

FATTY ACIDS PROFILE IN THE EGG YOLKS AND LIVER OF EMBRYOS AT DIFFERENT LEVELS OF CAROTENOIDS IN THE DIET OF CHICKENS

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ABSTRACT

It is established that there is a certain correlation between the level of carotenoids in the ration of breeding hens during the reproductive period and the content of unsaturated fatty acids in lipids of egg yolks. The increased content of carotenoids in the diet of chickens during the reproductive period from 8 to 32 mg per kg of fodder contributed to the reduction of content of saturated fatty acids in egg yolks and increased of the content of unsaturated fatty acids in comparison with the control group. It was found increased content of polyunsaturated fatty acids: linoleic (C18:2 ω -6), docosapentaenoic (C22:5 ω -3), docosahexaenoic (C22:6 ω -3), eicosapentaenoic (C20:5 ω -3) and eicosatrienoic (C20:3 ω -9). The inverse relationship between the content of unsaturated fatty acids in the lipid composition of egg yolks and the intensity index of lipid metabolism in the liver of 19-day-embryos was observed.

Key words: hens, embryos, liver, carotenoids, fatty acids, egg yolk

INTRODUCTION

Carotenoids and xanthophylls in particular play an important role in the processes of fertilization of birds' eggs [Fisinin et al. 1990, Tarique et al. 2013, Surai et al. 2016, Zaheer 2017]. They positively affect absorption of proteins and lipids by embryos, contribute to the development of embryonic membranes, increase hatching and survival of chicks. Therefore, the content of carotenoids along with vitamin A in the yolk is considered as a test that determines the quality of hatching eggs [Kurtyak and Yanovych 2004].

It should be noted that the lipid metabolism during embryonic development of fowl is characterized by certain features. In particular, the chicken's embryo, which is contained in the yolk of a fertilized egg within 21 days, develops in closed environment [Fisinin et al. 1990, Surai et al. 2016]. During the embryonic development complex physiological and biochemical processes between the embryo, yolk, protein and shell occur in the egg. At

the initial stages of development embryo assimilates substances of yolk, including lipids, and uses them as plastic material and energy source [Powell et al. 2004].

Almost all lipids, fat-soluble vitamins, over half of proteins and a significant part of the water-soluble vitamins of egg are located in the yolk [Kurtyak and Yanovych 2004, Yadgary et al. 2013]. The effect of yolk on chicken's embryo is revealed mainly after full usage of protein by embryo that is at the end of embryogenesis, from the 16–17th day and continues during early postnatal period when the chick uses nutrients from the vestigial yolk [Speake et al. 1998].

The bulk of the dry egg yolk substances is made up of the lipids (more than 60%) which are the main energy source for embryos. However, it should be mentioned that liver plays an important role in the lipid metabolism of fowl's embryos as lengthening and shortening of the carbon chains of fatty acids, their dehydrogenation, β -oxidation, synthesis and breakdown of lipids occur there [Kononsky 2004, Kurtyak and Yanovych 2004].

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Therefore, scientific and practical interest is in the research of lipid changes and in particular fatty acid composition of embryo egg yolks and embryos' liver depending on the level of carotenoid supplementation in the hens of parent herd.

MATERIAL AND METHODS

The experiment was conducted in 4 groups of analogue 220 days Shaver-579 hens on the base of breeding poultry farm Chortkiv, Ternopil region. Hens were kept in cages with free access to fodder and water. Main parameters of indoor climate were as following: 17°C air temperature, 65% relative humidity, 17 hours per day lighting with the intensity of 17 lux. In each group there were 10 hens and 1 cock. Hens of the first (control) group received standard mixed fodder, balanced according to the nutrition norms [Ryabokon 2005] without supplements of carotenoids in the ration. Concentrated feed for hens contained in %: corn – 15; wheat – 34.6; barley – 25; sunflower meal – 7; yeast feed – 4; grass meal – 6; meat-bone meal – 5; chalk – 2; salt – 0, 4; premix – 1. In 100 g concentrated feed contained: metabolizable energy – 1132 KDJ and in %: protein – 16.3; fat – 2.2; fiber – 4.2; Ca – 3.0; P – 0.75, Na – 0.30; lysine – 0.78; methionine – 0.30; cystine – 0.27. Hens of the second experimental group received 8 g of carotenoids additionally, of the third one – 16 g, and of the fourth one – 32 g of carotenoids per 1 tonne of mixed fodder, that stands accordingly for 0.92 mg, 1.84 mg, 3.68 mg for one unit of fowl.

