0.44	0.36	0.28
Dl- methionine	0.19	0.20	0.20	0.23	0.24	0.11	0.13	0.13	0.16	0.18	0.07	0.09	0.09	0.11	0.14
L-threonine	0.07	0.06	0.06	0.05	0.05	0.04	0.03	0.03	0.01	0.02	0.04	0.03	0.03	0.02	0.02
Vitamin mineral premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Common salts	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.33	0.35	0.35	0.35	0.35	0.35
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Antimycotoxin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritive value															
CP	23.00	23.16	23.20	23.00	23.05	21.52	21.50	21.52	21.50	21.51	19.52	19.53	19.54	19.52	19.50
EE	2.69	3.56	3.56	4.44	5.31	2.84	3.74	3.74	4.62	5.50	3.07	3.94	3.94	4.82	5.69
ME	3000.16	3000.38	3000.23	3000.33	3000.61	3100.91	3100.32	3100.74	3100.15	3100.41	3200.36	3200.30	3200.72	3200.08	3200.77
Calcium	0.96	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79	0.79
Available phosphorous	0.45	0.45	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40
N-3: N-6 ratio**	1:31.81	1:14.96	1:3.56	1:1.83	1:1.20	1:37.65	1:16.31	1:3.86	1:1.98	1:1.30	1:45.06	1:17.79	1:4.18	1:2.13	1:1.4

*Premixes including vitamins and minerals that correspond to the nutritional needs for broiler chicks as indicated in Aviagen (2019), made by the Multivita company. **Various ratios of n-3 to n-6 fatty acids determined according to Gunstone (1996).

The weight of the lymphoid organs (bursa of Fabricius, spleen, and thymus) were obtained at the end of the experiment from the slaughtered birds of each group to compute the ratio of organ weight. According to Anon. (1971), the Hemagglutination inhibition for Newcastle vaccination antibody titer was used to test the humoral immune response to Newcastle virus antibodies.

At the end of the experiment, an automated blood analyzer was used to determine the total leukocyte count of non-coagulated blood samples.

Serum biochemical parameters

The serum samples were tested for total cholesterol levels as recommended by

Naito and Kaplan (1984), triglycerides in accordance to Wahlefeld and Bergmeyer (1974), serum high-density lipoprotein-cholesterol (HDL-C) corresponding to Burstein and Scholnick (1973), serum low-density lipoprotein-cholesterol (LDL-C) in relation to Friedewald *et al*. (1972), serum very low-density lipoprotein (VLDL), total serum protein corresponding to Grant *et al*. (1987), albumin in regards to Doumas and Biggs (1971). Koracevic *et al*. (2001) techniques were used to measure serum Total Antioxidant Capacity (TAC) and glutathione peroxidase (GSH-Px).

The fatty acid content of homogenized freeze-dried breast flesh was determined using Folch *et al*. (1957) technique, and fatty acid methyl esters were produced using gas chromatography (GC) as reported by Ichihara and Fukubayashi (2010).

Histopathological examination

The typical samples from jejunum were obtained according to the procedure of Drury and Wallington (1967) for histopathological investigation of intestine to study wall thickness of intestine (MT), villous height, crypts depth CD, villous height: Crypt depth ratio, goblet cell proliferation, villus width (VW), villus perimeter calculated as (2π × (average villi width/2) × VH) and villus surface area (VA) calculated as villus perimeter× VH. samples from jejunum of slaughtered birds were fixed in 10% formalin and processed using standard paraffin embedding histopathology techniques, cut into 5 µm thick sections, and processed for hematoxylin and eosin staining, according to methods described by (Bancroft and Gamble, 2008). Morphometric examination of the tissue sections was done using a computer-assisted microscopic image analysis system. Throughout the trial, the mortality ratios for each trial were computed.

Economic study

In accordance with El-Kerdawy (1997), an economic study was done to assess the live cost of production of birds raised through different experimental protocols, where an economic efficiency (EE) was calculated according to the following equation: Y= (A-B)/B x 100 where A is the selling cost of the obtained gain and B is the feeding cost of this gain.

Statistical analysis

The prior data's mean, standard error, and coefficient of variation will be determined using Snedecor and Cochran's (1994) standard statistical formula. The data will be evaluated using ANOVA one-way classification in a totally randomized manner to determine the significance of differences between treatment groups followed by the Duncan's test as a post hoc analysis then descriptive option.

