CHAPTER 11

Fatty acid composition of total lipids of liver and thigh muscle broiler chickens under the influence of separate and complex action of vitamins E and C

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Abstract

The section presents the research results devoted to studying the effect of separate and complex inclusion of vitamins E and C in the diet of broiler chickens on the fatty acid composition of total lipids of their liver and skeletal muscles at 41 days of age. Four groups of broiler chickens were formed for the experiment. The control group received standard compound feed (SC); the first experimental group of chickens received vitamin E in addition to SC; the second experimental group received vitamin C; the third experimental group received vitamins E and C simultaneously. The results of the study proved significant differences in the fatty acid composition of total lipids of the liver and thigh muscles of 41-day-old broilers under the influence of separate and complex effects of vitamins E and C. The addition of vitamin E caused an increase in the content of individual saturated fatty acids and a moderate increase in the ω-3 PUFA level in liver tissues. The addition of vitamin C to the chicken diet contributed to an even more pronounced increase in saturated fatty acids, but at the same time a significant decrease in the total PUFA level, especially ω-6. Instead, the combined effect of vitamins E and C led to the most pronounced changes – a significant increase in the total PUFA level (44.06%), a sharp increase in ω-6 (up to 38.3%) and at the same time a decrease in ω-3, which was accompanied by an increase in the ω-6/ω-3 ratio (up to 6.65). In the muscle tissues of chickens receiving vitamin E (groups I and III), a faster increase in the ω-3 PUFA content compared to ω-6 was found against the background of a decrease in the SFA content and MUFA, which contributed both to providing these tissues with a set of necessary PUFA and to a significant increase in the biological value of meat due to optimization of the ω-6/ω-3 PUFA ratio. In chickens to which vitamin C was added, an increase in the antioxidant activity of the tissues

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was accompanied by an increase in the total SFA content, and the ω-6/ω-3 PUFA ratio remained at the level of the control group (35.84).

Keywords

Broiler chickens, liver, lipid peroxidation, fatty acid composition, vitamins E and C, ω-6 and ω-3 polyunsaturated fatty acids.

11.1 The problem of antioxidant protection of broiler chickens and the role of vitamins E and C in its provision

Poultry meat remains the main type of meat product on the domestic Ukrainian market. Poultry farming provides a significant share of the population's needs for balanced animal proteins. At the same time, even in wartime, the export of poultry meat, primarily chicken, is an essential activity of the Ukrainian agriculture, as Ukraine occupies one of the leading positions among the main exporters of this product [1]. Poultry meat is characterized by high nutritional value, digestibility and relative affordability, which determines stable demand for this product [2]. The full-scale invasion of the Russian Federation in 2022 radically changed the landscape of Ukrainian poultry farming. In addition to the direct destruction of capacity, the industry faced many other problems associated with disruption of logistics processes, energy instability and limited refrigeration infrastructure capabilities. However, despite many negative factors, the level of poultry production during the 4 years of the war decreased by only 7.2% [3] and now the efforts of scientists and specialists in the agricultural sector are aimed at the restoration of poultry farming and its further development.

Industrial crosses of broilers are characterized by a high level of metabolic processes, which is accompanied by intensification of lipid peroxidation (LPO) in poultry tissues and the accumulation of reactive oxygen species (ROS) [4]. These changes are more pronounced during critical periods of poultry ontogenesis and are caused by the influx of stress factors (vaccination) and intensive growth [5, 6]. The processes of raising poultry are associated with a number of stress factors, from hatching to slaughter. Excessive formation of reactive oxygen species and oxidative stress are the main negative factors that cause most of the losses of poultry. Therefore, the development of a system of optimal antioxidant supplements to maintain effective antioxidant protection and redox balance in the poultry body is an urgent task.

Polyunsaturated fatty acids (PUFA) of the ω-6 (linoleic, arachidonic, eicosatrienoic) and ω-3 (linolenic, eicosapentaenoic, docosahexaenoic) families play a key role and occupy a prominent place in the structure of cell membranes and the regulation of metabolic processes. The high PUFA content in phospholipids improves the fluidity and functionality of cell membranes, which is important for the normal functioning of poultry organs and systems. On the other hand, the content and ratio of ω-6 and ω-3 PUFA in muscles and liver significantly affect the nutritional value and quality of poultry products. Increasing the proportion of ω-3 PUFA in meat lipids increases its biological value for humans, as these acids have antiatherogenic, cardioprotective and anti-inflammatory properties. It has been proven that ω-3 PUFA are important modulators of immune function and the nervous system, and a high ω-6 PUFA content without sufficient ω-3 intake is associated with an increased risk of metabolic and inflammatory disorders [7, 8]. A low ω-6/ω-3 ratio (close to 4:1) is considered optimal for human nutrition, while an excess of linoleic acid against the background of a deficiency of ω-3 PUFA can cause undesirable health consequences [9]. In birds, an increase in the ω-3 PUFA content in tissues also has a positive effect: there is evidence that moderate enrichment of the diet with ω-3 PUFA enhances antioxidant status (glutathione peroxidase activity) and reduces the level of lipoperoxidation in chickens. In addition to their antioxidant and anti-inflammatory roles, ω-3 fatty acids are thought to regulate platelet homeostasis and reduce the risk of thrombosis. Conversely, the presence of a large amount of ω-6 PUFA in tissue lipids with insufficient levels of antioxidants leads to intensive fat oxidation and accumulation of lipid peroxidation products, which leads to a deterioration in the quality of the resulting meat [10, 11]. Therefore, in modern poultry farming, considerable attention is paid to optimizing the fatty acid composition (FAC) of the broiler diet – in particular, adding sources of ω-3 PUFA (linseed oil or fish oil) – in combination with appropriate antioxidant provision. This allows for a significant reduction in the ω-6/ω-3 ratio in poultry fats and obtaining meat enriched with beneficial ω-3 acids and capable of contributing to the prevention of cardiovascular and metabolic diseases in humans [7].

