THE EFFECT OF BIOMASS FROM GREEN ALGAE OF CHLORELLA GENUS ON THE BIOCHEMICAL CHARACTERISTICS OF TABLE EGGS

ВЛИЯНИЕ НА БИОМАСА ОТ ЗЕЛЕНИ ВОДОРАСЛИ ОТ РОД CHLORELLA ВЪРХУ БИОХИМИЧНИТЕ КАЧЕСТВА НА ЯЙЦАТА ЗА КОНСУМАЦИЯ

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ABSTRACT

An analysis was made of the fatty-acid content of the dry biomass from green algae of Chlorella genus cultivated in Bulgaria, with the aim of establishing its effect on the content of total lipids, cholesterol, phospholipids and the fatty-acid content of the table eggs.

The fatty-acid composition of the dry biomass from green microalgae of Chlorella genus was characterized by its high content of α linolenic acid – 36,5 %, palmitic acid – 20,4 %, linoleic acid – 15 % and oleic acid – 10,3 % of the total amount of fatty acids in the product. Omega-3/Omega-6 fatty acids ratio in the biomass was 0,4.

When adding 2 % and 10 % of alga biomass to the forage for the laying hens the total cholesterol content in 100 g of yolk decreased in the experimental groups compared to the control one, however, the differences were statistically insignificant.

The supplement of 2 % and 10 % of the studied product exerted an effect on the fatty-acid content of the egg yolk and it led to the increase of the amount of palmitic and linoleic acids and to the decrease of the docosatetraenic acid.

Key words: alga biomass, Chlorella, fatty acids, cholesterol, egg quality.

DETAILED ABSTRACT

Направен е анализ на мастнокиселинния състав на суха биомаса от зелени водорасли от род Chlorella, култивирани у нас, с цел установяване влиянието й върху съдържанието на общи липиди, холестерол, фосфолипиди и мастнокиселинния състав на яйцата за консумация.

Проведени бяха два опита с кокошки-носачки от хибридната комбинация Bovans Braun съответно на 26 седмична възраст и на 74 седмична възраст след период на принудително линеене, като по време на първия опит птиците от опитната група получаваха 2% добавка биомаса зелени водорасли,а по време на втория – 10% от изпитвания продукт. В края на I и II опит беше определено съдържанието на общи липиди, фосфолипиди, холестерол и мастнокиселинен състав на яйцата.

Съдържанието на холестерол беше определено по модифицирания от Sperry и Webb метод на Schoenheimer – Sperry (1950). Съдържанието на фосфолипиди беше определено по метода на Bligh и Dyer(1959). Мастнокиселинния състав беше определен с помощта на газхроматограф Pwe 304 след естерифициране на общите липиди.

Мастнокиселинният състав на сухата биомаса от зелени микроводорасли от род Chlorella се характеризира с високо съдържание на линоленова киселина – 36,5%, палмитинова – 20,4%, линолова – 15% и олеинова – 10,3% от общото количество на мастните киселини в продукта. Съотношението Омега 3/

Омега 6 мастни киселини на биомасата е 0,4.

При добавка на 2% и 10% биомаса от водорасли във фуража на кокошки-носачки, съдържанието на общ холестерол в 100g жълтък намалява в опитните групи в сравнение с контролната, но разликите са статистически недостоверни.

Включването на 2% и 10% от изпитвания продукт оказва влияние върху мастнокиселинния състав на яйчния жълтък и води до увеличаване съдържанието на палмитинова и линолова киселина, и до намаляване на докозатетраеновата киселина.

Ключови думи: биомаса от водорасли, Chlorella, мастни киселини, холестерол, качество на яйцата

INTRODUCTION

When feeding the animals, fats provide the animal organism with the essential fatty acids. Those are fatty acids with two and more double bonds, which could not be synthesized in the animal organism, but they are absolutely necessary for its functioning, that is why they should be provided with the food. The forages contain linoleic and linolenic acids, which can be transformed into other unsaturated fatty acids in the animal organism, including ones having 4, 5 and 6 double bonds. Depending on the position of the first double bond towards the methyl group, the unsaturated fatty acids are divided into Omega-3 and Omega-6 fatty acids. When there is a deficiency of Omega-3 fatty acids in the human organism people are inclined to diseases of the cardio-vascular system such as atherosclerosis, high blood pressure, heart attack, infarct, etc.

A number of authors confirmed experimentally that it was possible to model the fatty-acid composition of the animal products, especially eggs, by preparing the diet in a way as it could be of high nutritive value and at the same time – healthy sound for the humans [2, 3, 4].

