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Dietary Supplementation with Different ω-6 to ω-3 Fatty Acid Ratios Affects the Sustainability of Performance, Egg Quality, Fatty Acid Profile, Immunity and Egg Health Indices of Laying Hens

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Abstract: The supplementation of different ω-6/ω-3 ratio to the diets of the laying hens has been studied to evaluate the effects on performance, egg quality, egg health indices, egg fatty acid profiles, and immune response. One-hundred and twenty, 50-weeks-old hens were divided into three groups fed diets with different ω-6/ω-3 polyunsaturated fatty acids (PUFA) at ratio: 16.7:1, 9.3:1, and 5.5:1, respectively. Each group contained eight replicates of five hens. Hens fed the diet with the highest ω-6/ω-3 ratio had significantly increased weight gain compared to those fed the 5.5:1 and 9.3:1 ω-6/ω-3 ratios. In contrast, hens fed the 9.3:1 ω-6/ω-3 ratios laid significantly more eggs, had increased egg mass, greater livability, and a better FCR than the control group. However, hens fed a ratio of 5.5:1 ω-6/ω-3 PUFA showed improved thrombogenic, atherogenic, hypocholesteremia, and hypocholesteremia/hypercholesteremia indices. In conclusion, laying hens of the 9.3:1 ω-6/ω-3 PUFA group showed improved laying performance, while a ratio of 5.5:1 enriched the ω-3 PUFA in eggs and boosted the immune response of hens.

Keywords: omega-3; omega-6; supplementation; egg production sustainability; egg quality; egg health index; egg fatty acids; immunity status

1. Introduction

The use of vegetable oils from sunflower, corn, soybean, and cottonseed in human and animal nutrition has increased the intake of ω-6 polyunsaturated fatty acids (PUFAs) and decreased that of ω-3 PUFAs, increasing the ω-6 to ω-3 ratio of the ingested lipids [1]. It is well known that a high ω-6/ω-3 PUFA ratio may contribute to an increase of the incidences of many emerging diseases, including heart disease, depression, and autoimmune disorders [2,3]. Therefore, keeping a suitable dietary ω-6/ω-3 ratio is more critical than the single fatty acid level [3,4]. The ω-6/ω-3 ratio regulates the composition of the cell membranes and, consequently, the quantities and types of eicosanoids that are formed. For instance, high ω-6/ω-3 PUFA ratios are pro-inflammatory: thus, a low ratio of ω-6/ω-3 fatty acids is a possible way for decreasing the risks of lipid-related nutritional diseases [5,6]. The ω-6/ω-3 ratio has also been linked to metabolic syndrome markers, including lipid profiles, adiposity, insulin sensitivity, and inflammation. However, the role of PUFAs in regulating energy balance in metabolic diseases has not been investigated systematically [7].

Furthermore, ω-3 PUFAs are essential components of cell membranes and play a significant role in lipid-related nutritional conditions affecting public health [8,9]. In addition to their anti-inflammatory and antioxidant properties, ω-3 fatty acids are believed to regulate platelet homeostasis and reduce the risk of thrombosis [10,11]. In general, thrombogenic, atherogenic, hypocholesteremic, as well as hypocholesteremic/hypercholesteremic indices are good predictors of human health, influenced by lipid type and level [4,5,10].

Recently, ω-6 and ω-3 PUFA have been assessed to determine the nutritional value of dietary lipids [10–12]. Increasing dietary consumption of ω-3 PUFA, especially the longer-chain docosahexaenoic (DHA; 22:6) acid and eicosapentaenoic (EPA; 20:5), may aid in the control and treatment of cancer, cardiovascular disease, and inflammatory disorders [1–3,10]. The literature suggests for patients to intake lipids with a ω-6 to ω-3 ratio between 1:1 and 5:1 [4,7]. The ω-6/ω-3 fatty acid ratio is crucial due to the competition between enzymes involved in the elongation and desaturation process of linoleic acid and linolenic fatty acids [13,14]. As recently reviewed by Alagawany et al. [4], the inclusion of oils as ω-3 source in poultry diets improved growth performance, feed conversion ratio, immune response, egg and meat quality. In addition, fatty acid supplementation, especially of ω-3 and ω-6, helps in improving fertility, semen quality and quantity of chickens [2,4].

The nutritional quality of meat and eggs and fatty acids concentrations have been linked to the lipid source in the diet [2,4,15]. Thus, the appropriate ratio between ω-6 and ω-3 in poultry nutrition is of great importance. An increased intake of ω-6 interferes with the metabolic pathway of α-linolenic acid (ALA) and increases the production of eicosanoids from arachidonic acid, including prostaglandins, thromboxanes, and leukotrienes. High levels of eicosanoids result in thrombus formation, allergic and inflammatory disorders, and eventually, a decline in bird performance [10–12]. Although the effect of ω-3 in diets on the quality of poultry products has been studied, the ω-6 to ω-3 ratio has received little consideration. Keeping in mind the importance of the ratio ω-6 to ω-3 and its role in immunity and disease prevention [1–5,10], the present study aimed to test the dietary impact of different ω-6/ω-3 PUFAs on egg health indices with altered dietary ω-6 to ω-3 ratios, egg laying performance and quality, egg fatty acid profile, and immune response of laying hens.

2. Materials and Methods

The Deanship of Scientific Research at King Abdulaziz University, Saudi Arabia, approved this research according to the protocol number RG-1-155-43 H. The protocol recommends general humane treatment of animals that does not cause pain, suffering, distress, or lasting harm, following the Royal Decree number M59 from 14/9/1431H and institutional approve code ACUC-22-1-2.

2.1. Animals, Housing, Management, and Experimental Setup

Following a two-week preliminary experimental period, a total of 120 Lohmann Selected Leghorn (LSL) laying hens aged 50 weeks were used in this study for eight weeks. The straight-run experimental design consists of three ω-6/ω-3 PUFAs treatments, each with eight replicates of five hens. Twenty-four cages were used to house the birds (5 birds/cage) in an environmentally controlled house. The light–dark cycle was 16 L: 8D for the duration of the experiment. The hens were provided with mash feed and had free access to feed and water 24 h per day. Hens were housed in an environmental controlled house with 23–25 °C and 50–60% RH. The outdoor temperature and relative humidity were 39 ± 4 and 52 ± 7, respectively. The space provided per hen was 520 cm^2.

2.2. Diet Formulation

The tested diets were formulated to have 16.7:1 (HR), 9.3:1(MR), and 5.5:1 (LR) ω-6/ω-3 fatty acids ratio, respectively. The determined ratios of w-6/w-3 PUFA in the experiment was based on the pervious published results by [4–7]. The ω-6/ω-3 ratio of the experimental diets was modified by adding varying amounts of soybean oil and flax

oil while maintaining isocaloric and isonitrogenous nutrient profiles. The ingredients and chemical–nutritional characteristics of the diets are presented in Table 1. The diets were formulated using the Lohmann LSL-Classical Layer Cage Housing breeder guide [16] and feedstuff printout values [17].