It is known that the most powerful natural antioxidants, vitamins E and C, perform complementary functions in the poultry body. Fat-soluble vitamin E (tocopherol) is integrated into the phospholipid bilayer of membranes and protects PUFA from peroxidation by uncoupling free radical chains. Due to this, tocopherol stabilizes cellular and subcellular membranes (in particular mitochondria), prevents oxidative damage to lipids and proteins, and supports the functional activity of various systems of the broiler body during stress [12, 13]. In addition to its direct antioxidant action, tocopherol also performs a number of other physiological functions. Modern studies show that vitamin E is an important regulator of cellular processes, an immunomodulator, an anti-inflammatory factor, and a neuroprotector, therefore it is a factor in the general adaptive resistance of the poultry body in conditions of intensive production [14, 15].

At the same time, vitamin C (ascorbic acid) is a water-soluble antioxidant that is synthesized in the body of chickens, but its reserves are quickly depleted under the influence of stress and high metabolic rate. In such cases, additional administration of ascorbic acid (200–250 mg/kg of feed) helps to maintain the normal course of metabolic processes, increase the productivity and quality of broiler meat by enhancing their antioxidant and immune potential. It is important that vitamin C restores the activity of vitamin E, regenerating its active form from the tocopherol radical and thereby prolonging the antioxidant effect of tocopherol in tissues [16, 17]. Many studies on broilers confirm that the complex use of vitamins E and C, as well as trace elements (Selenium, Zinc) provides better protection against oxidative stress than the use of each of these substances separately [18]. In particular, combined supplementation of the diet with high doses of vitamin E and coherent antioxidants significantly reduces the in vivo concentration of lipid peroxidation products and improves the preservation and quality of broiler meat during storage. Antioxidant vitamins also indirectly affect lipid metabolism in poultry. It has been established that increasing the tocopherol level in the diet of broilers contributes to the normalization of lipid profile indicators – reducing the content of cholesterol and triacylglycerols in meat, and can also modulate the relative content of individual fatty acids in tissues. According to R. Voloshyn et al. [19], an increase in the concentration of vitamin E in the feed of broilers by 4–16 times compared to the norm caused a dose-dependent increase in the proportion of arachidonic acid (ω-6 PUFA) in liver lipids (by 1.3–1.5 times compared to the control) with a simultaneous decrease in the level of stearic acid, which indicates stimulation of the biosynthesis of arachidonic acid by tocopherol.

Our previous studies [20, 21] confirmed that the level of lipid hydroperoxides and end products of lipoperoxidation in the blood plasma of broilers sharply increases during increased growth processes in poultry and in the period after vaccination. Excessive accumulation of LPO products is one of the factors of damage to cell membranes and deterioration of both poultry productivity and meat quality after slaughter. Additional introduction of natural antioxidants into the poultry diet can restrain these negative processes. It has been established that increased doses of vitamins E and C in the diet of broilers cause a decrease in the content of intermediate and final products of lipid peroxidation in their tissues and enhance the antioxidant defense of the bird's body, especially under conditions of stress factors. It has been proven [22] that increased levels of tocopherol (0.1 g/kg) and ascorbic acid (0.25 g/kg) in the diet of broiler chickens significantly reduce the accumulation of lipid hydroperoxides and TBA-active products in the blood of 41-day-old broilers, and the lowest levels of lipid peroxidation were observed in chickens that received vitamin E and C supplements at the same time. This indicates the effectiveness of antioxidant prevention of oxidative stress in fast-growing broilers and the possible synergistic effect of the antioxidant effect of vitamins E and C under the action of stress factors. At the same time, questions regarding the influence of these vitamins on the fatty acid spectrum of lipids in broilers remain unclear. Therefore, the aim of our research was to determine the effect of individual and combined effects of vitamins E and C on the fatty acid composition of total lipids in liver and skeletal muscle in 41-day-old broiler chickens.

