

DRY BIOMASS OF FRESH WATER ALGAE OF CHLORELLA GENUS IN THE COMBINED FORAGES FOR LAYING HENS

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ABSTRACT

Dry biomass of algae is a good source of nutrients and biologically active substances, which in the last years attracted the interest of the specialists in their search for natural, ecologically and healthy sound foods for the animals. The aim of the present study was to characterize the chemical composition and the nutritive value of the dry biomass of fresh water algae of *Chlorella* genus cultivated in Bulgaria and to establish its effect on the laying hen productivity and the morphological characteristics of the table eggs. The tested product was analyzed for its crude protein content – 55 % to available wet, crude fats – 9,6 %, crude fibres – 6,4 %, xanthophylls – 0,6 g/kg, essential amino acids: lysine – 5,5 %, methionine – 1,2 %, triptophan – 1,2 %.

Adding 2 % and 10 % of dry biomass of fresh water algae of *Chlorella* genus to the combined forages for laying hens led to the improvement of the bird productivity and the morphological characteristics of the eggs and the egg yolk pigmentation was more intensive by 2,5 units by the Roche's scale.

KEY WORDS: alga biomass, green algae, *Chlorella*, hen feeding, egg qualities.

DETAILED ABSTRACT

Сухата биомаса от водорасли е добър източник на хранителни и биологичноактивни вещества, което през последните години засили интересът на специалистите към нея, продиктуван от търсенето на натурални, екологично чисти и здравословни храни за животните. Целта на настоящата изследователска работа е да се направи характеристика на химичния състав и хранителната стойност на сухата биомаса от сладководни водорасли от род CHLORELLA култивирани у нас и да се установи влиянието ѝ върху продуктивността на кокошките-носачки и морфологичните характеристики на яйцата за консумация. На изпитвания продукт беше направен анализ за съдържание на суров протеин – 55% към налична влага, сурови мазнини – 9,6%, сурови влакнини – 6,4% ксантофили – 0,6 g/kg, незаменими аминокиселини: лизин – 5,5%, метионин – 1,2%, триптофан – 1,2%. Проведени бяха два опита с кокошки-носачки от хибридна комбинация Bovans Braun съответно на 26 седмична възраст и на 74 седмична възраст след период на принудително линееене. По време на опитите бяха контролирани ежедневно носливостта на птиците и периодично: яйчно тегло, индекс на формата, тегло и дебелина на яйчната черупка, цвят на жълтъка. Първият опит беше проведен със 131 броя кокошки разделени в две групи, като тези от опитната група получаваха фуражна смеска с добавка на 2% от изпитвания продукт. Разликата между контролната и опитна група по отношение на носливостта е статистически достоверна ($P \leq 0,01$) в полза на опитната група. Статистически недостоверни са получените разлики по отношение на морфологичните характеристики на яйцата.

Вторият опит бе проведен с 24 птици разделени в две групи по 12, като птиците от опитната група получаваха 90% комбиниран фураж + 10% биомаса от сладководни водорасли от р. CHLORELLA. Бе установена тенденция за по-висока носливост и достоверност на разликите между контролната и опитна групи по отношение на яйчното тегло ($P \leq 0,05$), индекса на формата и цвят на жълтъка ($P \leq 0,001$) в полза на опитната група.

Включването на 2 и 10% суха биомаса от сладководни водорасли от род Chlorella в комбинираните фуражи за кокошки носачки води до подобряване продуктивността на птиците и морфологичните характеристики на яйцата, а пигментацията на яйчния жълтък е по-интензивна с 2,5 единици по скалата на Рош.

КЛЮЧОВИ ДУМИ: биомаса, зелени водорасли, Chlorella, кокошки, качества на яйцата.

INTRODUCTION

Dry biomass of algae is a good source of nutrients and biologically active substances for the animals. Thanks to the modern equipment and the analytical methods of research, in the last years the specialists' interest in it increased, dictated by their search for natural, ecologically and healthy sound foods for the animals, which could have a positive effect on the quality of the animal produce.

Studies have been carried out with biomass of different alga species, obtained by the respective biotechnological methods. Those two factors were the reason for the diversity in the chemical composition of the biomass, its nutritive value and the biochemical effect of its application.

Sanches et al. (1995), [7] determined the content of the major nutrient substances in the biomass of *Elodea olensa* algae. The authors established the presence of 14,66 % of protein; 14,68 % of fibers; 1,60 % Ca; 0,19 % P; 14 MJ/kg metabolizable energy and the maximal participation of *Elodea densa* in the combined forages for laying hens – 15 %. The biomass of red algae of *Porphidium* sp. was of a similar chemical content: protein – 15 %, sugars – 65 %, lipids – 10 % and ashes – 10 %. When adding 5 % and 10 % of the product to the forage for laying hens, the authors did not established any significant difference between the experimental and the control groups concerning the productivity indices. Only the yolk color in the group fed on 5 % of algae was 2,4 times darker compared to the control [4]. When adding 1,27 % and 1,77 % of biomass of sea algae of *Schizochytrium* sp. to the combined forages for laying hens, no differences were established between the groups in the laying capacity - 95,3 %, 94,6 % and 94,5 %; egg weight - 60,4; 59,9 and 59,9 g and forage consumption rate - 115, 114 and 114 g/hen daily in the experimental and the control birds, respectively [3].