RESULTS AND DISCUSSION

Effect of linseed oil dietary supplement on growth performance

The main effect of dietary linseed oil on growth response of the broiler chicks is presented in Table 2. The final live weight and weight gain ($P<0.05$) increased with dietary linseed oil treated groups but the best BW and BWG was recorded in group 1% and 2% LO, then 1% SBO. These results agree with Jones *et al*. (2018) who demonstrated that broilers fed a diet enriched with linseed oil exhibited significantly improved growth performance compared to the control group. Also, Smith *et al*. (2018) results showed that the inclusion of linseed oil in the diet significantly improved the growth rate of broilers compared to the control group. The mechanisms by which dietary linseed oil improves broiler body weight are not fully understood. However, some studies have suggested that increased growth rate due to the higher availability of omega-3 fatty acids, which play a crucial role in promoting muscle development and protein synthesis (Sakomura and Rostagno, 2019).

No significant difference observed in FI between control and LO treated groups and the highest value recorded in SBO group. Likewise, Wang *et al*. (2019) found no significant effects on feed intake. This may be due to the five exploratory meals were isocaloric and isonitrogenous and the study of Abdel-Hafeez *et al*. (2016) mentioned that many authors for example, (Peterson *et al*., 1954; Mraz *et al*., 1957; Golian and Maurice, 1992; Lesson *et al*., 1993; Kamran *et al*., 2008) showed that chicks attempt to eat to satisfy their energy requirements but are unsuccessful

Table 2: Effect of linseed oil supplementation in chicks diets on growth performance.

Growth performance	Dietary linseed oil supplementation%				
	0% oil	1% SBO	1 % LO	2% LO	3% LO
BW/ g bird	2253.15^{b}±88.10	2355.89^{ab}±63.32	2445.42^{ab}±11.31	2504.54^{a}±66.39	2337.17^{ab}±41.00
BWG, g bird	2209.68^{b} ± 88.02	2312.42^{ab} ± 63.33	2402.17^{ab} ± 11.36	2461.13^{a} ± 66.44	2293.81^{ab} ± 40.93
FI/ g bird	3247.15 ± 143.90	3341.94 ± 88.50	3317.95 ± 72.62	3234.85 ± 18.32	3121.84 ± 40.13
FCR	1.47^{a} ± 0.03	1.45^{ab} ± 0.00	1.38^{bc} ± 0.03	1.32^{c} ± 0.03	1.36^{c} ± 0.01

a, b, c means ± standard error in the same row with different superscripts are significantly different ($P<0.05$). BW=body weight, BWG=body weight gain, FI=feed intake, FCR=feed conversion ratio.

at low dietary ME concentrations. Our results about FCR showed that all treatments containing linseed oil significantly reduced FCR compared to control treatment which does not have and the best results in group fed 2% then 3% then 1% inclusion rate.

Studies with similar results, as Shawn by Silva *et al.* (2020) who found that dietary supplementation with linseed oil can improve the FCR in broilers. Other studies have shown similar results that dietary linseed oil has positive effects on broiler FCR, including reducing FCR and improving efficiency of nutrient utilization for instance study conducted by Wang *et al.* (2019). The enhanced FCR can be attributed to the improved nutrient utilization and digestion efficiency associated with linseed oil supplementation. Also, the higher bioavailability of omega-3 fatty acids which may enhance nutrient utilization and metabolism, leading to reduced feed wastage and improved FCR (Khatun *et al.*, 2021).

Effect of linseed oil dietary supplement on carcass composition

Table 3 revealed that the dressing percentages were the highest (P<0.05) in the linseed oil-treated groups than control. Thigh yield percentage was significantly the highest in the 1% LO with no significant difference observed in breast yield percentage. Also, significant (P<0.05) reduced abdominal fat percent in the linseed oil than control groups. Also, improvement of thymus weight with linseed oil groups than control groups and bursa improved with linseed oil supplement but spleen weight didn't reveal any improvement with linseed oil addition. This agreed with Ibrahim *et al.* (2018) and El-Zobier *et al.* (2016) who presented that the use of oil or fat in diets for broilers may alter both the composition and the quality of the carcass. This may be due to the dietary fortification of omega-3 fatty acids that improves carcass by modulating lipid metabolism, resulting in the improved deposition of lean muscle tissue and a healthier carcass composition (Hosseini-Vashan *et al.*, 2022). Also, reducing the abdominal fat installation in broilers (Nafees and Pagthinathan, 2017). In addition, PUFA-enriched di*et al*so may provide higher energy availability for muscle growth and the decrease in abdominal fat in these groups due to the shift in energy use for muscle growth rather than deposition in the abdominal cavity Alagawany *et al.* (2019).