11.2 Materials and methods of research

Experimental studies were conducted in a farm in Zolochiv district, Lviv region, on broiler chickens of the Ross-308 cross from 1 to 41 days of age, kept on the floor on deep litter, with free access to feed and water. Technological parameters of broiler farming met all zootechnical requirements. The experiment was conducted on 4 groups of broiler chickens of 100 heads each. The control group was fed standard compound feed (SC) in accordance with the existing standards recommended for the ROSS-308 cross. The first experimental group of chickens, in addition to SC, received vitamin E 100 mg/1 kg of compound feed. The second experimental group received vitamin C 250 mg/1 kg of compound feed. The third experimental group received vitamin C 250 mg/1 kg and vitamin E 100 mg/1 kg of compound feed in addition to the diet. The composition and nutritional value of compound feed for broiler chickens is given in **Table 11.1**.

Chickens were vaccinated according to the preventive vaccination schedule on the farm: against infectious bronchitis at 11 days of age; against Newcastle disease at 13 days of age; against infectious bursal disease at 15 days of age. After slaughtering chickens at 41 days of age, liver and thigh muscle samples were taken for biochemical studies. Before slaughter, broilers were kept for 6 hours without feed and 3 hours without water. Liver and muscle samples were frozen and stored in liquid nitrogen, then ground into a powder, which was used for further studies. The fatty acid composition (FAC) of total lipids was determined in the liver and muscle according to DSTU ISO 5508-2001 "Animal and vegetable fats and oils". Sample preparation was carried out according to the method of DSTU ISO 5509-2002 "Animal and vegetable fats and oils". Chromatographic analysis of pre-methylated fatty acids was performed on a Trace Ultra gas chromatograph with a flame ionization detector, on a highly polar capillary column SP-2560 (Supelco). The method limit is 0.01%. Identification of fatty acids is carried out using the analytical mixture of fatty acids SupelcoTM 37 Compone FAME MIX, 100 mg Nea [22].

Table 11.1 Composition and nutritional value of compound feed

100 grams of compound feed contains, %				
Metabolic energy, Kcal	303.41	314.46	323.33	328.11
Crude protein	22.20	20.21	19.00	18.00
Crude fat	6.28	8.85	9.78	10.14
Crude fat extracted	5.55	8.08	9.02	9.39
Crude fiber	3.19	4.09	4.19	4.17
Crude ash	6.10	5.11	4.51	4.29
Moisture	11.44	9.75	9.98	10.18
Lysine	1.38	1.28	1.16	1.07
Methionine	0.63	0.60	0.54	0.50
Methionine + cystine	0.99	0.93	0.85	0.80
Threonine	0.88	0.84	0.77	0.72
Tryptophan	0.27	0.24	0.22	0.21
Isoleucine	0.94	0.85	0.79	0.74
Arginine	1.46	1.33	1.26	1.19
Valine	1.06	1.00	0.91	0.85
Chlorine	0.25	0.26	0.25	0.25
Potassium	0.98	1.07	0.99	0.93
Sodium	0.14	0.14	0.14	0.14

The obtained digital data were statistically processed using the computer program "Microsoft Excel". The degree of probability of comparative data was assessed by the Student's test (t). The difference was considered significant at ($p < 0.05$–0.001).

Studies on broiler chickens were carried out in compliance with the provisions of the Council of Europe Convention of (04.08.1997) and the resolution of the Cabinet of Ministers of Ukraine of 24.08.2002, No. 1256.

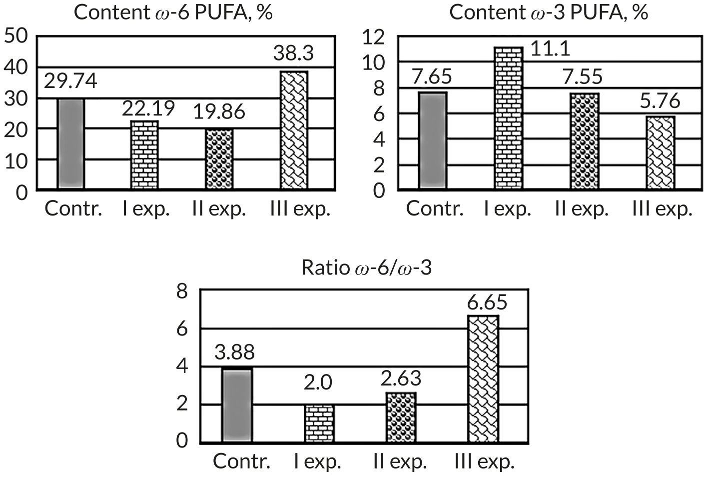
11.3 Results of the studies and their discussion

