



PAVEL ENCHEV TODOROV

*Influence of biologically active substances on the meat quality in lambs of  
aboriginal breeds raised in the Middle Rhodopes*

**ABSTRACT**

**of a dissertation for the acquisition of an educational and scientific degree  
"PhD"**

Professional direction: 6.3. Stock Breeding

Field of higher education:

6. Agricultural Sciences and Veterinary Medicine

Doctoral program: Sheep and goat breeding

Scientific supervisors:

Assoc. Prof. Tsonka Atanasova Odzhakova PhD

Assoc. Prof. Krum Vladimirov Nedelkov PhD

Reviewers:

Prof. Lilian Krumov Sotirov DSc

Prof. Zaprianka Nikolaeva Shindarska PhD

STARA ZAGORA,  
2022

*I would like to hearty thank Assoc. Prof. Tsonka Odzhakova PhD for the guidance, hard work and support provided for the development of the dissertation. I would also like to express my gratitude to the scientific supervisors Prof. Staika Laleva PhD and Assoc. Prof. Krum Nedelkov PhD and to the other colleagues from the Agricultural Institute - Stara Zagora and from the Scientific Center for Livestock and Agriculture - Smolyan for the help and advice given. I also express my gratitude to the members of the Scientific Jury for the objective and useful recommendations.*

*I thank my family for their patience, love and faith in me.*

The dissertation consists of 150 pages, 52 tables and 13 figures. The reference list consists of 236 literary sources, of which 97 are in Cyrillic and 139 are in Latin.

The numbering of the sections, tables and figures in the Abstract does not correspond to those in the dissertation.

The dissertation defense will take place on .....2023 from .....h in the Meeting Hall of the Agricultural Institute - Stara Zagora. The corresponding materials are available to the Scientific Secretary of the Agricultural Institute - Stara Zagora.

Scientific Jury:

Prof. Yovka Miteva Popova PhD

Prof. Petia Koleva Slavova PhD

Prof. Lilian Krumov Sotirov DSc

Prof. Zaprianka Nikolaeva Shindarska PhD

Assoc. Prof. Gancho Ganchev Ganchev PhD

The reviews and opinions prepared by the members of the Scientific Jury, as well as the Abstract, are published on the website of the Agricultural Institute - Stara Zagora:

<http://www.szinstitute.com/>

## I. INTRODUCTION

Effective management of livestock genetic resources is primary to ensuring global and sustainable food security. The erosion of genetic resources in farm animals has been analyzed by many researchers, and within a few decades most of the extremely valuable animal genetic resources may be lost.

Centuries of selection have led to changes in the genome of sheep breeds, in response to environmental challenges and human needs, related to various important economic characteristics, such as quality and quantity of wool, milk and meat, and the ever-increasing need for food in recent years created a demand for colossal food production. As well as in response to modern trends for healthy nutrition of both animals and humans, and in relation to the ever-increasing population, the world needs more food than it can grow and harvest.

On the other hand, local breeds can be a source for development and production of functional foods. This is mainly related to feeding management, which makes it possible to increase or decrease the ratio of biologically active components in meat and milk.

In this regard, there is a need for research and analysis of the productive characteristics and meat qualities of the Middle Rhodope and Karakachan breeds, as well as a contemporary assessment of the factors influencing the quality indicators of meat and consumer expectations.

## I. AIM AND TASKS

*The aim of the current dissertation was to study the influence of the biologically active supplement All-G Rich on the meat qualities of lambs from aboriginal breeds - Middle Rhodope and Karakachan breeds raised in the Middle Rhodopes.*

***The following tasks arose from the set main goal:***

- 1. To study the main productive traits of the Karakachan and Middle Rhodope sheep breeds.*
- 2. To study the weight development of lambs of both breeds at birth, on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age.*
- 3. To study the influence of the biological supplement All-G Rich on the fattening and meat qualities in lambs of both breeds.*
- 4. To study the influence of the biological supplement All-G Rich on the linear measurements in light carcasses and cutt off of the left half of the carcass.*
- 5. To study the influence of the biological supplement All-G Rich on the technological properties of the meat in light carcasses.*
- 6. To study the influence of the biological supplement All-G Rich on the chemical and fatty acid composition of the meat in lambs of the studied breeds.*

## II. MATERIAL AND METHODS

### 1. Material

The study was carried out in the period 2018–2020 and included ewes and their progeny of the Karakachan and Middle Rhodope sheep breeds. Studied sheep were raised outdoor during the period from May to September and indoor from October to April.

Two scientific experiments were conducted to investigate the productive qualities of the studied breeds and to determine the influence of the biologically active supplement All G - Rich.

➤ First experiment - the research was conducted in 2020 for the period of 60 days and the lambings of 130 ewes at second lactation were studied, including 67 ewes of the Karakachan breed and 63 ewes of the Middle Rhodope breed, clinically healthy and in good condition. They gave birth to 112 lambs divided into two groups by breed. The live weight of the progeny of the Karakachan and Middle Rhodope breeds was controlled at birth, as well as on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age.

➤ Second experiment – it was conducted to study the influence of the bioactive supplement All-G Rich on the fattening and meat qualities of male and female lambs from the Karakachan and Middle Rhodope breeds. The experiment was carried out in the sheep farm of the Scientific Center for Livestock and Agriculture - Smolyan for the period April - June 2020 with 24 weaned lambs obtained within the first experiment and separated into 4 groups (4x6), as follows:

- two control groups of the Karakachan and Middle Rhodope breeds;
- two experimental groups of the Karakachan and Middle Rhodope breeds to study the influence of 1% of the bioactive supplement All-G Rich.

Animals were matched by breed, sex, age, live weight and body development. The groups were formed according to the method of analogues - an equal number of male and female lambs and an equal number of singles and twins. The lambs were weaned when they reached 60 days of age and live weight of 12-14 kg.

The experiment started at an average live weight of 12,883 kg for the Karakachan breed and 12,950 kg for the Middle Rhodope breed. The animals of the groups were raised free in boxes on permanent bedding, according to the requirements of Ordinance No. 44. Constant access to fresh and clean water was ensured.

Feeding was carried out in combined mangers, and the lambs fed ad libitum with combined feed for lambs (by prescription - KF 125), certified and produced by the feed plant of the Agricultural Institute - Stara Zagora.

The composition of the food ration included concentrate mixture (**Table 1**) and alfalfa hay for both Middle Rhodope and Karakachan breed groups.

**Table 1. Composition of the concentrate mixture for the control group of lambs**

<i>Components</i>	<i>Content, %</i>
<i>Corn</i>	76,50
<i>Sunflower meal</i>	20,00
<i>Calcium carbonate – CaCO<sub>3</sub></i>	2,50
<i>Salt</i>	0,50
<i>Premix (lambs)</i>	0,50

In the experimental groups, in addition to the ration according to Table 1, 1% of the bioactive supplement All-G Rich was added to the concentrate mixture.

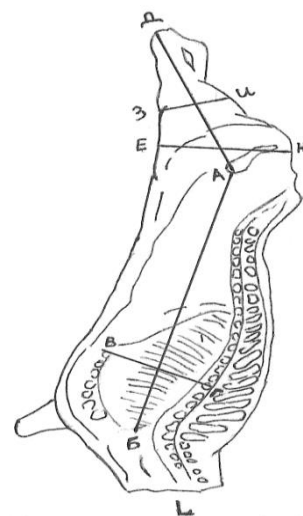
The lambs of the groups were fed ad libitum (+ 5 to 10% residue) with a concentrate mixture and alfalfa hay, according to a ration previously adjusted to their age, satisfying their needs for nutrients and biologically active substances. Feed consumption was daily recorded using an electronic scale, based on the difference between the amount of the given feed and the residues of the following day.

The experimental period lasted 60 days and until reaching 23 kg average live weight, the control of the live weight of lambs was carried out every 15 days.

To establish levels of the slaughter traits, a slaughter analysis was performed after the male lambs reached 23 kg average live weight. From each group, 3 animals with a live weight close to the average for the group were slaughtered. The slaughter was carried out in accordance with the requirements of Ordinance No. 27 (published in the State Newspaper 99/1999) in a licensed slaughterhouse in the town of Smolyan. After slaughter, the internal organs were weighed and the dry and wet cleaning of the carcasses was carried out. Carcasses were chilled at 4°C for 24 h, then weighed again to determine the slaughter yield as well as chilling losses. They were then halved, by cutting along the spine, and a cut off was made.

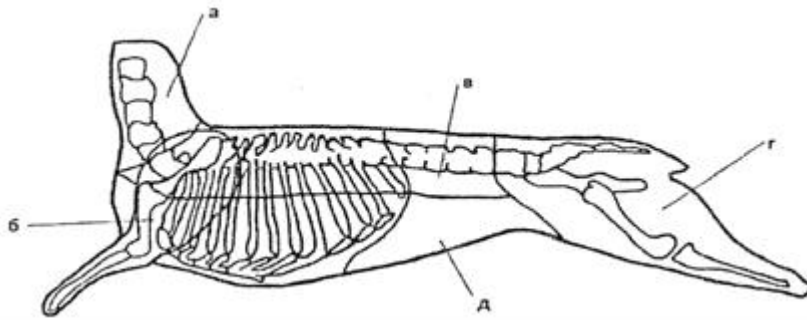
On the chilled and weighed carcasses, the following linear measurements were performed (**Figure 1**) on the left half of the carcass, according to the methodology proposed by **Zahariev and Pinkas (1979)**.

- Carcass length (cm) - the distance from the front end of the pelvic symphysis to the middle of the front end of the first rib (**Figure 1** line A - Б);
- Thigh length (cm) - the distance from the hock joint to the front end of the pelvic symphysis (**Figure 1** line A - Д);
- Thigh girth (cm): large girth - the widest part of the thigh (**Figure 1** line E - Ж); small girth - in the middle of the line determining the length of the thigh (A - Д) (**Figure 1** line 3 - И);
- *Chest width (cm)* - at the level of the 5<sup>th</sup> thoracic vertebra. We measured the distance from the 5<sup>th</sup> thoracic vertebra to the caudal end of the sternum on the ventral side (**Figure 1** line B - Г).



**Figure 1. Linear measurements of the carcass according to Zahariev and Pinkas (1979)**

The carcasses were cut according to the method of **Marinova and Popova (2011)** BSS 4348-1978 in the following parts: neck, lamb shoulder, outlet, thigh and loins (**Figure 2**). After cutting the carcass, the individual meat cuts were weighed using an electronic scale.



**Figure 2. Meat cut off of a lamb carcass (Marinova and Popova, 2011)**

*Musculus Longissimus lumborum* and *Musculus Semimembranosus* samples were taken from the carcass of each slaughtered animal to study the chemical composition, technological qualities and fatty acid composition of meat of the lambs of the studied breeds.

## **2. Methods of analysis**

The analysis of the meat samples was carried out in the laboratory of Trakia University - Stara Zagora.

Studies were carried out according to the chosen methodology, as follows:

### **2.1. Determination of the chemical composition and technological qualities of meat.**

#### **2.1.1. Determination of the chemical composition of meat**

##### **2.1.1.1. Determination of the water content according to BSS 15437:1982.**

**2.1.1.2. Determination of the protein content (of general nitrogen) of meat, Kjeldahl method according to BSS 9374:1982.**

##### **2.1.1.3. Determination of the lipid content according to BSS 8549:1992**

##### **2.1.1.4. Analysis of the fatty acids in lamb meat –**

Samples from *m. Longissimus Dorsi* and *m. Semimembranosus* were prepared for total lipid extraction after mechanical processing (grinding). 5 g of ground tissue was used and treated with chloroform and methanol in a ratio of 2:1. Methylation of the extract was carried out with a mixture of methanol and sulfuric acid. Fatty acid methyl esters were separated and quantified using a gas chromatography. Fatty acid analysis was performed using a GS/MS Clarus 500 Gas Chromatograph (PerkinElmer, USA) equipped with a flame ionization detector and an automatic injection system. A TG-WAXMS capillary column (Thermo Scientific, USA) filled with polyethylene glycol (PEG) with dimensions - 60 mm × 25 mm × 0.50 μm was used. The carrier gas was hydrogen. A temperature program started at 160 °C for 2 min, then increased by 10 °C/min to 240 °C and the maintained for 15 min until the process was completed. Results were calculated in g/100g fat.

##### **2.1.1.5. Analysis of the fatty acid composition of feed and bioactive supplement**

**All G – Rich** – extraction of total lipids was performed by the method of Bligh & Dyer (AOAC, 1959) with chloroform and methanol in a ratio of 1:2. Fatty acids methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kyoto, Japan). Analysis was performed with a CP7420 capillary column (100 m x 0.25 mm i.d., 0.2 μm, Varian Inc., Palo Alto, CA), with carrier gas hydrogen and make-up gas nitrogen. A five-step program was used to program the gas chromatograph oven. A four-step oven mode was programmed - the initial column temperature was 51° C/min, which was maintained for 8 min, then increased by 10° C/min to 170°C and

maintained for 20 min, followed by a further increase of 4°C/ min up to 186°C for 19 minutes and up to 220° C with 4° C/min until the process was finished. Results were calculated in g/100g fat.

**2.1.1.6. Determination of the mineral substance content** according to ISO 936:1998.

### **2.1.2. Determination of the technological qualities of meat**

**2.1.2.1. Determination of the pH values** using a pH-meter Testo 205 on the 24<sup>th</sup> h after slaughter of *Musculus Longissimus lumborum* and *Musculus Semimembranosus*.

**2.1.2.2. Determination of the water-holding capacity of meat (WHC)** using the classical method of **Grau and Hamm (1953)**, described by **Zahariev and Pinkas (1979)** with modifications according to **Petrov (1982)**.

**2.1.2.3. Determination of the water-absorbing capacity of meat (WAC)** according to the methodology of **Kyosev and Danchev (1979)**.

**2.1.2.4. Determination of the tenderness of meat** using a penetrometer DSD VEB Feinmess (Dresden, Germany). The meat sample was pre-treated with a physiological solution before performing the analysis to measure the tenderness of the meat in °P.

#### **2.1.2.5. Determination of the heat treatment losses (baking)**

The meat sample was baked at a temperature of 150°C for 20 min in a forced convection oven. The percentage of losses was determined as the difference in the mass of the samples before and after baking, expressed as a percentage.

