Influence of the biologically active additive All G-Rich on the fatty acid composition of meat in lambs from Midle Rodopian and Karakachan aboriginal breeds

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Citation: Todorov, P. (2022). Influence of the biologically active additive All G-Rich on the fatty acid composition of meat in lambs from Midle Rodopian and Karakachan aboriginal breeds. *Zhivotnovadni Nauki*, *59*(1), 59-66 (Bg).

Abstract

The purpose of this study was to determine the effect of the biologically active supplement All-G Rich on the fatty acid composition of *m. Semimembranosus* by male and female lambs from the Middle Rhodopian and Karakachan aboriginal breeds.

The object of the study are 24 lambs of Karakachan and Middle Rhodopian breed, rearing in the sheep farm of Research Center of Stockbreeding and Agriculture – Smolyan in the period April–June 2020. The lambs were divided into 4 groups – 2 control and 2 experimental. The control group was fed concentrated fodder. The experimental group was fed with concentrated fodder and 1% All G-Rich supplement. All G-Rich is a source of protein, fat and energy. Improves the quality of meat due to the high content of docosahexaenoic acid (DHA).

The total content of saturated and monounsaturated fatty acids is higher in the control group of *m. Semimembranosus* in Karakachan than in the Middle Rhodopian lambs, as follows 49.33 per 48.07 g/100 g fat and 46.67 per 44.34 g/100g fat.

The use of the All-G Rich supplement in the diet of lambs on two breeds leads to an inverse correlation in the results between the control and experimental group of Karakachan and Middle Rhodopian lambs for the total content of saturated, mono- and polyunsaturated fatty acids.

The ratio between omega-6 and omega-3 fatty acids is higher in the control compared to the experimental group lambs of the both breeds, but decreases twice when using the feed supplement All-G Rich.

Key words: Omega-3, omega-6, *m. Semimembranosus,* supplement, male, female

Влияние на биологично активната добавка All G-Rich върху мастно-киселинния състав на месото при агнета от аборигенните породи Среднородопска и Каракачанска

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Резюме

Целта на проучването е да се установи влиянието на биоактивната добавка All G-Rich върху мастно-киселинния състав на *m. Semimembranosus* на мъжки и женски агнета от Среднородопска и Каракачанска породи.

Обект на изследването са 24 броя агнета от Каракачанска и Среднородопска порода, отглеждани в овцевъдната ферма на НЦЖЗ – Смолян в периода април–юни 2020 година. Агнетата бяха разпределени в 4 групи – 2 контролни и 2 опитни. Контролната група е хранена с концентриран фураж. Опитната група е изхранвана с концентриран фураж и 1% добавка на All G-Rich. Биоактивната добавка All G-Rich е източник на протеин, мазнини и енергия. Подобрява качеството на месото поради високото съдържание на докозахексаенова киселина (DHA).

Общото съдържание на наситените и мононенаситените мастни киселини е по-високо при контролната група от *м. Semimembranosus* при Каракачанските в сравнение със Среднородопските агнета, както следва 49,33 на 48,07 g/100 g мазнина и 46,67 на 44,34 g/100 g мазнина.

Влагането на добавката All G-Rich в храненето на агнета от две породи води до обратна корелация при резултатите между контролната и опитната група Каракачански и Среднородопски агнета за общото съдържание на наситени, моно- и полиненаситени мастни киселини.

Съотношението между омега-6 и омега-3 мастните киселини е по-високо при контролните в сравнение с опитните групи агнета и от двете породи, но намалява двукратно при влагането на хранителната добавка All-G Rich

Ключови думи: омега-3, омега-6, *m. Semimembranosus,* добавка, мъжки, женски

Introduction

The in-depth research and analysis of productive characteristics and quality indicators of meat obtained from lambs by aboriginal breeds will give us important guidelines for working with them. It is necessary to develop methods for obtaining high and quality productivity in local breeds of lambs and sheep.

Inclusion of long-chain DHA and EPA n-3 FA contained in *Schizochytrium sp.* at animal nutrition increase the presence of both fatty acids in fat depots and skeletal muscle. This directly reflects the low levels of biohydrogenation of these fatty acids in the rumen of sheep and is therefore considered a response to increased dietary levels of DHA in the diet, similar to that observed by Boeckaert et al. (2007). The lack of biohydrogenation of DHA and EPA was found by Maia et al. (2007) with the inclusion of dietary products such as fish oil and microalgae, which may have a toxic effect on certain species of bacteria in the rumen by ruminants, in particular the *Butyrivibrio* group, which are responsible for the biohydrogenation of fatty acids. They suggest that the presence of a double bond in unsaturated fatty acids may prolong the time of biohydrogenation, therefore reducing the metabolism of these fatty acids in the rumen and accumulating in ruminant tissues.

