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EFFECT OF ADDING DIFFERENT SOURCES OF PROBIOTICS TO DIETS CONTAINING OXIDIZED PALM OIL ON SOME PRODUCTIVE AND REPRODUCTIVE TRAITS IN GIMMIZAH LAYING HENS

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SUMMARY

This study was carried out to evaluate the effects of different sources a probiotics (dry yeast, *Aspergillus awamori or Lactic acid bacteria*) as natural antioxidant agents to diets containing oxidized palm oil on some productive and reproductive traits, some blood components, egg quality, egg yolk cholesterol and fatty acids profile, economic efficiency and relative economic efficiency of Gimmizah laying hens. A total number of 168 (126 females and 42 males) Gimmizah laying hens at 25 weeks of age were weighed and randomly distributed into six experimental groups; each group contained 28 birds (21 females and 7 males). The first group was fed a basal diet contains 16.29% crude protein, 2730 ME kcal/ kg diet. The second group was fed basal diet contained 2% oxidized dietary palm oil supplementation. While, the birds of the third, fourth and fifth groups were fed a diet of the second group, adding 1% of different sources of probiotics (dry yeast, Aspergillus awamori or Lactic acid bacteria), respectively. As for the sixth group, they were fed a diet of the second group with the addition of 0.5% of each of: dry yeast, Aspergillus awamori and lactic acid bacteria. The results showed a significant improvement (at the 0.05 level) in the production rate, number, egg mass and feed conversion rate by adding 0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% lactic acid bacteria to the basal diet supplemented with 2% oxidized palm oil (group sixth). The sixth treatment birds also recorded the highest significant improvement in the quality of the eggshell (shell thickness and strength) and the highest value of the egg shape index at the age of 37 weeks. Significant improvement was also observed for some characteristics of egg yolk and Haugh units as a result of the additives used. It was found that the birds fed the diets supplemented with probiotics produced eggs with low cholesterol concentration, and the concentration of: total cholesterol, total triglycerides and low-density lipoprotein in the blood serum was significantly decreased, while the concentration of high-density lipoprotein was significantly increased in the blood serum of these birds. Saturated fatty acids decreased in egg yolk, while unsaturated fatty acids increased in all treatments added to probiotics, either alone or in combination, compared to the basal diets without or with oxidized palm oil. The addition of the probiotic mixture (the sixth treatment) led to a significant improvement in fertility and hatchability and a significant decrease in the percentage of embryonic mortality (early and later). **Conclusion**: The addition mixture of probiotics as (dry yeast, *Aspergillus awamori* or *Lactic acid bacteria*) as antioxidant agents had a positive effect on performance and reproductive traits of Gimmizah laying hens under experimental conditions.

***Keywords**:* Oxidized palm oil, *Saccharomyces cervices*, *Aspergillus awamori*, *Lactic acid bacteria*, performance, reproductive traits, egg quality, blood parameters, fatty acids composition and laying hens**.**

INTRODUCTION

Heated oils contain various amounts of peroxidation products (Zhang *et al.,* 2012), such as 4-hydroxynonenal, hydroperoxide, malondialdehyde, and 2,4-heptadienal (Choe and Min, 2007), which influence oil odor, palatability and quality (Smyk, 2015). Lipid oxidation is a serious problem to both the food industry and the consumer, because it shortens the shelf life of eggs and carcass of poultry, and increases the development of rancid odours and flavours. In addition, free radicals formed during oxidation may be a potential health risk to consumers (Lindblom, 2017).

Several studies reported that the consumption of oxidized fats affects metabolism in several ways (Skufca, *et al.,* 2003), in multiple animal species (Ehr *et al.,* 2015), oxidized oils decrease feed intake, depress growth and even cause disease. Feeding oxidized oils impaired the growth performance and induced oxidative stress in broiler and laying hens (Wang *et al.,* 2015). Feeding oxidized soybean oil impaired growth broilers and antioxidant activities (Tan *et al.,* 2018). In poultry, oxidative stress may occur due to several factors include: a) Feed such as, high concentration of PUFA], contamination with fungal toxins, prolonged storage and antioxidant deficiency (Chung, *et al.,* 2005), b) Environmental such as, heat, high stocking density, transportation, and vaccination (Panda, *et al.,* 2008) and c) pathological conditions such as, ascites, fatty liver haemmorrhagic disease syndrome arthritis and coccidiosis (Iqbal, *et al.,* 2002).

Probiotics as "live micro-organisms which" when administered in adequate amounts, confer a health benefit on the host. Probiotics are live microbial culture, such as bacteria, yeast and fungi, which influence the health and nutrition of the host by improving its intestinal microbial balance (Zarei *et al.,* 2018). Currently many species are being used in probiotic preparations as *Aspergillus awamori*, a variant of *Aspergillus niger*, is one of the fungi had long been used for processing foods because it safe, produce enzymes such as amylase, protease, pectinase and lipase, and thus enhances the digestion of carbohydrates, proteins and lipids. Also, feasible yeast feedstuffs are normally included into poultry diets microorganisms used in food fermentations and health promotion; numbers produced nutrition and health benefits with regards to nutrient digestibility, gut microbiota, immunity, improving lipid metabolism and reducing cholesterol (Xu *et al.,* 2020). Interestingly, Seal *et al*. (2018) reported and argued on the use of *Lactic acid bacteria* (LAB)-probiotics in promoting the growth and reproduction performances and the survival rate and health status of animals. Also, dry yeast (*Saccharomyces cerevisiae*) has biologically valuable proteins, vitamin B-complex, mannan oligosaccharide, important trace minerals and several unidentified growth promoting factors. Yeast contains digestible proteins especially in the form of free amino acids and peptides, functional nucleic acids and natural immune enhancer such as β-glucan and mannan oligosaccharides.

Inclusion of probiotic in the diet has been found to improve egg production and feed conversion ratio (Macit *et al.* (2021). Furthermore, giving probiotics to laying hens has been found to improve eggshell quality (Sobczak and Kozłowski, 2015). The supplementation of probiotics to diet of laying hens may play an important role in altering the lipid metabolism of chickens. Many investigators have pointed out that probiotics could reduce the cholesterol content of egg yolk (Lokapirnasari *et al.,* 2020). Also, Arpášová *et al.* (2016) reported that fatty acid composition in egg yolk could be altered by probiotic supplementation into diets of laying hens and reduce cholesterol level in serum (Ali *et al.,* 2020). Therefore, this study was conducted to investigate the effects of adding different sources of probiotics (dry yeast, *Aspergillus awamor or LAB*) as natural antioxidant agents to diets containing oxidized palm oil on the productive, reproductive traits of Gimmizah laying hens.

MATERIALS AND METHODS

The present study was conducted at a private farm in Tanta, Gharbia Governorate, Egypt during the period from September to November 2020. A total number of 168 (126 females and 42 males) Gimmizah laying hens, at 25 weeks of age with an average initial body weight of 1172 g for females and 1286 g for males were used. Birds were allotted at random to six equal dietary treatments each group contained 28 birds (21 females and 7 males). Feed and water were provided *ad-libitum* during the experimental period. Artificial light was used beside the normal day light to provide 16 hour/ day photo period.