Comparative analysis of the fatty acids of liver lipids of 41-day-old broiler chickens of the control and I experimental groups shows that under the action of vitamin E supplements (**Table 11.2**) there was a redistribution of fatty acids in the direction of increasing the content of saturated fatty acids by 12.6%, mainly due to an increase in the content of palmitic acid (by 25.4%, $p \leq 0.01$) with a simultaneous decrease in the total PUFA content by 11.0%. At the same time, the MUFA content in chickens of the I experimental group remained at a stable level. Within PUFA, a decrease in the

ω-6 PUFA content by 25.4% was established, which occurred mainly due to a decrease in the content of essential linoleic acid (18:2, by 37.6%). At the same time, the content of longer-chain ω-6 arachidonic acid (20:4) increased by 33.5%. Under the action of vitamin E, against the background of a decrease in the ω-6 PUFA content, an increase in the ω-3 PUFA content by 45.1% was established, including linolenic (18:3) by 15.2% ($p \le 0.01$), docosapentaenoic (22:5) by 2.19 times, docosahexaenoic (22:6) by 33.6%. Such changes in FAC, caused by an increase in the content of vitamin E in the diet of chickens of the I experimental group, were accompanied by a significant decrease in the ratio of ω-6/ω-3 PUFA compared to this indicator in broilers of the control group (by 1.94 times), (**Fig. 11.1**), which is evidence of an increase in the FAC biological value of liver lipids under the action of vitamin E.

Table 11.2 Fatty acid composition of liver lipids of 41-day-old broiler chickens under the influence of vitamin E and C supplements to the diet, % ($M \pm m$; $n = 3$)

Fatty acid	Groups of broiler chickens			
	C	I E	II E	III E
C14:0	0.18 ± 0.006	$0.21 \pm 0.006^{*}$	$0.30 \pm 0.006^{***}$	0.19 ± 0.006
C16:0	17.18 ± 0.038	$21.5 \pm 0.121^{***}$	$20.80 \pm 0.344^{***}$	$19.78 \pm 0.288^{***}$
C16:1 ω-9	0.40 ± 0.008	0.42 ± 0.012	$0.31 \pm 0.006^{**}$	$0.31 \pm 0.006^{**}$
C17:0	0.047 ± 0.003	0.053 ± 0.003	0.047 ± 0.003	$0.016 \pm 0.003^{*8}$
C18:0	14.4 ± 0.130	14.20 ± 0.026	$19.51 \pm 0.155^{***}$	$9.35 \pm 0.112^{***}$
C18:1 ω-9	29.64 ± 0.061	29.77 ± 0.44	$30.77 \pm 0.12^{**}$	$25.22 \pm 0.049^{***}$
C18:2 ω-6	24.58 ± 0.035	$15.33 \pm 0.072^{***}$	$13.5 \pm 0.032^{***}$	$30.47 \pm 0.348^{***}$
C20:0	0.39 ± 0.012	$0.31 \pm 0.006^{**}$	$0.47 \pm 0.012^{**}$	$0.50 \pm 0.006^{**}$
C20:1 ω-9	0.39 ± 0.006	$0.43 \pm 0.007^{*}$	$0.48 \pm 0.006^{***}$	$0.57 \pm 0.009^{***}$
C18:3 ω-3	2.83 ± 0.021	$3.26 \pm 0.006^{***}$	$2.33 \pm 0.009^{***}$	$2.49 \pm 0.012^{***}$
C20:3 ω-6	1.20 ± 0.008	$1.96 \pm 0.012^{***}$	$1.92 \pm 0.015^{***}$	$2.81 \pm 0.017^{***}$
C20:4 ω-6	3.28 ± 0.035	$4.38 \pm 0.038^{***}$	$3.59 \pm 0.018^{**}$	$4.17 \pm 0.031^{***}$
C22:2 ω-6	0.68 ± 0.012	$0.52 \pm 0.012^{***}$	$0.85 \pm 0.015^{***}$	$0.85 \pm 0.0015^{***}$
C20:5 ω-3	0.77 ± 0.006	$1.69 \pm 0.036^{***}$	$1.14 \pm 0.009^{***}$	0.76 ± 0.009
C22:5 ω-3	0.90 ± 0.012	$1.94 \pm 0.111^{***}$	0.94 ± 0.012	$0.65 \pm 0.009^{***}$
C22:6 ω-3	3.15 ± 0.023	$4.21 \pm 0.012^{***}$	3.14 ± 0.018	$1.86 \pm 0.014^{***}$
SFA	32.2	36.27	41.12	29.83
MUFA	30.43	30.62	31.56	21.10
PUFA	37.39	33.29	27.41	44.06


Content ω-6 PUFA, %

Content ω-3 PUFA, %

Ratio ω-6/ω-3

Fig. 11.1 Changes in the content of ω-6 and ω-3 PUFAs and their ratio in the composition of total lipids in the liver of broiler chickens of the experimental groups compared to the control

Comparative analysis of the fatty acid composition of liver lipids of broilers of the control and II experimental groups shows that under the action of ascorbic acid supplements, the total level of saturated fatty acids increased by 27.7% with a simultaneous decrease in the total PUFA content by 26.7%, mainly due to ω-6 PUFA, the content of which in the liver of broilers of the II experimental group was 33.2% lower than the corresponding indicator in the control group. At the same time, the content of essential ω-6 linoleic (18:2) acid in the liver of broilers under the action of vitamin C decreased by 45.1%. Such a significant decrease in the ω-6 PUFA content against the background of a stable ω-3 PUFA level contributed to a decrease in the ratio of ω-6/ω-3 PUFA by 32.2% compared to the corresponding indicator in broilers of the control group.