Table 1. Constituents and chemical profile of the experimental diets.

Ingredients, g/kg	ω-6/ω-3 Ratio		
	16.7:1	**9.3:1**	**5.5:1**
Yellow corn	612.0	588.3	481.2
Soybean meal (44% CP)	245.0	274.0	293.0
Flax oil	-	36.0	80.0
Soybean oil	40.0	-	-
Calcium diphosphate	13.0	13.0	13.0
Calcium carbonate	80.0	80.0	80.0
Sodium chloride	3.0	3.0	3.0
Vit. and Min. Premix [1]	3.0	3.0	3.0
DL-Methionine	1.0	0.7	0.8
L-lysine	1.0	0.0	0.0
Sodium carbonate	1.0	1.0	1.0
Choline chloride	1.0	1.0	1.0
Washed building sand	-	-	44.0
Determined values and calculated analysis			
Dry matter, g/kg [2]	894.1	897.3	892.3
Crude protein, g/kg [2]	168.0	170.0	170.0
ME, kcal/kg diet [3]	2954	2885	2937
Ether extract, g/kg [2]	66.2	61.8	102.3
Crude fiber, g/kg [2]	29.5	30.7	29.5
Calcium, g/kg [3]	35.1	35.2	35.3
Phosphorus available, g/kg [3]	3.04	3.07	3.01
Ash, g/kg [2]	142.6	145.7	186.8
Methionine, g/kg [3]	4.39	4.23	4.28
Methionine + Cysteine g/kg [3]	7.22	7.21	7.19
Lysine, g/kg [3]	8.80	8.75	9.02
SFA, % [2]	16.25	16.10	15.24
Unsaturated fatty acids, % [2]	76.45	76.65	72.87
MUFA, % [2]	23.04	22.45	20.27
PUFA, % [2]	53.42	54.17	52.59
ω-6 PUFA, % [2]	50.4	48.9	44.5
ω-3 PUFA, % [2]	3.02	5.27	8.09
ω-6/ω-3 ratio [3]	16.7:1	9.3:1	5.5:1

[1] Three kg of vitamin–mineral premix per ton of feed supplied each kg of diet with Vit. A 12,000 IU; Vit. D_3 2000 IU; Vit. E. 10 mg; Vit. k_3 2 mg; Vit.B_1 1 mg; Vit. $B_2$4 mg; Vit. B_6 1.5 mg; pantothenic acid 10 mg; Vit.B_{12} 0.01 mg; folic acid 1 mg; niacin 20 mg; biotin 0.05 mg; choline chloride (50% choline) 500 mg; Zn 55 mg; Fe 30 mg; I 1 mg; Se 0.1 mg; Mn 55 mg; ethoxyquin 3000 mg. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; [2] determined values; [3] calculated analysis.

2.3. Laying Performance

Body weight gain (BWG), rate of laying, egg weight, egg mass, feed intake, FCR, and survival rate were measured and expressed per cage throughout the experiment. The egg mass was estimated by multiplying the average egg weight (g) by the number of eggs laid during the eight weeks per hen. The feed conversion ratio was estimated as the ratio between feed intake and egg mass.

2.4. External Egg Quality

Egg quality measurements were determined using 24 fresh eggs per treatment as 3 eggs per replicate. The egg shape index was determined according to Romanoff and Romanoff [18], and shell percentage was determined by dividing shell weight by egg weight

and multiplying by 100. The shell thickness was estimated using a micrometer (S-6428, BC Ames, Melrose, MA, USA) in three regions: blunt, sharp, and equatorial. The shell weight per unit of surface area (SWUSA, mg/cm^2) was calculated as stated by Carter [19].

2.5. Internal Egg Quality

The eggs' internal quality, including Haugh units, yolk index, and total yolk solids, was calculated using the Haugh [20] and Funk [21] methods. The chemical composition of the experimental diets and yolk dry matter were measured according to AOAC [22]. The egg yolk color was assessed using the Hoffman La Roche color fan Vuilleumier [23].

2.6. Fatty Acids Profile

The fatty acid profile of the lipids in the experimental diets (n = 1/treatment) and egg yolk (n = 6/treatment) was determined by extracting the lipid according to Folch et al. [24]. The quantitative analysis of fatty acids was performed by a gas chromatography method, according to Radwan [25].

The following equations were utilized to estimate the atherogenic (AI) and thrombogenic indices (TI) [26]:

$$AI = (4 \times C14{:}0) + C16{:}0/(\Sigma MUFA + \Sigma PUFA - \omega\text{-}6 + \Sigma PUFA - \omega\text{-}3)$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/0.5 \times \Sigma MUFA + 0.5 \times \Sigma\,(\omega\text{-}6) + 3 \times \Sigma(\omega\text{-}3) + \Sigma(\omega\text{-}3)/\Sigma(\omega\text{-}6)$$

where ΣMUFA is the sum of monounsaturated fatty acids and ΣPUFA is the sum of polyunsaturated fatty acids

The hypocholesterolemic index (HI) was estimated using the following equation [27]:

$$HI = (C18{:}1 + C18{:}2 + C18{:}3 + C20{:}3 + C20{:}4 + C20{:}5 + C22{:}4 + C22{:}6)/(C14{:}0 + C16{:}0)$$

The hypocholesterolemic/hypercholesterolemic index was calculated following the equation [28] = [(C18:1 ω-9 + C18:1 ω-7 + C18:2 ω-6 + C18:3 ω-6 + C18:3 ω-3 + C20:3 ω-6 + C20:4 ω-6 + C20:5 ω-3 + C22:4 ω-6 + C22:5 ω-3 + C22:6 ω-3)/(C14:0 + C16:0)]

2.7. Immune Indices

At 58 weeks of age, blood samples (n = 8/treatment as 1 per replicate) were collected from the wing vein of hens in the heparinized blood type group (5 mL/sample) for blood plasma biochemistry. The serum was separated after centrifugation of blood at 1500× *g* for 10 min and then kept at −20 °C until analyses. The haemagglutination inhibition test was used to measure the humoral immune response, such as antibody production against the Newcastle disease virus (NDV), as described by King and Seal [29]. The phagocytic assay was used to measure cell-mediated immune response, as defined by Kawahara et al. [30]. In addition, the lymphocyte transformation test, serum bactericidal activity, and serum lysosomal activity were estimated according to the procedures described by Balhaa et al. [31], Rainger and Rowley [32], and Engstad et al. [33], respectively.