#### **2.1.2.6. Determination of the color characteristics of meat**

Muscle color was determined according to the CIE L\*a\*b\* system. A "Minolta CR-400" colorimeter of the company Konica Minolta (Osaka, Japan) was used for this purpose, using D65 illumination and an observation angle of 2°. The values of L\*, a\* and b\* coordinates were determined on the 24<sup>th</sup> h post mortem.

Working procedure. Before starting the measurements, the apparatus was pre-calibrated using a CR-A43 white plate (with illuminance characteristics D65: Y=84.4; x=0.3200 and y=0.3365). To determine the color characteristics, measurements were taken of the cross-sectional area of the muscle. A minimum of five measurements were made with the colorimeter on all the examined samples. For L\*, a\* and b\* values, the arithmetic mean of the five measurements was taken.

### **2.1.3. Statistical analysis**

A software product – Statistica program for Excel 2016 was used for processing and statistical layout of the obtained results.

The statistical information about the studied breeds, necessary for the development of the present dissertation, was obtained from the Association for Breeding of Middle Rhodope, Karakachan and Rhodope Cigai Sheep - city of Smolyan.

## **III. RESULTS AND DISCUSSION**

### **1. Production indicators**

#### **1.1. Productive characteristics of the Karakachan sheep breed**

Karakachan sheep are bred mainly in the mountainous and semi-mountainous settlements of Bulgaria, mainly in the southwestern and southern regions of the country.

Economic conditions in the field of agriculture in recent years have given rise to the tendency in areas poorer in terms of natural resources to breed animals with lower requirements for rearing and feeding conditions, which determines the increased interest in local sheep breeds, respectively sheep of the Karakachan breed.

A study was carried out to indicate the main productive characteristics of the Karakachan sheep breed controlled by the Association for Breeding of Middle Rhodope, Karakachan and Rhodope Cigai Sheep - Smolyan in 2015-2018. The results for the Karakachan breed are presented in **Table 2**.

**Table 2. Mean values of the main productive traits of the Karakachan sheep (2015 – 2018)**

Traits	Female				Male			
	n	$\bar{x}$	S x	C %	n	$\bar{x}$	S x	C %
Live weight at birth, kg	96	2,95	0,073	2,49	75	3,08	0,113	3,76
Live weight at 2 months of age, kg	96	15,45	0,17	1,05	75	16,01	0,25	1,56
Live weight at weaning, kg	854	17,600	0,270	6,12	135	18,086	0,221	5,38
Live weight 18 months of age, kg	96	36,83	0,77	2,09	75	41,35	0,87	2,12
Live weight 2,5 years of age, kg	854	37,036	0,121	6,561	135	53,692	0,450	10,770
Wool production, kg	854	2,125	0,027	30,882	135	3,560	0,114	12,421
Staple length, cm	854	26,603	0,089	8,313	135	31,754	1,180	14,932
Biological prolificacy, %	854	106,041	0,213	4,882	-	-	-	-
Milk yield, l	854	40,680	0,022	5,514	-	-	-	-

**Odzhakova (1994)** reported that the average live weight of lambs of the Karakachan breed at weaning varied from 16,828 to 17,467 kg. Live weight of 2,5-year-old ewes in the same study ranged from 40,345 to 41,683 kg.

In a study conducted in the period 1980-1996, **Kafedzhiev (1997)** found 18,170 kg of average live weight of Karakachan lambs at weaning, and variation in weaning weight from 16,500 to 19,810 kg.

The same author reported that the live weight of ewes at 2,5 years of age ranged from 33,220 to 41,370 kg for the observed period, as the average live weight at 2,5 years was 38,630 kg.

In a study from 2015 – 2018 (**Table 3**), an average live weight of 17,600 kg and 18,086 kg for female and male Karakachan lambs, respectively, was found at weaning. The results are close to those of **Kafedzhiev (1997)**, but are lower than those obtained by **Tyankov et al. (2003)** and **Boykovski et al. (2005)**. The average live weight of ewes at 2,5 years of age was 37,036 kg, and of rams - 53,692 kg, results being close to those found by **Alexieva et al. (1989)** and **Kafedzhiev (1997)** - 38,400 kg and 38,630 kg, respectively. **Genkovski (2002)** obtained higher values for this trait - 40,000 kg respectively. Our results were higher than those in the studies of **Hlebarov (1942)**, **Balevska and Petrov (1970)** – 33,0 kg and 25-30,0 kg, respectively, but are lower than the results obtained for the ewes at 2,5 years by **Odzhakova (1994)** and **Staykova and Yosifov (2014)** - 41,683 kg and 48,603 kg, respectively. The obtained results for wool production and staple length, as follows: 2,125 kg (3,560 kg - rams) and 26,60 cm (31,75cm - rams), were higher than those obtained by **Alexieva (1979)** and **Tyankov et al. (2003)** - 19,27 cm and 21,94 cm, respectively, but are close to those obtained by **Kafedzhiev (1997)** and **Genkovski (2002)** – 23,10 cm and 24,68 cm.

## 1.2. Productive characteristics of the Middle Rhodope sheep breed



**Table 3** shows the mean values of the main productive characteristics of the Middle Rhodope sheep.

The live weight of the Middle Rhodope female lambs at weaning was 19,390 kg, and of the male lambs - 20,830 kg. The obtained data were close to those reported by **Odzhakova (2014)** - 20,100 kg average live weight of Middle Rhodope lambs at weaning, but were higher than the results obtained by **Marinov (1973)** and **Vasilev et al. (2000)** - 12,950 and 15,430 kg, respectively.

**Table 3. Mean values of the main productive traits of the Middle Rhodope sheep (2015 – 2018)**

Traits	Female				Male			
	n	$\bar{x}$	S x	C %	n	$\bar{x}$	S x	C %
Live weight at birth, kg	209	2,69	1,05	39,03	186	3,02	1,07	35,43
Live weight at 2 months of age, kg	124	12,95	1,82	14,05	75	14,81	1,25	8,44
Live weight at weaning, kg	1126	19,390	1,06	9,8	256	20,830	0,88	16,34
Live weight 18 months of age, kg	125	23,73	1,57	6,62	40	29,15	1,00	3,43
Live weight 2,5 years of age, kg	1126	39,192	0,180	14,56	256	68,103	0,145	6,78
Wool production, kg	1126	2,273	0,008	12,44	256	3,488	0,010	9,59
Staple length, cm	1126	16,03	0,062	12,35	256	17,04	0,065	12,25
Biological prolificacy, %	1126	108,31	1,446	3,27	-	-	-	-
Milk yield, l	1126	32,3	0,035	6,52	-	-	-	-

The average live weight of 2,5-year-old ewes was found 39,192 kg, and of rams -68,103 kg. The results were higher than those obtained by **Madrov (1936)** and **Marinov (1973)** - 26,700 kg and 23,180 kg, respectively, but were close to those obtained by **Odzhakova et al. (2019)** - 39,650 kg average live weight of ewes at 2,5 years of age.

Wool production of ewes from the Middle Rhodope breed was 2,273 kg, and of rams - 3,488 kg. The average staple length was 16,03 cm for ewes and 17,04 cm for rams.

Biological prolificacy was found 108,31%, and milk yield - 32,3 l. The average prolificacy of ewes according to **Marinov (1973)** was 102-103%, and according to **Hristova (1990)** – 100,5%. According to **Marinov (1973)**, in the studied flock only 8-10% of the ewes had given birth to twins. In our study, the results were higher than those obtained by **Marinov (1973)** and **Hristova (1990)**, the reason for this being the better feeding and rearing conditions of the animals. Milk yield was studied by **Marinov (1973)**, who reported 61,75 l an average milk yield, but with large variations within the flocks. **Hristova (1990)** found 34,9 l milk yield per milking period, and **Odzhakova (2014)** – 32,3 l milk yield, varying within individual flocks from 20,0 to 40,0 l.

### 1.3. Live weight of Karakachan lambs at birth, at 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age

Live weight of lambs was studied at two stages: from birth to weaning and post-weaning.

The first stage gives us an idea of the individual growth abilities and is also an indicator of the milk yield of the ewes, and the second stage gives us an idea of the individual growth abilities and feed digestibility. Data on growth abilities are important characteristic of the breed and a suitable basis for organizing the selection process.

**Table 4** presents the data on the live weight of the Karakachan lambs at birth, as well as on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age:

**Table 4. Live weight of Karakachan lambs at birth, on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age**

Traits	Breed/group								Significance
	Karakachan female				Karakachan male				
	n	$\bar{x}$	$\pm Sx$	C	n	$\bar{x}$	$\pm Sx$	C	
Live weight at birth, kg	26	2,801	0,116	21,11	35	3,009	0,117	23,05	-
Live weight on the 10 <sup>th</sup> day of age, kg	26	5,031	0,224	22,70	35	5,466	0,174	18,88	-
Average daily gain, kg/day	26	0,223	0,026	59,38	35	0,246	0,018	42,90	-
Live weight on the 30 <sup>th</sup> day of age, kg	26	8,392	0,358	21,73	35	9,449	0,303	19,00	*
Average daily gain, kg/day	26	0,186	0,015	40,16	35	0,215	0,011	30,29	-
Live weight on the 70 <sup>th</sup> day of age, kg	26	15,546	0,458	15,03	35	17,769	0,405	13,50	***
Average daily gain, kg/day	26	0,182	0,007	19,36	35	0,211	0,006	16,83	***

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

Male lambs of the Karakachan breed were born 3,009 kg and had a higher live weight than females - 2,801 kg (by 7,4%), but the difference was not mathematically proven. The coefficient of variation at birth was 21,11% (females) and 23,05% (males).

Data on the live weight at birth were similar to those obtained by **Odzhakova (1999)** – 3,03 kg. **Genkovski (2002)**, in his study of the weight development of Karakachan breed found an average value of the trait of 2,690 kg at birth of the lambs with a variation of 13,14 %.

Values of the variation coefficient of the trait live weight of the female lambs ranged from 15,03% to 22,70%, and for males from 13,50% to 23,05%, which is an indicator for a high level of flock equality.

The average daily gain of male lambs of 0,246 kg/day on the 10<sup>th</sup> day was higher than that of female lambs - 0,223 kg/day. The difference between groups in terms of live weight on the 30<sup>th</sup> day of age increased to 13,5%, and was proved mathematically with a low level of significance ( $P \leq 0,05$ ). This trend was observed for the average daily gain on the 30<sup>th</sup> day of age as well, with male lambs having 13,48% higher average daily gain compared to females and reaching 0,215 kg/day.

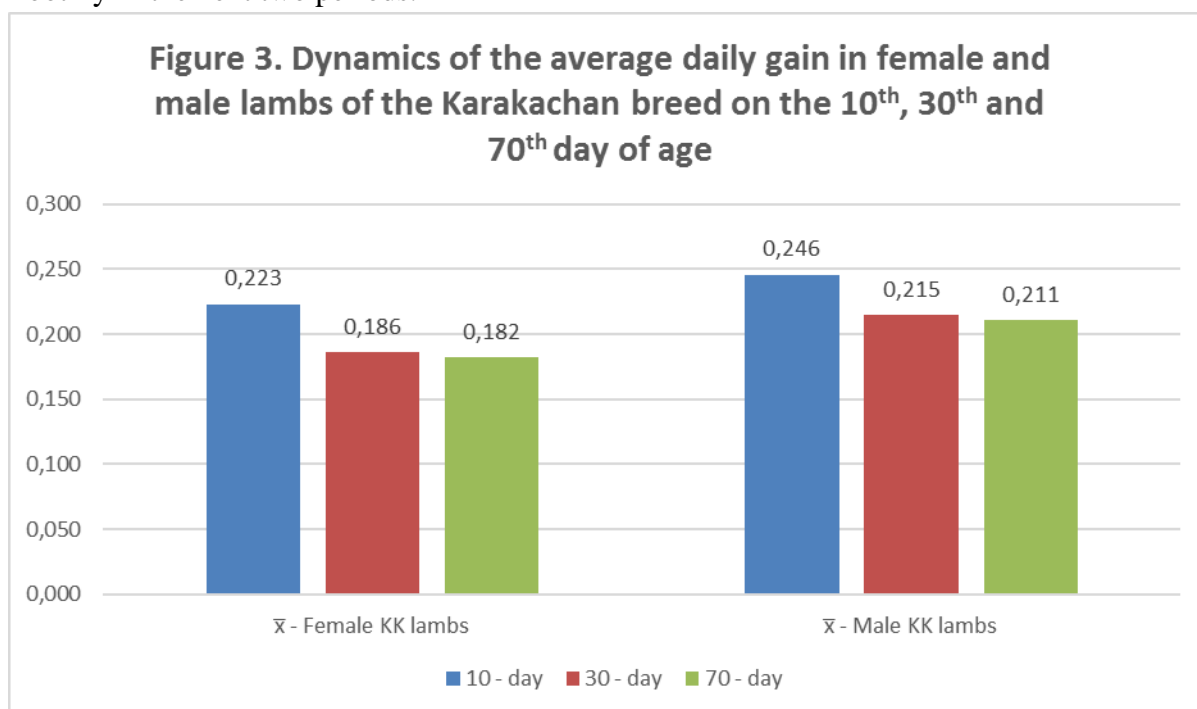
Highly significant differences ( $P \leq 0,001$ ) were found for the live weight on the 70<sup>th</sup> day of age in male versus female lambs of the Karakachan breed. We found the same trend in terms of the average daily gain on the 70<sup>th</sup> day of age ( $P \leq 0,001$ ). The intensity of growth and the changes in the average daily gain of the progeny during the separate reported periods are shown in **Figure 3**.

Male Karakachan lambs had a higher average daily gain compared to females during all considered periods.

The obtained data corresponded to those of **Genkovski (2002)** and **Yankov (1999)**, reporting a higher growth intensity of male Karakachan lambs compared to female lambs, with an average of 7,89% and 6,53%, respectively.

The obtained growth intensity data for the three periods reported ranged from 0,182 – 0,223 kg/day for female and 0,211 – 0,246 kg/day for male lambs. As can be seen in the figure

(Figure 3), growth intensity is the highest during the first period (10<sup>th</sup> day) and then decreased smoothly in the next two periods.