Effect of linseed oil dietary supplement on meat quality

Supplementation with linseed oil (n-3 PUFA's) significantly increased levels of both USFA and N-3 matched to control diet as Shawn in Table 4. This result agreed with Newman (2000) and Baiao and Lara (2005) who mentioned that in birds, body fat composition is related to the composition of the fat from the diet. More studies for instance, linseed oil supplementation has been found to enhance the meat's nutritional value by increasing omega-3 fatty acid content (Kralik *et al.*, 2018). These results may be due to altering the n-6/n-3 FA ratios in the diets may augment the concentrations of long chain n-3 PUFA and modify the n-6/n-3 FA ratio in chicken meat resulting in healthy meat products Mandal *et al.* (2014). Also, feeding broiler chickens high concentrations of n-3 PUFA can boost accumulation of these fatty acids in the meat tissues (Al-Khalifa, 2017). So, the improved omega-3 fatty acid profile can contribute to healthier meat products with potential human health benefits, including cardiovascular protection and anti-inflammatory properties.

Table 3: Effect of linseed oil supplementation in chicks' diets on carcass traits.

Carcass traits	Dietary linseed oil supplementation%				
	0% oil	**1% SBO**	**1 % LO**	**2% LO**	**3% LO**
Body weight/g	2241.67±7.26	2358.33± 4.41	2470.0 ±11.55	2606.67±17.64	2525.00±58.38
Carcass weigh/g	1520.00± 2.89	1614.33 ±2.33	1806.33 ± 5.93	1916.00±35.64	1843.33±56.00
Carcass %	$67.81^{b} \pm 0.18$	$68.45^{b} \pm 0.08$	$73.13^{a} \pm 0.25$	$73.52^{a} \pm 1.71$	$72.98^{a} \pm 0.55$
Liver %	$2.06^{a} \pm 0.05$	$2.36^{b} \pm 0.04$	$2.38^{b} \pm 0.05$	$2.39^{b} \pm 0.06$	$2.35^{b} \pm 0.09$
Heart weight, %	0.44 ± 0.06	0.47 ± 0.03	0.44 ± 0.04	0.43 ± 0.01	0.43 ± 0.05
Spleen %	0.14 ± 0.00	0.17 ± 0.06	0.10 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
Gizzard weight %	$1.46^{a} \pm 0.10$	$1.15^{c} \pm 0.05$	$1.39^{ab} \pm 0.03$	$1.28^{abc} \pm 0.02$	$1.25^{bc} \pm 0.07$
Total fat weight %	$2.89^{a} \pm 0.12$	$2.80^{ab} \pm 0.40$	$2.05^{c} \pm 0.10$	$1.70^{c} \pm 0.07$	$2.15^{bc} \pm 0.16$
Bursa weight %	$0.04^{b} \pm 0.00$	$0.04^{b} \pm 0.00$	$0.04^{b} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.04^{b} \pm 0.00$
Thymus weight %	$0.26^{b} \pm 0.04$	$0.28^{b} \pm 0.01$	$0.36^{a} \pm 0.01$	$0.37^{a} \pm 0.02$	$0.31^{ab} \pm 0.01$
Intestine weight %	$2.57b^{c} \pm 0.19$	$2.50^{c} \pm 0.08$	$2.92^{ab} \pm 0.09$	$3.08^{a} \pm 0.05$	$2.38^{c} \pm 0.15$
P. muscle weigh %	29.07 ± 0.23	29.82 ± 0.70	29.35 ± 0.13	28.78 ± 0.76	29.33 ± 0.99
Thigh, drum weight %	$27.51^{a} \pm 0.12$	$27.91^{a} \pm 0.35$	$28.54^{a} \pm 0.02$	$24.94^{b} \pm 0.96$	$25.46^{b} \pm 0.75$

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05).