2.8. Statistical Analysis

The influence of the ω-6/ω-3 fatty acid ratio was investigated using a one-way analysis of variance, and the replicate was the experimental unit (the cage) [34]. Mean differences were compared using the Tukey post hoc test when a significant probability value ($p < 0.05$) was obtained [34]. The following statistical model was implemented:

$$Yij = \mu + Ai + eij$$

where: μ is the general mean, Ai is the effect of types of diet, and eij is the random error.

Before executing the statistical analysis, the data distribution was normalized by transforming all percentages to the Log 10 form. Then, the survival rate was analyzed using a Chi-square test [34].

3. Results

Table 2 illustrates the impact of the ω-6 to ω-3 PUFA ratio on the performance of LSL laying hens. Hens fed the HR and MR diet gained 13.1 and 9.8% more weight, respectively, than the LR group ($p < 0.05$). The MR group showed a higher ($p < 0.01$) egg production rate and egg mass than the HR, but both groups were not different from the LR group. The feed intake was higher ($p < 0.01$) in the HR than in the other groups. The feed conversion ratio was more favorable in MR group compared to the HR but was not different from the LR group.

Table 2. Effect of ω-6/ω-3 PUFA ratio on laying performance of the hens.

Treatments	BWG g	EP %	EW G	EM g/h/d	FI g/h/d	FCR g/g
HR	173 ^{a}	81.3 ^{b}	58.6	47.6 ^{b}	115 ^{a}	2.42 ^{a}
MR	168 ^{a}	86.1 ^{a}	58.1	50.0 ^{a}	111 ^{b}	2.22 ^{b}
LR	153 ^{b}	83.1 ^{ab}	57.6	47.9 ^{ab}	112 ^{b}	2.34 ^{ab}
SEM	14.3	1.48	0.34	0.82	1.13	0.09
p value	0.01	0.001	0.462	0.001	0.001	0.001

^{a,b} means within a column within each factor not sharing similar letters are significantly different $p < 0.05$, SEM: standard error of means; BWG: body weight gain; EP: egg production; EW: egg weight; EM: egg mass; FI: feed intake; FCR: feed conversion ratio; HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 3 displays the effect of ω-6/ω-3 PUFA ratio on eggshell quality measurements of LSL laying hens. The diet affected the egg shape index and shell percentage by varying ω-6/ω-3 ratios. However, LSL hens fed a HR diet produced eggs with greater shell thickness than the other groups (+3.58 and +4.92% compared to MR and LR groups, respectively). In the same way, the HR group has a higher ($p < 0.05$) value of SWUSA than the other groups (+2.76 and +4.38% compared to the MR and the LR groups, respectively).

Table 3. Effect of ω-6/ω-3 PUFA ratio on external egg quality traits of LSL laying hens.

Treatments	ESI %	SW %	ST µm	SWUSA mg/cm^2
HR	73.5	9.45	405 ^{a}	85.7 ^{a}
MR	72.2	9.35	391 ^{b}	83.4 ^{b}
LR	73.2	9.18	386 ^{b}	82.1 ^{b}
SEM	0.53	0.128	13.1	0.90
p value	0.356	0.421	0.038	0.027

^{a,b} means within a column within trait not sharing similar letters are significantly different $p < 0.05$; SEM: standard error of means; ESI: egg shape index; SW: shell weight; ST: shell thickness; SWUSA: Shell weight per unit of surface area, HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 4 illustrates the effect of the ω-6/ω-3 ratio on the egg yolk quality of LSL laying hens. According to the results, the ω-6/ω-3 PUFA ratio did not significantly affect the percentage, index, total lipids, or yolk to albumen ratio. However, the intensity of the yolk color progressively increased from HR to LR group ($p < 0.01$).

Table 4. Effect of ω-6/ω-3 ratio on egg yolk quality of laying hens.

Treatments	Yolkweight %	Yolkindex %	Yolk Total Solid %	Yolk: Albumen	Yolk Color
HR	31.6	44.9	52.9	0.532	5.58 [c]
MR	31.3	45.9	51.4	0.527	6.08 [b]
LR	31.1	45.3	52.3	0.523	6.83 [a]
SEM	0.19	0.33	0.71	0.01	0.09
Pvalue	0.551	0.354	0.412	0.652	0.001

[a–c] means within a column within trait not sharing similar letters are significantly different $p > 0.05$, SEM: standard error of means HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 5 represents the effect of the ω-6/ω-3 ratio on the egg albumen quality of LSL laying hens. Except for meat and blood spots, the other albumen quality traits were not significantly influenced by the ω-6/ω-3 PUFA ratio in the diets. The meat and blood spots were the highest ($p = 0.01$) in the HR treatment.

Table 5. Effect of ω-6/ω-3 ratio on egg albumen quality of laying hens.

Treatments	AW %	pH	ATS %	HUS	MBS %
HR	59.0	8.36	11.3	82.3	0.15 [a]
MR	59.4	8.31	11.3	81.4	0.10 [b]
LR	59.7	8.29	11.0	82.3	0.10 [b]
SEM	0.323	0.093	0.213	1.12	0.047
p value	0.523	0.426	0.725	0.834	0.010

[a,b]: means within a column within trait not sharing similar letters are significantly different; $p < 0.05$; SEM: standard error of means; AW: albumen weight; ATS: albumen total solids; HUS: Haugh unit score; MBS: meat and blood spots; HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 6 displays the influence of the ω-6/ω-3 ratio on the egg health indices of LSL laying hens. Significant influences of various ω-6/ω-3 PUFA ratios on egg health indices were observed. Due to the low ω-6/ω-3 ratio, the atherogenic, thrombogenic, hypocholesteremic, and hypocholesterolemic/hypercholesterolemic indexes were improved ($p < 0.01$). The best (lowest) indices came from hens fed the LR diet.

Table 6. Effect of ω-6/ω-3 ratio on egg health indices from LSL laying hens.

Treatments	AI	TI	Hypo CI	Hypo/Hyper CI
HR	0.60 [a]	1.06 [a]	2.11 [c]	2.10 [c]
MR	0.46 [b]	0.66 [b]	2.91 [b]	2.91 [b]
LR	0.39 [c]	0.46 [c]	3.51 [a]	3.52 [a]
SEM	0.04	0.11	0.25	0.25
p Value	0.001	0.001	0.001	0.001

[a–c] means within a column within trait not sharing similar letters are significantly different $p < 0.05$, SEM: standard error of means; AI: atherogenic index; TI: thrombogenic index; HypoC: hypochoelsterolemic index; Hypo/hyper CI: hypo/hypercholesterolemic index; HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 7 illustrates the effect of the ω-6/ω-3 PUFA ratio on the fatty acid profile of the yolk of LSL laying hens. The birds fed LR and MR diets produced eggs with lower ($p < 0.01$) SFA, mainly 14:0 and 16:0 fatty acids, and higher ($p < 0.01$) UFA and PUFA compared to the HR group. The UFA/SFA of the LR group is higher ($p < 0.01$) than that of the HR group. The ω-6 fatty acids were unaffected by dietary treatments, while the ω-3 progressively increased ($p < 0.01$) from HR to LR group, and, as a consequence, an opposite trend was observed for the ω–6/ω–3 PUFA ratio, which progressively decreased from HR to LR group. The ω-3 yield of MR and LR groups was higher ($p < 0.01$) than that of the HR group.

