

Article

Effects of Dietary Mixture Enriched in Polyunsaturated Fatty Acids and Probiotic on Performance, Biochemical Response, Breast Meat Fatty Acids, and Lipid Indices in Broiler Chickens

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Abstract: This study evaluated the effects of a dietary mixture based on extruded linseed and pea (ELP; 20:80 *w/w*) and probiotics (*L. acidophilus*) on the performance, biochemical responses, breast muscle fatty acids (FA) profile, and lipid indices in broiler chickens. A total of 480 one-day-old Ross 308 broilers were assigned into four groups in a 2 × 2 factorial arrangement with two levels of ELP (0% and 30%) at the expense of soybean meal, corn, and vegetable oil and two levels of probiotic (0 and 20 g ton^{−1} feed). There were no effects of ELP diet or probiotic supplementation on performance and carcass traits. Feeding the ELP diet increased plasma total protein, urea nitrogen (PUN), and creatinine (Cre) levels with no changes in the PUN/Cre ratio. A probiotic addition lowered the total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and the TC/HDL-C and LDL-C/HDL-C ratios. The ELP diet improved the breast muscle FA profile by lowering total saturated FA (SFA) and increasing total polyunsaturated FA (PUFA), omega-3 (n-3), unsaturated FA (UFA), PUFA/SFA, and UFA/SFA ratios. Probiotics increased total PUFA, omega-6 (n-6) and decreased the n-6/n-3 ratio, total UFA, and UFA/SFA ratio. Dietary treatment interaction exhibited a synergistic effect for total PUFA and an antagonistic effect for n-3 PUFA and n-6/n-3 PUFA ratio in breast muscle. Probiotics reduced some lipid indices (PUFA/SFA ratio, desirable FA and nutritive value index) only when ELP0 was fed. In conclusion, based on these results, using ELP30 alone in broiler diets is recommended to improve meat's nutritional value for consumers.

Keywords: broiler meat; extruded linseed and pea mix; fatty acids; lipid indices; plasma response; probiotic



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1. Introduction

The potential contribution of nutritionally fortified foods to consumer health has been emphasized globally [1]. Health interventions are influenced by the cost-effectiveness and the potential risks of chronic diseases. As a result, enriching animal feed to improve nutritional imbalance and obtain a good quality product has become increasingly important and widely recognized [2].

The finding, which stated that consuming foods rich in polyunsaturated fatty acids (PUFAs) diminishes some dysfunctions that cause sickness, marked a turning point in scientific research [3]. PUFAs provide molecules that are used for synthesizing hormones that regulate blood clotting, arterial wall contraction and relaxation, and inflammation, and slow down the loss of immune function, brain development and visual function etc. [3]. The role

of PUFAs in mitigating or preventing cardiovascular diseases, autoimmune diseases, and certain forms of cancer has already been demonstrated [4].

The production of functional foods and/or value-added products (e.g., meat) by enriching animal diets with bioactive compounds such as PUFA, particularly n-3 PUFA, has gained attention due to their health effects [5,6]. Meat quality is influenced by birds' genotype [7,8], production systems [9], and by dietary composition, ingredients and additives [10–12]. Dietary manipulation has been widely recognized for how animal tissues reflect the intake of desirable healthy components, which develop positive biochemical, nutritional, and functional aspects [6,9]. Over time, several oilseeds [13–20] and legumes [21–29] were proposed as natural alternative solutions to enrich the diet with bioactive compounds (fatty acids, amino acids, natural antioxidants, soluble fibre, etc.).

Linseed (*Linum usitatissimum* L.) is a feed ingredient recognized for its high oil (35–45%) content with a unique PUFAs profile, especially α -linolenic acid (ALA, up to 50% of total oil content), but also protein (20–30%), soluble fibre, lignans, vitamins, and minerals [13,30,31]. However, the use of linseed in broilers is limited due to its antinutritional factors (ANF; e.g., mucilage, linatine, cyanogenic glycosides, trypsin inhibitor, and phytic acid), which can negatively affect the intestinal health, nutrient digestibility, and well-being of the muscular mass of broiler [32,33]. Several studies have stated that ANF content from linseed could be reduced by the extrusion process [13–15,34,35]. It was shown that chickens could metabolize dietary-extruded linseed throughout the digestive tract, absorbing most of the nutrients in the small intestine, and then further synthesize some of the ALA, elongating and desaturating the ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [13,36].

Pea (*Pisum sativum* L.) is a valuable source of protein (20–30%) with a nutritional value comparable to soybean meal (SBM) [22,23,37]. The genetic improvements of new pea cultivars minimized the content of certain antinutritional factors (e.g., protease inhibitors, tannins and phytates); however, the still high non-starch-polysaccharides (NSP) content may impair nutrients utilization [21]. With the increased public concerns about genetically modified SBM, known as a conventional protein source mainly imported from non-European countries at a higher price, pea seeds have become a viable alternative in poultry diets in Europe [38]. Laudadio and Tufarelli [23] investigated the beneficial effects of pea as a protein source and natural antioxidants in broilers' diets on meat quality and oxidative stability.

In response to emerging public health concerns about food safety and regulations, several studies have investigated the functional feed additives such as probiotics to enhance the natural immunological capacity and their role in maintaining or improving poultry gut health and performance [26,39–41]. One of the most used lactic acid bacterium as probiotics is *Lactobacillus acidophilus* (*L. acidophilus*), from the *Lactobacillaceae* family, due to its non-pathogenicity and beneficial effects on the host metabolism [42]. According to Dev et al. [43], dietary symbiotic supplementation (prebiotic and *L. acidophilus*) had hypocholesterolemic and hyperlipidemic effects improving serum health indices in broiler chickens. A study of Theilmann et al. [44] demonstrated the *L. acidophilus* capability to metabolize the glycosides from plant-derived compounds by bioconversion in the human gut.

Although some studies pointed out the effects of individual extruded linseed [13,14,18–20], raw pea seed [25,27,38,45], or micronized pea seed [22,23,46] on broiler performance, blood profile, intestinal health, oxidative stability, and meat quality, there are no previous studies that investigated the combined effects of extruded linseed and pea supplemented with *L. acidophilus* D2/CSL on performance, blood metabolites, breast muscle FA profile and dietary lipid indices in broilers. We hypothesized that the broilers' blood biochemical response, meat lipid quality, and the health-related index would be positively affected by a dietary mixture rich in PUFA and fibre, bioactive compounds that may have an additional prebiotic effect on *L. acidophilus* probiotic improving health status. Therefore, this study evaluated the effects of a dietary mix based on extruded linseed and pea (ELP; 20:80 w/w)

and *L. acidophilus* D2/CSL on the productive performance, biochemical responses, breast muscle FA profile, and lipid indices in broiler chickens.

2. Materials and Methods

2.1. Ethical Approval

The broilers were handled in agreement with the EU principles of Directive 63/2010/EU and Romanian Law 43/2014 concerning protecting the animals used for experimental and other scientific purposes [47]. The Local Ethics Committee authorized all procedures used during the trial at the National Research Development Institute for Biology and Animal Nutrition (INCDBNA) Balotesti, Romania (Protocol no. 3203/2019).