The growth intensity of Karakachan lambs was studied by **Stankov (1999)**, **Odzhakova (1999)**, **Staykova (2005)** and **Genkovski (2002)**, who established 0,203, 0,216, 0,213 and 0,207 kg/day, respectively. Their results corresponded with our data. **Nedelchev and Stoyanov (2004)** reported an average daily gain of Karakachan lambs of 0,180 kg/day, and these results were lower compared to those of our study and those reported by the other authors. Despite the low live weight at birth, the lambs developed very well and in a period of 120 days reached a live weight of 25–27 kg (**Kafedzhiev et al., 1992**; **Nedelchev et al., 1995**).

#### 1.4. Live weight of Middle Rhodope lambs at birth, on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age

Data on the live weight of lambs of the Middle Rhodope breed at different ages during the study period are presented in **Table 5**.

Male lambs were born 3,968 kg and females- 3,739 kg. The difference of 0,229 kg (5,77%) is not significant.

**Odzhakova (2014)**, in a study of the live weight of lambs in three farms in the Middle Rhodopes obtained similar results - from 2,86 kg to 3,76 kg for females, and from 3,59 to 4,13 kg for male animals. The data obtained in our study and by **Odzhakova (2014)** were higher than the results established by **Marinov (1970)** and **Vasilev et al. (2000)**, respectively 2,694 kg and 3,034 kg, which was due to improved feeding and rearing conditions.

On the 10<sup>th</sup> day of age, the difference between male and female lambs in terms of live weight reached 0,337 kg or 5,71%, but the result was not significant. Male lambs had a 4,62% higher average daily gain than females on the 10<sup>th</sup> day of age, but the difference was not mathematically proven.

The female lambs of the Middle Rhodope breed grew intensively in the second period until the 30<sup>th</sup> day of age and reached a live weight of 13,896 kg, while the male lambs reached 13,900 kg, and both groups were equal in terms of live weight in the period until the 30<sup>th</sup> day of age. **Marinov (1970)** and **Vasilev et al. (2000)** obtained the following results for the live weight of 30-

day-old lambs, 6,899 kg and 7,820 kg, respectively, which is 50,4% and 43,72% lower value of the live weight trait, compared to those obtained by us. The high live weight on the 30<sup>th</sup> day of age in the studied female and male lambs of the Middle Rhodope breed was most likely due to the improved conditions and rearing technology, combined with proper nutrition and manifestation of the genetic potential of the breed.

**Table 5. Live weight of Middle Rhodope lambs at birth and on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age**

Traits	Breed/ group								Significance
	Middle Rhodope Female				Middle Rhodope Male				
	n	$\bar{x}$	$\pm Sx$	C	n	$\bar{x}$	$\pm Sx$	C	
Live weight at birth, kg	23	3,739	0,086	11,02	28	3,968	0,118	15,69	ns
Live weight on the 10 <sup>th</sup> day of age, kg	23	6,013	0,327	26,11	28	6,350	0,214	17,83	ns
Average daily gain, kg/day	23	0,227	0,031	65,59	28	0,238	0,013	28,31	ns
Live weight on the 30 <sup>th</sup> day of age, kg	23	13,896	0,636	21,96	28	13,900	0,324	12,34	ns
Average daily gain, kg/day	23	0,339	0,021	29,13	28	0,331	0,010	15,26	ns
Live weight on the 70 <sup>th</sup> day of age, kg	23	18,761	0,491	12,56	28	19,257	0,268	7,37	ns
Average daily gain, kg/day	23	0,215	0,007	15,54	28	0,218	0,004	8,51	ns

The results obtained on the 70<sup>th</sup> day of age at weaning for the lambs of Middle Rhodope breed were 18,761 kg for the female and 19,257 kg for the male lambs. **Odzhakova (2014)**, in a study of the live weight after weaning of lambs reported a variation in live weight from 17,960 to 19,660 kg, and for males from 20,830 to 22,450 kg. Our results and those obtained by **Odzhakova (2014)** were higher than the results reported by **Marinov (1970)** and **Vasilev et al. (2000)**, respectively 12,950 and 14,810 kg for the live weight at weaning of Middle Rhodope lambs.

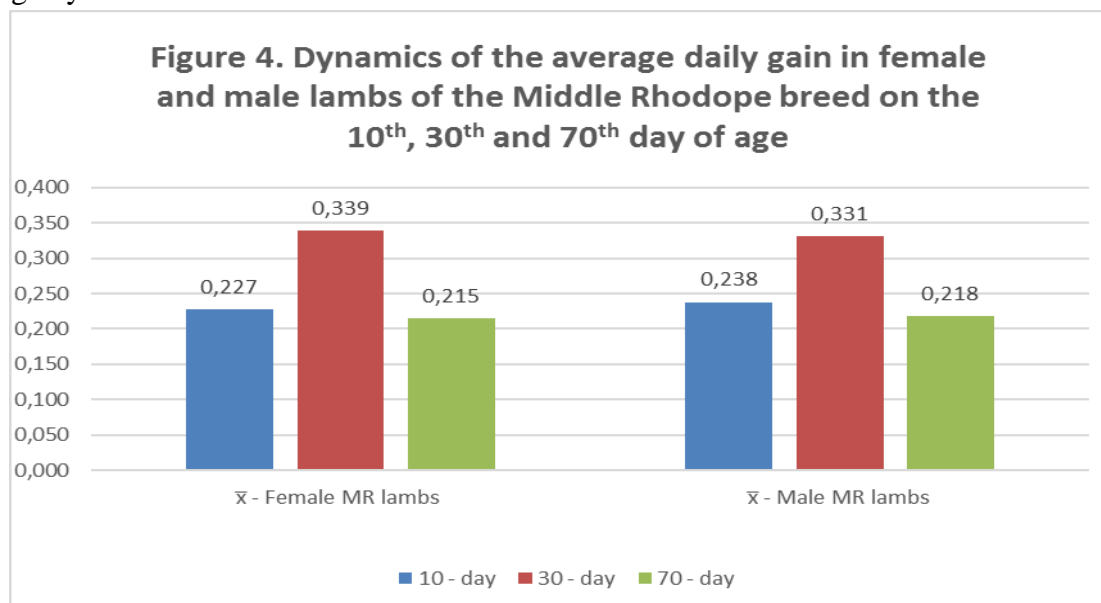
The value of the trait average daily gain is shown on **Figure 4**. In female Middle Rhodope lambs from the 10<sup>th</sup> day of age to weaning, it was within the range of 0,215 – 0,339 kg/day, and in males from 0,218 – 0,331 kg/day.

During the first period up to the 10<sup>th</sup> day, the group with the male lambs reached 0,238 kg/day average daily gain, and the female lambs - 0,227 kg/day, which represents a 4,62% higher average daily gain of the male compared to the female lambs of the Middle Rhodope breed, but the difference was not mathematically proven.

During the second period up to 30<sup>th</sup> day of age, the trend was reversed, female lambs had a higher average daily gain of 0,339 kg/day compared to males - 0,331 kg/day, or 2,42% higher average daily gain, but the difference was not mathematically proven. In his study, **Marinov (1970)** reported an average daily gain of 0,171 kg/day from birth to the 30<sup>th</sup> day of age. Comparing with our results, almost 2 times higher values were found.

The data obtained for the average daily gain on the 70<sup>th</sup> day of age of 0,218 kg/day in male lambs and 0,215 kg/day in female lambs showed an almost equal levels of male and female lambs for this trait in the period up to the 70<sup>th</sup> day. In his study of the growth of lambs and ewe and ram lambs from the Middle Rhodope breed, **Marinov (1970)** reported an average daily gain of 0,163

kg/day from birth to weaning (2,5-3 months). The results obtained by us were again higher than those reported by **Marinov (1970)**, but close to those obtained by **Odzhakova (2014)** for female lambs - 0,209 kg/day and for male lambs - 0,244 kg/day. An interesting trend was observed in the variation of the average daily gain (**Figure 4**) - in both groups the average daily gain increased in the period up to the 30<sup>th</sup> day of age, then decreased in the last period up to the 70<sup>th</sup> day of age of 0,163 kg/day.



The values of the coefficient of variation of the live weight trait for female lambs ranged from 11,02% to 26,11%, and for males - from 7,37% to 17,83%. The value of the coefficient of variation reported by **Odzhakova (2014)** for the live weight varied from 8,76 to 21,76 % for female and from 11,34 to 21,34 % for male lambs of the Middle Rhodope breed. The low values of the coefficients of variation are indicative of the relative equality of the groups of both studies.

#### **1.5. Comparative assessment of the live weight at birth, on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age of the lambs of both breeds**

The comparative analysis between breeds for female lambs is presented in **Table 6**. Female lambs of the Middle Rhodope breed were born with higher live weight ( $P \leq 0,001$ ) compared to the lambs of the Karakachan breed. The difference between breeds was 0,938 kg or 25,09 %. In her study of other aboriginal breeds, such as the Middle Stara Planina breed and Koprivshitsa breed, **Markova (2020)** reported an average live weight at birth for the lambs of the Middle Stara Planina breed of 3,110 kg and 3,330 kg for the lambs of the Koprivshitsa breed. Comparing them with our research we can conclude that the female Karakachan lambs were born with a much lower live weight than in the other aboriginal breeds.

The coefficient of variation in the Karakachan lambs was 21,11% and was higher than that in the Middle Rhodope lambs - 11,02%.

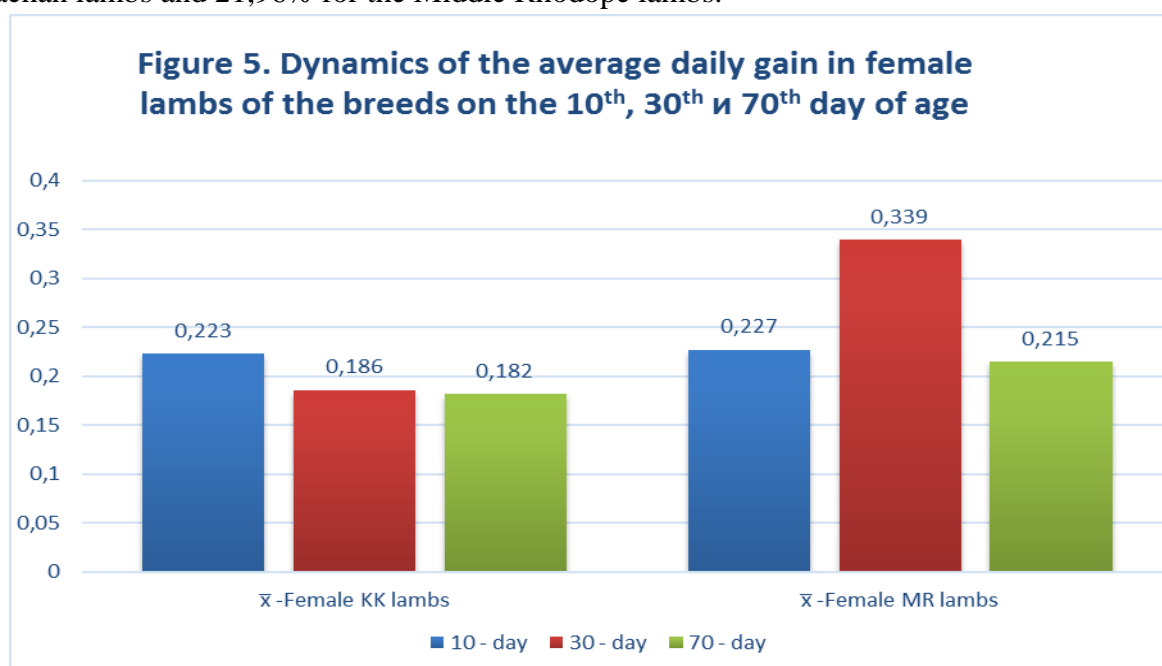
The analysis of the trait live weight on the 10<sup>th</sup> day of age shows that the Karakachan lambs grew more intensively and reached 5,031 kg, and the Middle Rhodope lambs reached 6,013 kg, but the difference had a low level of significance ( $P \leq 0,05$ ). The result between breeds in terms of live weight at birth and on the 10<sup>th</sup> day decreased from 25,09% to 16,33%. No significant differences were observed in terms of growth intensity from birth to the 10<sup>th</sup> day of age. Karakachan lambs realized 0,223 kg/day, and Middle Rhodope lambs - 0,227 kg/day, and no differences between breeds were observed. The coefficients of variation almost equalized, being 22,70% for the Karakachan lambs and 26,11% for the Middle Rhodope lambs.

**Table 6. Live weight of female lambs of the Karakachan and Middle Rhodope breeds at birth and on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age**

Traits	Breed/ group										Significance
	Karakachan Female					Middle Rhodope Female					
	n	$\bar{x}$	$\pm S\bar{x}$	SD	C	n	$\bar{x}$	$\pm S\bar{x}$	SD	C	
Live weight at birth, kg	26	2,801	0,12	0,59	21,11	23	3,739	0,09	0,41	11,02	***
Live weight on the 10 <sup>th</sup> day of age, kg	26	5,031	0,22	1,14	22,70	23	6,013	0,33	1,57	26,11	*
Average daily gain, kg/day	26	0,223	0,03	0,13	59,38	23	0,227	0,03	0,15	65,59	-
Live weight on the 30 <sup>th</sup> day of age, kg	26	8,392	0,36	1,82	21,73	23	13,896	0,64	3,05	21,96	***
Average daily gain, kg/day	26	0,186	0,01	0,07	40,16	23	0,339	0,02	0,10	29,13	***
Live weight on the 70 <sup>th</sup> day of age, kg	26	15,546	0,46	2,34	15,03	23	18,761	0,49	2,36	12,56	***
Average daily gain, kg/day	26	0,182	0,01	0,04	19,36	23	0,215	0,01	0,03	15,54	***

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The comparative analysis between breeds in terms of the trait live weight on the 30<sup>th</sup> day of age shows that the Karakachan lambs slowed down their growth intensity and reached a live weight of 8,392 kg, while the Middle Rhodope lambs reached 13,896 kg and the difference was proven with a high level of significance ( $P \leq 0,001$ ). The difference between the breeds was most clearly expressed in terms of live weight on the 30<sup>th</sup> day, reaching 39,61%. In terms of growth intensity from birth to the 30<sup>th</sup> day, significant changes were observed in the data. The intensity of growth in Karakachan lambs decreased to 0,186 kg/day, and in the Middle Rhodope lambs it increased to 0,339 kg/day (Figure 5), and the difference was proven with a high degree of significance ( $P \leq 0,001$ ). The coefficients of variation of the trait equalized, being 21,73% for the Karakachan lambs and 21,96% for the Middle Rhodope lambs.