Table 7. Effect of ω-6/ω-3 ratio on egg yolk fatty acids profile (%) of laying hens.

Fatty Acids	HR	MR	LR	SEM	*p* Value
C12:0	0.063	0.058	0.055	0.0045	0.123
C14:0	0.411 ^{a}	0.351 ^{b}	0.321 ^{b}	0.013	0.001
C16:0	27.49 ^{a}	21.85 ^{b}	19.14 ^{c}	0.493	0.002
C18:0	9.41	9.45	8.75	0.664	0.453
SFA	37.37 ^{a}	31.71 ^{a}	28.27 ^{b}	0.725	0.001
C16:1	3.97 ^{a}	3.65 ^{ab}	3.27 ^{b}	0.112	0.002
C18:1	36.4	35.8	36.15	4.15	0.632
MUFA	40.17	39.45	39.42	4.17	0.376
C18:2	19.82	21.63	20.54	3.91	0.485
C18:3	0.871 ^{c}	4.13 ^{b}	8.15 ^{a}	0.023	0.003
C20:4	1.04 ^{b}	1.69 ^{a}	1.63 ^{a}	0.027	0.001
C20:5	0.013 ^{b}	0.016 ^{b}	0.052 ^{a}	0.011	0.01
C22:5	0.061 ^{b}	0.081 ^{b}	0.211 ^{a}	0.013	0.004
C22:6	0.661 ^{c}	1.29 ^{b}	1.73 ^{a}	0.059	0.001
PUFA	22.46 ^{b}	28.84 ^{a}	32.31 ^{a}	4.187	0.031
UFA	62.63 ^{b}	68.29 ^{a}	71.73 ^{a}	0.853	0.004
UFA/SFA	1.68 ^{b}	2.15 ^{ab}	2.54 ^{a}	0.072	0.002
ω-6	20.92	23.4	22.38	4.12	0.524
ω-3	1.55 ^{c}	5.44 ^{b}	9.93 ^{a}	0.075	0.001
ω-6:ω-3	13.50 ^{a}	4.30 ^{b}	2.25 ^{c}	1.037	0.001
ω-3 yield	4.46 ^{c}	9.30 ^{a}	10.96 ^{a}	1.63	0.001

$^{a–c}$: means within a row within each factor not sharing similar letters are significantly different $p > 0.05$. SFA: saturated fatty acids, UFA: unsaturated fatty acids, UFA/SFA: unsaturated to saturated fatty acids ratio, PUFA: polyunsaturated fatty acids. SEM: standard error of means, ω-3 yield = ω-3 in egg/ω-3 intake. HR: group fed diet with 16.7:1 ω-6/ω-3 PUFA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

Table 8 illustrates the effect of the ω-6/ω-3 PUFA ratio on the immune indices and survival rate of LSL laying hens. Results showed a significant enhancement in lymphocyte transformation test (LTT) and lysozyme activity (LA) due to offering 5.5:1 and 9.3:1 ω-6/ω-3 PUFA ratios compared to 16.7:1 ω-6/ω-3 PUFA ratio. The hens of MR group showed the highest LTT value, while those of LR group showed the best LA. The survival rate of laying hens was not significantly different among the three ω-6/ω-3 PUFA ratios.

Table 8. Effect of ω-6/ω-3 ratio on immune responses indices and survival rate of laying hens.

Treatments	PA %	PI %	LTT %	BA %	LA IU%	HINDV Log2	Survival Rate %
HR	17.5	1.67	16.1 ^{c}	40.0	0.03 ^{b}	3.78	95.0
MR	18.9	1.62	18.6 ^{a}	39.9	0.03 ^{b}	4.01	97.5
LR	19.7	1.69	17.4 ^{b}	41.7	0.05 ^{a}	4.41	100
SEM	0.24	0.07	0.241	0.53	0.003	0.16	0.63
p value	0.523	0.431	0.010	0.731	0.001	0.153	0.321

$^{a–c}$: means within a column within each factor not sharing similar letters are significantly different $p < 0.05$. PA: phagocytic activity, PI: phagocytic index, LTT: lymphocyte transformation test, BA: bactericidal activity, LA: lysozyme activity, HINDV (Log_2): antibody titration against Newcastle disease virus (hemagglutination inhibition (HI)), IU: international unit. SEM: standard error of means HR: group fed diet with 16.7:1 ω-6/ω-3 PUA; MR: group fed diet with 9.3:1 ω-6/ω-3 PUFA; LR: group fed diet with 5.5:1 ω-6/ω-3 PUFA.

4. Discussion

The low BWG of LSL hens fed LR diet shows that decreasing ω-6/ω-3 PUFA of the diets from 16.7:1 to 5.5:1 has a great effect on bird metabolism, also considering that the feed intake of LR group was not different from that of MR, who had a similar BWG than HR group. A possible explanation of this aspect is that a lower ω-6/ω-3 ratio may inhibit lipid synthesis and increase the fatty acid oxidation and/or energy expenditure [35]. It was

observed that the BW of broiler breeders increased as the ω-6/ω-3 PUFA ratio in their diets increased [36].

The high BWG of hens 153–173 g observed herein during 8 wk experimental period could be explained by the lower egg production and egg mass outputs compared to the target production by LSL management guide. Considering energy metabolism, it is known that energy is used for egg production, birds' activity, and body weight gain. In the current study, as laying hens were housed in cages, their movements were restricted by the space allowed. Thus, energy surpassing the production process can be used for BWG, which is most likely to be fat deposition [37–39].

Except for the egg weight, most aspects of laying performance were significantly affected by the ω-6/ω-3 ratio. The hens exhibit, in general, higher laying performance with a ω-6/ω-3 ratio of 9.3:1. Thus, the enhancement in egg performance (egg mass and laying rate) could be attributed to oil supplementation, which acts as a source of essential fatty acids [39]. The present study demonstrated that hens fed the diet with the medium ratio of ω-6/ω-3 had a better FCR than the control (HR group). This result could be explained by the enhanced laying rate and decreased feed intake. Increasing flax oil supplementation to 8% in the group receiving the lowest ω-6/ω-3 ratio (LR diet) resulted in similar laying performance to the HR group. In the literature, compared to hens fed a 7.6:1 ω-6/ω-3 PUFA ratio, hens fed a 0.6:1 ω-6/ω-3 PUFA ratio showed a marked increase in laying rate but a decrease in egg weight. In contrast, egg mass did not vary significantly [40]. In addition, laying hens' diets supplemented with bio-omega-3 positively affected the hens' overall laying performance, showing higher laying persistence [41]. The feed intake decreased when the ω-6/ω-3 PUFA ratio was dropped to 9.3:1 or 5.5:1, indicating a similar inhibitory effect of the MR and LR diets without adverse effects on laying performance. The improvements in feed efficiency over the control (HR group) were similar and accounted for 8.26 and 3.31%, respectively. This improvement in laying performance due to flax oil supplementation at 3.6% indicated that this level can be considered satisfactory in laying hens' diets: in fact, a further increase to 8% did not have further effects. Like the present results, when the ω-6/ω-3 ratio decreased, feed intake decreased, and FCR improved [42–44]. Other authors [45] found that feeding different ω-6/ω-3 ratios did not affect hens' feed intake and FCR. These disparities in published results reflect different laying strains, dietary regimens, and environmental conditions.