A comparative assessment of the effect of vitamins E and C on the directions of the main changes in FAC in the liver of broilers of groups I and II shows that in both groups of broilers the increase in the antioxidant status of the tissues [23] occurred against the background of an increase in the content of saturated FA with a stable MUFA level. At the same time, the increase in the level of saturated fatty acids in chickens of groups I and II of the study occurred with a simultaneous increase ($p < 0.001$) in the content of palmitic (16:0), and in broilers of group II of the study also stearic (18:0) fatty acids. Regarding changes in the PUFA content, in both experimental groups (I and II) the decrease in ω-6 PUFAs was mainly due to a decrease in the content of linoleic ($p < 0.001$) acid, respectively by 37.6 and

45.1% against the background of a simultaneous increase in the content of longer-chain ω-6 arachidonic acid (by 33.5% ($p < 0.001$) and 9.5% ($p < 0.01$) respectively). At the same time, a significant increase in the content of all identified ω-3 PUFA was found in the liver of chickens of experimental group I, and in the liver of chickens of experimental group II the content of ω-3 acids generally remained at a stable level (linolenic acid even significantly decreased).

The main differences in the changes in the lipid composition of the liver of chickens of the I and II experimental groups are that under the influence of vitamin E supplementation, oppositely directed changes in the ω-6 PUFA content (decrease) and ω-3 PUFA (increase) occurred, which contributed to a decrease in the ratio of ω-6/ω-3 PUFA in the liver of broilers of the I experimental group to the lowest value in this experiment. In chickens of the II experimental group, the addition of vitamin C to the diet did not cause significant changes in the ω-3 PUFA content, but a decrease in the ω-6 PUFA content contributed to an improvement in the ratio of ω-6/ω-3 PUFA.

Thus, the conducted studies have shown that the addition of vitamin C to the diet of chickens has a lesser effect on the fatty acid profile in the liver, but its presence is necessary to maintain the pro-oxidant-antioxidant balance in the bird's body, especially under the influence of stress factors.

When studying the total lipid composition of liver tissues of chickens of III experimental group, which received a complex supplement of vitamins E and C, changes were recorded in a different direction than under the separate action of these vitamins. In particular, in the liver tissues of chickens of the 3rd experimental group, the level of saturated fatty acids and MUFA decreased by 7.4 and 30.7%, respectively, mainly due to a decrease in the content of stearic (18:0) and oleic (18:1) acids. At the same time, against the background of an increase in the total PUFA content (by 17.8%), an increase in the content of all ω-6 PUFA by 28.8% was established, including linoleic (18:2) by 24.0% ($p \leq 0.001$) and arachidonic (20:4) by 27.1% ($p \leq 0.001$). At the same time, in the liver of broilers of this experimental group, a decrease in the total ω-3 PUFA level by 24.7% was found due to a significant decrease in the content of linolenic (18:3), docosapentaenoic (22:5) and docosahexaenoic (22:6) fatty acids, which led to an increase in the ratio of ω-6/ω-3 PUFA by 71.4%.

The nutritional value of broiler chicken meat is largely determined by the fatty acid composition of total lipids, in particular the content and quality of PUFA. Analysis of the fatty acid composition of broiler chicken thigh muscles of the control group (**Table 11.3**) shows that 65.6% of the mass of all fatty acids are SFA and MUFA. Among PUFA (33.66%), only 0.93% is ω-3PUFA, and their main part is linoleic ω-6 acid. Accordingly, in terms of the ω-6/ω-3 PUFA ratio, the thigh muscles of chickens in the control group do not meet the recommendations of scientists and require correction of the FAC by additional enrichment of their lipid component with ω-3 PUFA (**Fig. 11.2**).

Increasing the level of vitamin E in the diet of broiler chickens of experimental group I caused a decrease in the relative level of SFA and MUFA, respectively by 17.5 and 8.2% and an increase in the relative PUFA content in the composition of thigh muscle lipids by 22.4%. The decrease in the SFA level in the composition of thigh muscle lipids of chickens of this group occurred mainly due to a decrease ($P < 0.001$) in the content of palmitic (16:0) and stearic (18:0) fatty acids, and MUFA – a decrease in the level of oleic (18:1) acid.