The between-breed analysis of the trait live weight on the 70<sup>th</sup> day of age shows that the intensity of growth of Karakachan lambs slightly decreased and they reached a live weight of 15,546 kg, while the Middle Rhodope lambs significantly slowed down their growth and reached 18,761 kg ( $P \leq 0,001$ ). Despite the high level of significance of the difference between breeds expressed in percentages (17,14%), it is the lowest for all observed periods. According to the indicator growth intensity from birth to the 70<sup>th</sup> day, for the female Karakachan lambs it decreased slightly from 0,186 kg/day to 0,182 kg/day, while for the Middle Rhodopes it decreased from 0,339 kg/day to 0,215 kg/day, the decrease being significant (**Figure 5**).

The difference between breeds remained with a high level of significance ( $P \leq 0,001$ ). The coefficients of variation of the trait decreased and the groups equalized, for the Karakachan lambs being 15,03% and for the Middle Rhodope lambs - 12,56%.

The comparative analysis between male lambs of the Karakachan and Middle Rhodope breeds is presented in **Table 7**.

Male lambs of the Middle Rhodope breed were born with a significantly higher live weight ( $P \leq 0,001$ ). The difference between breeds was estimated to 0,959 kg or 24,17%, with significantly higher live weight at birth in favour of the male Middle Rhodope lambs. By the 10<sup>th</sup> day of age, male lambs of the Karakachan breed grew intensively and reached live weight of 5,466 kg, while the Middle Rhodope lambs reached 6,350 kg, and the difference between the breeds decreased to 0,884 kg or 13,92%, remaining with a high degree of significance ( $P \leq 0,01$ ).

**Table 7. Live weight of male lambs of the Karakachan and Middle Rhodope breeds at birth and on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age**

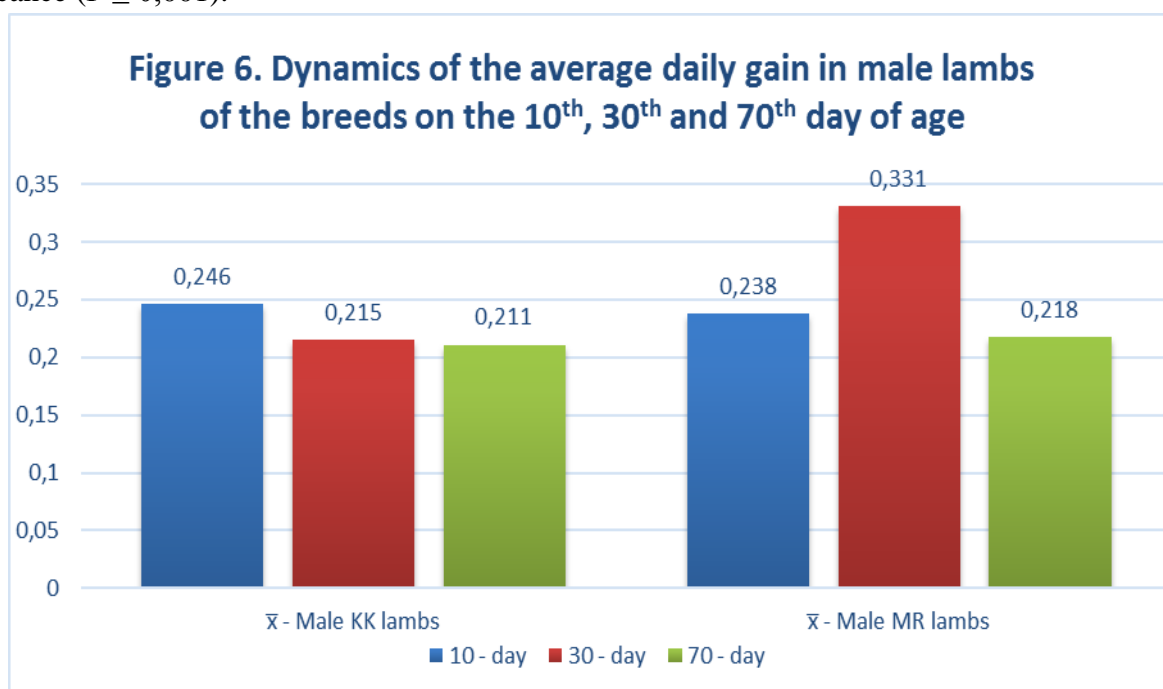
Traits	Breed/ group										Significance
	Karakachan Male					Middle Rhodope Male					
	n	$\bar{x}$	$\pm S\bar{x}$	SD	C	n	$\bar{x}$	$\pm S\bar{x}$	SD	C	
Live weight at birth, kg	35	3,009	0,12	0,69	23,05	28	3,968	0,12	0,62	15,69	***
Live weight on the 10 <sup>th</sup> day of age, kg	35	5,466	0,17	1,03	18,88	28	6,350	0,21	1,13	17,83	**
Average daily gain, kg/day	35	0,246	0,02	0,11	42,90	28	0,238	0,01	0,07	28,31	-
Live weight on the 30 <sup>th</sup> day of age, kg	35	9,449	0,30	1,79	19,00	28	13,900	0,32	1,71	12,34	***
Average daily gain, kg/day	35	0,215	0,01	0,07	30,29	28	0,331	0,01	0,05	15,26	***
Live weight on the 70 <sup>th</sup> day of age, kg	35	17,769	0,41	2,40	13,50	28	19,257	0,27	1,42	7,37	**
Average daily gain, kg/day	35	0,211	0,01	0,04	16,83	28	0,218	0,00	0,02	8,51	-

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

This trend can be clearly seen for the trait average daily gain from birth to the 10<sup>th</sup> day of age (**Figure 6**). Karakachan lambs reached 0,246 kg/day average daily gain, and Middle Rhodope

lambs - 0,238 kg/day, or 3,36% difference in favour of the Karakachan breed was found, but the difference was not mathematically proven.

The analysis of the trait live weight on the 30<sup>th</sup> day of age for both breeds shows that the growth intensity of Karakachan lambs decreased and they reached live weight of 9,499 kg, while the Middle Rhodope lambs reached 13,900 kg and the difference was proven with a high level of significance ( $P \leq 0,001$ ). Difference between breeds was most clearly expressed in terms of live weight on the 30<sup>th</sup> day of age and reached 31,61%, but it was lower than that achieved by the female Middle Rhodope lambs - 39,61%. In terms of the trait growth intensity from birth to the 30<sup>th</sup> day of age, the opposite trend was observed in favour of the Middle Rhodope lambs. The intensity of growth in Karakachan lambs decreased to 0,215 kg/day, while in the Middle Rhodope lambs it increased to 0,331 kg/day (**Figure 6**), the difference being proved with a high level of significance ( $P \leq 0,001$ ).



The analysis of the trait live weight on the 70<sup>th</sup> day of age shows that the intensity of growth in Karakachan lambs slightly slowed down, as in females, and lambs reached a live weight of 17,769 kg, while in the Middle Rhodope lambs it significantly decreased and lambs reached 19,257 kg ( $P \leq 0,01$ ). Despite the high level of significance of the difference between breeds (expressed as a percentage of 7,73%), it is the lowest for all observed periods and for all groups. According to the indicator of growth intensity from birth to the 70<sup>th</sup> day of age, the growth intensity of the female Karakachan lambs slightly decreased from 0,215 kg/day to 0,211 kg/day, and of the Middle Rhodope lambs it decreased significantly from 0,331 kg/day to 0,218 kg/day (**Figure 6**). The difference between breeds in terms of the average daily gain from birth to the 70<sup>th</sup> day of age was within 3,21% in favour of the Middle Rhodope breed, but it was not mathematically proven.

Low values of the coefficients of variation for live weight in all three periods could be noted. For the Middle Rhodope lambs it varied from 7,37% to 17,83%, and for the Karakachan lambs from 13,50 to 23,05%, which is an indication for the equality of the selected groups.

## **2. Influence of biological supplement All-G rich on the lambs of Karakachan sheep breed**



The study on the average daily gain of Karakachan lambs started at an average live weight of 13,167 kg for the control group and 12,833 kg for the experimental group. The live weight results at the beginning of the experiment and after a 60-day fattening period are presented in **Table 8**. Karakachan lambs of the control group showed a higher growth intensity compared to the lambs of experimental group. The average daily gain during the considered period was 0,186 kg for the control group of Karakachan lambs, while in the lambs from the experimental group it was 7,6% lower (0,172 kg). The obtained differences between two groups were not significant.

**Table 8. Live weight at the beginning and at the end of the experiment, absolute and average daily gain of Karakachan lambs for a 60-day fattening period (kg)**

Breed/ group	Traits											
	Live weight at the beginning of the experiment, kg					Live weight at the end of the experiment, kg					Absolute weight gain, kg	Average daily gain, kg/day
	n	$\bar{x}$	$\pm S_x$	Sd	C	n	$\bar{x}$	$\pm S_x$	Sd	C		
Karakachan control group	6	13,167	0,29	0,72	5,47	6	24,300	0,613	1,50	6,18	11,133	0,186
Karakachan experimental group	6	12,883	0,24	0,58	4,51	6	23,200	0,517	1,27	5,46	10,317	0,172
Significance		ns					ns				ns	ns

**Alexieva (1979)** found an average daily gain of 0,200 kg/day in Karakachan lambs for a 65-day fattening period, and **Raichev et al. (1984)** reported 0,180 kg/day average daily gain over a 60-day fattening period of Karakachan lambs. **Cafedzhiev et al. (1992)** obtained 0,183 kg/day average daily gain in lambs of the Karakachan breed when fattening up to 25 kg, and 0,181 kg/day when fattening up to 35 kg. **Yankov (1999)** reported an average daily gain of 0,212 kg/day in male and 0,199 kg/day in female lambs of the Karakachan breed as a result of an experiment to determine the intensity of growth up to the age of 100 days. **Stankov (1999)** found 0,203 kg/day average daily gain in intensive fattening of lambs of the Karakachan breed and 0,156 kg/day in pasture fattening of lambs of the Karakachan breed. **Genkovski (2002)** obtained an average daily gain of 0,205 kg/day in male and 0,190 kg/day in female lambs in his study of growth intensity over a 75-day fattening period in male and female lambs of the Karakachan breed. When fattening Karakachan lambs up to 30 kg live weight, **Boykovski et al. (2005)** reported 0,213 kg/day average daily gain.

The established results for average daily gain in our study (**Table 8**) were higher than those obtained by **Stankov (1999)** – 0,156 kg/day in pasture fattening of Karakachan lambs. They were very close to those obtained by **Alexieva (1979)** - 200 g, **Raychev et al. (1984)** – 180 g, **Kafedzhiev et al. (1992)** – 183 g and **Stankov (1999)** – 203 g, and were lower compared to those obtained by **Yankov (1999)** - 212 g, **Nedelchev et al. (1994)** – 249 g and **Boykovski et al. (2005)** – 213 g.

After the 24-hour fasting period, the pre-slaughter live weight of lambs of the control group was 1,69% higher than that of the experimental group. The same tendency between the groups was observed for the trait weight of the chilled carcass, a 5,34% higher value of the trait was calculated in favour of the control group. The highest difference was calculated for the weight of the hot carcass, 6,27% in favour of the control group. All established differences have not been

mathematically proven. The slaughter yield in the control group of Karakachan lambs (**Table 9**) corresponded to that obtained by **Nedelchev et al. (1994)** – 43,23%, but it was lower than that found by **Kafedzhiev et al. (1992)** – 48,30% and **Vuchkov (2020)** – 50,55% and was higher than that found by **Yankov (1999)** – 37,11% and **Genkovski (2002)** – 39,53%. The results for the slaughter yield in the experimental group of Karakachan lambs fed All-G rich supplement were 1,9% lower than those obtained for the control group, but were higher than those found in the research of **Genkovski (2002)** and **Yankov (1999)**.

**Table 9. Pre-slaughter live weight, hot carcass weight, chilled carcass weight, slaughter yield and chilling losses of lambs of the Karakachan breed**

Breed	n	Pre-slaughter live weight, kg				Hot carcass weight, Kg				Chilled carcass weight, kg				Slaughter yield %	Chilling losses %
		$\bar{x}$	$\pm S_x$	SD	C	$\bar{x}$	$\pm S_x$	SD	C	$\bar{x}$	$\pm S_x$	SD	C		
Karakachan control group	3	21,867	0,48	0,83	3,81	9,647	0,19	0,38	3,39	9,407	0,19	0,32	3,42	44,12	2,49
Karakachan experimental group	3	21,500	0,99	1,71	7,93	9,078	0,56	0,97	10,6	8,930	0,58	1,00	11,2	42,22	1,63
Significance		ns				ns				ns					

Carcass chilling losses represent the amount of water removed from the carcass during chilling, normally ranging for sheep from 2,00 to 2,80% (**James and James, 2002**). Losses during chilling of the carcass were 2,49% for the control group of Karakachan lambs, and 1,63% for the experimental group, so they were lower in the experimental group of lambs compared to the control group. The obtained results for chilling losses were lower than those reported by **Genkovski (2002)** – 3,31 % for Karakachan lambs' carcasses.

All established differences between the values of slaughter traits for both groups have not been mathematically proven, and the addition of 1% biological supplement All-G Rich to the concentrated feed did not significantly affect the change in slaughter traits of Karakachan lambs.

Results of the weight measurements of individual parts of the carcass cut are presented in **Table 10**.