It is worth noticing that the low production performance of LSL hens observed herein during the experimental period compared to the objectives published in the LSL management guide could be attributed to harsh weather conditions associated with high outdoor temperature and relative humidity 39 ± 4 and 52 ± 7, respectively during the experimental period. In addition, the production of chickens outside the selection region is always lower than that in the organ of selection. This could be due to different environmental, management, husbandry, and nutrition regimens [6,37–39].

The improvement in eggshell quality observed in hens fed a diet containing 16.7:1 ω-6/ω-3 may indicate increased calcium/mineral availability for eggshell formation. Similarly, Peebles et al. [36] found that feeding layers with a 24.7 ω-6/ω-3 ratio resulted in a significant positive impact on the eggshell weight of laying hens compared to feeding with a 13.4 ω-6/ω-3 ratio. In addition, increasing the ratio of ω-6/ω-3 PUFA from 6 to 8 to 14:1 increased the percentage of shell weight in brown dwarf hens [45]. Breeder hens fed diets containing a higher ω-6/ω-3 PUFA produced eggs with a higher eggshell percentage and specific gravity than those fed diets with lower ratios [45]. However, not all the researchers reached the same conclusions. In another study, the shell thickness of hens provided a diet with 0.6:1 ω-6/ω-3 PUFA ratio was greater than that of hens fed a 7.6:1 ω-6/ω-3 PUFA ratio, while there were no marked variations in shell weight [46]. The decrease in eggshell quality of laying hens in MR and LR groups could result from increased flax oil supplementation from 3.6% to 8%: increasing oil/fat supplementation increased calcium soap formation, hence decreased calcium absorption and limited calcium availability for eggshell formation [47].

As expected, the dietary treatments strongly influenced the inner quality of the eggs, particularly the yolk quality. The color of the yolk becomes darker from the HR to LR diet. Similarly, dietary flax oil at 2% (with a ω-6/ω-3 ratio of 1.4) increased the yolk color index compared to diets with 13 or 17 ratios [47]. The positive influences of dietary fats on carotenoid, lutein and zeaxanthin absorption in the intestines has been attested [48]. In partial agreement with the present findings, the yolk weight and color of hens fed 0.6:1 and 7.6:1 ω-6/ω-3 PUFA ratios did not differ significantly [39]. Yannakopoulos et al. [40] found that hens fed a diet enriched with bio-ω-3 produced more yolk total solids (e.g., fatty acids) than hens provided the control (HR) diet. The yolk lipid profile reflects the dietary ω-6/ω-3 PUFA ratio changes that modify lipid metabolism in laying hens [47]. According to Guenter et al. [49], increasing the linoleic acid (ω-6) content in hens' diets led to greater egg yolk lipid content than when hens were fed lower levels of linoleic acid. However, increasing ω-3 PUFA in the diet could limit the availability of the lipids for yolk formation [50] due to the increased lipid oxidation and decreased de novo lipid synthesis [32]. Yalcin et al. [51] found that the ω-6/ω-3 ratio of 24.5:1 or 1:1 did not affect the egg yolk health index, including total cholesterol. Similar results were also observed by other authors [52].

The efficiency of ω-3 transfers from feeds to eggs in the present trial was calculated to be 4.46, 9.3, and 10.96%, respectively, for HR, MR, and LR diets. These results indicate a gradual increase in yolk ω-3 deposition, reflecting the dietary increase in ω-3 intake by laying hens. In accordance with the present results, the fatty acid profile of the egg yolks of hens receiving diets with vegetable oils changes according to the dietary oil fed [4,53–57]. Moreover, a gradual effect of the ω-6/ω-3 PUFA ratio in feed and egg yolk (15.99 and 2.34; 16.72 and 4.34, respectively) revealed that 3% of tuna oil supplementation was a good predictor of the ω-6/ω-3 PUFA ratio in yolk [55]. Additionally, the ω-6/ω-3 PUFA ratio in the egg yolk is directly related to the type of oil (soybean vs. linseed) and ω-6/ω-3 ratio (10.17 vs. 0.83) [56]. Irawan et al. [57] reviewed the published literature and revealed a stepwise positive relationship among the levels of α-linolenic acid, total ω-3 PUFA, and the dietary ω-6/ω-3 ratio with the formation of EPA, DHA, total ω-3 PUFA, and the ω-6/ω-3 ratio in the eggs with various magnitudes. The intercept and the slope estimated for the dietary composition of ω-3 PUFA were 15.12 and -0.15, respectively, on ω-3-PUFA in eggs. Furthermore, ω-3 PUFAs positively affected the digestibility of nutrients and the gut morphology of hens farmed under stress conditions [58]; however, they cannot reduce the adverse influences observed under stress.

Recently, the eggs produced to have high levels of ω-3 demonstrated a nutritional value comparable to the ω-3 capsules [59]. In the present trial, decreasing the ω-6 to ω-3 ratio of the diets improved all the health indices of the eggs (AI, TI, HypoCI, Hypo/HyperCI), according to Vlaicu et al. [60]. This is very interesting and could open new perspectives in terms of egg production for human consumption. The egg is a food with two essential characteristics: it has a low cost and is a good source of nutrients such as protein, vitamins, minerals, essential fatty acids, phospholipids, sphingomyelin, lutein, zeaxanthin, antioxidants, and choline [61,62]. However, a single egg can supply 186–230 mg of cholesterol, which raised suspicion that egg consumption can be related to increased cardiovascular disease (CVD) risks and other problems. The role of egg cholesterol in human blood cholesterol concentration is not well understood yet [63]. Although a recent review [64] stated that there are inconsistent results regarding the possible relationship between the number of consumed eggs and the incidence of CVD, diabetes, and other pathologies, several researchers indicated an association between egg consumption and cause-specific mortalities in humans [65–67]. In contrast, other studies stated the contrary [68,69]. To better clarify the relationship between egg consumption and CVD, it is mandatory to well understand the fate of egg cholesterol in the gastrointestinal tract, including the role of the intestinal microbiota, as hypothesized by Sanlier and Ustun [64]. Waiting to improve knowledge on this topic, increasing egg health indices is a possible tool to encourage the consumption of eggs and reassure consumers about the possible risks.