Table 11.3 Fatty acid composition of total lipids of thigh muscles of 41-day-old broiler chickens under the influence of dietary supplements of vitamins E and C, % ($M \pm m$; $n = 3$)

Fatty acid	Groups of broiler chickens			
	C	I E	II E	III E
(8:0)	0.07 ± 0.006	0.05 ± 0.006	0.07 ± 0.006	0.04 ± 0.003*
(10:0)	0.11 ± 0.006	0.37 ± 0.265	0.13 ± 0.006	0.10 ± 0.006
(12:0)	0.26 ± 0.005	0.23 ± 0.006*	0.21 ± 0.006**	0.2 ± 0.006**
(14:0)	0.63 ± 0.012	0.49 ± 0.0012**	0.58 ± 0.003*	0.52 ± 0.008**
(15:0)	0.2 ± 0.006	0.17 ± 0.006*	0.22 ± 0.006	0.19 ± 0.006
(16:0)	21.32 ± 0.187	17.13 ± 0.052***	23.16 ± 0.056***	18.54 ± 0.055***
(16:1)	2.69 ± 0.015	1.9 ± 0.012***	3.16 ± 0.032***	2.20 ± 0.012**
(17:0)	0.12 ± 0.003	0.1 ± 0.006	0.11 ± 0.003	0.1 ± 0.006
(18:0)	11.26 ± 0.08	9.48 ± 0.180***	10.35 ± 0.027***	8.36 ± 0.132***
(18:1)	28.3 ± 0.29	26.53 ± 0.14**	25.25 ± 0.10***	25.87 ± 0.02***
(18:2) ω-6	30.44 ± 0.30	38.00 ± 0.295***	32.14 ± 0.055**	38.33 ± 0.054***
(20:0)	0.11 ± 0.007	0.09 ± 0.006	1.17 ± 0.009**	0.13 ± 0.009
(18:3) ω-3	0.10 ± 0.008	2.18 ± 0.036***	0.19 ± 0.006**	1.43 ± 0.045***
(20:1)	0.3 ± 0.012	0.34 ± 0.006*	0.20 ± 0.003**	0.50 ± 0.015***
(20:3) ω-6	0.33 ± 0.009	0.31 ± 0.006	0.41 ± 0.006**	0.41 ± 0.006**
(20:4) ω-6	1.96 ± 0.054	1.54 ± 0.072**	2.57 ± 0.034***	1.54 ± 0.072**
(20:5) ω-3	0.16 ± 0.006	0.2 ± 0.006**	0.12 ± 0.006**	0.23 ± 0.009**
(24:1)	0.26 ± 0.006	0.20 ± 0.009**	0.30 ± 0.009*	0.26 ± 0.009
(22:5) ω-3	0.21 ± 0.009	0.30 ± 0.009***	0.26 ± 0.006*	0.34 ± 0.006***
(22:6) ω-3	0.46 ± 0.009	0.66 ± 0.009***	0.41 ± 0.006*	0.71 ± 0.006***
SFA	34.08	28.11	36.0	28.18
MUFA	31.55	28.97	28.91	28.83
PUFA	33.66	41.19	34.1	42.99

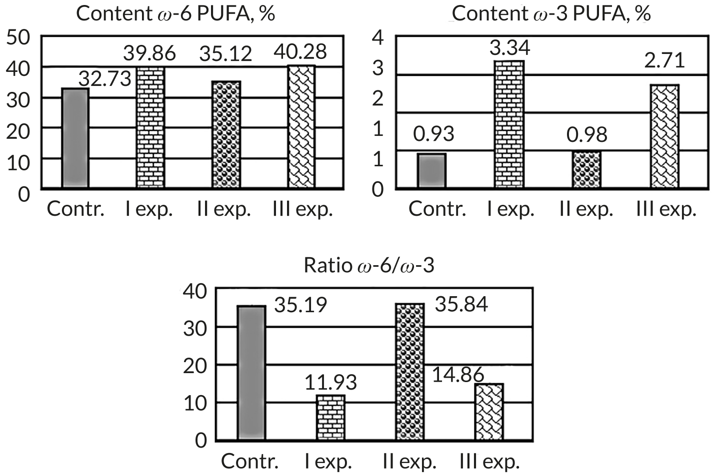


Fig. 11.2 Changes in the content of ω-6 and ω-3 PUFA and their ratio in the composition of total lipids in the thigh muscles of broiler chickens of the experimental groups compared to the control

Additional enrichment of the diet of chickens with vitamin E caused a significant increase in the PUFA level in the composition of thigh muscle lipids. Against the background of an increase in the total ω-6 PUFA content by 21.8%, including the content of linoleic acid by 24.8% ($p < 0.001$), the total ω-3 PUFA content increased by 3.5 times. At the same time, a higher ($p < 0.01$–0.001) content of linolenic (18:3), eicosapentaenoic (20:5), docosapentaenoic (22:5) and docosahexaenoic (22:6) fatty acids was recorded in the lipid composition of the thigh muscles of chickens of this group compared to the control group. Such a PUFA redistribution under the influence of vitamin E contributed to a significant reduction in ω-6/ω-3 PUFA and an approach of this indicator to the recommended level [24].