Herring oil contains 9,1 % docosahexaenoic acid (DHA) of the total content of fatty acids, eicosapentaenoic acid (EPA) – 13,5 %, linoleic acid – 1,1 %, all of them belonging to the group of Omega-3 fatty acids and the sea alga Schizochytrium sp. contains 7,44 DHA [7].

By adding 1,27 % and 1,77 % of dry biomass from the sea alga Schizochytrium sp. to the combined forage for laying hens given to the birds for 28 days, the eggs produced contained DHA in the yolk, amounting to 134, 196 and bjbjon of total lipids, cholesterol, phospholipids and the fatty-acid content of the table eggs.

MATERIAL AND METHODS

The content of the major nutrient substances and the fatty-acid content of lipids in the dry biomass of microalgae were analyzed in Lareal Laboratory of Evialis company, France. The methods applied were in compliance with the European Directives: 03 – 030, NF EN I SD 5509, NF EN ISO 5508. For detecting the fatty-acid content capillary gas chromatography was used after preliminary esterification.

An experiment was carried out with 131 laying hens, 26 weeks old, of the hybrid combination Bovans Braun, raised in three-storey battery cages. The birds were divided into two groups and the experiment went on for 37 days, two weeks being the preparatory period and 23 days – the experimental one. The birds in the control group were fed on standard combined forage for laying hens containing 17,7 % of crude protein, 0,88 % of lysine, 0,67 % of methionine plus cystine, 3,85 % of calcium, 0,44 % of absorbable phosphorus and 2680 kcal/kg of exchangeable energy (Table 1), without alga biomass supplemented. Those in the experimental group received the same forage plus 2 % of dry biomass from

Table 1 INGREDIENTS AND CHEMICAL COMPOSITION OF THE COMBINED FORAGE FOR HENS. I EXPERIMENT

INGREDIENTS, %	**GROUP** **I – Control**	**II - Experimental**
Maize	20,00	20,00
Wheat	36,90	37,40
Fish meal	2,00	2,00
Soybean meal	17,00	14,50
Sunflower meal	12,00	12,00
Alga Biomass	-	2,00
Sunflower Oil	1,50	1,50
Limestone	8,20	8,20
Dicalcium Phosphate	1,50	1,50
Salt	0,30	0,30
Vitamin Premix	0,25	0,25
Lysine HCL	0,20	0,20
DL - Methionine	0,15	0,15
TOTAL:	100	100
	CONTENT, %	
Crude Protein	17,7	17,8
Lysine	0,88	0,90
Methionine + Cystine	0,67	0,67
Calcium	3,85	3,85
Phosphorus, available	0,44	0,44
Metabolisable Energy, kcal/kg	2680	2680

green microalgae added to their food.

A second experiment was conducted with 24 laying hens, 74 weeks old, of the same hybrid combination, after forced molting, divided into two groups, 12 hens in each, raised in three-storey battery cages. The study went through a ten-day preparatory period and 37-day experimental one. The birds in the control group received the same combined forage as those in the first experiment and the hens in the experimental group – 90 % of the control mixture plus 10 % of dry biomass from algae. In both experiments the feeding rate was fixed to 120 g/hen daily.

At the end of the first and at the end of the second period the amounts of total lipids, total phospholipids, total cholesterol and the fatty-acid content of the table eggs were determined: in the first experiment – as a mixed sample, 3 eggs in a group, and, in the second experiment – individually for each egg laid in the group on the last day of the experiment.

The cholesterol content was determined by the method of Schoenheimer – Sperry (1950) modified by Sperry and Webb [8]. The content of phospholipids was determined by the method of Bligh and Dyer (1959), [1].

The fatty-acid content was detected by Pye 304 gas chromatographer with capillary columns /30 m x 0,32 mm, 0,25 thick/ and a flame-ionizing detector, after esterification of the total lipids.

For statistical evaluation of the results Anova is used.

RESULTS AND DISCUSSION

When analyzing the dry biomass from algae of Chlorella genus, the following results were obtained: 5,8 % of wet, 55 % of protein, 9,6 % of fats, 6,4 % of fibres, 8,7 % of mineral substances and 0,60 g/kg of xanthophylls.