Carotenoids preparation “ORO GLO 20 DRY” of “Kemin Europa NV” (Belgium) was used in our experimental as a fodder additive. The content of the xanthophylls (lutein and zeaxanthin) in the “ORO GLO” was 20 g per kg. The experiment lasted for 90 days. The objects of the study were hatching eggs yolks, liver tissues of embryos at 19 days of growth. The homogenate was prepared from liver tissues (1:10) on the basis of saline. Fatty acid composition of egg yolk lipids and liver's embryos was determined by gas-liquid chromatography on chromatograph Chrom-4 under the following conditions: column (240 cm × 3 mm) filled with chromosorb 60–80 mesh, covered with 15% polyethylene glycol succinate, temperature of evaporator 240°C, column thermostat – 180°C [Rivis and Fedoruk 2010]. Statistical analysis of the data was performed using Statistica software (StatSoft Inc., version 10.0). The significance of differences was tested using one-way ANOVA and Tukey's multiple range test.

RESULTS AND DISCUSSION

It is known that egg is formed in the reproductive organs of hens as a germ cell that is provided with all necessary

nutrients for the development of the embryo. That is why full development of the embryo depends on the content of essential nutrients in the egg yolk [Yadgary et al. 2013]. The data given in Table 1 show that the total content of saturated fatty acids in egg yolks lipids which were received from hens of research groups was reduced and the content of unsaturated fatty acids was increased in comparison with the hens in control group.

At the same time the changes of the content of saturated fatty acids in egg yolk lipids occur mainly at the expense of stearic (C18:0), palmitic (C16:0) and arachidonic (C20:0) fatty acids and the changes in the content of polyunsaturated fatty acids at the cost of linoleic (C18:2), docosapentaenoic (C22:5), docosahexaenoic (C22:6), eicosapentaenoic (C20:5) and eicosatrienoic (C20:3) fatty acids.

The content of saturated fatty acids in the lipid composition of egg yolks in the 2nd, 3rd and 4th research groups was lower by 2.4, 2.8 and 2.9% respectively compared with the control group and the content of unsaturated fatty acids was higher by 1.3, 1.5 and 1.6% correspondingly.

Fatty acid composition of lipids in the liver of 19-day-embryos undergoes certain quantitative changes due to the influence of Carotenoid supplementation to the ration of hens in reproductive period (Table 2). At the same time the amount of saturated fatty acids in the composition of liver lipids in embryos of the research groups is characterized by insignificant tendency to their reduction in comparison with the control group.

Thus, the amount of saturated fatty acids in the composition of liver lipids in embryos of the 2nd, 3rd and 4th research groups was lower than in the control group by 2, 9 and 12% respectively. In particular, the reduction of saturated fatty acids occur mostly at the expense of stearic (C18:0) and palmitic (C16:0) fatty acids as their amount was significantly reduced.

The amount of unsaturated fatty acids in the composition of liver embryo lipids was higher in the research groups compared with the control group. Thus, the amount of unsaturated fatty acids in the composition of liver lipids in embryos of the 2nd, 3rd and 4th research groups of hens was higher than in the control group by 0.7, 3 and 4% respectively. Changes in the amount of unsaturated fatty acids in the composition of liver lipids in embryos occur mainly due to linoleic (C18:2), arachidonic (C20:4), docosahexaenoic (C22:6) and oleic fatty acids (C18:1).

The increased amount of the unsaturated fatty acids in liver lipid composition may be associated with the impact of carotenoids on activation of desaturase enzymes in hepatocytes. There is evidence [Syed et al. 1984] that points changes in activity Δ^9 - and Δ^6 - desaturase in the liver under the influence of β -carotene.

Table 1. The content of fatty acids code in the egg yolks, % (mean \pm SD, n = 4)

Tabela 1. Zawartość kwasów tłuszczowych w żółtku jaj, % (średnia \pm SD, n = 4)