**Table 10. Influence of biological supplement All-G rich on the weight of individual parts and % of the weight of the left half in lambs of the Karakachan breed**

Parts of the cut off	Breed, group								Significance
	Karakachan								
	Control n=3				Experimental n=3				
	$\bar{x}$ , kg	$\pm S_x$	SD	%	$\bar{x}$ , kg	$\pm S_x$	SD	%	
Neck mass, kg	0,300	0,012	0,020	6,64	0,360	0,012	0,020	7,71	**
Shoulder mass, kg	1,967	0,035	0,061	43,51	2,147	0,199	0,345	46,00	ns
Thigh mass, kg	1,680	0,053	0,092	37,17	1,507	0,093	0,162	32,28	ns
Cutlet mass, kg	0,340	0,020	0,035	7,52	0,400	0,042	0,072	8,57	ns
Mass of the loins, kg	0,233	0,013	0,023	5,16	0,227	0,024	0,042	4,86	ns

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\* -  $P \leq 0,001$

The established results of the cut of the left half of the carcass show that the shoulder had the highest weight and the largest relative share of the half in both studied groups (**Table 10**). Lambs from the experimental group showed higher values for this indicator – 2,117 kg, compared to 1,967 kg for the control group, constituting respectively 46% and 43,51% of the weight of the halves. The control group had a 10,3% higher thigh weight (1,680 kg) compared to the experimental group – 1,507 kg, with a relative proportion of the thigh of the weight of the left half of 37,17% and 32,28%, respectively. The results for shoulder mass were similar to the data obtained by **Genkovski (2002)** – 1,967 kg, but they were lower for the thigh mass indicator - 2,995 kg (**Genkovski, 2002**) and 3,080 kg (**Yankov, 1999**).

According to **Pilar et al. (2002)** factors such as genetics, nutrition, slaughter weight and gender are responsible for differences in cut off weight between individual carcasses. **Markova (2020)** found that the relative share of the thigh in autochthonous Koprivshitsa and Middle Stara planina lambs was higher than that of the shoulder, regardless of gender, while the experiment with lambs from the autochthonous Karakachan breed revealed the opposite trend - the relative share of the shoulder was higher than that of the thigh.

The data obtained for the neck mass indicator of 0,300 kg in the control group of Karakachan lambs was significantly lower by 16,67% compared to 0,360 kg found for the experimental group ( $P \leq 0,01$ ). A slight preponderance of the experimental group was also observed in the mass of the cutlet, 0,400 kg against 0,340 kg for the control group, which is a 15% difference, but it was not mathematically proven. The results for the mass of the loins in both groups were almost the same with a slight advantage of the control group compared to the experimental group.

The data from the linear measurements of the left half are shown in **Table 11**. The linear measurements of the left halves of the control and experimental groups were characterized by a low significant difference ( $P \leq 0,05$ ) in terms of the thigh girth indicator (in the middle) in favour of the experimental group with 14,11%.

**Table 11. Influence of biological supplement All-G rich on the linear measurements of the left half in lambs of the Karakachan breed**

Linear measurements	Breed, group								Significance	
	Karakachan									
	Control n=3				Experimental n=3					
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C		
Carcass length, cm	50,00	0,58	1,00	2,00	50,50	1,26	2,18	4,32	ns	
Thigh length, cm	33,50	0,29	0,50	1,49	33,33	0,73	1,26	3,77	ns	
Thigh girth, cm	Widest	33,83	0,67	1,15	3,41	33,83	1,92	3,33	9,84	ns
	In the middle	24,33	0,44	0,76	3,14	28,33	1,20	2,08	7,35	*
Chest width, cm 5th thoracic	17,00	-	-	0,00	16,83	0,93	1,61	9,55	ns	

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\* -  $P \leq 0,001$

No significant differences were found for the other indicators between groups. The carcass length (50,0 cm for the control and 50,5 cm for the experimental group) was lower in both groups than that found in the studies of **Yankov (1999)** – 59,5 cm and **Vuchkov (2020)** – 54,0 cm. Thigh length did not differ significantly between groups, being 33,5 cm in the control group and 33,3 cm

in the experimental group, respectively. The thigh length measured by us was higher than those found by **Yankov (1999)** – 29,83 cm, **Boykovski et al. (2005)** – 30,7 cm and **Vuchkov (2020)** – 29,66 cm. According to the thigh girth indicator, the values measured by us corresponded to those measured in the studies of **Boykovski et al. (2005)** and **Vuchkov (2020)**. The remaining indicators did not change significantly in the lambs of Karakachan breed. The coefficients of variation were lower than 9,84%, which was an indicator of the equality within the groups.

## 2.1. Influence of biological supplement All-G rich on the lambs of Middle Rhodope sheep breed

The experiment started at an average live weight of 14,150 kg and the results obtained after a 60-day fattening period are presented in **Table 12**.

**Table 12. Live weight at the beginning and at the end of experiment, absolute and average daily gain for a 60-day fattening period (kg)**

Breed/ group	Traits											
	Live weight at the beginning of experiment, kg					Live weight at the end of experiment, kg					Absolute weigh gain, kg	Average daily gain, kg
	n	$\bar{x}$	$\pm Sx$	Sd	C	n	$\bar{x}$	$\pm Sx$	Sd	C		
Middle Rhodope control	6	12,933	0,112	0,27	2,11	6	24,833	0,385	0,94	3,80	11,900	0,198
Middle Rhodope experimental	6	12,950	0,401	0,98	7,59	6	23,333	0,421	1,03	4,42	10,383	0,173
Significance		ns					*				**	**

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The control group achieved a 1,500 kg higher average live weight at the end of the experiment compared to the experimental group. The difference between groups for this trait was proven with the lowest level of significance ( $P \leq 0,05$ ). The difference of 1,5 kg remained for the trait absolute gain as well, with the control group achieving a 12,75% higher absolute gain compared to the experimental group. The control group achieved a 12,63% significantly ( $P \leq 0,01$ ) higher growth intensity of 0,198 kg/day compared to 0,173 kg/day for the experimental group. The coefficient of variation for both groups was low, which was an indicator of their equality. In a study of the productive characteristics of the Middle Rhodope sheep, **Vassilev et al. (2000)** reported 12,955 kg (female) and 14,810 kg (male) live weight of lambs at weaning, respectively. **Marinov (1967)** started an experiment for intensive fattening at two different levels of protein in the rations of first generation lambs of Middle Rhodope x Tsigai for 74 days at an average live weight of the lambs of 13,380 kg. In the same study, the author also calculated the average daily gain in the groups - 0,182 kg and 0,214 kg, and the absolute gain of 13,500 kg in the first and 15,850 kg in the second group of lambs.

**Marinov (1970)** reported that the Middle Rhodope lambs were weaned at 12,970 kg average live weight. In the same study, the author found a strong decrease in growth intensity of 0,038 kg/day in pasture fattening of Middle Rhodope lambs after weaning. Much lower results were established than those in our study, and the author noted in his publication that the lambs

were not getting enough feed. The pre-slaughter live weight of lambs from the control group was 2,32% higher than that of the experimental group of Middle Rhodope lambs. A slight predominance in the weight of the hot carcass was found in the control group compared to the experimental group. The difference in chilled carcass weight between groups decreased to 1,26% in favour of the control group. The results were not mathematically proven. The data on slaughter yield and chilling losses are presented in **Table 13**. The slaughter yield of lambs was 43,14% for the experimental group and 42,44% for the control group. A slight advantage of the experimental group was found, but the difference was less than 1% and not mathematically proven.

**Table 13. Pre-slaughter live weight, hot carcass weight, chilled carcass weight, slaughter yield and chilling losses in lambs from the Middle Rhodope sheep breed**

Breed	n	Pre-slaughter live weight, kg				Hot carcass weight, kg				Chilled carcass weight, kg				Slaughter yield, %	Chilling losses, %
		$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C		
Control	3	23,067	0,86	1,50	6,51	9,790	0,55	0,95	9,75	9,533	0,52	0,91	9,5	42,44	2,62
Experimental	3	22,533	0,29	0,50	2,23	9,720	0,17	0,29	2,97	9,413	0,16	0,28	2,98	43,14	3,16
Significance		NS				NS				NS				NS	NS

It is striking, the reverse trend of losses during chilling of the carcass. We reported a higher percentage of losses in the experimental group, 3,16%, compared to the control group – 2,62%. According to **James and James (2002)**, carcass chilling losses normally range from 2,00 to 2,80% in sheep. **Ivanov (2021)**, in his study of Bulgarian dairy synthetic population (BDSP) and their crosses with specialized meat breeds (Ile de France and Mouton Charolais), reported the highest losses during storage in refrigerated conditions of the crosses of BDSPxMouton Charolais – 3,52%, in second place were lambs from BDSP with 3,06%, and with the lowest losses - animals from BDSPxIle de France (2,71%). Compared with the values in our study, we can conclude that the carcass chilling losses found were within the range for sheep.

The coefficients of variation showed low values from 2,23% to 9,5%, which was an indicator of the equality within the groups of the Middle Rhodope breed. The change in the slaughter traits after adding of the bioactive preparation All-G rich in lambs of the Middle Rhodope breed is shown in **Table 14**.

Comparing the weight of the hot carcass as a percentage of the live weight, we observe a slight difference in favour of the experimental group 43,14 % (9,720 kg), against 42,44 % (9,790 kg) for the control group. In terms of carcass weight after 24<sup>-hour</sup> chilling, compared to the live weight before slaughter, a slight advantage was again observed for the lambs from the experimental group – 41,78% (9,413 kg), while for those from the control group it was 41,33% (9,533 kg). Differences between groups were not mathematically proven.

The weight of the left half was 4,780 kg in the experimental group (21,21 % of the live weight before slaughter) and 4,540 kg in the control group (19,68 % of the live weight before slaughter). The difference of 0,240 kg for this trait between groups was in favour of the experimental group compared to the control group, representing 5,02%, but the result was not mathematically proven.

**Table 14. Influence of biological supplement All-G rich on the slaughter traits in lambs of the Middle Rhodope breed**

Slaughter traits		Breed, group								Significance	
		Middle Rhodope									
		control n=3				experimental n=3					
Measure units		$\bar{x}$ , kg	$\pm Sx$	SD	% of live weight before slaughter	$\bar{x}$ , kg	$\pm Sx$	SD	% of live weight before slaughter		
Live weight immediately before slaughter, kg		23,067	0,87	1,50		22,533	0,29	0,50		NS	
Carcass, kg	hot	9,790	0,55	0,95	42,44	9,720	0,17	0,29	43,14	NS	
	chilled	whole	9,533	0,52	0,91	41,33	9,413	4,78	0,00	41,78	NS
		left half	4,540	0,35	0,61	19,68	4,780	0,16	0,28	21,21	NS
Head, kg	with skin	1,148	0,15	0,26	4,98	1,445	0,08	0,14	6,41	NS	
	skinned	0,802	0,07	0,12	3,48	1,095	0,08	0,15	4,86	*	
	skin	0,347	0,08	0,14	1,50	0,350	0,01	0,02	1,55	NS	
Skin, kg		3,065	0,27	0,47	13,29	2,552	0,05	0,09	11,32	NS	
Liver and lungs, kg	liver	0,458	0,03	0,05	1,99	0,417	0,01	0,02	1,85	NS	
	lungs	0,350	0,06	0,11	1,52	0,397	0,02	0,03	1,76	NS	
Heart, kg		0,133	0,04	0,06	0,58	0,122	0,01	0,02	0,54	NS	
Spleen, kg		0,050	0,00	0,01	0,22	0,055	0,01	0,01	0,24	NS	
Kidneys, kg		0,097	0,02	0,03	0,42	0,093	0,00	0,01	0,41	NS	

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

A low-significant difference ( $P \leq 0,05$ ) was found in terms of the weight of the skinned head in favour of the experimental group – 1,095 kg, compared to the control group – 0,802 kg.

Differences between groups were found in terms of skin and liver weight: 3,065 and 0,458 kg in the control group and 2,552 and 0,417 kg in the experimental group, respectively. The differences were 16,74% and 9% in favour of the control group, but they were not mathematically proven.

For the rest of the slaughter traits, the values by group were close, and the differences were small.

The established results of the cut of the left half in the Middle Rhodope lambs are presented in **Table 15**. A small difference of 4,57% was reported for the neck mass in favour of the control group - 0,307 kg, against 0,293 kg in the experimental group of lambs.

It was established that the largest percentage of the weight of the half belonged to the shoulder, respectively 43,61% in the control group and 47,28% in the experimental group. The value of this indicator was 1,980 kg for the control group, which is less than the mass of the shoulder of the experimental group, respectively 2,260 kg. This represents a 12,39% difference in favour of the experimental group and this result was close to significance  $P \leq 0,05$ . The measured thigh mass in the control group was 1,673 kg (36,86% of the weight of the left half) and in the

experimental group it was 1,707 kg (35,70% of the weight of the half). For thigh mass, the difference between groups was 2% in favour of the experimental group.

**Table 15. Influence of biological supplement All-G rich on the weight of individual parts and % of the weight of the left half in lambs of the Middle Rhodope breed**

Slaughter traits	Breed, group								Significance
	Middle Rhodope								
	control n=3				experimental n=3				
	$\bar{x}$ , kg	$\pm Sx$	SD	%	$\bar{x}$ , kg	$\pm Sx$	SD	%	
Neck mass, kg	0,307	0,027	0,046	6,75	0,293	0,077	0,133	6,14	NS
Shoulder mass, kg	1,980	0,139	0,240	43,61	2,260	0,110	0,191	47,28	NS
Thigh mass, kg	1,673	0,122	0,212	36,86	1,707	0,048	0,083	35,70	NS
Cutlet mass, kg	0,387	0,071	0,122	8,52	0,353	0,007	0,012	7,39	NS
Mass of the loins, kg	0,200	0,012	0,020	4,41	0,247	0,024	0,042	5,16	**

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

We reported an 8,18% difference in favour of the control group compared to the experimental group in terms of the mass of the cutlet.

For the mass of the loins, the results found were 0,200 kg in the control group and 0,247 kg in the experimental group. The difference of 19,02% between groups was highly significant ( $P \leq 0,01$ ), again in favour of the experimental group of lambs.

The data from the studies on the effect of the biological supplement All-G rich in lambs of the Middle Rhodope breed on the linear measurements of the left half are shown in **Table 16**.