The first group was fed a basal diet (Table 1) contains 16.26% crude protein, 2737 ME kcal/ kg diet (the basal diet without oxidized palm oil), the second group was fed basal diet contained 2% oxidized dietary palm oil supplementation. While, the birds of the third, fourth and fifth groups were fed a diet of the basal diet with 2% oxidized palm oil (the second group), adding 1% of different sources of probiotics (dry yeast, *Aspergillus awamori* or *LAB*), respectively. As for the sixth group, they were fed a diet of the second group with the addition of 0.5% of each of: dry yeast, *Aspergillus awamori* and *LAB.* Oxidized palm oil, (200 mEq of peroxide number/ kg oil) was prepared at laboratories of Agriculture research center, Ministry of Agriculture, by aeration the refind palm oil at 145^{o}C for 24 hours (Dror *et al.,* 1976).
The peroxide value was calculated as follow:

Table (1): Ingredients and chemical analysis of the experimental laying diets.

Ingerdiets	Basal diet (%)	Oxidized palm oil diet (%)
Ground yellow corn (8.5%).	65.06	58.30
Soybean meal (44%).	23.91	23.60
Wheat bran (15%).	0.83	5.90
Oxidized palm oil.	-	2.00
Limestone ground.	7.50	7.50
Di-calcium phosphate.	1.93	1.93
Vitamin and mineralmi mixture[1].	0.30	0.30
Dl-Methionine[2].	0.14	0.14
Sodium cholorid (salt).	0.33	0.33
Total	100	100
Caculated values[3]:		
Crude protien, %.	16.29	16.29
ME, kcal/ kg diet.	2730	2730
C/P ratio.	168	168
Lysine, %.	0.82	0.82
Methionine, %.	0.4	0.4
Calcium, %.	3.36	3.36
Av. Phosphorus, %.	0.47	0.47
Determined values, %:		
Dry matter	90.36	90.21
Crude proteine	16.24	16.26
Ether extract	3.01	3.51
Crude fiber	3.12	3.46
Calcium	3.44	3.40
Av. Phosphorus	0.50	0.51

[1]*Vitamin and Mineral mixture at 0.30% of the diet supplies the following per kilogram of the diet: Vitamin A 12,000 IU, vitamin D_3 3,000 IU, vitamin E 40 mg, vitamin K_3 3 mg, vitamin B_1 2 mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 0.02 mg, niacin 45 mg, biotin 0.075 mg, folic acid 2 mg, pantothenic acid 12 mg, manganese 100 mg, zinc 600 mg, iron 30 mg, copper 10 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg.*
[2]*DL – Methionine: 98% feed grade (98 % Methionine).*
[3]*Calculate according to NRC (1994).*

Peroxide number (mEq/ kg oil) = Volume of thiosulphate × Standard (0.01) × 1000 ÷ Weight of sample. While, the probiotic microbial strains used in this study were obtained from Microbiological Research Center (Cairo, Egypt). The probiotic consists of *Aspergillus awamori* spores was about 25 x 10^4 g. Also, dried active yeast *S. cerevisia* factory strain, used in this study, was provided by a commercial company (The Egyptian Company for Starch, Yeast and Detergents, Co., Ltd.; Alexandria, Egypt). The number of *Saccharomyces cervices* F- 7 visiae factory strain 27 spores was CFU 2 × 10^{10} g and *LAB* ; *Lactobacillus planetarium* F – 456 CFU 1.8 × 10^8 g. Probiotics and dry yeast were in dried powder form and were thoroughly mixed in the negative diet. Mixing of oxidized palm oil supplemented diets was done weekly and nutrient requirements were calculated recommend by the National Research Council (NRC, 1994).

Individual body weights were recorded on the first day of the experiment and monthly, thereafter. Feed intake (FI) was recorded and feed conversion (FC) was determined as a gram of FI per gram of egg mass produced (g of feed/ g egg mass). Egg production (EP) traits including hen day production percent, egg weight (EW), egg number (EN) and egg mass (EM) were recorded and calculated on a daily basis throughout the laying period. Representative egg samples (15 eggs) were randomly collected from each dietary treatment groups were at 37 weeks of age for the determination some egg quality traits. Egg shape and yolk index were determined according to Funk (1948) and Romanoff and Romanoff (1949) as follows:

Egg shape index (%) = (width / length) × 100.

Yolk index (%) = (height / diameter) × 100.

Eggshell thickness (ST), including shell membranes, was measured using a micrometer at the equator.

Eggshell strength (ES - measured with a device recording breaking strength with the Haugh units were applied from a special chart using EW and albumen height which was measured using a tripod micrometer according to Haugh (1937), Kotaiah and Mohapatra (1974) and Eisen *et al.* (1962) as follows:
Haugh units = 100 log (H + 7.57 - 1.7 $w^{0.37}$), where: H = Albumen height (mm) and W = Egg weight (g).

The egg yolk visual color was determined by matching the yolk with one of the 15 bands. The Roche yolk color fan-an instrument for measuring yolk color by (Rauch, 1961).

Total cholesterol and fatty acids profile were determined in fat separated via extraction from the egg yolk with a mix of chloroform and methanol (2:1 vol: vol; according to Folch *et al.,* 1957). Cholesterol was separated from fat after saponification with KOH according to the modified method of the International Dairy Federation (1992).

The samples were subjected to chromatographic analysis in a PU-4600 (PyeUnican, Cambridge, UK) chromatograph with flame ionisation detectors, using the following conditions: the length of a glass column, 1 m; internal diameter, 4 mm; temperature: in detector, 300 C; at injector, 290 C; in column, 260 C; carrier gas, argon; flow rate, 50 cm 3/min and internal standard Dotriacontane (Sigma, St. Louis, MO). Egg yolk cholesterol was calculated and expressed as milligrams/gram of yolk. Fatty acids profile was identified based on retention times and expressed as a percentage (wt/wt) of total FA.

Fertility and hatchability traits were measured by collecting eggs for 7 days during the laying period at 37 weeks of age using artificial insemination methods, then stored and transferred to hatchery for incubation, data were recorded and parameters were calculated.

At the end of the experiment (37 weeks), blood samples were taken from the wing vein from 3 laying hens per treatment without anticoagulant and kept at room temperature for one hour to colt. Tubes were centrifuged at 300 rpm for 15 minutes to separate clear serum and determine serum total protein (Henry *et al.,* 1974), total cholesterol (Bogin and Keller, 1987), triglycerides (Allain *et al.,* 1974), High–density lipoprotein; HDL cholesterol (Siedel, 1983) and the transaminase enzymes activities of serum alanine aminotransferase (ALT) and asparate amino transferase (AST), were determined by calorimetric method of Reitman and Frankel (1957). These biochemical determinations of blood serum were performed calorimetrically by using commercial kits (spectrum diagnostics which was manufactured at 2006 by MDSS Gmbh, schiffgraben 41, 30175 Hannover, Germany). The compositions of the experimental basal diets were based in tabulated values for feed stuffs NRC (1994). Feed samples of the experimental basal diets were taken for proximate analysis. Moisture, crude protein, ether extracet, crude fiber and ash were determined according to the official methods of AOAC (2005). Egg yolk samples were analysed for dry matter content (DM) by drying samples at 1050C for 24 h in forced air oven. The crude protein was determined by Kjeldahl method and crude fat was determined by Soxhlet method (AOAC (2005).