2.2. Broilers and Housing

Four hundred and eighty Ross 308 one-day-old mixed-sex broilers (46.71 ± 0.45 g) procured from a commercial hatchery station (Dambovită county, Romania) were raised for 42 days under standard conditions in a controlled poultry house at research Biobase INCDBNA-Balotesti (Ilfov County, Romania). Broilers were wing-tagged and grown in floor pens on permanent litter (10 cm thick of wood shaving), each pen being equipped with experimental facilities such as feeders and automatic nipple drinkers. The light program and intensity used were 23L: 1D with a light intensity of 35 lux from 1 to 7 d, and 20L: 4D with a light intensity of 5–10 lux from 8 to 42 d. The broiler veterinary protocol included immunizations for Marek's, Newcastle, Gumboro and Infectious Bronchitis diseases. During the trial, birds had free access to water and feed. Feed was administrated in mash form.

2.3. Dietary Treatments

The broilers were randomly assigned into four dietary groups ($n = 120$ broilers/group, six replicates of 20 broilers each) in a 2×2 factorial arrangement with 2 levels of ELP mix (0 and 30%) and 2 levels of *L. acidophilus* D2/CSL (1.0×10^9 CFU/kg feed) freeze-dried live cells (0 and 20 g ton^{-1} feed).

The three-phase (starter, 1 to 10 d; grower, 11 to 24 d; and finisher, 25 to 42 d) formulated feeds (Table 1) were isocaloric, isonitrogenous, and met the nutritional requirements of the Ross hybrid guide [48]. The bacterial count analyses confirmed that lactobacilli supplemented diets contained 1.0×10^8 CFU/kg feed.

Table 1. Ingredients and chemical composition of broilers feed.

Ingredients (% As-Fed)	Starter (1 to 10 d)		Grower (11 to 24 d)		Finisher (25 to 42 d)	
	ELP0	ELP30	ELP0	ELP30	ELP0	ELP30
Corn	58.07	38.82	60.33	40.71	64.34	44.74
Soybean meal	30.00	20.00	28.00	18.00	24.00	14.00
ELP mix	0	30.00	0	30.00	0	30.00
Corn gluten meal	5.50	5.50	5.00	5.00	4.00	4.00
Vegetable oil	1.50	1.00	2.30	2.10	3.50	3.30
Monocalcium phosphate	1.30	1.30	1.00	1.10	1.00	1.00
Calcium carbonate	1.48	1.33	1.38	1.20	1.20	1.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.49	0.38	0.39	0.27	0.36	0.25
DL-Methionine	0.29	0.30	0.23	0.25	0.23	0.24
Premix choline	0.06	0.06	0.06	0.06	0.06	0.06
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin-mineral premix ¹	1.00	1.00	1.00	1.00	1.00	1.00
<i>L. acidophilus</i> ²	(–)/(+)	(–)/(+)	(–)/(+)	(–)/(+)	(–)/(+)	(–)/(+)

Table 1. Cont.

Ingredients (% As-Fed)	Starter (1 to 10 d)		Grower (11 to 24 d)		Finisher (25 to 42 d)	
	ELP0	ELP30	ELP0	ELP30	ELP0	ELP30
Calculated composition						
Metabolizable energy ³ (MJ/kg)	12.82	12.69	13.00	12.98	13.46	13.44
Av. phosphorus (%)	0.48	0.48	0.44	0.44	0.40	0.40
Dig. Lysine (%)	1.28	1.28	1.15	1.15	1.03	1.03
Dig. Methionine + Cysteine (%)	0.95	0.95	0.87	0.87	0.80	0.80
Analyzed composition (%)						
Dry matter	89.92	89.48	89.26	89.29	89.36	89.21
Crude protein	22.59	22.42	21.30	21.20	19.45	19.40
Lysine	1.44	1.44	1.29	1.29	1.16	1.16
Methionine + Cysteine	1.08	1.08	0.94	0.94	0.88	0.88
Calcium	0.96	0.96	0.87	0.87	0.79	0.79
Crude fat	3.69	3.81	4.63	5.12	5.27	5.78
Crude fibre	3.51	4.26	4.40	4.96	3.94	5.14
Crude ash	5.50	5.69	5.24	5.37	5.10	5.21
Fatty acids composition (g/100 g FAME)						
C16:0	14.97	10.68	12.06	10.75	13.84	12.41
C18:0	3.56	3.60	3.30	3.29	3.58	3.68
C18:1n-9	24.34	22.58	25.51	23.38	23.26	22.37
C18:2n-6	51.70	40.11	52.8	42.45	52.65	43.45
C18:3n-3	3.80	21.29	4.37	18.50	5.12	16.36
C20:2n-6	0.17	0.17	0.20	0.19	0.15	0.16
C20:4n-6	0.32	0.31	0.29	0.21	0.45	0.36
C22:2n-6	0.14	0.18	0.25	0.22	0.19	0.17
Σ SFA	18.53	14.28	15.36	14.04	17.42	16.09
Σ MUFA	24.34	22.58	25.51	23.38	23.26	22.37
Σ PUFA	56.13	62.06	57.91	61.57	58.56	60.5
Σ n-6 PUFA	52.33	40.77	53.54	43.07	53.44	44.14
Σ n-3 PUFA	3.80	21.29	4.37	18.50	5.12	16.36
n-6: n-3 ratio	13.77	1.91	12.25	2.33	10.44	2.70
Fatty acids composition (g/100 g feed)						
Σ SFA	0.55	0.44	0.57	0.58	0.73	0.74
Σ MUFA	0.72	0.69	0.94	0.96	0.98	1.03
Σ PUFA	1.66	1.89	2.14	2.52	2.47	2.80
Σ n-6 PUFA	1.54	1.24	1.98	1.76	2.25	2.04
Σ n-3 PUFA	0.11	0.65	0.16	0.76	0.22	0.76

¹ Vitamin-mineral premix (provided/kg of diet): vitamin A, 4.47 mg; vitamin D₃, 0.12 mg; vitamin E, 80 mg; vitamin K₃, 4 mg; thiamine, 4 mg; riboflavin, 9 mg; pyridoxin, 4 mg; cobalamin, 0.02 mg; calcium-pantothenate, 15 mg; niacin, 60 mg; folic acid, 2 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg. ² *L. acidophilus* D2/CSL (1.0×10^9 CFU/kg feed) = 20 g ton⁻¹ feed; ³ calculated based on regression equations NRC [49]. ELP0, 0% extruded linseed: pea mix (control diet); ELP30, 30% extruded linseed: pea mix (20:80 w/w); FAME, fatty acids methyl ester; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

The dietary mix used in this study was based on extruded linseed and pea (20:80 w/w; ELP mix) and partly replaced soybean meal, corn, and vegetable oil. The pea seeds were procured from a local producer (Arges County, Romania), and extruded linseed was purchased from a commercial supplier (Noack Romania). The proportion of these feed ingredients was established based on literature and our previous studies [18,25,45] that investigated the dietary effects of these ingredients individually. We expected that a mixture of extruded linseed, as a rich source of PUFA, with peas, as a rich source of essential amino acids (EAA), at a ratio of 20:80 (w/w), has a complementarily beneficial nutritional effect.

After the two ingredients were dosed, grounded, and homogenized, the ELP mix was sampled for analysis.