**Table 16. Influence of biological supplement All-G rich on the linear measurements of the left half of lambs of the Middle Rhodope breed**

Linear measurements	Breed, group								Significance	
	Middle Rhodope									
	control n=3				experimental n=3					
	$\bar{x}$	$\pm Sx$	SD	C %	$\bar{x}$	$\pm Sx$	SD	C %		
Carcass lenght, cm	51,50	0,29	0,50	0,97	51,17	0,17	0,29	0,56	NS	
Thigh length, cm	34,50	1,50	2,60	7,53	35,00	0,58	1,00	2,86	NS	
Thigh girth, cm	Widest	33,83	0,83	1,44	4,27	32,33	1,20	2,08	6,44	NS
	In the middle	23,33	1,17	2,02	8,66	25,33	2,33	4,04	15,95	NS
Chest width, cm – 5 <sup>th</sup> thoracic	16,83	0,60	1,04	6,18	17,67	0,33	0,58	3,27	NS	

No significant differences were found during conducted research. The highest difference was found for the thigh girth – 33,83 cm for the control group vs 32,33 cm for the experimental group. The difference between groups was 4,43% in favour of the control group. The other measurement with a slight advantage of the control group was that of the carcass length - 51,50 cm for the control group and 51,17 cm for the experimental group. The difference between groups, however, was less than 1% in favour of the control group.

The highest difference in favour of the experimental group was found for the thigh girth (in the middle) - 23,33 cm for the control group vs 25,33 cm for the experimental group, representing a 7,9% difference in favour of the experimental group. For the other two measurements, the differences were smaller, as follows: 4,75 % (when measuring chest width) and 1,43 % (when measuring thigh length), again the predominance was in favour of the experimental group of lambs, but the differences were small and insignificant.

The highest coefficient of variation found referred to the thigh girth measurement (in the middle) in the experimental group – 15,95%, indicating that the group was relatively uniform in terms of this indicator. The remaining coefficients of variation were low and varied from 0,56 to 8,66%, which was an indicator for a high uniformity of the groups according to these indicators.

### 3. Influence of biological supplement All-G rich on the physico-chemical parameters and fatty acid composition of *m. Longissimus* and *m. Semimembranosus*.

#### 3.1. Physico-chemical parameters of the meat

##### 3.1.1. Karakachan breed

**Table 17** presents the results obtained for the influence of biological supplement All-G rich on the chemical composition of the meat.

**Table 17. Influence of biological supplement All-G rich on the chemical composition of the meat of *m. Longissimus* and *m. Semimembranosus* in Karakachan lambs**

Traits name	Muscle	Karakachan								Significance
		Control group, n-3				Experimental group, n-3				
		$\bar{x}$	$\pm S_x$	SD	C	$\bar{x}$	$\pm S_x$	SD	C	
Water, %	LD	75,09	0,36	0,62	0,83	74,66	1,01	1,75	2,35	NS
	SM	74,67	0,46	0,80	1,07	74,89	0,74	1,27	1,70	NS
Lipids, %	LD	2,65	0,56	0,97	36,53	2,99	0,81	1,41	47,05	NS
	SM	2,90	0,39	0,67	23,14	2,25	0,31	0,54	23,99	NS
Mineral substances, %	LD	1,05	0,08	0,14	13,34	1,17	0,06	0,11	9,25	NS
	SM	1,09	0,03	0,06	5,04	1,14	0,04	0,07	5,82	NS
Dry matter, %	LD	24,91	0,36	0,62	2,51	25,34	1,01	1,75	6,92	NS
	SM	25,33	0,46	0,80	3,16	25,11	0,74	1,27	5,08	NS
Protein, %	LD	21,20	0,42	0,72	3,40	21,18	0,27	0,47	2,20	NS
	SM	21,33	0,46	0,80	3,75	21,72	0,57	0,99	4,54	NS

No significant differences were observed in the studied traits during analysis, both between the groups and between the two examined muscles. A small difference in water content was reported between both groups of muscles in the control group - 75,09% was measured in *m.*



*Longissimus dorsi* vs 74,67% in *m. Semimembranosus*. A difference in the lipid content was observed in favour of the control group of lambs - 2,90% vs 2,25% for the experimental group. Differences were also reported for the mineral substances in both muscles, as follows: for the control group of Karakachan lambs in *m. Longissimus dorsi* – 1,05% and in *m. Semimembranosus* - 1,09%, vs the results in the experimental group of lambs - in *m. Longissimus dorsi* – 1,17% and in *m. Semimembranosus* - 1,14%. The reported differences were not mathematically proven. For the rest of the traits, no significant differences were found - the values were close and the differences were small.

The results that we obtained for the water content in meat corresponded to those reported by **Yankov (1999)** – 74,41%, **Genkovski (2002)** – 74,82% and **Boykovski et al. (2005)** – 73,41%, respectively. The protein content in meat in our study corresponded to the those of **Williams (2007)** – 21,9% and **Genkovski (2002)** – 20,55%, but was higher than reported by **Yankov (1999)** – 19,79% and **Boykovski et al. (2005)** – 19,77%. In our study, lipid content was lower than those reported by the other authors: **Yankov (1999)** – 4,7%, **Williams (2007)** – 4,7%, **Boykovski et al. (2005)** – 5,71% and **Genkovski (2002)** – 3,94%.

The obtained results give us a reason to conclude that the addition of 1% All-G rich did not significantly affect the chemical composition of meat in lambs of the Karakachan breed.

The laboratory tests of the technological properties of the samples of *m. Longissimus dorsi* and *m. Semimembranosus* of lambs of the Karakachan breed are shown in **Table 18**.

**Table 18. Influence of biological supplement All-G rich on the chemical and physico-chemical characteristics of meat in *m. Longissimus* of lambs of the Karakachan breed**

Traits		Karakachan								Significance
		Control group KK, n-9				Experimental group KK, n-9				
Name	muscle	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
WHC, %	LD	22,14	1,29	3,88	17,53	22,14	0,99	2,98	13,46	NS
	Sm	20,74	0,83	2,50	12,06	14,97	1,40	4,19	27,96	**
WAC - saline, %	LD	18,15	1,12	3,37	18,56	17,36	1,67	5,01	28,85	NS
	Sm	12,87	1,30	3,90	22,45	17,57	2,04	6,12	34,82	NS
WAC - distilled water, %	LD	12,05	1,13	3,38	28,02	9,57	1,83	5,50	57,43	NS
	Sm	9,66	0,62	1,85	19,10	10,61	2,02	6,07	57,22	NS
pH <sub>24</sub>	LD	5,55	0,01	0,04	0,69	5,53	0,01	0,04	0,72	NS
	Sm	5,54	0,01	0,05	0,96	5,66	0,02	0,07	1,18	***
Tenderness, °P	LD	382,73	7,18	27,81	7,27	299,40	19,40	75,14	25,10	***
	Sm	240,67	10,11	39,16	16,27	280,93	12,81	49,62	17,66	*
Cooking losses (baking), %	LD	48,36	1,43	4,28	8,84	39,24	2,21	6,64	16,91	**
	Sm	41,78	2,05	6,15	14,72	36,84	1,27	3,80	10,32	*

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

One of the important traits characterizing the quality of meat is the ability of the muscles to hold part of the chemically unbound water (WHC), which is directly related to the taste, tenderness and color, and these traits determine the quality of the meat. No difference in water holding capacity of *m. Longissimus dorsi* was observed between groups. In the other muscle group *m. Semimembranosus* significant differences were found ( $P \leq 0,01$ ) in the water-holding capacity of the meat in favour of the control group of lambs compared to the experimental group. An

interesting trend was observed when comparing the two muscle groups, in both the control and experimental groups, the water holding capacity was higher in *m. Longissimus Dorsi*, compared to *m. Semimembranosus*. In the experimental group, a high level of significance was established at  $P \leq 0,01$ . The results obtained for the WHC of meat were close to those reported by **Yankov (1999)** – 27,79%, **Genkovski (2002)** – 24,42% and **Boykovski et al. (2005)** – 27,75%. The results obtained were lower than the water holding capacity found by **Kafedzhiev et al. (1992)** – 35,92 % for *m. Longissimus Dorsi* and 36,73% for *m. Semimembranosus*, and by **Nedelchev et al. (1994)** – 39,82% for *m. Longissimus Dorsi* in Karakachan lambs. In the estimation of the water absorption capacity of meat (WAC) using physiological solution, the differences between the control and the experimental groups were small and insignificant.

After analyzing the samples from both groups of muscles, the water absorption capacity of *m. Longissimus Dorsi*, when using physiological solution was significantly higher ( $P \leq 0,01$ ) compared to that of *m. Semimembranosus* in the control group of lambs. When using distilled water instead of saline, no significant differences were observed for WAC, both between groups and between studied muscle groups.

According to the methodology used by **Pinkas and Marinova (1984)**, the quality of meat is optimal or very good at a pH of 5,5 to 5,90, average - at a pH of 6,0 to 6,6, and poor - at a pH of higher than 6,6. The mean values of pH<sub>24h</sub> post mortem found in our study for both muscle groups ranged from 5,53 to 5,66. The obtained results can be defined as optimal, predetermining the quality of meat as very good, according to the indicated methodology.

A highly significant difference was found ( $P \leq 0,001$ ) between the control and experimental groups when examining the pH of *m. Semimembranosus*. The obtained results for the control group were pH<sub>24</sub> - 5,54, and for the experimental - pH<sub>24</sub>-5,66, a difference of 2% was in favour of the experimental group. When examining the meat samples from *m. Longissimus Dorsi* no differences were found between the control and experimental groups.

Our results were higher than those established by **Nedelchev et al. (1994)** for pH<sub>24</sub> – 5,32 in samples from *m. Longissimus Dorsi* in purebred Karakachan lambs and pH<sub>24</sub> – 5,42 in F1 crosses of Karakachan\*Dreisdel. They corresponded with the results obtained by **Boykovski et al. (2005)** for pH<sub>24</sub> – 5,64 and **Genkovski (2002)** for pH<sub>24</sub> – 5,75, but were lower than those reported by **Yankov (1999)** for pH<sub>24</sub> – 5,83.

The tenderness of *m. Longissimus Dorsi* decreased significantly ( $P \leq 0,001$ ) by 28% in the experimental group, while of *m. Semimembranosus* it increased significantly ( $P \leq 0,05$ ) by 17% in the experimental vs control group.

Technological processing of the meat to a ready-to-eat product led to significantly higher losses in both muscles in the control group compared to the experimental group. Cooking losses (after baking) in *m. Longissimus Dorsi* were significantly higher ( $P \leq 0,01$ ) for the control group compared to the experimental group. The same trend of significantly higher cooking losses was found for *m. Semimembranosus* in the control group compared to the experimental group, but the difference has a lower level of significance ( $P \leq 0,05$ ). Our results corresponded to those obtained by **Boykovski et al. (2005)** - 45% and **Ivanov (2021)** - from 41,75% to 45,02% in both muscles.

### 3.1.2. Middle Rhodope breed

The water content in *m. Longissimus Dorsi* in the experimental group decreased slightly compared to the control group, while in *m. Semimembranosus* this result did not change significantly. For lipids, no substantial differences were found in *m. Longissimus Dorsi* between

groups. The analysis of *m. Semimembranosus* showed that fat increased significantly ( $P \leq 0,01$ ) in the control group and was 4,02 %, while in the experimental group it was 2,60 %. The experimental group accumulated a higher amount of mineral substances in both groups of muscles compared to the control group of lambs (**Table 19**). Close to significance ( $P \leq 0,05$ ) was the difference between groups in samples of *m. Semimembranosus* in terms of mineral content in favour of the experimental group.

**Table 19. Chemical composition of the meat in *m. Longissimus* and *m. Semimembranosus* of Middle Rhodope lambs**

Traits Name	Muscle	Middle Rhodope								Significance
		Control group MR, n-3				Experimental group MR, n-3				
		$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
Water, %	LD	76,28	1,01	1,75	2,30	75,05	0,38	0,67	0,89	NS
	SM	74,03	0,32	0,56	0,75	74,35	0,21	0,36	0,48	NS
Lipids, %	LD	2,99	0,16	0,29	9,54	2,93	0,19	0,34	11,42	NS
	SM	4,02	0,24	0,41	10,14	2,60	0,22	0,39	14,85	**
Mineral substances, %	LD	1,19	0,04	0,07	5,74	1,21	0,03	0,05	4,23	NS
	SM	1,00	0,05	0,08	8,17	1,11	0,02	0,03	2,38	NS
Dry matter, %	LD	23,72	0,70	1,21	5,11	24,95	0,38	0,67	2,67	NS
	SM	25,97	0,32	0,56	2,15	25,65	0,21	0,36	1,39	NS
Protein, %	LD	19,54	0,88	1,53	7,81	20,80	0,31	0,53	2,57	NS
	SM	20,95	0,46	0,80	3,80	21,94	0,41	0,70	3,21	NS

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\* -  $P \leq 0,001$

No significant differences were found for the other studied traits.

The results of the study on the physico-chemical characteristics of the samples are shown in **Table 20**. The WHC of both types of muscles decreased in the experimental group compared to the control group, and statistically significant changes were found in *m. Semimembranosus* ( $P \leq 0,05$ ).

The WAC of both muscle groups after using saline increased significantly ( $P \leq 0,01$ ) in the experimental group compared to the control group. When studying the same trait using distilled water, a slight advantage of the experimental group over the control group was observed in both muscles, but the differences were not mathematically proven. The mean values of pH<sub>24h</sub> post mortem, for both groups in both muscles, ranged from 5,50 to 5,59. They can be defined as optimal, predetermining the quality of meat as very good, according to the classification that we use (**Pinkas and Marinova, 1984**).

The measurements of pH<sub>24h</sub> post mortem revealed a significant difference between the groups ( $P \leq 0,001$ ) in favour of the experimental group for this characteristic. For *m. Longissimus Dorsi* in the control group pH<sub>24</sub> - 5,50 was measured, and in the experimental group it was pH<sub>24</sub> - 5,56. In the other group of muscles *m. Semimembranosus*, the same trend was observed, but with a lower level of significance ( $P \leq 0,01$ ). The predominance was again in favour of the experimental group pH<sub>24</sub>-5,59, and in the control group it was pH<sub>24</sub>-5,51.

The tenderness of *m. Longissimus Dorsi* decreased by 12,5% in the experimental group compared to the control group, but the difference was not mathematically proven. While in the other group of muscles *m. Semimembranosus* it decreased significantly ( $P \leq 0,001$ ) by 23,8% in the experimental compared to the control group.