In many countries there is a great interest in the blue-green algae *Spirulina platensis*. Their unique biochemical composition is the reason for using them as biologically active additive to the forages for different animal species and categories. They contain up to 70 % of protein, which is represented by all the essential amino acids and it is very rich in triptophan, threonine, isoleucine and valine. By content of lysine the blue-green algae are surpassed only by the leguminous and the absorption of their protein is 85-90 % [1].

In Bulgaria there is a production of dry biomass of fresh

water microalgae representatives of *Chlorella* genus, cultivated in the area of Rupite, between the towns of Sandanski and Petrich. That is one of the few places in the world having a favorable climate. The location has the biggest number of sunny days for our country. It is ecologically clean, with mineral waters rich in carbon dioxide, which enables the cultivation of top quality microalgae. After their filtration they are dried following a special technology that guarantees the preservation of the biologically active substances.

The aim of the present research was to characterize the chemical composition and the nutritive value of the dry biomass of fresh water algae of *Chlorella* genus and to establish its effect on the productivity of laying hens and on the morphological characteristics of the table eggs.

MATERIAL AND METHODS

An experiment was carried out with 131 laying hens of the hybrid combination Bovans Braun, 26 weeks old, under the conditions of battery cage breeding, in a three-storied battery cage and controlled microclimate and light regime in the premises. The birds were divided into two groups – the first one control and the second one – experimental. The layers of the control group were fed on combined forage without alga biomass supplement, and, those of the experimental group – with 2 % of the tested product added to the forage. The forage mixtures for both groups contained equal amounts of the major nutrient substances (Table 1). The experiment went on for 38 days in total, 15 days – a preparatory period and 23 days – an experimental period.

The following indices were controlled during the study: forage consumption, laying capacity, egg weight and morphological characteristics of the eggs such as shape index, eggshell thickness and yolk color. An analysis of the tested product was made about the content of dry matter, crude protein, crude fibers, crude fats, crude ash and essential amino acids, pigments.

In another experiment 24 laying hens of the same hybrid combinations, 74 weeks old, after a period of forced molting, were divided into two groups, 12 birds in each. The individuals from the control group were given the standard industrial mixture for laying hens and those from the second group – 90 % of the mixture plus 10 % of biomass of fresh water algae of *Chlorella* genus. The aim of the second experiment was to study the effect of the higher biomass rate on the morphological characteristics of eggs. The experiment went on for 5 weeks. For statistical evaluation of the results ANOVA is used.

RESULTS AND DISCUSSION

Table 2 presents the results of the chemical analysis of the alga biomass. They show high content of crude protein – 55 % to available wet. Concerning that index the product surpasses even the best soybean groats (maximum 50 %) and it gets close to the protein sources of animal origin – meat-and-bone and fish powder. Taking into consideration the prohibition of using whole body carcass meal due to the danger of “mad cow” disease and the limited use of fish meal as a protein source in the combined forages, the dry alga biomass is a promising alternative. The crude fibre content was within the acceptable limits in the young poultry diet and it was not a problem for the adults. The crude fats amounted to 9,6 %. However, their fatty acid content was of interest. But those studies and the effect of crude fats on the cholesterol in the egg yolk and the fatty acid content of eggs obtained by laying hens fed on combined forages with alga biomass supplement, were an object of study of another publication (Grigorova et al., 2005 – unpublished).

The next Table 3 presents the data about the essential amino acid content. Lysine was within 5,5 % at 2,75 % in the soybean meal and 5,26 % in fish meal containing 70 % of protein. Methionine amounted to 1,2 % at 0,63 % in the soybean meal and 1,73 % in the fish meal. By its triptophan content the product also surpassed the soybean meal and the fish meal – 1,2 %; 0,59 % and 0,72 %, respectively. Along with stimulating the protein synthesis, the amino acids, which the alga biomass is rich in, played an important role for poultry productivity, especially their laying capacity and the egg weight (lysine, methionine), for stimulating the immunity system (lysine, arginine).

The green alga biomass was also a source of pigments. The biggest amount was the chlorophyll, which according to the data by the producer, at a minimum requirement of 1,5 % was 1,7 %. In the batch used for conducting the experiments the established content of xanthophylls was 0,6 g/kg.