Economic efficiency for EP was calculated from the input – output analysis (Heady and Jensen, 1954), according to the price of the experimental diets and egg production. Values of economic efficiency were calculated as the net revenue per unit of total costs (Soliman and Abdo, 2005).

The experiment was conducted using a completely randomized design using SPSS (2011) program and the difference among treatment means were determined using Duncan's multiple range test (Duncan, 1955). Percentages were transformed to the corresponding arcsine values before performing statically analysis.

Statically model: $Yij = \mu + \alpha i + Eij$.

Where: Yij = observed traits, μ = overall mean, αi = effect of treatment (i = 1, 2, 3,…..6),
Eij = experimental random error.

RESULTS AND DISCUSSION

The effects of feeding probiotics as natural antioxidant agents to diets containing oxidized palm oil on the performance of Gimmizah laying hens is presented in Table (2). Experimental data indicated that dietary treatments T_3, T_4, T_5 and T_6 recorded that both Gimmizah males and females body weight at 37 wks were significantly differ. It is observed that body weight was significantly increased ($P \leq 0.05$) with the combination of probiotics supplementation, T_6 (0.5% dry yeast + 0.5% *Aspergillus awamori* + 0.5% *LAB*) of males (1893 g) and females (1706 g) in a respective order compared to the positive and negative control groups (1729 and 1639 g) and (1579 and 1440 g) for males and females, respectively. The increase in body weight suggests that combination of probiotics beneficial effects of *Saccharomyces cerevisiae* is considered as one of the live microorganisms probiotic that, when administered through the digestive tract, have a positive impact on the hosts health through its direct nutritional effect (Shareef and Dabbagh, 2009), *Aspirgulus awamori* appears to result

Table (2): Productive performance of Gimmizah chickens as affected by dietary oxidized palm oil and probiotics (Means ± SE).

Item	Dietary treatment[1]						Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Male body weight 25 wks (g).	1287 ± 7.39	1283 ± 7.00	1289 ± 4.29	1288 ± 4.62	1284 ± 5.91	1284[2,3] ±5.83	NS
Male body weight 37 wks (g).	1729[c] ± 3.71	1639[d] ±3.59	1802[b] ± 9.78	1823[b]± 4.69	1857[ab] ± 5.98	1893[a] ±7.21	*
Female body weight at 25 wks (g).	1171 ± 5.81	1173 ± 6.61	1168 ± 7 .36	1174 ± 6.08	1170 ± 3.95	1172 ± 6.53	NS
Female body weight at 37 wks (g).	1579[d] ± 5.04	1440[e] ± 5.32	1590[c] ± 7.38	1634[b] ± 5.30	1676[a] ± 5.87	1706[a] ± 5.19	*
Egg number (per hen/ 25 - 37 wks).	49.73[d] ± 062	44.17[e] ± 0.85	51.27[c] ± 0.48	53.48[ab] ± 0.53	53.85[b] ± 1.06	54.91[a] ± 0.49	*
Egg production, % (hen-day).	59.20[c] ± 1.22[c]	52.58[d] ± 0.67	61.03[ab] ± 0.94	63.67 [ab] ± 1.22	64.11[b] ± 0.61	65.36 [a] ± 0.79	*
Egg weight (g).	50.01[c] ± 0.19	48.87[d] ± 0.24	50.86 [b] ± 0.42	51.76[a] ± 0.44	52.00[a] ± 0.19	53.11[a] ± 0.35	*
Egg mass (g/ hen/ d).	24.87[c] ± 0.77	21.48[d] ± 0.57	26.01[b] ± 0.75	27.69[b] ± 0.82	28.04[b] ± 0.88	29.54[a] ± 0.78	*
Feed intake (g/ hen/ d).	109.86[d] ± 0.89	106.00[e] ± 0.79	111.68[c] ± 0.90	115.33[b]± 1.03	116.19[ab] ± 0.89	117.34[a] ±0.78	*
Feed conversion (g feed/ g egg mass).	3.70 [b] ± 0.08	4.12[a] ± 0.07	3.60[bc] ± 0.07	3.50[c] ± 0.08	3.49[d] ± 0.06	3.38[e] ±0.08	*

[1]*T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori,*
T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria).
[2]*Means ± S.E. of 3 replicates/ treatments.*
[3]*Means followed by different superscripts in the same row are significantly different (P ≤ 0.05).*
**Significant (P ≤ 0.05). NS, Not significant.*

from an increase in the feed efficiency of laying hens and metabolisable energy from the diet. The *Aspirgulus awamori* possesses the ability to digest raw starches according to (Amsal *et al.*, 1998) and to produce cellulase and xylanase, which are required for the digestion of soluble non-starch polysaccharides and improved the nutritional quality of soybean meal because the high level of trypsin inhibitor contained in unprocessed soybean is degraded by *Aspergillus spp.* (Hong *et al.*, 2004). Also, this improvement could be attributed to the effect of *lactobacilli* culture on improving intestinal microbial balance, adhering to the intestinal mucosa, secreting active metabolites, and its ability to antagonize and competitively exclude some pathogenic bacteria in chickens (Trela *et al.*, 2020). Interestingly, Seal *et al.* (2018) reported and argued on the use of *LAB* probiotics in promoting the growth and reproduction performances and the survival rate and health status of animals. These results are in agreement with those obtained by Zhang and Kim (2014) found that diets supplemented with multi strain probiotics significantly improved body weight. There are some studies that report depressed growth and impaired health in birds fed oxidised oil (Wang *et al.*, 2015 and Ali *et al.*, 2020). On the other hand, Sakine *et al.* (2014) reported that there were no significant effects on live body weight of laying hens fed diets supplementation with single or combination probiotics.

The data in Table (2) showed that there is significant effect of probiotic (natural antioxidant) on production traits. Hens fed T_6 (basal diet with 2% oxidized palm oil +0.5% dry yeast + 0.5% *Aspergillus awamori* + 0.5% *LAB*) recorded significantly increased EN, percentage of EP, EWand EM. The lowest value of EN (44.17), percentage of EP (52.58%), EW (48.87g) and EM (21.48 g/ hen/ d) for second treatments (T_2 , basal diet with 2% oxidized palm oil). The differences between the results of the present study and those of previous studies may be due to the species and age of the birds, dietary nutrient composition, type, dosage and composition of yeast and probiotic in the diets and environmental conditions. *Saccharomyces spp* supplementation increased EP and its total weight, this may be due to the fact that the present of probiotic in the digestive tract of poultry may improve digestibility of nutrients, particularly of protein and minerals (Bidura *et al.*, 2012). Results reported herein are in harmony with these obtained by others researchers reported EN, EM and EP were increased by the dietary supplementation of by using various levels and sources of probiotic of laying hens (Hassan *et al.*, 2019). While, Lee *et al.* (2019) found a significant reduction in EP by dietary oxidized oil. The inconsistence results have been reported by Elnagar (2013) and Tapingkae *et al.* (2018) who found that there were no significant differences in EP and EM due to probiotics supplementation to the diets.