The technological processing of the meat to a ready-to-eat product led to significantly higher cooking losses in *m. Longissimus Dorsi* ( $P \leq 0,001$ ) and *m. Semimembranosus* ( $P \leq 0,01$ ) for the control group compared to the experimental group.

**Table 20. Physico-chemical characteristics of the meat in *m. Longissimus* of lambs of the Middle Rhodope breed**

Traits		Middle Rhodope								Significance
		Control group MR, n-9				Experimental group MR, n-9				
Name	muscle	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
WHC, %	LD	17,75	1,85	5,54	31,20	15,79	1,81	5,44	34,45	NS
	Sm	20,22	1,11	3,32	16,42	13,51	2,42	7,25	53,67	*
WAC - saline, %	LD	17,24	0,88	2,63	15,25	20,62	0,64	1,93	9,37	**
	Sm	16,75	1,00	3,00	17,90	20,90	0,73	2,19	10,47	**
WAC - distilled water, %	LD	13,04	1,55	4,64	35,56	13,74	0,85	2,55	18,54	NS
	Sm	13,72	0,82	2,47	18,03	16,79	1,45	4,36	25,97	NS
pH <sub>24</sub>	LD	5,50	0,01	0,03	0,52	5,56	0,01	0,06	1,01	***
	Sm	5,51	0,02	0,08	1,46	5,59	0,03	0,11	1,88	**
Tenderness, °P	LD	385,80	6,71	25,97	6,73	342,87	21,76	84,26	24,58	NS
	Sm	275,80	9,41	36,46	13,22	212,13	12,56	48,64	22,93	***
Cooking losses (baking), %	LD	47,86	1,24	3,72	7,78	40,92	1,07	3,22	7,86	***
	Sm	38,54	1,56	4,69	12,17	31,00	1,51	4,53	14,60	**

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

### 3.2. Fatty acid composition of the meat

#### 3.2.1. Karakachan breed

The fatty acid composition of the *m. Longissimus Dorsi* muscle of lambs of the Karakachan breed is presented in **Table 21**. The use of supplement All-G Rich during fattening of lambs led to a slight increase in lauric, myristic, palmitic and linoleic fatty acids. Stearic fatty acid increased with a low level of significance ( $P \leq 0,05$ ) compared to the control group, while oleic acid decreased significantly ( $P \leq 0,01$ ) in the experimental group compared the control group. The addition of All-G rich in the diet of Karakachan lambs resulted in a significant increase of docosahexaenoic fatty acid from 0,27 to 0,50 g/100g fat ( $P \leq 0,01$ ), while in decosapentaenoic acid the increase was insignificant from 0,24 to 0,34 g/100g fat.

In their study, **Gerchev et al. (2018)** reported the fatty acid composition of milk from Tsigai and Karakachan sheep and of meat of their lambs F1 crosses with Awassi. The authors obtained similar to our results for the fatty acid composition of Karakachan lamb meat - for Palmitic acid (C16:0) – 27,89 g/100 g, and higher results for Oleic acid (C18:1) – 44,22 g/ 100 g, Linoleic acid (C18:2) – 6,70 g/100 g and Arachinic acid (C20:0) – 0,76 g/100 g. The authors obtained lower results for Myristic acid (C14:0) – 2,61 g/100 g and Stearic acid (C18:0) – 11,57 g/100 g.

**Table 21. Fatty acid composition of *m. Longissimus Dorsi* of Karakachan lambs in g/100 g of fat**

Fatty acids	Control group KK, n-3				Experimental group KK, n-3				Significance
	$\bar{x}$	$\pm S_x$	SD	C	$\bar{x}$	$\pm S_x$	SD	C	
C-10:0	0,39	0,04	0,11	27,60	0,35	0,02	0,04	11,14	NS
C-12:0	0,75	0,00	0,01	0,69	0,78	0,03	0,06	8,27	NS
C-14:0	4,20	0,22	0,53	12,58	4,39	0,14	0,34	7,72	NS
C-14:1	0,19	0,02	0,05	24,91	0,24	0,02	0,04	17,13	NS
C-15:0	0,77	0,03	0,07	8,53	0,74	0,03	0,07	10,11	NS
C-16:0	27,44	0,51	1,25	4,54	28,01	0,43	1,05	3,74	NS
C-16:1	1,77	0,03	0,08	4,49	1,77	0,05	0,12	6,77	NS
C-17:0	1,39	0,05	0,13	9,68	1,31	0,05	0,12	8,80	NS
C-17:1	0,40	0,03	0,07	16,62	0,36	0,02	0,04	10,83	NS
C-18:0	17,30	0,14	0,35	2,04	18,61	0,44	1,08	5,80	*
C-18:1	40,34	0,50	1,23	3,05	38,48	0,27	0,65	1,70	**
C-18:2	3,85	0,08	0,18	4,80	4,00	0,25	0,60	15,09	NS
C-18:3	0,86	0,04	0,09	10,56	0,87	0,04	0,09	9,84	NS
C-20:0	0,40	0,03	0,08	18,86	0,56	0,02	0,06	10,33	NS
C-22:5n3	0,24	0,01	0,01	6,01	0,34	0,06	0,15	43,38	NS
C-22:6n3	0,27	0,02	0,06	20,81	0,50	0,04	0,11	21,94	**

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\* -  $P \leq 0,001$

The total content of saturated fatty acids increased with a low level of significance ( $P \leq 0,05$ ) (**Table 22**), while polyunsaturated fatty acids showed a slight increase in their content on the account of a low significant decrease ( $P \leq 0,05$ ) in the concentration of monounsaturated fatty acids in the experimental group compared to the control group of lambs of the Karakachan breed. The ratio between Omega-6 and Omega-3 fatty acids decreased by 16,37% in *Longissimus Dorsi* muscle samples of the experimental group and was getting closer to the ratio that would have a healthy effect on human nutrition.

It is noteworthy that **Gerchev et al. (2018)** reported an Omega-6/Omega-3 ratio of 8,21 in meat from Karakachan crossbred lambs, while in the supplemented Karakachan breed lambs in our study it was significantly lower than 2,35. In their study on fatty acids by group in meat from Karakachan F1 crosses \* Awassi, **Gerchev et al. (2018)** found SFA 42,07 g/100 g fat, MUFA – 44,22 g/100 g fat and PUFA – 10,32 g/100 g fat in *m. Longissimus Dorsi*.

**Table 22. Total content of fatty acids in m. Longissimus Dorsi of Karakachan lambs in g/100 g of fat**

Fatty acids	Control group KK, n-3				Experimental group KK, n-3				Significance
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
<i>SFA</i>	52,65	0,64	1,56	2,97	54,75	0,36	0,89	1,63	*
<i>MUFA</i>	42,69	0,53	1,30	3,05	40,85	0,27	0,65	1,60	*
<i>PUFA</i>	5,22	0,10	0,25	4,84	5,70	0,27	0,66	11,56	NS
$\Sigma n-3$	1,37	0,02	0,06	11,48	1,70	0,07	0,18	21,86	NS
$\Sigma n-6$	3,85	0,10	0,25	5,27	4,00	0,28	0,68	14,00	NS
$\Sigma n-6/\Sigma n-3$	2,81	0,52	1,28	13,68	2,35	0,80	1,96	31,81	NS

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The fatty acid composition of the *Semimembranosus* muscle in lambs of the Karakachan breed is presented in **Table 23**. The use of All-G Rich supplement during fattening of lambs led to a slight increase in lauric, myristic, oleic and docosopentaenoic acids, while capric and docosahexaenoic fatty acids increased significantly ( $P \leq 0,05$ ). The remaining fatty acids decreased in the Karakachan lambs of the experimental group. Biologically active fatty acids did not have particular changes. Oleic acid decreased from 41,73 to 40,67 g/100g fat, while linoleic and linolenic acids increased from 4,10 to 5,23 g/100g fat and from 0,94 to 1,06 g/100g fat, respectively.

Our results corresponded with those obtained by **Abril et al. (2000)** and **Boeckert et al. (2007b)**. The authors concluded that supplementing feed with additives such as *Schizochytrium spp* microalgae increased the DHA content of the feed and therefore feeding animals with the supplemented feed could lead to enrichment in ruminant tissues. This may be due to the limited biohydrogenation of these fatty acids in the digestive system of ruminants fed these feeds (**Maia et al., 2007**).

**Hopkins et al. (2014)**, when treating lambs with 2% DM microalgae of the *Schizochytrium* species, obtained similar data to those obtained in the present study. The authors found a significantly higher content of EPA+DHA in meat and lamb could be considered a "good source" of Omega-3, but the concentration of vitamin E decreased after the addition of algae. This was an indicator of increased lipid peroxidation, but the color of the meat did not change and the expiry date for consumption was not reduced.

**Table 23. Fatty acid composition of *m. Semimembranosus* in lambs of the Karakachan breed in g/100 g of fat**

Fatty acids	Control group KK,				Experimental group KK,				Significance
	n-3				n-3				
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	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	NS
C-14:0	3,40	0,03	0,07	2,17	2,64	0,34	0,83	31,25	NS
C-14:1	0,32	0,00	0,01	3,74	0,29	0,09	0,23	77,18	NS
C-15:0	0,68	0,01	0,03	4,58	0,64	0,07	0,16	25,28	NS
C-16:0	25,94	0,96	2,36	9,10	23,64	0,35	0,87	3,68	NS
C-16:1	1,98	0,15	0,36	18,40	1,37	0,31	0,76	55,92	NS
C-17:0	0,96	0,01	0,03	3,18	1,87	0,31	0,77	40,90	NS
C-17:1	1,18	0,01	0,03	2,17	1,21	0,04	0,10	8,61	NS
C-18:0	17,32	0,66	1,61	9,29	17,91	1,12	2,74	15,31	NS
C-18:1	41,73	0,03	0,08	0,19	40,67	1,62	3,97	9,77	NS
C-18:2	4,10	0,11	0,28	6,84	5,23	0,25	0,61	11,73	NS
C-18:3	0,94	0,04	0,09	9,55	1,06	0,03	0,07	6,56	NS
C-20:0	0,36	0,06	0,15	41,71	1,04	0,17	0,41	39,89	NS
C-22:5n3	0,14	0,01	0,02	13,46	0,34	0,07	0,16	48,50	NS
C-22:6n3	0,05	0,01	0,01	28,32	0,27	0,03	0,06	23,26	*

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The total content of saturated and monounsaturated fatty acids slightly decreased (**Table 24**), while polyunsaturated fatty acids increased significantly ( $P \leq 0,05$ ) in the experimental compared to the control group of Karakachan lambs. The use of supplement resulted in a decrease in the ratio of Omega-6 to Omega-3 fatty acids ( $P \leq 0,05$ ) in the *Semimembranosus* muscle samples of the experimental group.

The use of 1% of bioactive supplement All G - Rich in the feed of lambs led to a decrease in the ratio between Omega-6 and Omega-3 fatty acids in the samples taken from the Karakachan breed, as in *m. Longissimus Dorsi* the difference between the groups was 16,37% in favour of the experimental group, and for *m. Semimembranosus* low significant difference of 15,97% was reported again in favour of the experimental group.

**Table 24. Total content of fatty acids in m. Semimembranosus of lambs of the Karakachan breed in g/100 g of fat**

Fatty acids	Control group KK,				Experimental group KK,				Significance
	n-3				n-3				
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
SFA	49,33	0,16	0,40	0,82	48,46	1,67	4,10	8,46	NS
MUFA	45,21	0,27	0,66	1,41	43,54	1,46	3,57	8,21	NS
PUFA	5,23	0,09	0,22	4,25	6,90	0,25	0,61	8,78	*
$\Sigma n-3$	1,13	0,02	0,06	5,11	1,67	0,06	0,16	9,31	NS
$\Sigma n-6$	4,10	0,08	0,19	3,76	5,23	0,24	0,59	9,44	NS
$\Sigma n-6/\Sigma n-3$	3,63	0,16	0,40	8,87	3,13	0,22	0,54	14,22	*

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

Meale et al. (2014), after conducting a fattening experiment with Canadian Arcott lambs at three different levels of concentration of *Schizochytrium spp.* Microalgae added to the concentrate, concluded that supplementation of DHA-rich feed with microalgae did not affect fattening and meat quality of lambs, as in our study. The authors proved that supplementation increased EPA and DHA concentration in meat, supporting our initial hypothesis that the inclusion of microalgae in lamb concentrate feed would change the fatty acid composition of the meat. Moreover, supplementation of DHA-rich foods increased the total Omega-3 content in all tissue types and therefore caused a decrease in the n-6:n-3 ratio.

### 3.2.2. Middle Rhodope breed

The fatty acid composition of *Longissimus Dorsi* muscle of lambs of the Middle Rhodope breed is presented in **Table 25**. The nutritional supplement All-G Rich used during fattening of lambs led to a significant decrease in capric ( $P \leq 0,05$ ), palmitic ( $P \leq 0,001$ ), palmitoleic ( $P \leq 0,01$ ), margaric acids ( $P \leq 0,05$ ) in the experimental group compared to the control group and also to a slight decrease in the concentration of lauric acid, on the account of significant increase in myristinoleic ( $P \leq 0,001$ ) and pentadecic ( $P \leq 0,001$ ) acids. Oleic and linoleic fatty acids increased significantly ( $P \leq 0,01$ ) in the experimental group compared to the control group. Docosahexaenoic acid increased significantly from 0,27 to 0,54 g/100g fat ( $P \leq 0,05$ ) as a result of All-G rich supplementation, while decosapentaenoic acid increased insignificantly from 0,35 to 0,47g/100g fat.

Accumulation of DHA and EPA in the meat of lambs fed 3% DM DHA-G (*Schizochytrium spp.*) in a 100 g portion of lamb could provide 21,4% of the recommended daily intake level for humans. The authors concluded that dietary supplements of algae, added up to 3% of the



concentrate feed, can contribute to a substantial increase in DHA and EPA in meat, and consumption of such enriched meat can serve to meet our daily needs for DHA and EPA.