Table 4: Effect of linseed oil supplementation in chicks' diets on breast muscle content of fatty acids (%).

Fatty acids % of breast muscle	Dietary linseed oil supplementation%				
	0% oil	1% SBO	1 % LO	2% LO	3% LO
SFA	1.98^{c}±0.16	57.84^{a}±4.47	40.83^{b}±3.21	36.49^{b}±2.97	36.78^{b}±2.97
USFA	3.04^{c}±0.25	48.11^{b}±3.72	62.90^{a}±4.95	69.96^{a}±5.69	69.01^{a}±5.57
Total n-3 fatty acids	0.08^{d}±0.01	2.16^{c} ± 0.17	6.68^{b} ± 0.53	8.44^{b} ± 0.69	11.03^{a}±0.89
Total n-6 fatty acids	1.57^{c}±0.13	14.14^{b}±1.09	19.22^{a}±1.51	22.75^{a}±1.85	20.88^{a}±1.68
n-6:n-3 ratio	21.79^{a}±1.80	6.97^{b} ± 0.54	3.00^{c} ±0.24	2.88^{c} ± 0.23	2.05^{c}±0.17

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05). SFA =saturated fatty acid; UFA = unsaturated fatty acid, n-6=omega 6, n-3=omega 3.

Table 5: Effect of linseed oil supplementation in chicks diets on serum biochemical parameters and total antioxidant capacity.

Serum biochemical parameters	Dietary linseed oil supplementation%				
	0% oil	1% SBO	1 % LO	2% LO	3% LO
Triglycerides (mg/dl)	57.16^{a} ± 1.96	48.74^{b} ± 1.40	44.78^{bc} ± 1.97	42.28^{c} ± 1.38	42.20^{c} ± 0.96
Total cholesterol (mg/dl)	141.71^{a} ± 6.98	131.92^{a} ± 7.93	109.79^{b} ± 0.40	108.96^{b} ± 3.87	101.63^{b} ± 6.56
HDL (mg/dl)	56.26^{b} ± 0.67	58.99^{a} ± 0.75	54.51^{b} ± 1.03	54.74^{b} ± 0.64	55.21^{b} ± 0.83
LDL (mg/dl)	71.45^{a} ± 7.60	60.93^{ab} ± 7.24	44.30b^{c} ± 1.70	43.76b^{c} ± 3.20	36.13^{c} ± 5.68
VLDL* (mg/dl)	11.43^{a} ± 0.39	9.75^{b} ± 0.28	8.96^{bc} ± 0.39	8.46^{c} ± 0.28	8.44^{c} ± 0.19
Cholesterol ester (mg/dl)	2.57^{a} ± 0.16	2.25^{ab} ± 0.12	2.03^{bc} ± 0.03	2.01b^{c} ± 0.07	1.85^{c} ± 0.10
Total protein (mg/dl)	3.24 ± 0.10	3.28 ± 0.09	3.64 ± 0.16	3.66 ± 0.22	3.47 ± 0.04
Albumin (mg/dl)	1.89 ± 0.07	1.93 ± 0.04	1.79 ± 0.05	1.78 ± 0.08	1.86 ± 0.09
Globulin(mg/dl)	1.35^{b} ± 0.03	1.35^{b} ± 0.09	1.85^{a} ± 0.13	1.89^{a} ± 0.23	1.61^{ab} ± 0.05
TAC (mM/l)	0.33^{a} ± 0.01	0.29^{ab} ± 0.04	0.18^{c} ± 0.01	0.16^{c} ± 0.03	0.26^{b} ± 0.01
GSH-Px (IU/mg)	48.99^{b} ± 1.89	50.98^{b} ± 4.87	72.24^{a} ± 2.76	73.96^{a} ± 4.51	75.56^{a} ± 3.36

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05). HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein, TAC=total antioxidant capacity, GSH-Px= Glutathione peroxidase. *Serum very low-density lipoprotein (VLDL) = Triglyceride/5