The analyzed nutrient composition of the ELP mix used in the feeding trial (Table 2) showed a higher crude protein (CP, 19.8%) content with a balanced AA profile consisting of 47% EAA, from which lysine represents ~8% and sulphury AA ~5% of CP content, and 41% non-essential AA. The crude fat content of the ELP mix had a favourable FA profile with 60.4% Σ PUFA from which n-6 PUFA represents 37.8%, and 22.6% n-3 PUFA composed mainly by ALA, and an n-6: n-3 PUFA ratio below 2: 1, which led to promising potential to enrich broiler meat. The dietary inclusion level of the ELP mix was set based on a various simulation to meet the specific nutrient requirements of diets.

Table 2. Analyzed composition of dietary-extruded linseed:pea mix.

Item (%)	ELP mix
Dry matter	88.2
Crude protein	19.8
Crude fat	4.50
Crude fibre	9.60
Crude ash	3.48
Nitrogen-free extract	50.8
Calcium	0.29
Phosphorus	0.47
Metabolizable energy (MJ/kg)	12.3
Amino acids (g/100 g)	
Lysine	1.60
Methionine + Cystine	0.99
Threonine	0.98
Valine	1.45
Leucine	0.85
Isoleucine	1.58
Arginine	0.93
Phenylalanine	0.59
Essential AA	9.46
Tyrosine	0.60
Serine	1.10
Glycine	0.97
Alanine	0.96
Aspartic acid	2.11
Glutamic acid	2.31
Non-essential AA	8.05
Fatty acids (g/100 g FAME)	
C12:0	0.56
C14:0	0.61
C16:0	12.8
C16:1	0.51
C18:0	4.69
C18:1n-9	19.5
C18:2n-6	37.3
C18:3n-3	22.1
C20:2n-6	0.30
C20:3n-6	0.15
C20:3n-3	0.54
Other FA	0.90

Table 2. Cont.

Item (%)	ELP mix
Σ SFA	18.7
Σ MUFA	20.1
Σ PUFA	60.4
Σ n-6 PUFA	37.8
Σ n-3 PUFA	22.6
n-6: n-3 ratio	1.67
Fatty acids (g/100 g ELP)	
Σ SFA	0.67
Σ MUFA	0.72
Σ PUFA	2.17
Σ n-6 PUFA	1.36
Σ n-3 PUFA	0.81

FAME, fatty acids methyl ester; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

2.4. Performance Measurements and Sample Collection

The performance parameters determined were body weight (BW) by individually weighing at 1 d and 42 d of age to calculate the body weight gain (BWG). The broilers' mortality was monitored daily. Feed intake (FI) per pen was registered daily as well. Feed conversion ratio (FCR) and production efficiency factor (PEF) were calculated for the entire phase (1–42 d).

At the end of the trial (42 d), 24 broilers (n = 6/group; 1 broiler/replicate), sex ratio 1:1, were selected for blood collection and carcass analyses after 12 h of feed withdrawal.

Blood was sampled from the brachial vein by using $23G \times \frac{3}{4}$ '-gauge needles in a 4-mL lithium-heparin vacutainer (Vacutest Kima, Italy).

After blood collection, the broilers were humanely slaughtered by cervical dislocation, bled, and the carcasses were manually depلمed and eviscerated. Carcasses were weighed, and carcass yield was calculated as a percentage of BW at slaughter. Breasts and legs were removed and weighed (with skin and bone), and their relative weights were expressed as a percentage of carcass weight. Breast muscle (*Pectoralis major*) samples (n = 6/group) without skin were collected, packed into plastic zip bags, labelled, and frozen at -20°C for chemical analyses. All breast muscle samples were analyzed in duplicate.

2.5. Blood Biochemical Analysis

After blood samples were centrifuged at $3000 \times g$ at 4°C for 15 min (Centrifuge 5804R, Eppendorf AG, Hamburg, Germany), plasma was transferred in tubes and stored at -20°C before analysis. The blood metabolites (TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; Glu, glucose; TP, total protein; Alb, albumin; PUN, plasma urea nitrogen; Cre, creatinine; UA, uric acid) were assessed by dry chemistry and solid-phase reagent (SP-4430 Spotchem EZ, Arkay Inc., Tokyo, Japan). All plasma samples were analyzed in duplicate.

The low-density lipoprotein cholesterol (LDL-C) was calculated by the formula [50]:

$$\text{LDL-C (mg/dL)} = \text{TC} - \text{HDL} - \text{TG}/5$$

The TC/HDL-C, LDL-C/HDL-C and TG/HDL-C ratios and the PUN/Cre ratio were calculated.

2.6. Analyses and Calculations

2.6.1. Chemical Composition

The ELP mix and feed samples were analyzed in duplicate for nutrients composition as follows: dry matter (gravimetric method; ISO 6496:2001), crude protein (Kjeldahl method; ISO 5983-2:2009), crude fat (organic solvents extraction; ISO 6492:2001), crude

fibre (intermediary filtration method; ISO 6865:2002), crude ash (gravimetric method; ISO 2171:2010), calcium (titrimetric method; SR ISO 6490-1:2006), and phosphorus (photometric method) following EU Regulation no. 152/2009 [51].

2.6.2. Amino Acids Determination

Amino acids profile of the ELP mix and feed samples were assessed in duplicate by the high-performance liquid chromatography method as described by Vărzaru et al. [52], by using an HPLC Surveyor Plus Thermo-Electron analyzer (Thermo-Electron, Waltham, MA, USA) and HyperSil BDS C18 column (250 mm × 4.6 mm × 5 µm; Thermo-Electron, Waltham, MA, USA), according to EU Regulation no. 152/2009 [51].

2.6.3. Fatty Acids Determination

Fatty acids (FA) profile of the ELP mix, feeds, and breast muscle were determined in duplicate after fat extraction (ISO 6492:2001) by the gas chromatography method (ISO/TS 17764-2:2008) by using a Perkin Elmer-Clarus 500 chromatograph (PerkinElmer, Shelton, WA, USA) connected to a flame ionization detector (FID) and a capillary separation column BPX70 GC (60 m × 0.25 mm × 0.25 µm; Agilent GC Columns, Santa Clara, CA, USA) as described Hăbeanu et al. [53]. The FA was identified by comparing their retention times with the known standards (SUPELCO 37 Component FAME mix; Sigma-Aldrich Co., Burlington, MA, USA) and were expressed as g/100 g of total FA methyl esters (FAME). The sums of the saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), and unsaturated (UFA) fatty acids were calculated based on the average quantity of each FA.

2.6.4. Microbial Analyses

Lactobacillus bacterial counts in feed samples were determined by conventional techniques, as previously described [18]. Briefly, 1 g of feed sample was diluted at 1:9 (wt/vol) in sterile water and mixed. After supernatants were 10-fold serially diluted, 1 mL of each dilution was inoculated on Petri plates on MRS agar media (Biokar Diagnostics, Solabia, Paris, France) and were anaerobically incubated at 37 °C for 48 h. The lactobacilli colony forming units (CFU) were counted in duplicate per sample (Scan 300, Interscience, Saint-Nom-la-Bretèche, France).