Results in Table (2) showed that FI (g/ hen/ day) was significantly increased by adding dry yeast, *Aspergillus awamori or LAB* compared to the basal diets without or with oxidized palm oil (T_1or T_2). Highest value of FI was (117.34g/ h/ d) in hens fed combination probiotics to basal diet with 2% oxidized palm oil comparsion with other treatments. Finding of Zhang and Kim (2014) and Özsoy *et al.* (2018) reported an increase in FI in chicken fed with probiotics and yeast addition to the diet. While, Ali *et al.*, 2020 reported that FI was not adversely affected by oxidised oil in layer and broiler. The results disagree with Saleh *et al.* (2017) and Hassan *et al.* (2019).

Data on the effect of dietary single or combination probiotics supplementation on FC are presented in Table (2). *Saccharomyces cerevisia*, *Aspergillus awamori and LAB* supplementation at levels of 1% being 3.60, 3.50 and 3.49g feed/ g EM for T_3, T_4 and T_5, respectively and T_6 (0.5% dry yeast + 0.5% *Aspergillus awamori* and 0.5% LAB), FC value was (3.38 g feed/ g egg mass). These results may be due to the better EP rate of these birds, and may be due to the ability of probiotics to improve the performance and FC ratio may be attributed to the improvement in nutrients absorption and utilization associated with adding yeast which reduces the proliferation of enteric harmful bacteria that responsible of mal-absorption (Tapingkae *et al.* 2016). These results are in agreement with those obtained by (El-Kaiaty *et al.*, 2019 and Macit *et al.*, 2021) who showed that FC ratio was significantly ($P \leq 0.05$) improved due to probiotic supplementation.

In this study, the absence of probiotic, FC ratios were 4.12 and 3.70 g feed/ g EM for T_2 and T_1, respectively. These results indicate that the use of antioxidants may prevent deterioration of productivity under the condition where oxidative damage is expected. These results are in line with the previous findings (Anjum *et al*., 2004); this might be due to the destruction of fat soluble vitamins in rancid oil that leads to reduced availability of nutrients as well as immunity, and consequently depressed growth performance (Cheeke, 1991). On the contrary, Tan *et al.* (2018) reported that oxidized oils cause's negative effects in FC ratio. While, Hassan *et al.* (2019) and Mikulski *et al.* (2020) found that yeast and probiotic supplementation had no effect on FC ratio in laying hens.

As shown in Table (3) egg quality parameters did show significant responses to the dietary supplementation of probiotics. Except for shell and yolk percent, the effects of probiotics to the basal diet with oxidized palm oil on other egg quality parameters were significant increased at 37 weeks of age. The addition of (0.5% dry yeast + 0.5% *Aspergillus awamori* and 0.5% *LAB)* to hen's diet significantly increased egg shape index, shell thickness and ST, respectively.

The improvement in ST and ES may be attributed to the enhancement of calcium absorption and retention associated with adding yeast into the diet (Tangendjaja and Yoon, 2002). Also, Abdelqader *et al.* (2013) mentioned that the development of ST was connected to the promoting effect of probiotics on metabolic processes as well as calcium utilization. Therefore, this variation might be due to trace mineral content and utilization with microbial supplementation in the diet of laying hens. Similar finding on egg shape index and ST were reported by Davis and Anderson (2002) obtained that eggshell quality were significantly ($P \leq 0.05$) increased by hens fed diets with a mixture of probiotic content *Lactobacillus* cultures.

Elnagar (2013); Saleh *et al.* (2017) and El-Kaiaty *et al.* (2019) found that eggshell quality was improve using probiotic in laying hens. While, Lee *et al.* (2019) reported that ST and ES was not affected by feeding of oxidized oil. Sakine *et al.* (2014) reported that yeast supplementation had no significant effect on the mean values of ST of laying hens. There were significant differences between treatments in percentage of albumen %, Haugh units, yolk index and yolk color. Whereas, there were insignificant effect in yolk percentage (Table 3) by addition of 0.5% dry yeast + 0.5% *Aspergillus awamori* + 0.5% *LAB* to basal diet with oxidized palm oil,

Table (3): Egg quality traits of Gimmizah hens as affected by dietary oxidized palm oil and probiotics at 37 weeks of age of the experimental period (Means ± SE).

Item	Dietary treatment[1]						Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Egg shape index (%).	73.60^{c} ± 0.50^{2}	71.44^{d} ± 0.44	75.09^{b} ± 0.48	77.82^{ab} ± 0.64	76.17^{b} ± 0.70	$79.66^{a2,3}$ ± 0.60	*
Shell (%).	10.66 ± 0.42	10.68 ± 0.34	10.66 ± 0.45	10.89± 0.38	10.94 ± 0.24	10.67 ± 0.24	NS
Shell thickness (mm).	0.348^{d} ± 0.005	0.339^{e} ±	0.368^{c} ± 0.010	0.362^{c} ± 0.010	0.375^{b} ±	0.393^{a} ± 0.005	*
Eggshell strength (N/cm3).	32.01^{d} ± 1.77	29.86^{d} ± 2.46	34.22^{ab} ± 1.96	33.90^{c} ± 2.62	35.78^{b} ± 1.87	43.52^{a} ± 2.87	*
Albumen (%).	56.06^{c} ± 0.53	56.14^{c} ± 0.52	57.16^{ab} ± 0.63	58.11^{a} ± 0.75	56.66^{b} ± 0.58	56.88^{b} ± 0.58	*
Haugh units.	79.44^{d} ± 0.63	75.02^{e} ± 0.64	83.22^{b} ± 0.79	82.87^{c} ± 0.60	83.05^{b} ± 0.64	84.36^{a} ± 0.84	*
Yolk index (%).	42.39^{b} ± 0.72	41.74^{c} ± 0.63	43.55^{a} ± 0.61	44.13^{a} ± 0.93	44.35^{a} ± 0.47	44.85^{a} ± 0.67	*
Yolk (%).	33.29^{c} ± 0.34	33.18^{d} ± 0.34	32.18^{b} ± 0.35	31.00^{b} ± 0.39	32.40 ^{a} ±	32.45^{a} ± 0.39	*
Yolk colour.	6.31^{c} ± 0.25	6.11^{d} ± 0.29	7.19^{a} ± 0.31	7.68^{a} ± 0.20	6.87^{b} ± 0.23	7.45^{a} ± 0.20	*
Egg yolk cholesterol (mg/ g yolk).	16.58^{b} ± 0.59	19.78^{a} ± 0.58	13.77^{dc} ± 0.38	15.11^{c} ± 0.57	12.08^{d} ± 0.55	10.72^{e} ± 0.55	*

[1]*T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori, T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria).*

[2]*Means ± S.E. of 3 replicates/ treatments.*

[3]*Means followed by different superscripts in the same row are significantly different (P≤0.05).*