Effect of linseed oil dietary supplement on serum biochemical parameters

Serum triglycerides, cholesterol, LDL and VLDL concentrations were significantly (P<0.05) reduced with linseed oil treatments, but serum HDL-c concentrations shown no significance with linseed oil treatment and 0 % oil group as shown in Table 5. Different studies conducted about effect of dietary linseed oil. For example, a study by Zhu *et al.* (2007) demonstrated that broilers fed with linseed oil-supplemented diets had lower levels of serum cholesterol and triglycerides compared to the control group. These results may be related to the role of omega-3 fatty acid in reduction of triglycerides, high elimination of VLDL by liver and higher excretion of bile via feces which can also reduce the serum of cholesterol and triglycerides concentrations Leaf and Weber (1988). Also, significant (P<0.05) increased serum content of total protein (TP) and globulin (GL) concentrations but decreased albumin (A) concentration. On the other hand, the concentration of TAC was significantly decreased (P < 0.05) in broiler groups fed diet supplemented with LO when compared with control group. Also, a significant increase (P < 0.05) in GSH-Px values. Ahmed *et al.* (2019) agreed with the obtained results and reported that improved antioxidant status and reduced oxidative stress markers in broilers receiving linseed oil supplementation. Alike, Bhattacharya *et al.* (2003) who settled that n-3 PUFA scavenge H_2O_2 and lipid peroxides and thus can enhance the activities of the hepatic antioxidant enzymes.

The effect of linseed oil dietary supplement on avian immune function

Table 6 revealed that weight of immune organ differs as a percentage of body weight with different linseed oil supplement as showed where improvement of thymus weight with linseed oil groups than control groups and bursa improved with 2% linseed oil supplement but spleen weight didn't reveal any improvement. This result agreed with Wang *et al.* (2000) who finished that at 4 wk, the chicks fed the three PUFA-rich diets (SO, LO, and FO) had significantly developed weights of thymus, bursa, and spleen as a percentage of body weight compared with the chicks fed the diet with a moderate level of PUFA (AO).

Table 6: Effect of linseed oil supplementation in chicks' diets on Newcastle vaccine antibody titter, total leucocyte counts and mortality rate.

	Dietary linseed oil supplementation%				
Exp. period (weeks)	**0% oil**	**1% SBO**	**1 % LO**	**2% LO**	**3% LO**
Antibody titer1st day	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33
Antibody titer at 14-day age	3.33^{b} ± 0.33	3.67^{ab} ± 0.67	4.33^{ab} ± 0.33	5.33^{a} ± 0.67	4.00^{ab} ± 0.58
Antibody titer at 26-day age	3.00 ± 0.00	3.33 ± 0.88	4.67 ± 0.88	4.33 ± 0.33	4.00 ± 0.58
Antibody titer at 35-day age	2.33 ± 0.88	3.33 ± 0.33	3.67 ± 0.88	3.33 ± 0.33	1.67 ± 0.67
Total leucocyte count	166.07^{c} ± 2.46	170.87^{bc} ± 3.01	180.97^{ab} ± 4.64	193.60^{a} ± 3.27	177.67^{bc} ± 6.42
Mortality rate	13.87 ± 2.79 ^{a}	8.32 ± 4.81 ^{ab}	0.00 ±0.00 ^{b}	0.00±0.00 ^{b}	0.00±0.00 ^{b}

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05).

Table 7: Effect of linseed oil supplementation in chicks' diets on intestinal morphology.

Intestinal morphology	Dietary linseed oil supplementation %				
	0% oil	**1% SBO**	**1 % LO**	**2% LO**	**3% LO**
VH at 100X	823.00 ± 7.51	896.00^{c} ± 4.04	881.87^{d} ± 1.95	1012.73^{b} ± 2.97	1154.27^{a} ± 3.46
CD at 100X	84.40^{c} ± 2.83	93.60^{b} ± 1.85	94.23^{b} ± 3.01	102.93^{a} ± 1.92	108.57^{a} ± 2.45
VH/CD at 100X	9.78^{ab} ± 0.42	9.58^{b} ± 0.15	9.38^{b} ± 0.33	$9.84a^{b}$ ± 0.16	10.64^{a} ± 0.27
Mucosa thickness at 40X	418.50^{e} ± 4.05	431.00^{d} ± 2.16	943.27^{c} ± 2.79	783.53^{b} ± 2.86	1045.83^{a} ± 2.74
wall thickness at 40X	492.67^{d} ± 2.90	472.47^{e} ± 1.55	1071.33^{b} ± 2.40	1035.00^{c} ± 2.31	1390.67^{a} ± 3.48
VW at 100X	47.83^{b} ± 1.17	73.28^{a} ± 1.09	73.70^{a} ± 1.27	78.93^{a} ± 5.70	77.80^{a} ± 2.46
Villus perimeter	12.36^{d} ± 0.19	20.63^{c} ± 0.23	20.42^{c} ± 0.36	25.12^{b} ± 1.83	28.22^{a} ± 0.96
Villus surface area	101.71^{d} ± 0.83	184.79^{c} ± 1.48	180.07^{c} ± 3.32	254.38^{b} ± 8.63	325.75^{a} ± 11.99