2.6.5. Lipid Indices Calculation

The values of health lipid indices in the breast muscle, i.e., PUFA/SFA ratio (P/S ratio) [54], desirable FA (DFA) [55], atherogenic indices (AI) [56], thrombogenic indices (TI) [56], hypercholesterolemic SFA (HSFA) [55], hypo/hypercholesterolemic ratio (h/H) [57], and nutritional value indices (NVI) [58], were estimated based on the FA profile by using the specific equations

$$P/S \text{ ratio} = (C18:2 \text{ n-6} + C18:3 \text{ n-3}) / (C12:0 + C14:0 + C16:0) \quad (1)$$

$$DFA = (C18:0 + \Sigma UFA) \quad (2)$$

$$AI = (C12:0 + (4 \times C14:0) + C16:0) / ((\Sigma PUFA) + (\Sigma MUFA)) \quad (3)$$

$$TI = (C14:0 + C16:0 + C18:0) / ((0.5 \times \Sigma MUFA) + (0.5 \times \text{n-6}) + (3 \times \text{n-3}) + (\text{n-3}/\text{n-6})) \quad (4)$$

$$HSFA = (C14:0 + C16:0) \quad (5)$$

$$h/H = ((C18:1\text{n-9} + C18:2\text{n-6} + C18:3\text{n-6} + C18:3\text{n-3} + C20:3\text{n-6} + C20:4\text{n-6} + C20:5\text{n-3} + C22:4\text{n-6} + C22:5\text{n-3} + C22:6\text{n-3}) / (C14:0 + C16:0)) \quad (6)$$

$$NVI = [(C18:0 + C18:1)] / C16:0 \quad (7)$$

2.7. Statistical Analysis

Data were analyzed by using IBM SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA) [59]. The normal distribution of data was verified with a Shapiro–Wilk's test.

The results were analyzed as a 2×2 factorial arrangement by the general linear model (GLM) procedure by using a two-way ANOVA according to the model

$$Y_{ijk} = \mu + \text{ELP}_i + P_j + (\text{ELP} \times P)_{ij} + e_{ijk},$$

where Y_{ijk} is the dependent variables, μ is the overall mean, ELP_i is the effect of dietary mixture (none, ELP), P_j is the effect of probiotic (no, yes), $(\text{ELP} \times P)_{ij}$ is the effect of interaction between treatments, and e_{ijk} is the residual error. The experimental unit for the performance parameters was replicate-pen, and each bird sample was considered the experimental unit for the other variables. All data are presented as mean values with standard error of the mean (SEM). The statistically significant mean differences were estimated by using the Tukey's post-hoc test at $p < 0.05$.

3. Results

3.1. Performance and Carcass Traits

Table 3 results showed that the ELP or probiotic addition did not significantly influence the performance (BWG, FI, FCR and PEF) and carcass traits (carcass yield, breast, legs, and abdominal fat percentage). There was no interaction concerning performance parameters ($p > 0.05$).

Table 3. Productive performance ¹ and carcass traits ² of broilers.

Dietary Groups					SEM ⁴	Main Effects ³				<i>p</i> -Value		
ELP0		ELP30		ELP		P ⁵						
Probiotic (P)	(−)	(+)	(−)	(+)		0	30	(−)	(+)	ELP	P	Interaction
Overall performance (1–42 days)												
BWG (g)	2577	2636	2450	2532	39.6	2606	2500	2513	2584	0.073	0.200	0.800
FI (g)	4569	4650	4462	4562	49.3	4609	4512	4515	4606	0.382	0.416	0.930
FCR (g: g)	1.77	1.75	1.82	1.80	0.013	1.77	1.81	1.80	1.78	0.077	0.125	0.636
PEF	349	368	332	359	6.71	359	346	341	364	0.332	0.101	0.721
Carcass traits												
Carcass yield ⁶	71.57	71.98	71.10	71.36	0.49	71.77	71.23	71.34	71.67	0.475	0.605	0.811
Breast ⁷	38.61	39.35	38.44	38.88	0.29	38.98	38.66	38.52	39.11	0.597	0.801	0.337
Legs ⁷	27.46	27.70	27.23	28.15	0.21	27.58	27.69	27.34	27.93	0.801	0.190	0.436
Abdominal fat ⁷	1.18	1.07	1.31	1.11	0.048	1.13	1.21	1.24	1.10	0.356	0.122	0.608

¹ Means of 120 broilers/group (20 broilers $\times$ 6 replicates each). ² Means of 6 samples/group. ³ Data were analyzed as a 2×2 factorial arrangement. ⁴ SEM, standard error of the mean. ⁵ *L. acidophilus* D2/CSL (1.0×10^9 CFU/kg feed). ⁶ Calculated as % of FBW. ⁷ Calculated as % of carcass weight. ELP0, 0% extruded linseed: pea mix (control diet); ELP30, 30% extruded linseed: pea mix; (−)/(+) without or with *L. acidophilus*; FBW, final body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; PEF, production efficiency factor.

3.2. Biochemical Plasma Profile

As shown in Table 4, feeding the ELP diet did not significantly affect the broilers' plasma lipids parameters (TC, HDL-C, LDL-C, TG, the ratios of TC/HDL-C, LDL-C/HDL-C, TG/HDL-C, and Glu) compared to control. Probiotics significantly lower the TC level, LDL-C, as well as the TC/HDL-C and LDL-C/HDL-C ratios. The plasma protein profile revealed that TP, PUN, and Cre concentrations increased in broilers fed the ELP diet with no significant changes in PUN/Cre ratio. Probiotic addition did not affect the plasma protein response ($p > 0.05$). No treatment interaction was found for plasma biochemical response ($p > 0.05$).

Table 4. Plasma biochemical profile ¹ of broilers.

Dietary Groups					SEM ³	Main Effects ²				<i>p</i> -Value		
ELP0		ELP30		ELP		P ⁴						
Probiotic (P)	(−)	(+)	(−)	(+)		0	30	(−)	(+)	ELP	P	Interaction
TC (mg/dL)	134	125	131	128	1.38	130	129	133 ^a	126 ^b	0.876	0.028	0.222
HDL-C (mg/dL)	66.0	67.0	68.0	68.0	1.04	66.5	68.4	67.0	67.9	0.392	0.683	0.964
LDL-C (mg/dL)	56.2	46.6	52.4	47.8	1.32	51.4	50.1	54.3 _a	47.2 _b	0.569	0.005	0.297
TG (mg/dL)	61.0	57.8	51.8	57.2	2.01	59.4	54.5	56.4	57.5	0.240	0.789	0.301
TC/HDL-C ratio	2.05	1.87	1.93	1.86	0.027	1.96	1.90	1.99 _a	1.87 _b	0.232	0.024	0.331
LDL-C/HDL-C ratio	0.86	0.70	0.78	0.69	0.026	0.78	0.74	0.82 _a	0.70 _b	0.401	0.020	0.470
TG/HDL-C ratio	0.93	0.87	0.76	0.84	0.032	0.90	0.80	0.84	0.85	0.133	0.938	0.283
Glu (mg/dL)	275	271	263	258	3.54	273	261	269	264	0.097	0.484	0.933
TP (g/dL)	2.84	2.64	3.08	3.14	0.068	2.74 _b	3.11 _a	2.96	2.89	0.005	0.559	0.283
Alb (g/dL)	1.18	1.16	1.20	1.18	0.029	1.18	1.19	1.19	1.17	1.000	0.757	0.757
Cre (mg/dL)	0.200	0.220	0.240	0.260	0.008	0.210 _b	0.250 _a	0.220	0.240	0.015	0.197	1.00
PUN (mg/dL)	2.20	2.40	2.60	2.80	0.097	2.30 _b	2.70 _a	2.40	2.60	0.040	0.286	1.00
PUN/Cre ratio	11.0	10.8	11.0	10.8	0.26	10.9	10.9	11.0	10.8	0.997	0.720	0.965
UA (mg/dL)	7.84	7.70	7.76	7.32	0.26	7.80	7.54	7.77	7.51	0.057	0.644	0.751

¹ Means of 6 samples/group. ² Data were analyzed as a 2 × 2 factorial arrangement. ³ SEM, standard error of the mean. ⁴ *L. acidophilus* D2/CSL (1.0 × 10⁹ CFU/kg feed). ^{a,b} Means with a different superscript in the same row differ at *p* < 0.05. ELP0, 0% extruded linseed: pea mix (control diet); ELP30, 30% extruded linseed: pea mix; (−)/(+) without or with *L. acidophilus*; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; Glu, glucose; TP, total protein; Alb, albumin; PUN, plasma urea nitrogen; Cre, creatinine; UA, uric acid.