Lamb meat is characterized by a higher fat and SFA content and a lower MUFA content than beef. The quality of local production obtained from small ruminants in terms of fatty acid composition can be improved by supplementation and dietary changes (Zervas and Tsiplakou, 2011, Popova et al., 2015).

There is a growing interest from consumers in to the so-called “functional foods” on recent years, which contain in addition to high quality protein, fat and carbohydrates and biologically active substances that have a positively affect various physiological processes and help detoxify the body (Abadzhieva and Kistanova, 2011).

Microalgae are an alternative source in animal nutrition to naturally improve the ratio of ω-6 to ω-3 fatty acids in meat and dairy products. They have a well-balanced chemical composition and a high content of polyunsaturated fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Microalgae were characterized by rapid accumulation of biomass during photosynthesis (Adarme-Vega et al., 2012). All the characteristics of microalgae provoke a number of studies related to their application in feed, nutrition and biofuel production (Chew et al., 2017).

Microalgae were used to improve the nutritional value of feed (Christaki et al., 2010) as a source of essential polyunsaturated fatty acids that were not synthesized by animals.

The use of small amounts of microalgae biomass in feed can be beneficial for animal physiology, improve the immune response, resistance to disease, antiviral and antibacterial action, intestinal function and to stimulate probiotic colonization (Souza et al., 2021). Valença et al. (2021) in their study found that seaweed flour of the genus *Schizochytrium* can be used in sheep feed as a source of protein and energy. It affects the chemical composition of meat mainly due to its higher DHA content and regular consumption of ω-3 fatty acid rich meat can reduce the risk of cardiovascular disease (Coates et al., 2009), but it is not widespread available (Dugan et al., 2015).

The purpose of the present study was to determine the effect of adding 1% of the biologically active additive All G-Rich to the concentrated feed on the fatty acid composition of *m. Semimembranosus* by male and female lambs from the Middle Rhodopian and Karakachan aboriginal breeds.

Material and methods

The object of the study are 24 lambs of the Karakachan and Middle Rhodopian breed rearing in purebred herds in the Middle and Western Rhodopes. The study was conducted in April–June 2020 at the Research Center of Stockbreeding and Agriculture – Smolyan. The lambs were matched by the method of analogues (sex, breed, age, live weight and body development with an initial average live weight by 12 ± 0.2 kg. The lambs were divided into 4 groups – 2 control and 2 experimental with six animals. Control group was fed with concentrated fodder. The experimental group was fed with concentrated fodder and the addition of 1% All G-Rich. Experiment was started with an average live weight for the Middle Rhodopian breed by 12.950 kg and an average live weight for the Karakachan breed by 12.883 kg. Lambs from 4 groups were rearing indoor at groups in boxes. They were fed ad libitum (+5 to 10% residue) with a concentrate mixture and alfalfa hay in a pre-age ration that met their nutrient and biologically active needs with constant access to fresh and clean water.

The experimental period lasted until reaching 23 kg average live weight. At the end of the experiment, a slaughter analysis of 3 lambs from each group were performed. Prior to slaughter, the lambs were deprived of food for 24 hours. The animals were slaughtered in a licensed slaughterhouse in the town of Smolyan.

The analysis of fatty acids in lamb meat was performed by fat extraction by the method of Bligh & Dyer (1951). The methyl esters were analysed on a Pye Unicam 304 gas chromatograph equipped with a flame ionization detector (FID) and an ECTM WAX capillary column (30 m x 0.25 mm, I. d.; 0.25 μm film) with hydrogen carrier gas (H_2).

The statistical data processing was performed using the statistics package of the Exel 2016 program.

Results and discussions

Karakachan breed

The fatty acid composition of *Semimembranosus muscle* obtained from Karakachan lambs is presented in Table 1. The use of the All-G Rich supplement in fattening lambs leads to a slight increase in lauric, myristic, oleic and docosapentaenoic acid, capric and docosahexaenoic acid were significant increase ($P \leq 0.05$). The remaining fatty acids decrease in the experimental lambs from Karakachan breed. Biologically active fatty acids do not undergo significant changes. Oleic acid decreases from 41.73 to 40.67 g/100 g fat, while linoleic and linolenic increase, respectively from 4.10 to 5.23 g/100 g fat and from 0.94 to 1.06 g/100 g fat in the control compared to the experimental group.