The eggs produced by LSL hens provided the HR feed had higher blood and meat spots percentages than those of the LR group, showing improved interior egg quality due to the lowering of ω-6/ω-3 ratio. However, the quality of albumen is generally unaffected by the ω-6/ω-3 ratio of the feeds, according to other authors [40]. This happens because albumen is mainly represented by proteins, without fats. However, other authors [41] observed that hens fed bio-ω3-enriched diets produced less albumen than those provided a control, unenriched diet. These differences in albumen quality traits may be attributed to hen age, strain, feeding regimen, and environmental conditions [46,47].

According to our results, laying hens offered feeds with ω-6/ω-3 PUFA ratios of 29.33, 56.72, 1.35, and 2.41 had total antibody titers of 7.36, 7.74, 6.87, and 8.07, respectively [58]. Dietary ω-3 PUFA decreases phagocytosis in broiler chickens [70]. The survival rate of laying hens fed LR and MR diets were not significantly different from laying hens fed the HR diet. These indicated that different ω-6/ω-3 PUFA ratios studied herein did affect the survival rate.

5. Conclusions

Laying hens fed the intermediate level of ω-6/ω-3 PUFA ratio (9.3:1) had improved laying performance compared to the hens fed the diet with the highest ratio (16.7:1). The intermediate level of ω-6/ω-3 seems to be the best ratio, as further increases in ω-3 as in the LR diet did not induce further improvements. Instead, the low ω-6/ω-3 ratio (5.5:1) in the laying hens' diet was the best level regarding the egg quality: in fact, egg health index, ω-3 deposition in the yolk, and immune response were strongly improved compared to the other ω-6/ω-3 levels. However, decreasing ω-6/ω-3 ratio in the diet of hens reduced the shell thickness. Thus, ω-3 can be increased in the laying hens diets up to an appropriate percentage, depending on the purpose of production (eggs for human consumption or incubation).

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References

1. Salem, N.; Simopoulos, A.P.; Galli, C.; Lagarde, M.; Knapp, H.R. Fatty acids and lipids from cell biology to human disease. *Lipids* **1996**, *31*, 326.
2. Arias-Rico, J.; Cerón-Sandoval, M.I.; Sandoval-Gallegos, E.M.; Ramírez-Moreno, E.; Fernández-Cortés, T.L.; Jaimez-Ordaz, J.; Contreras-López, E.; Añorve-Morga, J. Evaluation of Consumption of Poultry Products Enriched with Omega-3 Fatty Acids in Anthropometric, Biochemical, and Cardiovascular Parameters. *J. Food Qual.* **2018**, 9620104.
3. Richardson, C.E.; Krishnan, S.; Gray, I.J.; Keim, N.L.; Newman, J.W. The Omega-3 Index Response to an 8 Week Randomized Intervention Containing Three Fatty Fish Meals Per Week Is Influenced by Adiposity in Overweight to Obese Women. *Front. Nutr.* **2022**, *9*, 810003. [CrossRef]

4. Alagawany, M.; Elnesr, S.S.; Farag, M.R.; Abd El-Hack, M.E.; Khafaga, A.F.; Taha, A.E.; Tiwari, R.; Yatoo, M.I.; Bhatt, P.; Khurana, S.K.; et al. Omega-3 and Omega-6 Fatty Acids in Poultry Nutrition: Effect on Production Performance and Health. *Animals* **2019**, *18*, 573. [CrossRef]
5. Simopoulos, A.P.; Norman, H.A.; Gillaspy, J.E. Purslane in human nutrition and its potential for world agriculture. *World Rev. Nutr. Diet.* **1995**, *77*, 47–74.
6. Klasing, K. *Comparative Avian Nutrition*; CAB International: Cambridge, UK, 1998.
7. Simopoulos, A.P. The omega-6/omega-3 ratio: The scientific evidence and the need to return the omega-3 fatty acids into eggs and other foods. In *The Amazing Egg: Nature's Perfect Functional Food for Health Promotion*; Sim, J., Sinwoo, H.H., Eds.; University of Alberta: Edmonton, AB, Canada, 2006; pp. 195–218.
8. Mazzuco, H.; McMurtry, J.P.; Kuo, A.Y.; Hester, P.Y. The effect of pre- and post-molt diets high in ω-3 fatty acids and molt programs on skeletal integrity and insulin-like growth factor-I of White Leghorns. *Poult. Sci.* **2005**, *84*, 1735–1749. [CrossRef]
9. Thanabalan, A.; Kiarie, E.G. Influence of feeding omega-3 polyunsaturated fatty acids to broiler breeders on indices of immunocompetence, gastrointestinal, and skeletal development in broiler chickens. *Front. Vet. Sci.* **2021**, *8*, 653152. [CrossRef]
10. Djuricic, I.; Calder, P.C. Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. *Nutrients* **2021**, *13*, 2421. [CrossRef]
11. Lee, S.A.; Whenham, N.; Bedford, M.R. Review on docosahexaenoic acid in poultry and swine nutrition: Consequence of enriched animal products on performance and health characteristics. *Anim. Nutr.* **2018**, *5*, 11–21. [CrossRef]
12. Keszthely, L. Nutritional Manipulation of the Fatty Acid Composition and Oxidative Stability of Table Egg. Summary of. Ph.D. Thesis, University of Veszprem Georgikon Faculty of Agricultural Science, Keszthely Deák Ferenc, Hungary, 2003.
13. Brandao, P.A.; Costa, F.G.P.; Barros, L.R.; Nascimento, G.A.J. Acidos graxos e colesterol na alimentação humana. *Agropec. Tecnic.* **2005**, *26*, 5–14.
14. Nobakht, A. Effects of using different levels of vegetable oils and their blends on egg yolk fatty acids profile of laying hens. *Adv. Environ. Biol.* **2011**, *5*, 2728–2731.
15. Sadish, S. Development and Evaluation of Omega-3 Fatty Acids Enriched Chicken Frankfurters. Master's Thesis, Dalhousie University Halifax, Nova Scotia in co-operation with Nova Scotia Agricultural College Truro, Halifax, NS, Canada, 2011.
16. Lohmann LSL-Classical Layer Cage Housing. Available online: https://www.winmixsoft.com/files/info/Lohman-lsl-classic-management-guide-eng.pdf (accessed on 15 April 2022).
17. NRC. *National Research Council. Nutrient Requirements of Poultry*, 9th ed.; National Academy of Sciences: Washington, DC, USA, 1994.
18. Romanoff, A.L.; Romanoff, A.J. *The Avian Egg*; John Wiley and Sons Inc.: New York, NY, USA, 1949.
19. Carter, T.C. Estimation of shell area and egg volume using measurements of fresh egg weight and shell length and breadth alone or in combination. *Br. Poult. Sci.* **1975**, *16*, 514–543. [CrossRef]
20. Haugh, R.R. The Haugh units for measuring egg quality. *Poult. Mag.* **1937**, *43*, 552–575.
21. Funk, E.M. The relation of the yolk index determined in natural position to the yolk index as determined after separating the yolk from the albumen. *Poult. Sci.* **1948**, *27*, 367–375. [CrossRef]
22. AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2007.
23. Vuilleumier, J.P. The roche yolk colour fan-An instrument for measuring yolk colour. *Poult. Sci.* **1997**, *48*, 767–779. [CrossRef]
24. Folch, J.; Lees, M.; Sloane Stanley, G.H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
25. Radwan, S.S. Coupling of two-dimensional thin layer chromatography with gas chromatography for the quantitative analysis of lipids classes and their constituent fatty acids. *J. Chromatog. Sci.* **1978**, *16*, 538–542. [CrossRef]
26. Omri, B.; Chalghoumi, R.; Izzo, L.; Ritieni, A.; Lucarini, M.; Durazzo, A.; Abdouli, H.; Santini, A. Effect of Dietary Incorporation of Linseed Alone or Together with Tomato-Red Pepper Mix on Laying Hens' Egg Yolk Fatty Acids Profile and Health Lipid Indexes. *Nutrients* **2019**, *11*, 813. [CrossRef]
27. Fernández, M.; Ordóñez, J.A.; Cambero, I.; Santos, C.; Pin, C.; De la Hoz, L. Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chem.* **2007**, *9*, 107–112. [CrossRef]
28. Fernandesa, C.E.; da Silva, M.A.; Marisildade, V.; Ribeirob, A.; Asfora, L.; Samara, S.; Cardoso, A.; Artur, A.; de Melo Filhoa, B. Nutritional and lipid profiles in marine fish species from Brazil. *Food Chem.* **2014**, *160*, 67–71. [CrossRef]
29. King, D.J.; Seal, B.S. Biological and molecular characterization of Newcastle disease virus (NDV) field isolates with comparisons to reference NDV strains and pathogenicity after chicken or embryo passage of selected isolates. *Avian Dis.* **1998**, *42*, 507–516. [CrossRef] [PubMed]
30. Kawahara, E.T.; Ueda, S.; Nomura, A. In vitro phagocytic activity of white-spotted shark cells after injection with Aermonas salmonicida extra cellular products. *Gyobyo Kenkyu* **1991**, *26*, 213–214. [CrossRef]
31. Balhaa, R.L.; Hinz, H.H.; Luders, H.; Siegmann, O. Clinical experiences with the drugs for lymphocyte transformation in chickens and turkey flocks. *Tierarztl. Umsch.* **1985**, *43*, 507–508.
32. Rainger, G.E.; Rowley, A.F. Antibacterial activity in the serum and mucus of rainbow trout, Oncorhynchus mykiss following immunization with Aeromonas salmonicida. *Fish Shellfish Immunol.* **1993**, *3*, 475–482. [CrossRef]
33. Engstad, R.E.; Robertsen, B.; Frivold, E. Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. *Fish Shellfish Immunol.* **1992**, *2*, 287–297. [CrossRef]