3.3. Fatty Acids Profile in Breast Muscle

Table 5 presents the effects of dietary treatments on fat content and the fatty acids profile in breast muscle of broilers. The breast meat fat content significantly decreases as effect of feeding ELP diet (1.10-fold) or probiotic supplementation (1.41-fold).

Dietary treatments had a major impact on the breast meat FA profile (Table 5). Compared to control, there was a significant effect of ELP diet on individual SFA by decreasing palmitic (C16:0; 1.1-fold), myristic (C14:0; 1.24-fold), arachidic (C20:0; 1.8-fold) acids and total SFAs (1.07-fold). Total MUFAs and the predominant oleic acid (C18:1n-9) tended to decrease. Total PUFAs increased (1.07-fold) by increasing the individually concentrations of CLA (C18:2; 1.8-fold), ALA (C18:3n-3; 2.3-fold), EPA (C20:5n-3; 2-fold), docosadienoic acid (C22:2n-6; 5.1-fold), DPA (C22:5n-3; 2.3-fold), and DHA (C22:6n-3; 1.5-fold). A significant decrease in LA was noticed (C18:2n-6; 1.13-fold). Therefore, the n-3 PUFA content increased by 2.14-fold, whereas the n-6 PUFA content and the n-6/n-3 ratio decreased by 1.12-fold and 2.4-fold. Obviously, a significant increase in the total UFAs (1.04-fold), PUFA/SFA (1.14-fold), and UFA/SFA (1.09-fold) ratios was noticed.

Probiotic supplementation led to increase the stearic (C18:0; 1.17-fold) and decrease arachidic (C20:0; 1.62-fold) acids with no significant changes in total SFAs. The oleic (C18:1n-9), palmitoleic (C16:1), and total MUFAs were significantly lower (1.07-fold). A significant increase in eicosatrienoic acids C20:3n-6 (1.4-fold), C20:3n-3 (1.43-fold), ARA (C20:4n-6; 1.62-fold), DPA (C22:5n-3; 1.74-fold), DHA (C22:6n-3; 1.5-fold), total PUFAs (1.02-fold), and n-6 PUFA content (1.03-fold) was found. The n-6/n-3 ratio decreased by 1.16-fold, as well as the total UFAs (1.02-fold) and UFA/SFA ratio (1.06-fold).

Table 5. Fatty acids profile (g/100 g FAME) ¹ in breast muscle.

Dietary Groups					SEM ³	Main Effects ²				<i>p</i> -Value		
ELP0		ELP30		ELP		P ⁴						
Probiotic (P)	(−)	(+)	(−)	(+)		0	30	(−)	(+)	ELP	P	Interaction
Fat (% DM)	2.10	1.47	1.89	1.36	0.085	1.78 ^a	1.62 ^b	1.99 ^a	1.41 ^b	0.050	<0.0001	0.555
C12:0	0.08	0.06	0.10	0.05	0.012	0.07	0.08	0.09	0.06	0.877	0.291	0.741
C14:0	0.60	0.65	0.53	0.46	0.022	0.62 ^a	0.50 ^b	0.56	0.55	0.001	0.754	0.064
C16:0	21.44 ^{ab}	22.38 ^a	20.24 ^{bc}	19.70 ^c	0.30	21.91	19.97	20.84	21.04	<0.0001	0.546	0.040
C18:0	6.10	7.13	6.34	7.36	0.15	6.62	6.85	6.22 ^b	7.25 ^a	0.072	<0.0001	0.763
C20:0	0.26	0.17	0.16	0.09	0.019	0.22 ^a	0.12 ^b	0.21 ^a	0.13 ^b	0.002	0.006	0.721
Σ SFA	28.48 ^b	30.39 ^a	27.37 ^b	27.67 ^b	0.33	29.44	27.52	27.92	29.03	<0.0001	0.089	0.030
C14:1	0.12	0.12	0.13	0.10	0.003	0.12	0.11	0.12	0.11	0.605	0.108	0.630
C16:1	3.74 ^b	3.82 ^b	4.14 ^a	3.54 ^c	0.061	3.78	3.84	3.94	3.67	0.277	<0.0001	<0.0001
C18:1n-9	31.65	29.33	30.50	28.86	0.29	30.49	29.68	31.07 ^a	29.09 ^b	0.061	<0.0001	0.079
Σ MUFA	35.51	33.26	34.77	32.49	0.32	34.39	33.63	35.14 ^a	32.88 ^b	0.082	<0.0001	0.924
C18:2 CLA	0.09	0.11	0.18	0.18	0.011	0.10 ^b	0.18 ^a	0.13	0.14	<0.0001	0.552	0.180
C18:2n-6 LA	28.74 ^a	26.33 ^b	23.14 ^c	25.73 ^b	0.52	27.53	24.44	25.94	26.03	<0.0001	0.546	<0.0001
C20:2n-6	0.13	0.21	0.17	0.23	0.016	0.17	0.20	0.15	0.22	0.279	0.088	0.654
C20:3n-6	0.27 ^c	0.42 ^a	0.28 ^c	0.35 ^b	0.017	0.34	0.32	0.28	0.39	0.079	<0.0001	0.003
C20:4n-6 ARA	1.25	1.99	1.13	1.87	0.049	1.62	1.50	1.19 ^b	1.93 ^a	0.178	<0.0001	0.995
C22:2n-6	0.11	0.08	0.50	0.41	0.049	0.09 ^b	0.46 ^a	0.30	0.25	<0.0001	0.079	0.373
C22:3n-6	0.05	0.08	0.09	0.04	0.009	0.07	0.06	0.07	0.06	0.823	0.725	0.083
Σ n-6 PUFA	30.54 ^a	29.12 ^b	25.32 ^c	28.64 ^b	0.50	29.83	26.98	27.93	28.88	<0.0001	<0.0001	<0.0001
C18:3n-3 ALA	3.09 ^d	3.72 ^c	8.90 ^a	6.78 ^b	0.61	3.40	7.84	5.99	5.25	<0.0001	0.077	<0.001
C20:3n-3	0.32	0.51	0.38	0.48	0.023	0.42	0.43	0.35 ^b	0.50 ^a	0.682	0.001	0.140
C20:5n-3 EPA	0.08	0.09	0.18	0.18	0.014	0.09 ^b	0.18 ^a	0.13	0.14	<0.0001	0.611	0.470
C22:5n-3 DPA	0.18	0.52	0.66	0.94	0.073	0.35 ^b	0.80 ^a	0.42 ^b	0.73 ^a	<0.0001	<0.0001	0.457
C22:6n-3 DHA	0.17	0.28	0.28	0.37	0.021	0.22 ^b	0.32 ^a	0.22 ^b	0.32 ^a	<0.0001	<0.0001	0.585
Σ n-3 PUFA	3.84 ^d	5.12 ^c	10.41 ^a	8.75 ^b	0.69	4.48	9.58	7.12	6.94	<0.0001	0.100	<0.0001
Σ PUFA	34.47 ^c	34.35 ^c	35.92 ^b	37.57 ^a	0.36	34.41	36.74	35.19	35.96	<0.0001	0.015	0.007
n-6/n-3 ratio	7.97 ^a	5.69 ^b	2.43 ^d	3.27 ^c	0.56	6.83	2.85	5.20	4.48	<0.0001	<0.0001	<0.0001
Σ UFA	69.99 ^a	67.61 ^b	70.69 ^a	70.06 ^a	0.32	68.8	70.37	70.33	68.84	<0.0001	<0.0001	0.005
PUFA/SFA	1.21 ^b	1.13 ^b	1.31 ^a	1.36 ^a	0.024	1.17	1.34	1.26	1.24	<0.0001	0.429	0.015
UFA/SFA	2.46 ^a	2.23 ^b	2.58 ^a	2.53 ^a	0.038	2.34	2.56	2.52	2.38	<0.0001	0.001	0.022
Other FA	1.53	1.99	1.94	2.27	0.097	1.76	2.11	1.75	2.13	0.086	0.069	0.813