The total content of saturated and monounsaturated fatty acids decreased slightly, while polyunsaturated fatty acids increased significantly ($P \leq 0.05$) in the experimental compared to the control group lambs of Karakachan breed. The ratio of omega-6 to omega-3 fatty acids decreased significantly ($P \leq 0.05$) in the *Semimembranosus muscle* at the experimental group (Table 2).

Studies with lambs for fattening in rations enriched with *Schizochytrium sp.* of DHA-Gold extract led to improved meat quality by increasing the content of omega-3 fatty acids and in particular EPA and DHA, and reducing the ratio of omega-6 / omega-3 fatty acids (Hopkins

Table 1. Fatty acid composition of *m. Semimembranosus* in Karakachan lambs, g/100 g fat

Fatty acids	Control group, n-3				Experimental group, n-3				Sign.
	x̄	± Sx	SD	C	x̄	± Sx	SD	C	
C-10:0	0.38	0.02	0.04	9.93	0.51	0.02	0.05	9.75	*
C-12:0	0.28	0.04	0.09	31.86	0.21	0.05	0.12	56.14	
C-14:0	3.40	0.03	0.07	2.17	2.64	0.34	0.83	31.25	
C-14:1	0.32	0.00	0.01	3.74	0.29	0.09	0.23	77.18	
C-15:0	0.68	0.01	0.03	4.58	0.64	0.07	0.16	25.28	
C-16:0	25.94	0.96	2.36	9.10	23.64	0.35	0.87	3.68	
C-16:1	1.98	0.15	0.36	18.40	1.37	0.31	0.76	55.92	
C-17:0	0.96	0.01	0.03	3.18	1.87	0.31	0.77	40.90	
C-17:1	1.18	0.01	0.03	2.17	1.21	0.04	0.10	8.61	
C-18.0	17.32	0.66	1.61	9.29	17.91	1.12	2.74	15.31	
C-18:1	41.73	0.03	0.08	0.19	40.67	1.62	3.97	9.77	
C-18:2	4.10	0.11	0.28	6.84	5.23	0.25	0.61	11.73	
C-18:3	0.94	0.04	0.09	9.55	1.06	0.03	0.07	6.56	
C-20:0	0.36	0.06	0.15	41.71	1.04	0.17	0.41	39.89	
C-22:5n3	0.14	0.01	0.02	13.46	0.34	0.07	0.16	48.50	
C-22:6n3	0.05	0.01	0.01	28.32	0.27	0.03	0.06	23.26	*

** – $P \leq 0.05$, ** – $P \leq 0.01$, *** – $P \leq 0.001$*

Table 2. Total fatty acid content in *m. Semimembranosus* in Karakachan lambs, g/100 g fat

Fatty acids	Control group, n-3				Experimental group, n-3				Sign.
	x̄	± Sx	SD	C	x̄	± Sx	SD	C	
SFA	49.33	0.16	0.40	0.82	48.46	1.67	4.10	8.46	
MUFA	46.67	0.27	0.66	1.41	43.54	1.46	3.57	8.21	
PUFA	5.23	0.09	0.22	4.25	6.90	0.25	0.61	8.78	*
Σ n-3	0.19	0.01	0.03	17.20	0.61	0.09	0.22	36.02	
Σ n-6	5.04	0.08	0.19	3.76	6.30	0.24	0.59	9.44	*
Σ n-6/Σ n-3	27.35	1.50	3.69	13.48	11.39	1.73	4.25	37.28	*

** – $P \leq 0.05$, ** – $P \leq 0.01$, *** – $P \leq 0.001$*

et al., 2014; Meale et al., 2014; Ponnampalam et al., 2016; Díaz et al., 2017). The application of high doses *Schizochytrium sp.* causes a negative impact, which worsens the quality of meat expressed in changes in its colour (Urrutia et al., 2016) and its oxidative stability (Urrutia et al., 2016; Ponnampalam et al., 2016; Díaz et al., 2017).

Middle Rhodopian breed

The fatty acid composition of *Semimembranosus muscle* obtained from lambs of the Middle Rhodopian breed is presented in Table 3. The All-G Rich nutritional supplement in lamb fattening leads to a slight decrease in capric, lauric, palmitoleic, linoleic and linolenic fatty acids. Oleic, docosapentaenoic and docosahexaenoic acid increase slightly.

The total content of saturated and monounsaturated fatty acids in the studied *Semimembranosus muscle* in lambs from the experimental group of the Middle Rhodopian breed is higher than in the control group, at the expense of polyunsaturated fatty acids.

The ratio between omega-6 and omega-3 fatty acids decreased twice in the experimental group of lambs of the Middle Rhodope breed (Table 4).