The increase in vitamin C in the diet of chickens of the experimental group II contributed to certain fluctuations in the content of individual acids, in particular, a probable increase in the content of linoleic (18:2) and arachidonic (20:4) fatty acids and a decrease in the level of oleic (18:1) acid in the composition of thigh muscle lipids. However, these changes did not significantly affect the total SFA content, MUFA and PUFA and the ratio of ω-6/ω-3 PUFA in the composition of muscles, which indicates a significant deficiency of ω-3 PUFA in these tissues.

With the combined addition of vitamins E and C to the diet of broiler chickens, the changes in the SFA content, MUFA and PUFA observed were similar to those in the experimental group I (application of vitamin E). In particular, in the composition of thigh muscle lipids of chickens of the experimental group III, a lower SFA content, MUFA and a higher PUFA content were recorded compared to the control.

The decrease in the relative level of SFA and MUFA in the composition of the muscles of chickens of this group was also similar to the experimental group I and occurred mainly with a simultaneous significant decrease in the content of palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids.

The PUFA content in the composition of the thigh muscles of chickens of the experimental group III was 9.3% higher than in the control group. The increase in the level of polyunsaturated fatty acids in the composition of lipids of the thigh muscles of chickens of this group was mainly due to an increase in the content of linoleic acid. Thus, the content of linoleic acid in the composition of lipids of the thigh muscles of chickens of the experimental group III was 7.9% ($p < 0.001$) higher than in the control group.

The increase in the level of vitamin C in the diet of chickens of the experimental group II contributed to certain fluctuations in the content of individual acids, in particular, a probable increase in the content of linoleic (18:2) and arachidonic (20:4) fatty acids and a decrease in the level of oleic (18:1) acid in the lipid composition of thigh muscles. However, these changes did not significantly affect the total content of SFA, MUFA and PUFA and the ratio of ω-6/ω-3 PUFA in the muscles of chickens of the experimental group II, which indicates a persistent deficiency of ω-3 PUFA in the muscles of chickens of this group. Therefore, taking into account previously published research results [25], the addition of vitamin C to the diet of broilers contributes to the inhibition of lipid peroxidation processes and an increase in the level of ω-6 PUFA. However, according to the ratio of ω-6/ω-3 PUFA in the muscles of chickens of the experimental group II, no increase in biological value was detected compared to the control, which proves the need for additional enrichment of the diet of this group of chickens with ω-3 PUFA.

With the combined addition of vitamins E and C to the diet of broiler chickens (experimental group III), changes in the SFA content, MUFA and PUFA observed in their thigh muscles were similar to those in experimental group I. The decrease in SFA, MUFA in the muscles of chickens of this group occurred mainly with a simultaneous significant decrease in the content of palmitic (16:0), stearic (18:0) and oleic (18:1) fatty acids. The PUFA content in the thigh muscles of chickens of experimental group III was 27.7% higher than in the control. At the same time, an increase in the ω-6 PUFA content in the muscles of chickens of this group by 23.1% was established (mainly due to the content of linoleic acid (by 25.9%, $p < 0.001$). At the same time, the ω-3 PUFA content in the muscles of chickens of the III experimental group exceeded the corresponding indicator of the control by 2.91 times. The content of all ω-3 PUFA (linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids) was higher ($p < 0.01–0.001$), than in the lipids of the thigh muscles of broilers of the control group. These data indicate a stimulating effect of vitamin E supplementation to the diet of chickens separately, as well as in combination with vitamin C, on the PUFA content in the lipids of the thigh muscles of chickens. The increase in the PUFA content, and especially linoleic and linolenic fatty acids in the lipids of the thigh muscles of chickens can be explained by the stimulating effect of tocopherol on the activity of enzyme systems involved in the synthesis of these fatty acids [26]. In addition, the positive side of the effect of vitamin E and C supplementation to the diet is the increase in the nutritional value of broiler chicken meat due to a decrease in the ratio of ω-6/ω-3 PUFA content relative to the control.