¹ Means of 6 breast samples/group. ² Data were analyzed as a 2 × 2 factorial arrangement. ³ SEM, standard error of the mean. ⁴ *L. acidophilus* D2/CSL (1.0 × 10⁹ CFU/kg feed). ^{a–d} Means with a different superscript in the same row differ at *p* < 0.05. ELP0, 0% extruded linseed: pea mix (control diet); ELP30, 30% extruded linseed: pea mix; (−)/(+) without or with *L. acidophilus*; DM, dry matter; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

There were significant interactions between treatments for certain FAs in breast muscle. Palmitic, as well as palmitoleic acid concentration, was most pronouncedly reduced in ELP30 when combined with the probiotic. Although the probiotic markedly increased the total SFA in ELP0, it had only a minor effect in ELP30 diet. A significant interaction was recorded for LA as well as total n-6 PUFA; aside from the LA and n-6 PUFA lowering effect of ELP30, probiotics decreased these concentrations on ELP0, whereas the supplementation increased it in the ELP30 diet. The C20:3n-6 concentration increased when the probiotic was fed. Unexpectedly, a significant interaction was noticed for ALA and total n-3 PUFA; these concentrations markedly increased in ELP30. A probiotic addition increased the ALA and total n-3 PUFA in ELP0 but decreased these concentrations in ELP30. The total PUFA increased in ELP30 with or without probiotics. Due to the influence of the treatments on n-6 and n-3 PUFA, the interaction for their ratio was also significant, with ELP30 having a lowering effect, whereas the probiotic addition increased the n-6/n-3 ratio only in ELP30. Obviously, the total UFA and the ratio between UFA/SFA had a significant interaction between treatments; these concentrations decrease as an effect of the probiotic only when fed ELP0.

3.4. Lipid Indices in Breast Muscle

As can be seen in Table 6, feeding broilers with the ELP diet led to a significant increase in health-related lipid indices such as P/S ratio (14.5%), DFA (2.4%), h/H ratio (12.2%), and NVI (7.6%) in breast muscle. As consequence, a significant decrease of the AI (16.6%), TI (28.1%), and HSFA (9.1%) compared to control were registered. The probiotic addition did not significantly alter the lipid indices in the breast meat of broilers. There were significant interactions between treatments for lipid indices, except for TI. Probiotics significantly reduced P/S ratio, DFA, and NVI, only when ELP0 was fed.

Table 6. Lipid indices ¹ in breast muscle.

Dietary Groups					SEM ³	Main Effects ²				p-Value		
ELP0		ELP30		ELP		P ⁴						
Probiotic (P)	(−)	(+)	(−)	(+)		0	30	(−)	(+)	ELP	P	Interaction
P/S ratio	1.44 ^{ba}	1.30 ^c	1.54 ^a	1.61 ^a	0.032	1.37	1.57	1.49	1.46	<0.0001	0.253	0.002
DFA (%)	76.0 ^b	74.7 ^c	77.0 ^a	77.4 ^a	0.29	75.4	77.2	76.5	76.1	<0.0001	0.084	0.004
AI (%)	0.34 ^a	0.37 ^a	0.32 ^b	0.31 ^b	0.007	0.36	0.30	0.33	0.34	<0.0001	0.200	0.020
TI (%)	0.63	0.65	0.44	0.48	0.024	0.64 ^a	0.46 ^b	0.53	0.56	<0.0001	0.097	0.250
HSFA (%)	22.0 ^a	23.0 ^a	20.8 ^b	20.2 ^{cb}	0.32	22.5	20.5	21.4	21.6	<0.0001	0.589	0.038
h/H ratio	2.99 ^b	2.77 ^{cb}	3.18 ^a	3.28 ^a	0.057	2.88	3.23	3.09	3.02	<0.0001	0.285	0.012
NVI (%)	1.76 ^a	1.64 ^b	1.82 ^a	1.84 ^a	0.024	1.70	1.83	1.79	1.74	<0.0001	0.074	0.023

¹ Calculated value. ² Data were analyzed as a 2 × 2 factorial arrangement. ³ SEM, standard error of the mean.

⁴ *L. acidophilus* D2/CSL (1.0 × 10⁹ CFU/kg feed). ^{a–c} Means with a different superscript in the same row differ at *p* < 0.05. ELP0, 0% extruded linseed: pea mix (control diet); ELP30, 30% extruded linseed: pea mix; (−)/(+) without or with *L. acidophilus*; P/S, polyunsaturated/saturated fatty acid ratio; DFA, desirable fatty acids; AI, atherogenicity index; TI, thrombogenicity index; HSFA, hypercholesterolemic saturated fatty acids; h/H, hypo/hypercholesterolemic fatty acids ratio; NVI, nutritive value index.

4. Discussion

This study investigated the effects of a 30% ELP dietary mixture based on extruded linseed and pea (20:80 *w/w*) rich in PUFA, supplemented with probiotics (*L. acidophilus* D2/CSL, 20 g ton^{−1} feed), on productive performance, plasma biochemical response, breast muscle FA profile, and dietary indices in broiler chickens.