Fatty acid code Kwas tłuszczowy, kod	Groups – Grupy			
	1	2	3	4
12:0	0.18 \pm 0.02	0.18 \pm 0.03	0.16 \pm 0.02	0.17 \pm 0.03
14:0	0.46 \pm 0.03	0.45 \pm 0.02	0.44 \pm 0.03	0.42 \pm 0.09
16:0	27.38 \pm 0.70	27.17 \pm 0.70	23.67 \pm 0.50**	22.81 \pm 0.80**
16:1 ω -7	0.48 \pm 0.21	0.51 \pm 0.17	0.63 \pm 0.12	0.87 \pm 0.10
18:0	9.83 \pm 0.53	9.72 \pm 0.41	9.23 \pm 0.32	8.96 \pm 0.35
18:1 ω -9	40.56 \pm 1.02	40.64 \pm 0.83	42.94 \pm 0.7	43.12 \pm 1.12
18:2 ω -6	13.28 \pm 0.93	13.44 \pm 0.86	14.61 \pm 0.82	15.21 \pm 0.71
18:3 ω -3	1.06 \pm 0.02	1.07 \pm 0.03	1.12 \pm 0.01*	1.13 \pm 0.02*
20:0	0.32 \pm 0.03	0.28 \pm 0.02	0.25 \pm 0.02	0.23 \pm 0.01*
20:1 ω -9	0.48 \pm 0.02	0.51 \pm 0.03	0.61 \pm 0.02**	0.62 \pm 0.03**
20:2 ω -9	0.23 \pm 0.02	0.24 \pm 0.04	0.24 \pm 0.02	0.25 \pm 0.03
20:3 ω -9	0.37 \pm 0.04	0.39 \pm 0.02	0.41 \pm 0.05	0.42 \pm 0.02
20:4 ω -6	1.91 \pm 0.08	1.93 \pm 0.08	2.04 \pm 0.05	2.03 \pm 0.13
20:5 ω -3	0.33 \pm 0.03	0.35 \pm 0.02	0.38 \pm 0.05	0.39 \pm 0.02
22:5 ω -3	0.45 \pm 0.01	0.47 \pm 0.03	0.51 \pm 0.02*	0.63 \pm 0.02***
22:6 ω -3	2.12 \pm 0.15	2.14 \pm 0.13	2.28 \pm 0.18	2.31 \pm 0.12
24:1 ω -9	0.56 \pm 0.02	0.51 \pm 0.03	0.48 \pm 0.02*	0.43 \pm 0.05
Saturated – Nasycone	38.17	37.80	33.75	32.59
Unsaturated – Nienasycone	61.83	62.20	66.25	67.41
Monounsaturated – Jednonienasycone	42.08	42.17	44.66	45.04
Polyunsaturated – Wielonienasycone	19.75	20.03	21.59	22.37

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$.

*Współczynniki istotne przy $P \leq 0,05$; **współczynniki istotne przy $P \leq 0,01$; ***współczynniki istotne przy $P \leq 0,001$.

It is considered [Arkhipov 1980] that the correlation of the content of palmitic acid to oleic acid (16:0/18:1) in fowl liver lipids characterizes the intensity index of lipid metabolism. According to the obtained data, the index of intensity of lipid metabolism in the liver of 19-day-embryos in the research groups is lower than in the control group.

In the tissues of animals and fowl oleic acid (C18:1) does not undergo desaturation and further metabolism provided there is sufficient amount of linoleic (C18:2) and linolenic (C18:3) fatty acids [Holman 1971]. Perhaps that is why in research groups of hens there were higher amounts of C18:1 in the composition of embryonic liver lipids and a relatively high level of C18:2 and C18:3.

The reverse correlation dependence between the amount of unsaturated fatty acids in the lipid composition of egg yolks and index of intensity of lipid metabolism (0.9) in the liver of 19-day-embryos was defined.

CONCLUSIONS

In general, the following conclusions come out from the received results: there is a certain connection between the level of carotenoids in the diet of breeding hens during the reproductive period and the content of fatty acids in lipids of egg yolks. In particular the increase of carotenoids in the fodder of chickens during the reproductive period from 8 to 32 mg per kg of fodder during the intense oviposition contributed to the reduction of total amount of saturated fatty acids in egg yolks and increased the amount of unsaturated fatty acids. Changes in the content of polyunsaturated fatty acids in the composition of lipids of egg yolks occur mainly at the expense of linoleic (C18:2), docosapentaenoic (C22:5), docosahexaenoic (C22:6), eicosapentaenoic (C20:5) and eicosatrienoic (C20:3) fatty acids. The reverse dependence is established between the content of unsaturated fatty acids in the lipid composition of egg yolks and the intensity index of lipid metabolism in the liver of 19-day-

Table 2. The content of fatty acids code in the liver of embryos at 19 days of growth, % (mean \pm SD, n = 4)

Tabela 2. Zawartość kodu kwasów tłuszczowych w wątrobie 19-dniowych zarodków, % (średnia \pm SD, n = 4)