Table 2 presents the fatty-acid content of the green microalga biomass. The highest percentage of the total amount of fatty acids was α linolenic acid – 36,5 %, which is one of the essential fatty acids for the animals. The following animal foods are rich in that fatty acid: lucerne – 37,7 %, soybean oil – 6,5 %, maize silage – 8,0 %, [9]. The content of α linolenic fatty acid in the rapeseed is 10,1 %, in the green leaves – 56 %, in the flaxseed – 45-60 %, in sea fish – 1 % and in freshwater fish – 1-6 %, [2]. The other essential fatty acid, the linoleic one, was 15 % of all the fatty acids in the product. In sunflower oil it amounts to 64,3 % , in soybean oil – 52 %, in maize – 56,3 %, in pig fat – 9,5 % of the total content of fatty acids [9]. The green alga biomass contains also oleic acid – 10,3 %, and the unsaturated palmitic acid – 20,4 %.

The fatty acid balance showed 24 % for the unsaturated fatty acids, 14,4 % for the monounsaturated ones and

Table 2 FATTY-ACID CONTENT OF DRY BIOMASS FROM *CHLORELLA* (IN PERCENTAGE OF THE TOTAL AMOUNT OF FATTY ACIDS)

FATTY ACIDS		%
Myristinic	$C_{14:0}$	0,4
Palmitic	$C_{16:0}$	20,4
Palmitooleic	$C_{16:1}$	3,9
Hexadecatrienic	$C_{16:3}$	2,7
Stearic	$C_{18:0}$	0,7
Oleic	$C_{18:1}$	10,3
Linoleic	$C_{18:2\ n\text{-}6}$	15,0
γ Linolenic	$C_{18:3\ n\text{-}6}$	0,8
α Linolenic	$C_{18:3\ n\text{-}3}$	36,5
Steradonic	$C_{18:4\ n\text{-}3}$	5,8
Arachidic	$C_{20:0}$	0,1
Docosadienoic	$C_{22:2\ n\text{-}3}$	0,3
Balance		
Omega – 3 fatty acids		42,3
Omega – 6 fatty acids		16,1
Ω-6/ Ω-3 ratio		0,4
Unsaturated fatty acids		24,0
Monounsaturated fatty acids		14,4
Polyunsaturated fatty acids		61,6

Table 3 CONTENT OF TOTAL LIPIDS, PHOSPHOLIPIDS AND CHOLESTEROL IN EGGS

Indices	**First Experiment**		**Second Experiment**	
	Groups		**Groups**	
	Control	**Experimental**	**Control**	**Experimental**
In 100 g of yolk,g				
Total lipids	**39,64**	**38,04**	**26,29 ± 2,42**	**26,81 ± 2,23**
Total phospholipids	**10,28**	**10,18**	**7,38 ± 0,73**	**7,88 ± 0,33**
Total cholesterol	**1,52**	**1,47**	**1,27 ± 0,023**	**1,14 ± 0,087**
In an egg, g				
Total lipids	**7,14**	**6,85**	**4,73 ± 0,44**	**4,83 ± 0,40**
Total phospholipids	**1,84**	**1,83**	**1,33 ± 0,13**	**1,42 ± 0,058**
Total cholesterol	**0,27**	**0,26**	**0,23 ± 0,006**	**0,21 ± 0,012**

Table 4 FATTY-ACID CONTENT OF THE TOTAL LIPIDS IN THE YOLK OF HEN EGGS (IN WEIGHT PERCENTAGE)

Fatty Acids	First Experiment with 2% of algae Group		Second Experiment with 10 % of algae Group	
	Control	Experimental	Control	Experimental
Myristinic $C_{14:0}$	0,43	0,39	$0,33^a \pm 0,018$	$0.44^a \pm 0,046$
Pentadecanoic $C_{15:0}$	0,06	0,07	$0,08 \pm 0,0057$	$0,08 \pm 0,0049$
Palmitic $C_{16:0}$	27,38	26,41	$24,56^a \pm 1,11$	$27,91^a \pm 2,53$
Margarine $C_{17:0}$	0,17	0,18	$0,22 \pm 0,011$	$0,22 \pm 0,059$
Stearic $C_{18:0}$	7,58	7,57	$7,62^a \pm 0,166$	$7,00^a \pm 0,239$
Oleic $C_{18:1}$	41,04	41,62	$43,47 \pm 0,53$	$41,30 \pm 1,74$
Linoleic $C_{18:2\ n\text{-}6}$	14,72	14,72	$16,13 \pm 0,613$	$15,18 \pm 0,422$
Linolenic $C_{18:3\ n\text{-}3}$	0,46	0,53	$0,36 \pm 0,022$	$0,47 \pm 0,091$
Arachidic $C_{20:4\ n\text{-}6}$	1,65	1,81	$1,67 \pm 0,060$	$1,52 \pm 0,102$
Docosatetraenoic $C_{22:4\ n\text{-}6}$	0,41	0,40	$0,43\ ^b \pm 0,033$	$0,26^b \pm 0,40$
Docosapentaenoic $C_{22:5\ n\text{-}3}$	0,09	0,09	$0,07 \pm 0,0088$	$0,08 \pm 0,0103$
Docosahexaenoic $C_{22:6\ n\text{-}3}$	0,50	0,59	$0,46 \pm 0,026$	$0,45 \pm 0,035$