a, b, c, d means ± standard error in the same row with different superscripts are significantly different (P<0.05). VH= Villous height, CD =Crypt depth, VH/CD= Villous height: Crypt depth, VW= Villous width.

TLC significantly(P<0.05) give best ratio in all linseed oil groups than control groups. This result agreed with a study by Al-Khalifa (2017) who revealed that the consumption of n-3 PUFAs particularly the long chain (>18 carbon atoms) fatty acids have been shown to have a pronounced effect on the health and immune status and rework the phenotypes of immune cells of different species including humans, rats and poultry.

However, dietary PUFA from a variety of sources can be a useful tool for altering immune response (Calder, 2001). Unsaturated FA increases cell flexibility in immune cells and modifies the activity of particular immune cells (Calder, 2007). Numerically antibody titer against Newcastle disease vaccine increased with linseed oil supplement groups than control groups (Table 6). This result agrees with research conducted by Smith *et al.* (2018) who demonstrated that broilers fed diets enriched with linseed oil exhibited elevated levels of immunoglobulins, such as IgG and IgM, compared to control groups. These findings suggest that linseed oil can enhance humoral immunity in broilers, leading to improved defense against pathogens.

Linseed oil supplementation significantly (P<0.05) decreased mortality ratio. But Carragher *et al.* (2015) showed that there was no effect of dietary treatment on the mortality of the broilers during the trial. Overall mortalities were 6.0 % in the Control diet fed broilers and 6.7% in the high ALA fed broilers. This may be due to supplementation of PUFAs, to poultry diets, has been strictly connected with immune regulatory effects on both the innate and adaptive immunity through various mechanisms de Pablo *et al.* (2002). Also, lower dietary levels of linseed oil may be advantageous for improvements in broiler immunity and recovery from immunological tasks (Kidd, 2004).

Effects of linseed oil dietary supplement on intestinal morphology

The recorded results in Table 7 and Figure 1 showed a significant improvement in intestinal morphology in linseed oil-based diets than control groups through significant improvement of villus height, crypt depth, mucosal thickness, wall thickness, villus width, villus perimeter and villus surface area. Several studies agreed with these results for example, Li *et al.* (2016) who conducted a trial and found that broilers receiving diets supplemented with linseed oil exhibited improved intestinal morphology, increased villus height, and decreased crypt depth in the jejunum and ileum of broilers. Another study found that dietary linseed oil increased villus height and crypt depth in the duodenum of broilers (Shamliyan *et al.*, 2019). The mechanisms by which dietary linseed oil improves broiler intestinal villi

Table 8: Effect of linseed oil supplementation in chicks' diets on economic efficiency of 35-days old broiler chickens.

Economic efficiency	Dietary linseed oil supplementation%				
	0% oil	1% SBO	1 % LO	2% LO	3% LO
Feeding cost of the obtained gain (LE)	18.52	18.93	19.18	20.40	20.68
Selling price of the obtained gain (LE/kg live weight)	23	23	23	23	23
Selling cost of obtained gain (LE)	50.82	53.19	50.82	53.19	55.93
Economic efficiency (EE) %	174.41	180.92	164.95	160.77	170.44

LE= Egyptian pound date of experiment= 1/3//2019.