Fatty acid code Kwas tłuszczowy, kod	Groups – Grupy			
	1	2	3	4
12:0	0.14 \pm 0.03	0.12 \pm 0.01	0.11 \pm 0.02	0.15 \pm 0.02
14:0	0.28 \pm 0.01	0.25 \pm 0.02	0.23 \pm 0.03	0.23 \pm 0.03
16:0	11.84 \pm 0.12	11.6 \pm 0.18	11.25 \pm 0.16*	10.39 \pm 0.11***
16:1 ω -7	0.73 \pm 0.03	0.71 \pm 0.05	0.65 \pm 0.02	0.64 \pm 0.02*
18:0	12.89 \pm 0.15	12.66 \pm 0.17	11.31 \pm 0.18***	11.23 \pm 0.23***
18:1 ω -9	48.74 \pm 0.21	48.94 \pm 0.32	50.2 \pm 0.26**	50.67 \pm 0.18***
18:2 ω -6	15.21 \pm 0.32	15.57 \pm 0.43	16.21 \pm 0.22*	16.4 \pm 0.25*
18:3 ω -3	1.31 \pm 0.08	1.32 \pm 0.07	1.35 \pm 0.05	1.36 \pm 0.04
20:0	0.1 \pm 0.01	0.11 \pm 0.02	0.12 \pm 0.01	0.11 \pm 0.02
20:1 ω -9	0.45 \pm 0.07	0.46 \pm 0.05	0.41 \pm 0.03	0.49 \pm 0.05
20:2 ω -9	0.22 \pm 0.02	0.23 \pm 0.03	0.23 \pm 0.02	0.24 \pm 0.01
20:3 ω -9	1.54 \pm 0.03	1.56 \pm 0.05	1.56 \pm 0.09	1.57 \pm 0.02
20:4 ω -6	2.28 \pm 0.04	2.21 \pm 0.02	2.1 \pm 0.05*	2.13 \pm 0.04*
20:5 ω -3	0.58 \pm 0.03	0.57 \pm 0.02	0.51 \pm 0.02	0.51 \pm 0.03
22:5 ω -3	0.68 \pm 0.01	0.65 \pm 0.03	0.61 \pm 0.02*	0.62 \pm 0.02*
22:6 ω -3	2.08 \pm 0.05	2.1 \pm 0.03	2.2 \pm 0.03	2.31 \pm 0.06*
24:1 ω -9	0.93 \pm 0.03	0.94 \pm 0.04	0.95 \pm 0.03	0.95 \pm 0.02
16:0/ 18:1	0.24	0.24	0.22	0.21
Saturated – Nasycone	25.25	24.74	23.02	22.11
Unsaturated – Nienasycone	74.75	75.26	76.98	77.89
Monounsaturated – Jednonienasycone	50.85	51.05	52.21	52.75
Polyunsaturated – Wielonienasycone	23.90	24.21	24.77	25.14

*Significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$.

* Współczynniki istotne przy $P \leq 0.05$; **współczynniki istotne przy $P \leq 0.01$; ***współczynniki istotne przy $P \leq 0.001$.

embryos. Changes in the content of unsaturated fatty acids in the composition of liver lipids in embryos occur mainly due to linoleic (C18:2), arachidonic (C20:4), docosahexaenoic (C22:6) and oleic (C18:1) fatty acids.

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PROFIL KWASÓW TŁUSZCZOWYCH W ŻÓŁTKACH JAJ I WĄTROBIE ZARODKÓW PRZY RÓŻNYCH POZIOMACH KAROTENOIDÓW W DIECIE KUR

STRESZCZENIE

Stwierdzono, że istnieje związek pomiędzy poziomem karotenoidów w dawce u kur niosek w okresie reprodukcyjnym a zawartością nienasyconych kwasów tłuszczowych w żółtkach jaj i wątrobie zarodków kurzych. Zwiększenie zawartości karotenoidów w diecie kur w okresie rozrodczym z 8 do 32 mg na kg paszy przyczyniło się do zmniejszenia zawartości nasyconych kwasów tłuszczowych i zwiększenia zawartości nienasyconych kwasów tłuszczowych w żółtkach jaj w porównaniu z grupą kontrolną. Zaobserwowano zwiększoną zawartość wielonienasyconych kwasów tłuszczowych: linolowego (C18:2 ω -6), docetapentaenowy (C22:5 ω -3), docaheksaenowy (C22:6 ω -3), eikozapentaenowy (C20:5 ω -3) i eikozatrieniczny (C20:3 ω -9). Zaobserwowano odwrotną zależność pomiędzy zawartością nienasyconych kwasów tłuszczowych w żółtkach jaj, a indeksem intensywności metabolizmu lipidów w wątrobie 19-dniowych zarodków.

Słowa kluczowe: kury, zarodki, wątroba, karotenoidy, kwasy tłuszczowe, żółtko