34. SAS Institute. *User's Guide, version 6.06*; SAS institute Inc.: Cary, NC, USA, 1990.
35. Sanz, M.; Florves, A.; Lopez, C.L. Effect of fatty acid saturation in broiler diets or abdominal fat and breast muscle fatty acid composition and susceptibility to lipid oxidation. *Poult. Sci.* **1999**, *64*, 602–1604. [CrossRef]
36. Peebles, E.D.; Zumwalt, C.D.; Doyle, S.M.; Gerard, P.D.; Latour, M.A.; Boyle, C.R.; Smith, T.W. Effects of breeder age and dietary fat source and level on broiler hatching egg characteristics. *Poult. Sci.* **2000**, *79*, 698–704. [CrossRef]
37. Daghir, N. *Poultry Production in Hot Climates*, 2nd ed.; CAB International: Cambridge, UK, 2008. [CrossRef]
38. Attia, Y.A.; Burke, W.H.; Yamani, K.A.; Jensen, L.S. Daily Energy allotments and reproductive performance of broiler breeders.: 2. Females. *Poult. Sci.* **1995**, *74*, 261–270. [CrossRef]
39. Attia, Y.A.; Burke, W.H.; Yamani, K.A.; Jensen, L.S. Energy allotments and performance of broiler breeders. *Poult. Sci.* **1993**, *72*, 42–50. [CrossRef]
40. Grobas, S.; Mendez, J.; Lazaro, R.; De Blas, C.; Manteos, G.G. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poult. Sci. J.* **2001**, *80*, 1170–1179. [CrossRef]
41. Yannakopoulos, A.; Tserveni-Gousi, A.; Christaki, E. Enhanced egg production in practice: The case of bio-omega-3 egg. *Int. J. Poult. Sci.* **2005**, *4*, 531–535.
42. Sijben, J.W.; Nieuwland, M.G.; Kemp, B.; Parmentier, H.K.; Schrama, J.W. Interaction and antigen dependence of dietary ω-3 and ω-6 polyunsaturated fatty acids on antibody responsiveness in growing layer hens. *Poult. Sci.* **2001**, *90*, 885–893. [CrossRef] [PubMed]
43. Sijben, J.W.; Schrama, J.W.; Nieuwland, M.G.; Hovenier, R.; Beynen, A.C.; Verstegen, M.W.A.; Parmentier, H.K. Interaction of dietary polyunsaturated fatty acids and vitamin E with regard to vitamin E status, fat composition and antibody responsiveness in layer hens. *Br. Poult. Sci.* **2002**, *43*, 297–305. [CrossRef] [PubMed]
44. Shang, X.G.; Wang, F.L.; Li, D.F.; Yin, J.D.; Li, J.Y. Effects of dietary conjugated linoleic acid on the productivity of laying hens and egg quality during refrigerated storage. *Poult. Sci.* **2004**, *83*, 1688–1695. [CrossRef] [PubMed]
45. Qota, E.M. Dietary linolenic acid alters ω-3 fatty acid metabolism, immunity and cholesterol level in laying hens and their progeny. *Egypt Poult. Sci.* **2007**, *27*, 457–471.
46. Brake, J. Effect of four levels of added fat on broiler breeder performance. *Poult. Sci.* **1990**, *69*, 1659–1663. [CrossRef]
47. Attia, Y.A.; Al-Harthi, M.A.; Abo El-Maaty, H.A.M. Calcium and cholecalciferol levels on late phase-laying hens' diets: Effects on productive and egg quality traits, blood biochemistry and immune responses. *Front. Vet. Sci.* **2020**, *7*, 389. [CrossRef]
48. Augustyn, R.; Barteczko, J.; Smulikowska, S. The effect of feeding regular or low α-linolenic acid linseed on laying performance and total cholesterol content in eggs. *J. Anim. Feed Sci.* **2006**, *1*, 103–106. [CrossRef]
49. Guenter, W.; Bragg, D.B.; Kondra, P.A. Effect of dietary linoleic acid on fatty acid composition of egg yolk, liver and adipose tissue. *Poult. Sci.* **1971**, *50*, 845–850. [CrossRef]
50. Van Elswyk, M.E. Nutritional and physiological effects of flaxseed in diets for laying fowl. *World's Poultry Sci. J.* **1997**, *53*, 253–264. [CrossRef]
51. Yalcın, H.; Unal, M.K.; Ünal, M.K.; Yoolu, H.B. The fatty acid and cholesterol composition of enriched egg yolk lipids obtained by modifying hens' diets with fish oil and flaxseed Grasas. *Y. Aceites* **2007**, *58*, 372–378.
52. Attia, Y.A.; Al-Harthi, M.A.; Korish, M.M.; Shiboob, M.M. Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. *Lipids Health Dis.* **2015**, *14*, 136. [CrossRef] [PubMed]
53. Oliveira, D.D.; Baião, N.C.; Cançado, S.V.; Oliveira, B.L.; Lana, Â.M.; Figueiredo, T.C. Effects of the use of soybean oil and animal fat in the diet of laying hens on production performance and egg quality. *Ciênc. Agrotec.* **2011**, *35*, 995–1001. [CrossRef]
54. Oliveira, D.D.; Baião, N.C.; Cançado, S.V.; Grimaldi, R.; Souza, M.R.; Lara, L.J.; Lana, A.M. Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. *Poult. Sci.* **2010**, *89*, 2484–2490. [CrossRef]
55. Diaz, G.J.; Cortes, M.V.; Cepeda, S.M. Effect of feeding hen's trout by-product meal or tuna fish oil on production parameters and yolk fatty acid profile. *Rev. Colomb. Cienc. Pecu.* **2011**, *24*, 609–616.
56. Pita, M.C.; de Carvalho, P.R.; Neto, E.P.; de Mendonça-Júnior, C.X. Vegetable and marine sources of supplementaries PUFAs in the diet of laying hens: Effect on lipid composition of the plasma and of the egg yolk and time of incorporation of omega 6 PUFAs. *J. Appl. Sci. Res.* **2011**, *7*, 654–671.
57. Irawan, N.; Hafizuddin, R.K.; Rusli, N.; Akhirini, W.; Setyon, A.; Jayanegara, C. Supplementary n-3 fatty acids sources on performance and formation of omega-3 in egg of laying hens: A meta-analysis. *Poult. Sci.* **2022**, *101*, 101566. [CrossRef]
58. Berenjian, A.; Sharifi, S.D.; Mohammadi-Sangcheshmeh, A.; Bakhtiarizadeh, M.R. Omega-3 fatty acids reduce the negative effects of dexamethasone-induced physiological stress in laying hens by acting through the nutrient digestibility and gut morphometry. *Poult. Sci.* **2021**, *100*, 100889. [CrossRef]
59. Sijben, J.W.; Groot, H.; Nieuwland, M.G.B.; Schrama, J.W.; Parmentier, H.K. Dietary linoleic acid divergently affects immune responsiveness of growing layer hens. *Poult. Sci.* **2000**, *79*, 1106–1115. [CrossRef]
60. Vlaicu, P.A.; Panaite, T.D.; Turcu, R.P. Enriching laying hens eggs by feeding diets with different fatty acid composition and antioxidants. *Sci. Rep.* **2022**, *11*, 20707. [CrossRef]
61. İskender, H.; Kanbay, Y. Üniversite Öğrencilerinin Yumurta Tüketim Alışkanlıklarının Belirlenmesi. *Van Vet. J.* **2014**, *25*, 57–62.
62. Zdrojewicz, Z.; Herman, M.; Starostecka, E. Hen's egg as a source of valuable biologically active substances. *Post. Hig. Medyc. Dosw.* **2016**, *70*, 751–759. [CrossRef] [PubMed]