Table 3. Fatty acid composition of *m. Semimembranosus* in lambs from Middle Rhodopian breed, g/100 g fat

Fatty acids	Control group, n-3				Experimental group, n-3			
	$\bar{x}$	± Sx	SD	C	$\bar{x}$	± Sx	SD	C
C-10:0	0.41	0.03	0.08	19.26	0.46	0.01	0.03	6.30
C-12:0	0.19	0.04	0.11	57.94	0.29	0.05	0.12	41.88
C-14:0	2.15	0.14	0.33	15.42	3.23	0.31	0.75	23.18
C-14:1	0.33	0.10	0.25	78.18	0.30	0.01	0.03	9.38
C-15:0	0.61	0.06	0.15	25.21	0.63	0.03	0.07	11.76
C-16:0	24.05	0.30	0.72	3.01	23.79	0.47	1.16	4.88
C-16:1	1.81	0.27	0.66	36.62	1.61	0.32	0.79	49.45
C-17:0	0.86	0.05	0.13	15.61	1.77	0.30	0.74	41.57
C-17:1	1.18	0.08	0.18	15.61	1.23	0.06	0.15	12.35
C-18.0	18.48	0.87	2.13	11.54	19.55	1.53	3.75	19.16
C-18:1	41.02	1.32	3.24	7.90	42.03	1.09	2.68	6.37
C-18:2	5.74	1.20	2.95	51.35	3.80	0.35	0.86	22.54
C-18:3	0.94	0.09	0.22	23.51	0.88	0.02	0.06	6.78
C-20:0	1.33	0.37	0.91	68.44	0.38	0.08	0.18	48.62
C-22:5n3	0.21	0.02	0.05	24.76	0.32	0.09	0.21	67.33
C-22:6n3	0.05	0.01	0.03	60.09	0.10	0.03	0.07	64.62

Table 4. Total fatty acid content in *m. Semimembranosus* in lambs from Middle Rhodopian breed, g/100 g fat

Fatty acids	Control group, n-3				Experimental group, n-3			
	$\bar{x}$	± Sx	SD	C	$\bar{x}$	± Sx	SD	C
SFA	48.07	0.65	1.59	3.30	50.09	1.09	2.67	5.33
MUFA	44.34	1.03	2.53	5.70	45.17	0.97	2.38	5.26
PUFA	6.94	1.26	3.08	44.34	5.10	0.25	0.62	12.22
Σ n-3	0.27	0.03	0.09	31.84	0.42	0.08	0.20	47.62
Σ n-6	6.67	1.29	3.16	47.40	4.68	0.34	0.82	17.58
Σ n-6/Σ n-3	30.17	10.37	25.39	84.18	14.35	4.25	10.42	72.64

*Comparative analysis between the two bre*eds

The fatty acid composition of *Semimembranosus muscle* obtained from lambs of Karakachan and Middle Rhodopian breeds is presented in Table 5. Muscles obtained from Karakachan lambs have a higher content of myristic and palmitic acid compared to muscles obtained from Middle Rhodopian lambs. The *Semimembranosus muscles* of the Middle Rhodopian lambs have a higher amount of stearic and linoleic fatty acids.

The change in fatty acids in both breeds after application of the All-G Rich supplement was not significant. A higher content of oleic acid in the *Semimembranosus muscle* was found in the Middle Rhodopian lambs – 42.03 g/100 g fat and a higher content of linoleic and linolenic in the Karakachan lambs, respectively 5.23 and 1.06 g/100 g fat. The content of docosapentaenoic and docosahexaenoic acids is higher in the muscles of Karakachan lambs – 0.34 and 0.27 g/100 g fat.

The total content of saturated and monounsaturated fatty acids is higher in the control analysed *Semimembranosus muscles* than the Karakachan in comparison with the Middle Rhodopian lambs, as follows 49.33 per 48.07 g/100 g fat and 46.67 per 44.34 g/100 g fat. The use of the All-G Rich supplement in the diet of lambs at two breeds leads to an inverse correlation in the results between the control and experimental group of Karakachan and Middle Rhodopian lambs for the total content of saturated, mono- and polyunsaturated fatty acids. Polyunsaturated fatty acids, omega-6 and the ratio between omega-6 and omega-3 are higher in the Middle Rhodopian lambs in the control groups. The ratio of omega-6 to omega-3 fatty acids was higher in the control compared to the experimental groups of lambs of both breeds, but decreased twice when using the All-G Rich food supplement (Table 6).