**Significant (P ≤ 0.05). NS, Not significant.*

respectively in comparison with other treatments. Similar finding were reported by Özek (2012) who found that dietary mannan oligosaccharide supplementation significantly modified albumen height and Haugh units. Arpášová *et al.* (2016) reported that probiotic supplementation had a significant effect on the Haugh units. The Haugh units, an indicator of the most widely accepted measure of internal egg quality, tended to decrease according to the elapsed time of storage (Williams, 1992). It is also suggested that dietary antioxidant nutrients and natural antioxidants are effective in improving the quality of eggs and meats during extended storage (Surai, 2000). Ayanwale *et al.* (2006) recorded that yolk weight was higher in laying hens fed diets having 7.5 g/ kg dried yeast. However, Maziar *et al.* (2007) did not find any effect on egg yolk of laying hens fed yeast and probiotic supplemented diet. These results disagreed with these finding of Chumpawadee *et al.* (2009), who reported that addition of yeast in commercial layer hens diet had not any positive effect on Haugh units. Saleh *et al.* (2017) observed that yolk color was synergistically increased by the combination of *Aspergillus awamori* and *LAB.* Hassan *et al.* (2019) stated that yolk color and Haugh units were not affected by probiotic treatments. Macit *et al.* (2021) reported that albumen index and Haugh units traits were not affected by dietary probiotic and humate.

Experimental results indicated that egg yolk cholesterol was significantly ($P \leq 0.05$) decreased with probiotics supplementation. Means of egg yolk cholesterol (mg/ g yolk) 13.77, 15.11,12.08 and10.72 for the T_3, T_4, T_5 and T_6 compared to T_1 and T_2, (16.58 and 19.78), respectively (Table 3).

This study has shown that probiotics could reduce cholesterol level in egg yolk (Lokapirnasari *et al.*, 2020). The decrease of cholesterol level by probiotic bacteria is due to the modulation of lipid metabolism, incorporation and assimilation of cholesterol in the cell membrane of the probiotics, intestinal conversion of cholesterol in coprostanol and inhibition of the expression of the intestinal cholesterol transporter Niemann–Pick C1 like 1 (NPC1L1) in the enterocytes (Reis *et al.*, 2017).

Gimmizah hens fed diets supplementation with different probiotics are presented in Table (4). Supplemental combination of probiotics to basal diet with oxidized palm oil during the laying period had significantly greater fertility than those fertile eggs without probiotic. The statistical analysis of incubated eggs showed that there were significant differences among treatments in fertility, hatchability of setting eggs percentages and chick weights. Also, values of hatchability of fertile eggs percentage and early embryonic mortality percentages were significantly differed ($P \leq 0.05$). The fertility percentages was improved being 95.14, 90.43, 89.82 and 96.95 % for T_3, T_4, T_5 and T_6 compared to thebasal diets without or with oxidized palm oil (T_1 and T_2; 84.45 and 80.76%), respectively. Both early and late embryonic mortality were significantly ($P \leq 0.05$) decreased being for probiotic supplementation compared to the positive and negative control. It can be suggested that the increase in hatchability may be due to the increased ST Roque and Soares (1994).

This is in accordance with Bozkurt *et al.* (2011), who reported that hatchability of total eggs set increased probiotic supplementation. Hajati *et al.* (2014) reported that probiotic supplementation had beneficial effects and numerically increased hatchability and decreased infertile eggs, as well as dead-in shells. There are several factors that affect hatchability. Breeder factors that affect hatchability include health, nutrition and egg size, weight and quality. Egg weight, shell thickness, and shape index are the most important factors among egg parameters that influence hatchability (King'ori, 2011). Mazanko *et al.* (2018) found that egg hatchability of birds was significantly improved by the supplementation of diets with probiotic. Similar improvements in hatchability have been observed in birds fed a yeast culture supplement (Mansoub McDaniel and Sefton, 1991).

Also, one reason for the observed improvement in egg hatchability of probiotic fed group could be due to improved haugh unit (Tona *et al.* 2002). Rizk *et al.* (2019) showed that addition of 0.3 and 0.5 g commercial probiotics (contains LAB (Lactobacillus lactis) 2.5×10^8 CFU, Bacillus subtilis 1.8×10^9 CFU/ g product) were significantly increased fertility and hatchability during rearing period as compared to the control diet. Darsi and Zhaghari (2021) showed that dietary supplementation of *Lactobacillus sporogenes* as probiotic in White Leghorn layer breeders diet significantly increased egg hatchability ($P \leq 0.05$). No effects of probiotic supplementation on hatchability was consistent with the results of the study conducted on laying quails by (Aalaei *et al.*, 2018), this was explained by Chesson (1994) that the effect of probiotics may be due to several aspects of the probiotic such as strains of bacteria, dose level, diet composition, feeding strategy, feed form and interaction with other dietary feed additives.

Table (5) shows the effect of feeding single or combination probiotic supplementation on fatty acid composition of egg yolk of basal diet with 2% oxidized palm oil laying hens. In this study, palmatic and stearic acids were decreased by feeding dry yeast, *Aspergillus awamori LAB* or their combination compared with basal diets without or with 2% oxidized palm oil. On the other hand, oleic, linoleic and arachidonic acids were found higher in the group trated basal diet + 2% oxidized palm oil with 0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria, (T_6) compared with other treatments. There were no differences in myristic, eicosenoic, eicosadienoic and eicosapentaenoic acids between treatments.

Table (4): Fertility and hatchability of Gimmizah hens as affected by dietary oxidized palm oil and probiotics at 37 weeks of age (Means ± SE).

Item	Dietary treatment1						Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Total egg set.	45	45	45	45	45	45	NS
Egg weight (g).	48.26	46.55	49.11	49.71	50.20	$50.87^{2,3}$	*
	± 0.29	± 0.18	± 0.34	± 0.51	± 0.46	± 0.56	
Fertility (%).	84.45^{c}	80.76^{d}	95.14^{a}	90.43^{b}	89.82^{b}	96.95^{a}	*
	± 0.20	± 0.14	± 0.14	± 0.29	± 0.17	± 0.56	
Hatchability of setting	75.85^{d} ±	64.00^{e}	79.75^{c}	78.93^{c}	82.41^{b}	83.33^{a}	*
eggs (%).	0.27	± 0.09	± 0.13	± 0.17	± 0.14	± 0.12	
Hatchability of fertile	79.79^{c} ±	68.65^{d}	83.61^{ab}	84.63^{ab}	86.52^{b}	90.22^{a}	*
eggs (%).	0.14	± 0.13	± 0.22	± 0.16	± 0.22	± 0.26	
Early embryonic	$1.12^{a\,\#}$	1.52^{a} .	0.77^{c}	0.75^{c}	0.82^{b}	0.72^{c}	*
mortality (%).	± 0.06	± 0.91	± 0.02	± 0.01	± 0.04	± 0.06	
Late embryonic	0.93^{b}	1.15^{a}	0.79^{d}	0.81^{c}	0.85^{c}	0.79^{d}	*
mortality (%).	± 0.04	± 0.04	± 0.02	± 0.02	± 0.01	± 0.01	
Chick weight (g)	32.52^{c}	30.09^{d}	33.10^{b}	33.73^{ab}	34.06^{a}	34.76^{a}	*
	± 0.21	± 0.14	± 0.21	± 0.25	± 0.40	± 0.20	

[1]*T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori,*
T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori +
0.5% Lactic acid bacteria).
[2]*Means ± S.E. of 3 replicates/ treatments.*
[3] *Means followed by different superscripts in the same row are significantly different (P ≤ 0.05).*
**Significant (P≤0.05). NS, Not significant.*

Table (5): Effect of feeding dietary oxidized palm oil and probiotics on yolk eggs fatty acid in Gimmizah laying hens, % (Means ± S.E).