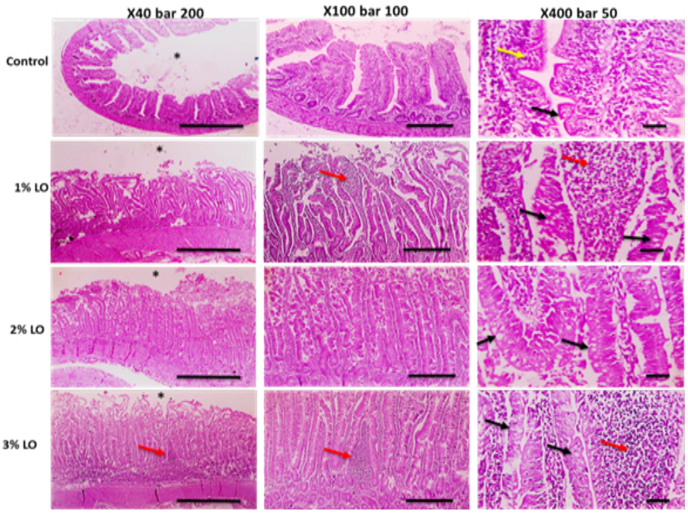

Figure 1: Microscopic pictures of H and E stained jejunal sections from groups supplemented with ascending percentages of LO showing gradual increase in villous height, numbers and size of goblet cells (black arrow), lymphocytic cells population in lamina propria (reds arrows) and decrease epithelial vacuolization (yellow arrows). (asterisks point to lumen of duodenum).

morphology are not fully understood. However, a study found that dietary linseed oil enhanced immune function in broilers challenged with *Salmonella* (Shamliyan *et al*., 2019). Additionally, linseed oil supplementation has been associated with enhanced intestinal barrier function and reduced inflammatory response in broilers (Wang *et al*., 2000). Also, Xu *et al*. (2017) demonstrated that linseed oil increased the abundance of beneficial bacteria such as Lactobacillus and Bifidobacterium, while decreasing the population of potentially harmful bacteria like *Escherichia coli*. These alterations in gut microbiota composition have been associated with reduced inflammation and improved gut health. What is more, research by Cheng *et al*. (2017) indicated that linseed oil supplementation elevated the production of immunoglobulins and cytokines, which are vital components of the immune defense mechanism. In addition, the anti-inflammatory properties of omega-3 PUFAs may help mitigate intestinal inflammation and alleviate stress-related immune responses. One potential mechanism by which dietary linseed oil may improve broiler health is through its anti-inflammatory effects due to decreased the levels of pro-inflammatory cytokines in the serum of broilers. Similarly, Shamliyan *et al*. (2019) found that dietary linseed oil reduced the expression of inflammatory markers in the intestines of broilers.

The economic evaluation of linseed oil dietary supplement The results presented in Table 8 showed that feeding and selling cost of the obtained gain had increased by increasing LO use. However, groups that received no oil had the lowest feeding cost. The highest economic efficiency was recorded in the group fed 1% SBO followed by 0% oil, 3% LO, 1% LO, and 2% LO, respectively. Likely, numerous studies had evaluated the influence of linseed oil supplementation on broiler growth performance. For instance, Smith *et al*. (2018) reported a significant improvement in body weight gain and feed conversion ratio in broilers fed a diet supplemented with linseed oil compared to the control group. The economic evaluation is specific to the study and market conditions at the time of the experiment and may not generalize to different settings.

An economic analysis was calculated according to the following equation: Y= (A-B)/B x 100 where A is the selling cost of the obtained gain and B is the feeding cost of this gain El-Kerdawy (1997).

CONCLUSIONS AND RECOMMENDATIONS

The supplementation of linseed oil in chick's diets significantly improved the growth performance, body composition, meat quality, immune response, serum biochemical parameters, intestinal morphology, and decreased the mortality rate. The most beneficial inclusion rate is 2% then 1% LO along the breeding period for the best FCR and immune response with improved meat quality. At the same time, the work is promising for practical implications for meat or egg poultry producers and future research in different dosages and durations. future research, including examining different sources of omega-3 fatty acids or varying supplementation levels, would provide a more comprehensive overview and may introduce further explanations and take our paper as a guide, for addition new results and benefits of using linseed oil as a feed supplement.

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NOVELTY STATEMENT

Supplementing chick feed with linseed oil improves growth performance, immunological response, body composition, serum biochemical parameters, and intestinal morphology while decreasing mortality ratio, according to the current study.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the current study.

Conflict of interest

The authors have declared no conflict of interest.

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