a– $p \leq 0,05$
b – $p \leq 0,01$

61,6 % for the polyunsaturated ones. The balance of Omega-3 and Omega-6 fatty acids – 42,3 % and 16,1 %, respectively, is also important. The studies in the last years showed that Omega-3 to Omega-6 fatty acid ratio was of great importance for the human health. That ratio also had an effect on the health and reproductive capacities of the animals. In the dry biomass of green algae that ratio was 0,4.

The results of the analyses about the content of total lipids, cholesterol and phospholipids in the egg yolk of the birds of the control and of the experimental group for both experiments are presented in Table 3.

Data from both experiments showed the same tendencies and there were no significant differences between the control and the experimental groups concerning the content of total lipids, total phospholipids and total cholesterol in the egg yolk. When supplementing 2 % of dry biomass from green algae, as well as when adding 10 % of the product to the forage mixture, the total cholesterol content in 100 g of yolk decreased by 3,3 % in the first experiment and by 10,24 % in the second experiment, in the groups fed on algae, compared to the control group, however, the differences were statistically insignificant. Such conclusions were also made by other authors [7], who considered that the cholesterol in the egg yolk changed very slightly or in many cases it did not change at all under the influence of genetic, pharmacological or nutritive factors. Ginsberg et al. [4] established 11 % and 28 % lower levels of the serum cholesterol in laying hens fed on forage supplemented with 5 and 10 % of red alga biomass in comparison with the control group, but they reported only about a tendency towards reduction of the cholesterol in the egg yolk.

Table 4 presents the fatty acid content of the total lipids in the yolk of eggs laid by hens receiving 2 % and 10 % of dry biomass from green microalgae added to the combined forages (first and second experiment). Similar results were obtained about the linolenic acid, which was contained in the highest percentage in the tested product.

When using the lower alga rate, that essential fatty acid increased in the egg yolk from 0,46 to 0,53 % and when applying the higher rate – from 0,36 to 0,47 %. In the first experiment the palmitic fatty acid, which was also well represented in the product (20,4 %), did nor change significantly, while when adding 10 % of alga biomass, it increased from 24,93 % to 27,91 % (the difference being significant at $p \leq 0,05$). Linolic acid content in the egg in both experiments remained unaffected. However, it was not the same concerning Omega-6 fatty acids, docosatetraenoic acid in particular, which did not change when using 2 % of alga biomass (0,41 – 0,40%), but at 10 % of the tested product it decreased significantly, from 0,43 % to 0,26 % (the difference being statistically proven at $p \leq 0{,}01$). Similar changes in the fatty acid profile of the egg yolk after adding sea algae in the forage for laying hens were also established by other authors [6, 11]. In the experiments carried out by them, the increase of the level of Omega-3 fatty acids in the forage led to the decrease of Omega-6 fatty acids in the yolk. It was suggested that Omega-3 fatty acids were preferred for combining with the biological membranes at the exhaustion of Omega-6 fatty acids [5].

Including green alga biomass in the dietary rate of laying hens did not affect the content of pentadecanic, margarine, stearic, linolic and docosapentaenic fatty acids.

The results of that study confirmed the results established by other authors stating that the combined forage content for feeding laying hens could hardly influence the cholesterol content in the egg yolk but it could model the fatty acid content of the eggs.

CONCLUSION

The fatty acid content of the dry biomass from green microalgae of Chlorella genus is characterized by high content of α-linolenic acid – 36,5 %, palmitic acid – 20,4 %, linoleic acid – 15,0 % and oleic acid – 10,3 % of the total amount of fatty acids in the product. Omega-3 fatty acids comprise 42,3 % and Omega-6 ones – 16,1 %. The ratio between them is 0,4 %.

When adding 2 and 10 % of alga dry biomass in the dietary rate of laying hens the content of the total cholesterol in 100 g of yolk decreased in the experimental groups compared to the control but the differences were statistically insignificant.

Adding 2 and 10 % of the tested product to the forage mixture exerted an effect on the fatty acid content of the egg yolk and led to the increase of the content of palmitic and linolenic acids and the decrease of the docosatetraenoic fatty acid (the differences between the control and the experimental group being statistically insignificant when using 2% of the dry biomass).

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