Item	Dietary treatment[1]						Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Myristic acid, C14:0	0.43 ± 0.01[2]	0.42 ± 0.04	0.44 ± 0.02	0.41 ± 0.01	0.43 ± 0.02	0.41 ± 0.01	NS
Palmitic acid, C16:0	25.73[b] ± 1.36	28.16[a] ± 1.74	22.48[c] ± 1.25	21.85[d] ± 1.28	22.85[c] ± 1.24	20.03[e2,3] ± 1.24	*
Palmitoleic acid, C16:1 ω7, 9	3.01[d] ± 0.85	2.58[e] ± 0.60	3.40[c] ± 0.62	3.73[b] ± 0.080	3.52[c] ± 0.66	3.84[a] ± 0.85	*
Stearic acid, C18:0	9.22[b] ± 0.43	10.76[a] ± 0.46	8.22[c] ± 0.45	7.90[d] ± 0.62	7.78[d] ± 0.87	6.52[e] ± 0.89	*
Oleic acid, C18:1, ω9	41.06[c] ± 1.53	37.74[d] ± 1.55	48.16[a] ± 1.53	46.11[ab] ± 1.75	44.66[b] ± 1.58	48.08[a] ± 1.56	*
Linoleic acid, C18:2, ω6	7.44[d] ± 0.97	5.22[e] ± 0.64	11.22[b] ± 1.01	9.87[c] ± 0.90	13.05[b] ± 1.14	14.36[a] ± 1.44	*
γ-linolenic acid,C18:3, ω6	0.040[c] ± 0.03	0.021[d] ± 0.04	0.065[b] ± 0.01	0.067[b] ± 0.03	0.086[a] ± 0.04	0.077[ab] ± 0.07	*
Eicosenoic acid, C20:1, ω9	0.31 ± 0.24	0.28 ± 0.24	0.32 ± 0.25	0.31 ± 0.29	0.32 ± 0.24	0.34 ± 0.25	NS
Eicosadienoic acid, C20:2, ω6	0.20 ± 0.02	0.16 ± 0.01	0.22 ± 0.03	0.23 ± 0.02	0.23 ± 0.03	0.24 ± 0.02	NS
Eicosatrienoic acid, C20:3, ω6	0.101[b] ± 0.04	0.070[c]. ± 0.04	0.116[b] ± 0.07	0.136[a]. ± 0.08	0.148[a] ± 0.07	0.157[a] ± 009	*
Arachidonic acid, C20:4, ω6	1.32[c] ± 0.32	1.20[d] ± 0.34	2.01[b] ± 0.04	1.93[b] ± 0	2.04[ab] ± 0.24	2.18[a] ± 0.:	*
Eicosapentaenoic acid, C 20:5, ω3	0.076 ± 0.01	0.069 ± 0.02	0.082 ± 0.32	0.093 ± 0.02	0.089 ± 0.02	0.095 ± 0.	NS
Docosahexaenoic acid C 22:6, ω3	1.17[c] ± 0.03	1.04[d] ± 0.01	1.30[b] ± 0.04	1.32[b] ± 0	1.27[b] ± 0.04	1.37[a] ± 0.(	*
Nervonic acid C 24:1, ω9	0.44[d] ± 0.03	0.38[c] ± 0.03	0.47[b] ± 0.03	0.52 [b]± 0	0.55[b] ± 0.06	0.59[a] ± 0.(	*
SFA[4]	35.47[b] ± 1.02	39.44[a] ± 1.0	31.20[bc] ± 0.94	30.22[c]± 0.96	31.15[cbc]± 0.94	27.03[d]± 0.	*
UFA[5]	55.09[c] ± 0.82	49.33[d] ± 0.832	67.28[b] ± 0.96	64.25[ab]± 0.92	65.87[ab]± 0.92	71.11[a] ± 0.98	*
MUFA[6]	44.96[d] ± 0.82	41.02[e] ± 0.94	52.17 [ab] ± 0.82	50.77[b] ± 0.89	49.16[c] ± 0.88	52.9[a] ± 0.8	*

PUFA[7]	10.66[e] ± 1.75	8.04[f] ± 1.82	15.33[c] ± 1.45	14.01[d] ± 2.33	17.98 [b] ± 2.02	18.66[a] ± 1	*
n-6[8]	8.80[d] ± 0.68	6.34[e] ± 0.65	13.30[b] ± 0.88	11.88[c] ± 0.67	15.19[ab] ± 0.75	16.86[a] ± 0.75	*
n-3[9]	1.50[d] ± 0.18	1.11[e] ± 0.18	2.44[c] ± 0.22	2.41[c] ± 0.19	2.78[b] ± 0.19	3.82[a] ± 0.29	*
UFA / SFA	1.55[c] ± 0.02	1.25[d] ± 0.04	2.16[b] ± 0.02	2.13[b] ± 0.04	2.11[b] ± 0.03	2.63[a] ± 0.03	*

[1]*T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori,*
T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria).
[2]*Means ± S.E. of 3 replicates/ treatments.*
[3]*Means followed by different superscripts in the same row are significantly different ($P \leq 0.05$).*
[4]*SFA = saturated fatty acid.;* [5]*UFA = unsaturated fatty acid.;* [6]*MUFA = monounsaturated fatty acid.;* [7]*PUFA = polyunsaturated fatty acid.;*
[8]*n-3 = omega-3 fatty acid.;* [9]*n-6 = omega-6 fatty acid. *Significant ($P \leq 0.05$, NS, Not significant.*

The saturated fatty acid (SFA) was decreased and unsaturated fatty acid (UFA) was increased in the dietary groups compared with control groups. Similarly, the addition of dry yeast, *Aspergillus awamori*, *LAB* individually or in combination to the negative diet layer hens increased PUFA ($P \leq 0.05$) and omega-3 fatty acid, respectively. Higher UFA and SFA ratio (UFA/SFA) was found in group treated with combination probiotics in the oxidized palm oil diet, (T_6; 2.63%) compared to other treatments (1.55, 1.25, 2.16, 2.13 and 2.11) for T_1, T_2, T_3, T_4 and T_5, respectively. Several studies have shown that oleic acid (n-9) and linoleic acid (n-6) are the most common unsaturated fatty acids produced by different types of probiotics and linoleic acid is a major constituent of fungal and bacteria lipid (Tsitsigiannis *et al.,* 2004). Thus, it is probable that the increase in oleic, linoleic and alpha-linolenic acids in the egg yolk may be due to the intestinal activities of *Aspergillus awamori and LAB* . This may also refer to that *Aspergillus awamori* produces desaturase which may change saturated fatty acid to unsaturated fatty acids (Saleh *et al.*, 2013). Similarly, Mikulski *et al.* (2012) reported that laying hens fed with diets including probiotics significantly increased the proportion of PUFA, including linoleic and linolenic acid. Also, Arpášová *et al.* (2016) reported that fatty acid composition in egg yolk could be altered by probiotic supplementation into diets of laying hens. The effects of probiotics on the performance parameters, egg quality traits, and fatty acid composition in egg yolk of laying hens may point out alteration depending on factors such as microbial species composition, supplemented dose, nutrient content of diet, applied method, environmental conditions in poultry house, and hen age. Hassan *et al.* (2019) found that SFA was decreased and UFA was increased in the dietary groups compared with the control.