4.1. Performance and Carcass Traits

The study results showed that Ross 308 broilers fed with dietary ELP and/or probiotic addition achieve similar growth performance responses. These could be attributed to the complementing effects of the dietary mixture and to the fact that experimental diets were well-balanced in essential amino acids that meet the nutritional requirements of broilers. The performance response was also supported by the intestinal microbial results beneficially affected by probiotic addition, decreasing pathogen populations (staphylococci and *E. coli*), and increasing the *Lactobacillus* spp. and lactobacilli: *E. coli* ratio (data not shown). Previously, Czerwiński et al. [60] studied the effects of pea with organic acid/or probiotic supplementation in Ross 308 broilers and reported that pea increases FI and decreases FCR with no significant effects on BWG, whereas probiotic or organic acid supplementation did not affect broiler performance. Laudadio and Tufarelli [22] found that Hubbard broilers fed pea as a protein source did not significantly change the growth performance parameters. Our previous work [25] has shown that feeding Cobb 500 broilers with 200 g/kg pea seed as a partial SBM substitute improved performance and breast yield. Conversely, Biesek et al. [27] obtained a reduced BWG and increased FCR in Ross 308 broilers as effects of fed pea as a protein source. On the other hand, studies by Anjum et al. [13] using up to 15% extruded linseed in Hubbard broilers, and Mridula et al. [61] using up to 10% linseed on Vencob broilers reported impaired growth performance that was attributed to the presence of antinutritional compounds in linseed and vitamin B6 antagonism [62]. In contrast, Kostadinović et al. [14] stated that 10% extruded linseed improved Ross 308 broilers' productive performance vs. 2.5, 5% extruded flaxseed and control diets. Skrivan et al. [19] found that a dietary combination of 6% extruded flaxseed and 4% hempseed increased BW

and FI of Ross 308 with no effects on FCR more than using these dietary seeds alone vs. control. Previous studies showed that feeding 10% or 15% whole linseed with or without enzymes [16] or up to 12% extruded linseed with or without *L. acidophilus* supplementation [18] had no significant effects on growth performance in Ross 308 broilers.

Our data concerning carcass traits showed no significant effects of the ELP diet or probiotics. The carcass trait results corroborate those previously reported by Laudadio and Tufarelli [22,23] who used micronized-dehulled pea as a partial substitute of SBM in Hubbard broilers, or Kiczorowska et al. [46] replacing 50% of SBM with a micronized pea in Ross 308 broilers. Our previous results have shown that extruded linseed levels (6 and 12%) and/or probiotic addition did not affect the carcass or cut-up part yields in Ross 308 broilers but lowered the abdominal fat percentage [18]. In contrast, Konieczka et al. [15], reported that using linseed or rapeseeds and fish oil in Ross 308 broilers had no effects on performance but improved slaughter parameters and decreased carcass abdominal fat percentage. Zając et al. [20], evaluating the impact of 15% camelina, linseed, or sunflower seeds in broiler diets, reported an improvement in performance and carcass traits, and they found a significantly lower fat digestibility in broilers fed camelina and linseed diets.

4.2. Biochemical Plasma Profile

Regarding the plasma biochemical response, our results revealed that the fed ELP diet did not significantly affect the broilers' plasma lipids but altered the plasma protein profile by increasing TP, PUN, and Cre concentrations with no significant changes in the PUN/Cre ratio. Similarly, Pirmohammadi et al. [35] reported no alteration of plasma lipid profile by feeding expanded linseed in broilers. In contrast, Kiczorowska et al. [46] found an improvement in the blood lipid of broilers fed micronized pea due to the increasing soluble fraction of pea fibre by micronization and ability to bind bile acids, thus controlling the cholesterol resorption. Our previous work has shown that peas in the broiler diet improved the plasma protein profile with no significant alterations on lipid profile [25]. Bingol et al. [24] also found that blood total protein and its globulin fractions increased in broilers fed pea protein, arguing that peas may stimulate the protein synthesis related to immunity. However, increased PUN and lower UA concentrations denote an increase in protein catabolism [63]. In turn, the probiotic supplementation has shown a hypocholesterolemic effect lowering the TC and LDL-C levels, as well as the TC/HDL-C and LDL-C/HDL-C ratios, without affecting the plasma protein response. Gheorghe et al. [18] found that dietary-extruded linseed level was correlated with a decrease in plasma total cholesterol, triglycerides, and very-low-density lipoprotein concentrations; meanwhile, the *L. acidophilus* addition was correlated with a decrease in plasma total cholesterol. Several studies reported that dietary supplementation with probiotics based on *Lactobacillus* strains lowers broilers' blood total cholesterol and triglycerides [64–68]. It was noticed that the addition of *L. acidophilus* and *L. casei* via feed or water decreases the gallbladder acids in the digestive process, which results in a reduction in fat digestion capacity and a decrease in blood lipids [69]. In vitro study has demonstrated that *L. acidophilus* may absorb cholesterol, decreasing the cholesterol levels in the medium [70]. The hypocholesterolemic effect of probiotic strains could be related to the ability to absorb cholesterol to their cell surface and incorporate it into cell membranes to increase cellular resistance against lysis [71].

4.3. Fatty Acids Profile in Breast Muscle

The fat content is one of the major attributes determining consumers' decisions about meat [72]. The present study has shown a decrease in breast muscle fat content as the effect of feeding the ELP diet and/or probiotic supplementation. These results are in line with previous research that highlighted a decrease in breast muscle fat content as a consequence of feeding linseed or pea in broilers, possibly due to a reduced digestibility of fat [16,20,23,73,74] and as an effect of probiotic supplementation by decreasing the activity of acetyl-CoA carboxylase, the rate-limiting enzyme in FA synthesis, and therefore lowering the blood lipids [65,69,71,75].

Fatty acids, including MUFA, PUFA, and SFA, are essential indicators of meat quality, nutritional value, and flavour characteristics [10]. Currently, the nutritional guidelines recommend decreasing SFA intake, particularly lauric, myristic, and palmitic acids, due to their potential to elevate total cholesterol and LDL cholesterol levels and the risk for cardiovascular disease (CVD). The most atherogenic acids are lauric and myristic, whereas stearic is thrombogenic with neutral atherogenicity [76].

In the present study, the breast muscle of broilers fed the ELP diet was characterized by a favourable decrease in total SFA and an increase in total PUFA, n-3 PUFA, UFA, PUFA/SFA, and UFA/SFA ratios. The ELP diet decreases palmitic (the predominant SFA), myristic and arachidic acids in the breast muscle, which is beneficial to human health. A similar trend of predominant SFA in broiler breast muscle has been reported previously by fed linseed diets [14,77] or pea diets [22,38,46]. The UFA and SFA ratios mainly determine the meat flavour, but UFA also has essential roles in supporting growth, removing free radicals, and modulating lipid metabolism [10]. Total MUFAs and oleic acid tended to decrease in breast muscle from broilers fed ELP diet. The results partially agree with Mirshekar et al. [78], who reported lower oleic and higher palmitic acid in broilers fed linseed diet, and other studies [22,79] that noticed a decrease in oleic acid in broiler chickens or guinea fowl broilers fed peas. Conversely, other research found an increase in oleic acid in broilers fed the linseed diet [77] or peas [38,46]. Our data have shown that the ELP diet significantly increased total PUFAs in broilers' breast muscle by increasing the concentrations of CLA, docosadienoic acid, and especially n-3 PUFA ALA, EPA, DPA, and DHA. At the same time, LA and n-6 PUFA content decreased. The decrease of n-6 FAs concentrations could be related to competition among n-3 and n-6 FAs for $\Delta 5$ - and $\Delta 6$ -desaturases implicated in the biosynthesis of PUFAs such as AA, EPA, and DHA [80]. Because the conversion rate of ALA into EPA and DHA in humans is lower [81,82], one of the most desired results is to increase EPA and DHA levels to improve the nutritional quality of meat for humans. This is due to the n-3 and n-6 FA competition for desaturation and elongation and the higher ALA oxidation with the increasing ALA intake [83]. Long-chain (LC) n-3 PUFA (EPA, DPA and DHA) have a beneficial effect on the optimal development of the cell, tissues, and organs [84], with the potential to lower CVD, hypertension, and inflammation [85]. An optimum conversion of ALA in LC n-3 PUFA depends on the n-6/n-3 ratio in the diet. Our results show that the ELP diet significantly decreased the n-6/n-3 ratio to 2.85:1, which is desirable for broiler meat. The recommended n-6/n-3 ratio is below 5:1 [86]. Overall, these findings are in line with previous reports regarding the alterations in total PUFAs, n-3 PUFA, and the n-6/n-3 ratio as effects of feeding linseed [14,19,77] or peas [22,46,79].