In general, the results of the conducted studies of the FAS of total lipids of liver tissues of 41-day-old broiler chickens indicate different changes in the content of individual fatty acids with separate and combined use of vitamin E and C supplements. The data on the PUFA content in the composition of total lipids of liver tissues of broiler chickens of the experimental groups deserve special attention. In particular, under the conditions of separate use of vitamin E and C supplements to the feed of broiler chickens, a significant decrease in the relative content of linoleic acid in the composition of total lipids of liver tissues was recorded. At the same time, the decrease in the content of linoleic acid in the liver of chickens of the experimental groups I and II during the specified period of research was accompanied by a simultaneous increase in the relative proportion of arachidonic acid. Linoleic acid is not synthesized in the poultry body, on the other hand, it is a precursor of arachidonic acid [4]. Thus, it is logical to assume that the decrease in the content of linoleic acid may also be associated with its use in the synthesis of longer-chain unsaturated arachidonic acid under the influence of the studied vitamin supplements. It was found that vitamin E increased the content of arachidonic acid (20:4 *n*-6) in the liver to a greater extent and simultaneously reduced the level of its precursor linoleic acid (18:2 *n*-6) – probably due to the activation of the enzymatic conversion of ω-6 PUFA to long-chain derivatives. On the other hand, in chickens of experimental group I, an increase in the ω-3 PUFA level (linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids) was observed, which is consistent with the results of studies [24], the authors of which noted that the addition of 200 mg/kg of vitamin E led to an increase in the ω-3 PUFA content and a decrease in the ω-6/ω-3 ratio in broiler meat. According to the researchers, tocopherol selectively protects the most unsaturated fatty acid molecules from oxidation, due to which more long-chain ω-3 PUFA (EPA, DHA) accumulate in the lipids of the muscle tissue of broilers and relatively fewer ω-6 derivatives, which compete with them for metabolic enzymes and inclusion in the phospholipids of cell membranes. At the same time, it is in the liver (as an organ with a high intensity of lipid metabolism) that the effects of vitamin E on the fatty acid composition are probably most pronounced. Vitamin C does not exert a significant effect on the fatty acid profile in the tissues, but its presence is necessary to maintain the antioxidant status and peroxide balance of the bird's body, especially under the influence of stress factors.

Thus, the results of the conducted studies indicate that feeding broiler chickens with supplements containing vitamins E and C during their intensive growth period contributes to an increase in lipid synthesis in their liver and the deposition of synthesized lipids in skeletal muscles. At the same time, these processes are accompanied by a redistribution of the ratio of individual lipid classes in the organs and tissues of chickens, which was shown in our previous works [20, 21].

The doses of vitamins used were determined based on the results of studies [22–25], which confirmed the dose-dependent effects of vitamin E and C supplements on growth performance, nutrient digestibility, and hematological parameters in broiler chickens.

The results of our studies are consistent with those of [26–28], which showed that increasing the vitamin E level (an additional 200 mg/kg) was accompanied by an increase in the ω-3 PUFA content and a decrease in the ω-6/ω-3 ratio in muscle. The effect of vitamin E supplementation on fatty acid dehydrogenase activity was also studied [29, 30]. However, most researchers believe that tissue FA is primarily determined by diet composition, and that maintenance of the fatty acid profile is ensured by antioxidant vitamins, primarily vitamin E, by reducing lipid peroxidation [31].

11.4 Conclusions

Significant differences in the fatty acid composition of total lipids of the liver and thigh muscles of 41-day-old broilers were established under the influence of separate and complex action of vitamins E and C. In the liver tissues of chickens under the individual influence of vitamins E and C, an increase in the SFA content was established with a simultaneous decrease in PUFA due to ω-6 PUFA. Under the joint action of vitamins E and C, oppositely directed changes in SFA and PUFA were established, which caused an increase in the ratio ω-6/ω-3 by 71.3% (up to 6.65). However, even at this level, this indicator remains within acceptable limits. In the muscle tissues of chickens receiving vitamin E (groups I and III), a more rapid increase in the ω-3 PUFA content compared to ω-6 was found against the background of a decrease in the SFA content and MUFA, which contributed both to providing these tissues with a set of necessary PUFAs and to increasing the biological value of meat by optimizing the ratio of ω-6/ω-3 PUFA.

Thus, the results of the studies suggest that the effect of vitamins E and C on FA has a certain tissue specificity. While the directions of FA changes in muscle tissue in groups I and II coincide, the different directions of changes in FAC in liver tissue are determined by the intensity of metabolic processes in this organ. Further work by scientists and producers should be aimed at developing diets for poultry and pets rich in omega-3 PUFA, vitamins and phytonutrients, but low in omega-6 PUFA, which will ultimately improve the health and well-being of consumers [28]. In addition, healthy nutrition requires ensuring the required composition of polyunsaturated fatty acids (PUFA), vitamins and microelements in meat throughout the entire processing and storage process – from farm to table. Omega-3 PUFA-rich diets for pets offer numerous economic, environmental, and social benefits for meat consumers.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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The study was performed without financial support.

Data availability

The data that support the findings of this study will be made available by the authors on reasonable request.

Use of artificial intelligence statement

The authors confirm that they did not use artificial intelligence technologies when creating this work

Authors' contributions

Ludmila Romanovich: Conceptualization, Literature review, Conducting experimental studies, Data analysis, Writing – original version.

Bohdan Kurtyak: Conceptualization, Methodology, Organization of the study, Formal analysis, Writing – review and editing.

Olena Danchenko: Theoretical framework, Literature review, Interpretation of results, Visualization, Writing – review and editing.

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