In another experiment, Yalçın *et al.* (2010) also stated that C18:1n9 and MUFA levels increased, and the other fatty acid parameters were not affected by yeast culture supplementation.

Macit *et al.* (2021) recorded that addition of probiotics in laying diets improved total unsaturated fatty acids compared with the control group. Data on some blood serum constituents of 37 wks – old laying hens fed diets supplemented with different levels of probiotics and oxidized palm oil are presented in Table (6). Supplementation of individual or combination of probiotics to layer diet with oxidized oil significantly ($P \leq 0.05$) increased serum total protein, and HDL. In this study, the concentration of plasma blood glucose had no significant differences between all dietary treatments at 37 weeks of age. AST was significantly ($P \leq 0.05$) increased. Total triglycerides, total cholesterol and LDL were significantly (P ≤

Table (6): Effect of feeding dietary oxidized palm oil and probiotics on some blood serum constituents in Gimmizah laying Hens at 37 weeks of age (Means ± SE).

Item	Dietary treatment[1]						Sig.
	T_1	T_2	T_3	T_4	T_5	T_6	
Total protein, g/ dl.	3.76[c] ± 0.08	3.29[d] ± 0.06	4.60[bc] ± 0.09	4.75[bc] ± 0.27	5.34[b] ± 0.19	5.92[a2,3] ± 0.07	*
Glucose, mg/ dl.	224.31 ± 0.61	222.54 ± 0.23	229.23 ± 0.79	225.62 ± 0.01	229.75 ± 0.46	227.42 ± 0.46	NS
AST, U/ L. [4]	59.33[d] ± 0.76	42.34[e] ± 0.49	65.24[b] ± 0.35	62.11[b] ± 0.39	69.32[ab] ± 0.36	72.15[a] ± 0.33	*
ALT, U/ L. [5]	23.21 ± 0.29	22.77 ± 0.26	21.71 ± 0.09	20.65 ± 0.76	22.81 ± 0.84	21.25 ± 0.79	NS
Total triglyceride, mg/ dl.	172.84[b] ± 0.46	187.4[a] ± 0.63	162.50[c] ± 0.44	156.81[e] ± 0.54	160.74[d] ± 0.54	151.27[e] ± 0.52	*
Total cholesterol, mg/ dl.	145.00[b] ± 0.36	154.68[a] ± 0.66	134.79[bc] ± 0.76	131.23[c] ± 0.46	128.70[d] ± 0.26	122.45[e] ± 0.66	*
HDL, mg/ dl. [6]	19.43[c] ± 0.28	16.33[d] ± 0.25	25.74[b] ± 0.18	34.33[ab] ± 0.19	34.22 [ab] ± 0.38	39.07[a] ± 0.48	*
LDL, mg/ dl. [7]	68.85[b] ± 0.38	72.10[a] ± 0.19	45.00[bc] ± 044	47.76[c] ± 0.61	43.98[bc] ± 0.49	37.01[d] ± 0.76	*

[1]T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori,
T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria).
[2]Means ± S.E. of 3 replicates/ treatments.
[3]Means followed by different superscripts in the same row are significantly different (P ≤ 0.05).
4Asparate amino transferase (AST), 5Serum alanine aminotransferase (ALT), 6High–density lipoprotein (HDL), 7Low–density lipoprotein (LDL).
**Significant (P ≤ 0.05). NS, Not significant.*

0.05) decreased by dietary *Saccharomyces cerevisia, Aspergillus awamori* or *LAB* or their combination supplementation compared with hens fed basal diet and basal die + 2% oxidized palm oil, respectively. The decrease of blood cholesterol concentrations in yeast-treated hens may be due to the incorporation and assimilating cholesterol into the cellular membrane of the microorganism thus, in turn reduces cholesterol absorption. Also, the decrease of blood cholesterol level could be attributed to bile salts deconjugations, which are less depressed and less absorbed in the gastro-intestinal tract (Guo and Zhang, 2010). The above results are agree with the results of El-Kaiaty *et al.* (2019) found that yeast adding in laying hen diets significantly (P ≤ 0.05) lowered serum total lipids, cholesterol and triglycerides levels compared to un-supplemented control group. Also, probiotic may contribute in the regulation of serum cholesterol concentrations conducted by deconjugated bile acids. As cholesterol is a precursor for bile acid formation and when deconjugated bile acids excretion is enhanced by probiotics supplementation, then more precursor molecules are needed for the recovery of bile acid formation (Ezema and Eze, 2015). Consequently, it may be expected that level of serum cholesterol decreases (Sutarpa *et al.*, 2011). Moreover, Klaver and Van Der Meer (1993) also suggested that co-precipitation with bile acids may be of importance in decreasing serum cholesterol concentrations. Moreover the observed hyperthyroidism associated with dietary probiotic could also explain the observed reduction in serum lipid profile. Sjofjan et al. (2021) noted that probiotics reduced cholesterol and LDL while elevated (P ≤ 0.05) HDL blood concentrations.

On the other hands, Sacakli *et al.* (2013) recorded that the serum levels of total triglyceride and cholesterol by the addition of yeast culture were no significant differences by probiotics supplementation. The economic efficiency of the experimental treatments is shown in Table 7. The highest economic efficiency 36.45% and relative economic efficiency (135.15) were obtained with the diet containing 0.5% *Saccharomyces cerevisia*, 0.5% *Aspergillus awamori* and 0.5% *LAB* (T_6). This may be due to the better FC ratio obtained in birds received the experimental diet compared to other diets. While, the lowest economic efficiency (16.13%) and relative economic efficiency (59.81) was found in the basal diet with 2% oxidized palm oil diet (T_2). The obtained results in the present study encouraging and indicated that adding mixture of probiotics as (dry yeast, *Aspergillus awamori* or *LAB*) as antioxidant agents supplementation had a positive effect on performance and reproductive traits, egg quality, egg yolk cholesterol and fatty acids profile, economic efficiency and relative economic efficiency of Gimmizah laying hens under experimental conditions.

Table (7): Effect of dietary oxidized palm oil and probiotic on economic efficiency of Gimmizh hens during the experiment period.