Probiotic supplementation led to an increase in the stearic and a decrease in arachidic acids with no significant changes in total SFAs. The oleic, palmitoleic, and total MUFAs were significantly lower. A significant increase in ARA, DPA, DHA, total PUFAs, and n-6 PUFA content was found. The n-6/n-3 ratio and the total UFAs decreased, as well as UFA/SFA ratio. To date, there are limited scientific literature reports about the dietary effects of probiotics on meat FA profile in broiler chickens. Our results are in line with previous findings using multi-strain probiotics in broiler chickens (including *L. acidophilus*) by Hossain et al. [87], who noticed an increase in n-3 PUFA and a decrease n-6/n-3 ratio in the breast meat and Hussein et al. [88] who found an improvement in total PUFA, n-3 PUFA, and the PUFA/SFA ratio, and the lower n-6/n-3 ratio in breast meat. Recently, Dev et al. [75] have shown that dietary probiotic and *L. acidophilus* supplementation in broilers improves the meat health indices (PUFA/SFA ratio, MUFA/SFA ratio, UFA/SFA ratio, saturation index, AI, TI, DFA, HFA, and h/H ratio) due to the influence of stearoyl CoA desaturase (SCD-1) gene expression.

4.4. Lipid Indices in Breast Muscle

Our breast meat fat components data highlighted the strong influence of FA lipid profile in breast meat from ELP and probiotics on health lipid indices. The nutritional

value of fat depends on the PUFA content and their n-6/n-3 ratio, which are the major determinants of the hypocholesterolemic index [89]. The P/S ratio evaluates the effects of diet on cardiovascular health, and the higher P/S values (>0.45) result in a more positive effect [85]. Feeding an ELP diet has been shown to increase the P/S index value in broiler breast meat (1.57 vs. 1.37), whereas probiotic addition did not alter the P/S value (1.46 vs. 1.49) significantly. The atherogenicity (AI) and thrombogenicity (TI) nutritional indices are commonly used to evaluate the composition of FA, demonstrating the potential to provide benefits to human health. The AI indicates the risk of CVD and is dominated by n-6 PUFA, whereas TI indicates the risk of blood clots forming in blood arteries and is regulated by n-3 PUFA [5,85]. Our results showed a significant decrease in AI (0.30), and TI (0.46) in breast muscle of broilers fed the ELP diet vs. control (0.36, respectively 0.64), whereas probiotic supplementation led to a similar AI (0.34 vs. 0.33) and TI (0.56 vs. 0.53) values. These results range in recommended values for a healthy human diet, below 1.0 for AI and below 0.5 for TI [85,86]. The hypo/hypercholesterolemic ratio (h/H) index evaluates the impact of FAs on human cholesterol levels [57]. Higher h/H combined with lower AI and TI contributed to a drop in cholesterol levels, ultimately reducing the risk of coronary heart disease [5,85]. In this study, the h/H was higher at 3.23 vs. 2.88 in breast muscle of broilers fed ELP, whereas probiotics led to a similar value (3.02 vs. 3.09). The decrease of total SFAs in the breast muscle of broilers fed ELP was reflected in the HSFA decrease (20.5 vs. 22.5), which is desirable for humans' health due to lowered blood cholesterol levels and the prevention of coronary heart disease [5]. Consequently, breast muscle of broilers fed ELP was characterized by higher DFA (77.2 vs. 75.4) and NVI (1.83 vs. 1.70) indices, whereas probiotic supplementation did not significantly alter these values. These findings agree with the FAO recommendations mentioning that DFA and NVI should be as high as possible due to their neutral or cholesterol-lowering effects [90].

Our study results showed a synergistic effect between ELP and probiotics only for total PUFA in breast muscle. Although a significant interaction between treatments was found for total SFA, palmitic and palmitoleic acids, LA, ALA, n-3 PUFA, and PUFA/SFA ratio, as well as for some meat quality lipid indices (P/S ratio, DFA, AI, HSFA, h/H ratio and NVI) the effects were not synergistic. They even show an antagonistic effect for n-3 PUFA and n-6/n-3 PUFA ratio. Based on these results, probiotics reduced the beneficial effects of ELP30 on n-3 PUFA and n-6/n-3 PUFA ratio in breast muscle; thus, it is recommended to use ELP alone in broiler diets more efficiently for improving the nutritional value of meat. There is a lack of literature studies regarding the interaction of FA and probiotics in broilers' breast muscle for comparison. Kankaanpää [91] studied the mechanisms by which PUFA affected the physicochemical and functional properties of probiotics and the functionality of epithelial cells in vitro and reported a bilateral interaction between dietary PUFA and probiotics. This study demonstrated that *Lactobacillus* spp. probiotics incorporated and interconverted exogenous PUFA in the growth medium into bacterial FA strain and PUFA dependently. Generally, higher concentrations of PUFA inhibited probiotics' growth and mucus adhesion. Conversely, low concentrations of specific LC PUFA were found to promote *Lactobacillus*'s growth and mucus adhesion. These effects were paralleled with only minor alterations in hydrophobicity and electron donor–electron acceptor properties of lactobacilli. The Kankaanpää [91] study also highlighted that PUFA alters the adhesion capacity of the intestinal epithelial cells; n-6 PUFA tended to inhibit the Caco-2 adhesion of probiotics, whereas n-3 PUFA had either no or minor effects, or even promoted the bacterial adhesion to PUFA treated Caco-2 cells.

5. Conclusions

The study results demonstrated that the use of 30% ELP in broilers' diet had no negative effects on productivity performance, improved plasma protein profile, and enhanced breast muscle of broilers with PUFA, improving its dietary value. A probiotic (*L. acidophilus*) supplementation positively affects plasma lipoproteins, alters breast muscle total PUFA, and significantly reduces some lipid indices (P/S ratio, DFA and NVI) only when ELP0

was fed. Dietary treatment interaction exhibited a synergistic effect for total PUFA and an antagonistic effect for n-3 PUFA and n-6/n-3 PUFA ratio in breast muscle. Thus, based on these results, it is recommended to use ELP alone in broiler diets to improve meat's nutritional value for consumers. Although the probiotic benefits in broilers' diets were evident (i.e., plasma lipoproteins, total PUFA in breast muscle), further studies are needed to understand the mechanisms of interaction between a dietary vegetable-rich PUFA mixture and probiotics on lipid profile and health lipid indices of broiler meat.

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