Valença et al. (2021) in their research found that seaweed flour of the genus *Schizochytrium* affects the chemical composition of meat due to the higher content of DHA. They found that the addition of algae at a concentration of up to 4% did not affect growth intensity, carcass yield

Table 5. Fatty acid content in *m. Semimembranosus* by lambs of Karakachan (KK) and Middle Rhodopian (MR) breeds, g/100 g fat

Fatty acids	Control group KK, n-3		Experimental group KK, n-3		Control group MR, n-3		Experimental group MR, n-3	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
C-10:0	0.38	0.04	0.51	0.05	0.41	0.08	0.46	0.03
C-12:0	0.28	0.09	0.21	0.12	0.19	0.11	0.29	0.12
C-14:0	3.40	0.07	2.64	0.83	2.15	0.33	3.23	0.75
C-14:1	0.32	0.01	0.29	0.23	0.33	0.25	0.30	0.03
C-15:0	0.68	0.03	0.64	0.16	0.61	0.15	0.63	0.07
C-16:0	25.94	2.36	23.64	0.87	24.05	0.72	23.79	1.16
C-16:1	1.98	0.36	1.37	0.76	1.81	0.66	1.61	0.79
C-17:0	0.96	0.03	1.87	0.77	0.86	0.13	1.77	0.74
C-17:1	1.18	0.03	1.21	0.10	1.18	0.18	1.23	0.15
C-18.0	17.32	1.61	17.91	2.74	18.48	2.13	19.55	3.75
C-18:1	41.73	0.08	40.67	3.97	41.02	3.24	42.03	2.68
C-18:2	4.10	0.28	5.23	0.61	5.74	2.95	3.80	0.86
C-18:3	0.94	0.09	1.06	0.07	0.94	0.22	0.88	0.06
C-20:0	0.36	0.15	1.04	0.41	1.33	0.91	0.38	0.18
C-22:5n3	0.14	0.02	0.34	0.16	0.21	0.05	0.32	0.21
C-22:6n3	0.05	0.01	0.27	0.06	0.05	0.03	0.10	0.07

Table 6. Total fatty acid content in *m. Semimembranosus* in lambs from Karakachan and Middle Rhodopian breeds, g/100 g fat

Fatty acids	Control group KK, n-3		Experimental group KK, n-3		Control group MR, n-3		Experimental group MR, n-3	
	x̄	SD	x̄	SD	x̄	SD	x̄	SD
SFA	49.33	0.40	48.46	4.10	48.07	1.59	50.09	2.67
MUFA	46.67	0.66	43.54	3.57	44.34	2.53	45.17	2.38
PUFA	5.23	0.22	6.90	0.61	6.94	3.08	5.10	0.62
Σn-3	0.19	0.03	0.61	0.22	0.27	0.09	0.42	0.20
Σn-6	5.04	0.19	6.30	0.59	6.67	3.16	4.68	0.82
Σn-6/Σn-3	27.35	3.69	11.39	4.25	30.17	25.39	14.35	10.42

and carcass weight, while at higher levels of 6% the fattening and slaughtering qualities deteriorated. Their research corresponds to the results obtained to improve the concentrations of EPA, DHA, Σn – 3 and CLA and reduce total cholesterol in meat.

Conclusions

• Adding the 1% All-G Rich to the diet ration improves the composition of fatty acids. Polyunsaturated fatty acids increased significantly ($P \leq 0.05$) in the experimental compared to the control group of Karakachan lambs. The ratio of omega-6 to omega-3 fatty acids decreased significantly ($P \leq 0.05$) in the *Semimembranosus muscle* in the experimental group.

• The use of the All-G Rich supplement in Karakachan lambs leads to an increase in lauric, myristic, oleic and docosapentaenoic acid, and capric and docosahexaenoic fatty acids increase significantly ($P \leq 0.05$). Biologically active fatty acids do not undergo significant changes. Oleic acid decreases from 41.73 to 40.67 g/100 g fat, while linoleic and linolenic acid increase from 4.10 to 5.23 g/100 g fat and from 0.94 to 1.06 g/100 g fat, respectively.

• The nutritional supplement from All-G Rich in the fattening of lambs of the Middle Rhodopian breed leads to a slight decrease in capric, lauric, palmitoleic, linoleic and linolenic fatty acids. Oleic, docosapentaenoic and docosahexaenoic acids increase slightly. The ratio between omega-6 and omega-3 fatty acids decreased twice in the experimental group.

• Higher content of oleic acid in *Semimembranosus muscle* was found in the Middle Rhodopian lambs – 42.03 g/100 g fat and higher content of linoleic and linolenic in Karakachan lambs, respectively 5.23 and 1.06 g/100 g fat. The content of docosapentaenoic and docosaohexaenoic acids is higher in the muscles of Karakachan lambs – 0.34 and 0.27 g/100 g fat.

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