63. Kuang, H.; Yang, F.; Zhang, Y.; Wang, T.; Chen, G. The impact of egg nutrient composition and its consumption on cholesterol homeostasis. *Cholesterol* **2018**, *22*, 6303810. [CrossRef]
64. Sanlier, N.; Üstün, D. Egg consumption and health effects: A narrative review. *Concise Rev. Hypoth. Food Sci.* **2021**, *86*, 4250–4261. [CrossRef]
65. Zhao, B.; Gan, L.; Graubard, B.I.; Männistö, S.; Albanes, D.; Huang, J. Associations of Dietary Cholesterol, Serum Cholesterol, and Egg Consumption With Overall and Cause-Specific Mortality: Systematic Review and Updated Meta-Analysis. *Circulation* **2022**, *145*, 1506–1520. [CrossRef] [PubMed]
66. Al-Ramady, O.; Latifi, A.N.; Treu, T.; Ho, Y.-L.; Seshadri, S.; Aparicio, H.J.; Cho, K.; Wilson, P.W.F.; Gaziano, J.M.; Djoussé, L. Egg consumption and risk of acute stroke in the Million Veteran Program. *Cl. Nutr. Espen* **2022**, *50*, 178–182. [CrossRef] [PubMed]
67. Mofrad, M.D.; Naghshi, S.; Lotfi, K.; Beyene, J.; Hypponen, E.; Pirouzi, A.; Sadeghi, O. Egg and Dietary Cholesterol Intake and Risk of All-Cause, Cardiovascular, and Cancer Mortality: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies. *Front. Nutr.* **2022**, *9*, 878979. [CrossRef]
68. Godos, J.; Micek, A.; Brzostek, T.; Toledo, E.; Iacoviello, L.; Astrup, A.; Franco, O.H.; Galvano, F.; Martinez-Gonzalez, M.A.; Grosso, G. Egg consumption and cardiovascular risk: A dose–response meta-analysis of prospective cohort studies. *Eur. J. Nutr.* **2021**, *60*, 1833–1862. [CrossRef]
69. Mohammadifard, N.; Taheri, M.; Haghighatdoost, F.; Grau, N.; Najafian, J.; Sadeghi, M.; Talaei, M.; Sarrafzadegan, N. Egg consumption and risk of cardiovascular events among Iranians: Results from Isfahan Cohort Study (ICS). *Eur. J. Clin. Nutr.* **2022**, *76*, 1409–1414. [CrossRef]
70. Al-Khalifa, H.; Givens, D.I.; Rymer, C.; Yaqoob, P. Effect of ω-3 fatty acids on immune function in broiler chickens. *Poult. Sci.* **2012**, *91*, 74–88. [CrossRef]