Item	Dietary treatment[1]					
	T_1	T_2	T_3	T_4	T_5	T_6
Price of Kg feed (L. E.)[2].	5.65	5.73	5.77	5.80	5.81	5.92
Total feed intake/ hen (Kg).	9.22	8.83	9.31	9.61	9.68	9.86
Total feed cost hen (L.E.)	52.09	50.59	53.71	55.73	56.24	57.89
Total number of eggs/ hen, egg).	49.73	44.17	51.27	53.48	53.85	54.91
Total price of eggs/ hen (L. E.)[3].	66.14	58.75	68.19	71.13	71.62	79.07
Net revenue/ hen (L. E.)[4].	14.05	8.16	14.48	15.40	15.38	21.18
Economic efficiency (%)[5].	26.97	16.13	26.96	27.63	27.35	36.45
Relative Economical efficiency[6].	100	59.81	99.96	102.45	101.41	135.15

[1]T_1: Basal diet, T_2: (Basal diet + 2% oxidized palm oil), T_3: (Basal diet + 2% oxidized palm oil)+ 1% dry yeast, T_4: (Basal diet + 2% oxidized palm oil)+ 1% Aspergillus awamori, T_5:(Basal diet + 2% oxidized palm oil) + 1% Lactic acid bacteria and T_6: (Basal diet + 2% oxidized palm oil) + (0.5% dry yeast + 0.5% Aspergillus awamori + 0.5% Lactic acid bacteria)

[2]Means ± S.E. of 3 replicates/ treatments. [2]Price of Kg oxidized palm oil, dry yeast, Aspergillus awamori and Lactic acid bacteria are (4.04, 8.00, 14 and 15 L. E.), respectively, according to Egyptian market, 2020.

[3]Assuming the price of one – egg was 1.33 L. E. (according to Egyptian market, 2020).

[4]Net revenue / hen, (L. E.) = Total price of eggs – Total feed cost. [5]Economic efficiency = (Net revenue ÷ Total feed cost) × 100. [6]Relative economic efficiency of control considered 100.

CONCLUSION

The addition mixture of probiotics as (dry yeast, *Aspergillus awamori* or *Lactic acid bacteria*) as antioxidant agents had a positive effect on performance and reproductive traits of Gimmizah laying hens under experimental conditions.

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تأثير إضافة مصادر مختلفة من البروبيوتيك إلى العلائق المحتوية على زيت النخيل المؤكسد على بعض الصفات الإنتاجية والتناسلية في دجاج الجميزة البياض

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أجريت هذه الدراسة لتقييم تأثير إضافة مصادر مختلفة من البروبيوتيك (الخميرة الجافة، الأسبرجلس أمورى أو بكتيريا حمض اللاكتيك) كعوامل طبيعية مضادة للأكسدة إلى العلائق المحتوية على زيت النخيل المؤكسد على بعض الصفات الإنتاجية والتناسلية، بعض مكونات الدم، جودة البيض، تركيز الكوليسترول والأحماض الدهنية في صفار البيض، الكفاءة الإقتصادية والكفاءة الاقتصادية النسبية في دجاج الجميزة البياض (Gimmizah). استخدم في هذه التجربة عدد 168 طائر جميزة (126 دجاجة و 42 ديك) عمر 25 أسبوع متماثلة تقريبا في وزن الجسم (بمتوسط 1172 و 1286جم للدجاجات والديوك على التوالي). قسمت الطيور عشوائيا إلى ستة مجموعات تجريبية، كل مجموعه احتوت على 28 طائر (21 دجاجة و7 ديوك). غذيت طيور المجموعة الأولى على العليقة الأساسية تحتوى على 16.26% بروتين خام، 2737 كيلو كالورى طاقة ممثلة/ كجم عليقة. طيور المجموعة الثانية غذيت على العليقة الأساسية المضاف إليها 2% من زيت النخيل المؤكسد. بينما غذيت طيور المجموعة الثالثة، الرابعة والخامسة على عليقة المجموعة الثانية مضاف إليها 1% من مصادر مختلفة من البروبيوتيك (الخميرة الجافة أو الأسبرجلس أمورى أو بكتريا حمض اللاكتيك) على التوالي. أما المجموعة السادسة غذيت على عليقة مجموعة المجموعة الثانية مع إضافة 0.5% من كل من: الخميرة الجافة، فطر الأسبرجلس أمورى وبكتريا حمض اللاكتيك. أظهرت النتائج تحسن معنوي في معدل إنتاج، عدد، كتلة البيض ومعدل تحويل الغذاء بإضافة 0.5% خميرة جافة + 0.5% فطر أسبرجلس أمورى + 0.5% بكتريا حمض اللاكتيك إلى العليقة الأساسية المضاف إليها 2% من زيت النخيل المؤكسد (المجموعة السادسة). كما سجلت طيور المعاملة السادسة أعلى تحسن معنوي في جودة قشرة البيضة (سمك القشرة وقوتها) وأعلى قيمة لدليل شكل البيضة عند عمر 37 أسبوعًا. كذلك لوحظ تحسن معنوي لبعض صفات صفار البيض ووحدات هاوف نتيجة الإضافات المستخدمة. اتضح أن الطيور المغذاة على العلائق المضاف إليها البروبيوتيك أنتجت بيضا منخفض فى تركيز الكوليسترول، كما انخفض معنوياً تركيز كل من: الكوليسترول الكلى، الدهون الثلاثية الكلية والبروتين الدهني منخفض الكثافة (LDL) – بينما زاد معنويا تركيز البروتين الدهنى عالي الكثافة (HDL) في سيرم دم هذه الطيور. انخفضت الأحماض الدهنية المشبعة في صفار البيض بينما زادت الأحماض الدهنية غير المشبعة (USFA) في كل المعاملات المضاف إليها البروبيوتيك سواء منفرداً أو خليطاً مقارنة بالطيور المغذاة على العليقة الأساسية الغير مضاف أو المضاف لها زيت النخيل المؤكسد ، كما أدت إضافة مخلوط البروبيوتيك (المعاملة السادسة) إلى تحسن معنوي في نسبه الخصوبة ونسبه الفقس وانخفاض معنوي في نسبة الميت أول والميت ثاني عند مستوى 0.05. كانت أفضل كفاءة اقتصادية وكفاءة اقتصادية نسبية للمعاملة السادسة (0.5% خميرة جافة + 0.5% فطر أسبرجلس أمورى + 0.5% بكتريا حمض اللاكتيك إلى العليقة الأساسية المحتوية على 2% زيت نخيل مؤكسد) مقارنة بباقي المعاملات. وبصفة عامة، من خلال النتائج المتحصل عليها أشارت إلى أن إضافة خليط البروبيوتيك المكون من 0.5% خميرة جافة + 0.5% فطر أسبرجلس أمورى + 0.5% بكتريا حمض اللاكتيك كعوامل طبيعية مضادة للأكسدة لها تأثير إيجابى على الأداء الإنتاجي والتناسلي ودون تأثيرات ضارة على الطيور تحت ظروف التجربة.