Data about the laying capacity of hens in the preparatory period when all of them were fed on the same forage mixture, showed good equality of the birds concerning the productivity index – 90,7 % and 91,1 % laying capacity of the control and the experimental groups, respectively (Table 4). At the end of the experimental period the values reached 89,9 % and 93,0 %, respectively. For the whole study period the laying capacity of the birds in control group changed from 90,7 % to 89,9 %, i.e. 0,8 units less, while in the experimental group the change was from 91,1 % to 93 %, i.e. 1,9 percentage units more. The difference was statistically significant ($P \leq 0,01$). Concerning egg weight, the index of egg shape and eggshell thickness (Table 4) the differences were insignificant ($p > 0,05$). The

Table 1 CONTENT AND CHEMICAL COMPOSITION OF THE COMBINED FORAGE FOR LAYING HENS. IEXPERIMENT

COMPONENTS, %	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
Metabolizable Energy, kcal/kg	2680	2680

Table 2 CHEMICAL COMPOSITION OF THE DRY BIOMASS FROM FRESH WATER ALGAE OF *CHLORELLA* GENUS (IN % TO THE AVAILABLE MOISTURE)

INDICES	%
Moisture	5,8
Crude Protein	55,0
Crude Fats	9,6
Crude Fibers	6,4
Crude Ash	8,7
Xanthophylls, g/kg	0,6

Table 3 ESSENTIAL AMINO ACIDS IN THE DRY BIOMASS FROM FRESH WATER ALGAE OF *CHLORELLA* GENUS

Essential Amino Acids	g/100 g of Biomass	g/100 g of Protein
Lysine	5,5	10,0
Methionine	1,2	2,2
Tryptophan	1,2	2,2
Threonine	5,0	9,1
Leucine	8,9	16,2
Isoleucine	3,8	6,9
Valine	6,7	12,2
Phenylalanine	4,3	7,8

Table 4 PRODUCTIVITY AND MORPHOLOGICAL CHARACTERISTICS OF THE EGGS. FIRST EXPERIMENT

INDICES	GROUP	
	I Control	II Experimental
Hens, number	70	61
Duration of the Experiment, days	26	26
Laying capacity at the beginning of the experiment, %	90,7±1,03	91,1±1,48
Laying capacity for the whole period, %	89,9±0,65a	93,0±0,72a
% to the first group	100	103,4
Egg weight, g	61,3±0,49	60,5±0,46
Shape index, units	77,0±0,26	77,2±0,26
Eggshell thickness, mm	0,362±0,102	0,347±0,108
Yolk color, units by the Roche's scale	6,0±0,32	6,5±0,34

^a P≤0,01

Table 5 PRODUCTIVITY AND MORPHOLOGICAL CHARACTERISTICS OF THE EGGS. SECOND EXPERIMENT

INDICES	GROUP	
	I Control	II Experimental
Hens, number	12	12
Duration of the Experiment, days	37	37
Laying capacity at the beginning of the experiment, %	83,5±1,92	84,2±2,04
Laying capacity for the whole period, %	81,5±2,12	85,5±1,80
% to the first group	100	105,1
Egg weight, g	65,9±0,52a	67,5±0,53a
Eggshell weight, g	6,35±0,208	6,75±0,223
Eggshell weight in % of the egg weight	9,68±0,78	9,02±1,39
Shape index, units	76,5±0,24 _B	75,2±0,27 _B
Eggshell thickness, mm	0,376±0,0052	0,382±0,108
Yolk color, units by Roche's scale	3±0,16 _B	5,5±0,31 _B

a- P≤0,05

b - P≤0,001

results of the study show more intensive yolk pigments of the eggs of the hens from the experimental group – 6,5 units on Roche 's scale against 6 units for the control group.

During the second experiment a tendency towards higher laying capacity was established at the presence of 10 % of biomass of Chlorella genus (Table5). The differences between the control and the experimental groups were significant concerning egg weight (P ≤0,05), the egg shape index and yolk color (P≤0,001) in favour of the experimental group. The results obtained were in agreement with the experiments published by other authors [2,3,5,6].

The analysis of the results obtained from the conducted studies gave the reason for the high evaluation of the quality of the dry biomass obtained in Bulgaria from

green algae of Chlorella genus, following modern biotechnological methods. It is a valuable source of protein with very good content of amino acids and a carrier of natural pigments. The product replaced successfully a part of the plant protein in the combined forages for laying hens, as a result of which the bird productivity and the morphological characteristics of the eggs were improved. The intensity of yolk color increased, meeting the requirements of the consumers.

CONCLUSIONS

The biomass produced in our country from green algae of Chlorella genus contained 55 % of crude protein to available wet, 9,6 % of fats, 6,4 % of fibers and 0,6 g/kg of xanthophylls.

It was also rich in essential amino acids – lysine – 5,5 %,

methionine 1,2 %, triptophan 1,2 %.

When adding 2 % and 10 % of dry biomass from fresh water algae of *Chlorella* genus in the combined forages for laying hens, the laying capacity and the morphological characteristics of the eggs improved and the yolk pigmentation became more intensive by 2,5 units by the Roche's scale.

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