**Table 25. Fatty acid composition of *m. Longissimus Dorsi* in lambs of the Middle Rhodope breed in g/100 g of fat**

Fatty acids	Control group MR, n-3				Experimental group MR , n-3				Significance
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
C-10:0	0,41	0,06	0,16	37,63	0,23	0,02	0,05	21,11	*
C-12:0	0,72	0,06	0,16	21,84	0,60	0,01	0,03	4,53	NS
C-14:0	4,67	0,02	0,04	0,80	4,65	0,26	0,64	13,73	NS
C-14:1	0,04	0,00	0,01	29,81	0,21	0,01	0,03	16,39	***
C-15:0	0,08	0,01	0,01	16,89	0,73	0,00	0,01	1,23	***
C-16:0	27,99	0,30	0,74	2,64	25,73	0,19	0,48	1,85	***
C-16:1	1,91	0,10	0,24	12,64	1,55	0,05	0,12	7,50	**
C-17:0	1,39	0,04	0,09	6,49	1,27	0,03	0,08	6,14	*
C-17:1	0,44	0,03	0,07	15,35	0,42	0,03	0,07	16,08	NS
C-18:0	18,14	0,23	0,57	3,16	18,19	0,60	1,46	8,02	NS
C-18:1	38,84	0,45	1,11	2,85	40,88	0,50	1,23	3,02	**
C-18:2	3,45	0,14	0,34	9,96	4,24	0,22	0,53	12,48	**
C-18:3	0,89	0,02	0,05	5,14	0,90	0,02	0,04	4,33	NS
C-20:0	0,35	0,04	0,09	25,56	0,46	0,02	0,06	12,14	NS
C-22:5n3	0,35	0,04	0,09	26,44	0,47	0,03	0,07	15,47	NS
C-22:6n3	0,27	0,02	0,06	28,15	0,54	0,12	0,29	53,75	*

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

In a study of the local Middle Stara planina breed and Koprivshitsa breed **Markova (2020)** found a lower content of capric acid (C10:0) – 0,160 g/100g fat in the Middle Stara planina breed (MSB) and 0,165 g/100g fat in the Koprivshitsa breed (KB), lauric acid (C12:0) – 0,340 g/100g fat in MSB and 0,380 g/100g fat in KB and stearic acid (C18:0) – 15,450 g/100g fat in MSB and 14,306 g/100g fat in KB. The results were similar for myristic acid (C14:0) – 4,45 g/100g fat in KB, palmitic acid (C16:0) – 25,410 g/100g fat in MSB and 25,640 g/100g fat in KB and arachidic acid (C20:0) – 0,410 g/100g fat in KB. Higher results were found for the pentadecanoic acid (C15:0) – 0,547 g/100g fat in MSB and 0,597 g/100g fat in KB and margaric acid (C17:0) – 1,620 g/100g fat in MSB and 1,634 g/100g fat in KB.

Significance of results ( $P \leq 0,001$ ) was found for saturated fatty acids, which decreased in the experimental group, and as a result mono ( $P \leq 0,05$ ) and polyunsaturated ( $P \leq 0,01$ ) fatty acids increased significantly. The ratio between Omega-6 and Omega-3 fatty acids decreased by 7,66% in the samples of *Longissimus Dorsi* muscle in favour of the experimental group of lambs of the Middle Rhodope breed (**Table 26**). **Markova (2020)** found similar results for PUFA – 5,34 g/100 g fat for MSB and 6,25 g/100 g fat for KB. A lower content of SFA – 53,52 g/100 g fat in MSB and 52,06 g/100 g fat in KB and a higher content of MUFA – 48,18 g/100 g fat in MSB and 6,25 g/100 g of fat in KB could be noted.

**Table 26. Total content of fatty acids in *m. Longissimus Dorsi* of lambs of the Middle Rhodope breed in g/100 g of fat**

Fatty acids	Control group MR,				Experimental group MR,				Significance
	n-3				n-3				
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
<b>SFA</b>	53,76	0,22	0,54	1,01	51,86	0,29	0,72	1,39	***
<b>MUFA</b>	41,22	0,42	1,02	2,47	43,06	0,45	1,11	2,57	*
<b>PUFA</b>	4,90	0,16	0,40	8,17	6,15	0,31	0,75	12,18	**
$\Sigma n-3$	1,44	0,06	0,14	26,16	1,91	0,12	0,29	28,69	NS
$\Sigma n-6$	3,45	0,12	0,30	6,86	4,24	0,20	0,49	9,63	NS
$\Sigma n-6/\Sigma n-3$	2,39	0,66	1,62	19,65	2,22	0,59	1,44	26,62	NS

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The ratio  $\Sigma n-6/\Sigma n-3$  of *m. Longissimus Dorsi* in both experimental groups of lambs was lower compared to that of the control groups, respectively for the Karakachan lambs it was 2,81 vs. 2,35 and for the Middle Rhodope lambs – 2,39 vs. 2,22. This gives us a reason to conclude that the addition of a minimal amount of the bioactive supplement All G – Rich improves meat quality and reduces the ratio of  $\Sigma n-6/\Sigma n-3$ . According to the **English Health Department (1994)**, the recommended ratio of  $\Sigma n-6/\Sigma n-3$  should be less than 5, in our study the two breeds used in the experiment had a ratio of  $\Sigma n-6/\Sigma n-3$ , which was lower than 5, and the addition of even just 1% of the bioactive supplement All G – Rich further reduced the ratio and brought us closer to the limit defined as healthy by the **English Health Department (1994)**.

Similar results were obtained by **Elmor et al. (2005)** in Suffolk crosses supplemented microalgae meal and fish oil in addition to the concentrated feed. The authors reported an increased content of omega-3 fatty acids in *m. Longissimus Dorsi* and reduced ratio of Omega-6/Omega-3 fatty acids in the supplemented lambs.

The fatty acid composition of the *Semimembranosus* muscle in lambs of the Middle Rhodope breed is presented in **Table 27**. The nutritional supplement All-G Rich used in the fattening of lambs led to a slight decrease in capric, lauric, palmitoleic, linoleic and linolenic fatty acids. Oleic, decosapentaenoic and docosahexaenoic acids slightly increased.

**Table 27. Fatty acid composition of *m. Semimembranosus* in lambs of the Middle Rhodope breed in g/100 g of fat**

Fatty acids	Control group MR,				Experimental group MR,				Significance
	n-3				n-3				
	$\bar{x}$	$\pm Sx$	SD	C	$\bar{x}$	$\pm Sx$	SD	C	
C-10:0	0,41	0,03	0,08	19,26	0,46	0,01	0,03	6,30	NS
C-12:0	0,19	0,04	0,11	57,94	0,29	0,05	0,12	41,88	NS
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	NS
C-15:0	0,61	0,06	0,15	25,21	0,63	0,03	0,07	11,76	NS
C-16:0	24,05	0,30	0,72	3,01	23,79	0,47	1,16	4,88	NS
C-16:1	1,81	0,27	0,66	36,62	1,61	0,32	0,79	49,45	NS
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	NS
C-18:0	18,48	0,87	2,13	11,54	19,55	1,53	3,75	19,16	NS
C-18:1	41,02	1,32	3,24	7,90	42,03	1,09	2,68	6,37	NS
C-18:2	5,74	1,20	2,95	51,35	3,80	0,35	0,86	22,54	NS
C-18:3	0,94	0,09	0,22	23,51	0,88	0,02	0,06	6,78	NS
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	NS
C-22:6n3	0,05	0,01	0,03	60,09	0,10	0,03	0,07	64,62	NS

\* -  $P \leq 0,05$ , \*\* -  $P \leq 0,01$ , \*\*\*-  $P \leq 0,001$

The total content of saturated and monounsaturated fatty acids in the studied *Semimembranosus* muscle in lambs from the experimental group of the Middle Rhodope breed was higher than this of the control group on the account of polyunsaturated fatty acids (**Table 28**).

The ratio between Omega-6 and Omega-3 fatty acids decreased by 38,78% in the experimental group of lambs of the Middle Rhodope breed (**Table 28**), but the differences were not mathematically proven.

**Table 28. Total content of fatty acids in *m. Semimembranosus* in lambs of the Middle Rhodope breed in g/100 g of fat**

<i>Fatty acids</i>	<b>Control group MR,</b>				<b>Experimental group MR,</b>				<b>Significance</b>
	<b>n-3</b>				<b>n-3</b>				
	$\bar{x}$	$\pm Sx$	<b>SD</b>	<b>C</b>	$\bar{x}$	$\pm Sx$	<b>SD</b>	<b>C</b>	
<i>SFA</i>	48,07	0,65	1,59	3,30	50,09	1,09	2,67	5,33	NS
<i>MUFA</i>	44,34	1,03	2,53	5,70	45,17	0,97	2,38	5,26	NS
<i>PUFA</i>	6,94	1,26	3,08	44,34	5,10	0,25	0,62	12,22	NS
$\Sigma n-3$	1,20	0,05	0,13	11,19	1,30	0,10	0,24	18,37	NS
$\Sigma n-6$	5,74	1,29	3,16	47,40	3,80	0,34	0,82	17,58	NS
$\Sigma n-6/\Sigma n-3$	4,77	0,79	1,94	35,89	2,92	0,53	1,31	34,90	NS

## V. SUMMARY

The Middle Rhodope and Karakachan breeds belong to the gene pool of Bulgarian local autochthonous breeds with a population of less than 11,000. In order to achieve the goals set in the study, the main productive characteristics of the two breeds were investigated. The obtained results can be the basis for the further selection activity within the breeds.

The main criteria in the implementation of the future selection activity is the preservation of the typicality of the animals. Therefore, the search for innovative approaches to increase the income from breeding local breeds should be the driving force of scientific work with both breeds.

As shown in the present thesis, the use of 1% additive in the concentrate feed used in our study resulted in a significant improvement in the ratio of Omega 6 to Omega 3 fatty acids. In this regard, a study and analysis of the productive characteristics and meat qualities of the Middle Rhodope and Karakachan breeds was carried out, as well as a modern assessment of the factors affecting the quality indicators of meat was made.

The use of biologically active supplements is also in response to the modern trends in healthy nutrition of both animals and humans, to achieve in a natural way an increase in the functionality of food produced from sheep farming and in particular from aboriginal sheep farming, in order to update the selection approaches applied to their conservation and breeding.

In conclusion, the search for approaches to increase the benefits of breeding endangered local and low-productive sheep breeds such as the Middle Rhodope and Karakachan breeds will contribute not only to the preservation of the biodiversity in Bulgaria and maintenance of the ecological balance in the mountainous regions, but it can be considered also as the main incentive to stop the processes of depopulation of entire areas of southern Bulgaria, providing a good income for farmers.

## VI. CONCLUSIONS AND RECOMMENDATIONS:

### 1. CONCLUSIONS

Based on the results obtained from the analysis of the productive indicators of the Middle Rhodope and Karakachan sheep breeds, as well as from the experiments carried out to identify the influence of bioactive supplement All-G Rich on the fattening, meat, technological qualities and fatty acid composition of the meat, the following conclusions can be drawn:

1. Lambs of the Middle Rhodope breed were born with a significantly higher live weight ( $p \leq 0,001$ ) compared to lambs of the Karakachan breed. The same trend was observed for the indicator of average daily gain in female lambs on the 30<sup>th</sup> day and on the 70<sup>th</sup> day of age.
2. Addition of 1% All-G Rich to the concentrate feed led to 5,20% less feeding units for growth per 1 kg weight gain in the Middle Rhodope lambs compared to Karakachan lambs. All-G rich supplementation had no effect on the average daily gain in both breeds.
3. The use of 1% of the biologically active supplement All-G rich did not affect both fattening and meat qualities, as well as slaughtering traits in both breeds.
4. The chemical composition of meat in lambs of the Middle Rhodope and Karakachan breeds was not significantly affected by the addition of 1% All-G rich.
5. The technological processing of meat to a ready-to-eat product led to significantly higher losses during cooking (baking) in *m. Longissimus Dorsi* and *m. Semimembranosus* in the control groups compared to the experimental groups from the Middle Rhodope and Karakachan breeds.
6. Addition of All-G Rich led to a 5,84% ( $p \leq 0,01$ ) increase in cooking (baking) losses of *m. Semimembranosus* in lambs of the Karakachan breed compared to the Middle Rhodope breed. The color of the meat according to the  $a^*$  and  $b^*$  scale significantly ( $p \leq 0,05$ ) increased in *m. Longissimus Dorsi* and *m. Semimembranosus* of lambs of the Middle Rhodope breed compared to lambs of the Karakachan breed.
7. The tenderness of *m. Semimembranosus* in the experimental groups significantly increased in lambs of the Karakachan breed ( $p \leq 0,001$ ) compared to that of the Middle Rhodope breed.
8. The total content of saturated and monounsaturated fatty acids in *m. Semimembranosus* of lambs from the experimental group of the Middle Rhodope breed was higher than in lambs of the control group, on the account of polyunsaturated fatty acids.
9. The bioactive supplement All-G Rich of 1% to the concentrated feed reduced the ratio of  $\Sigma n-6/\Sigma n-3$  fatty acids in *m. Longissimus Dorsi*: respectively for the Karakachan breed with 16,37% (experimental group) and with 7,66% for the Middle Rhodope breed (experimental group).
10. The influence of biologically active supplement All G - Rich on the ratio of  $\Sigma n-6/\Sigma n-3$  fatty acids in *m. Semimembranosus* was determined. It decreased by 15,97% for the Karakachan breed ( $p \leq 0,05$  experimental group) and by 38,78% for the Middle Rhodope breed (experimental group).
11. The total content of monounsaturated fatty acids in *m. Longissimus Dorsi* of lambs of the Middle Rhodope breed ( $P \leq 0,01$ ) was affected to a higher extent by the addition of 1% bioactive supplement All G – Rich to the concentrated feed compared to lambs of the Karakachan breed.

## 2. RECOMMENDATIONS

1. Deepening the research of the productive characteristics of the Middle Rhodope and Karakachan breeds could serve as a basis for updating the standards when preparing the breeding programs of the two breeds.

2. Due to the low productivity of animals of the Middle Rhodope and Karakachan sheep breeds, it is necessary to look for additional ways to increase interest in breeding them. One of these ways is to add small amounts of the bioactive supplement All G – Rich to the concentrated feed after weaning of lambs. This will improve the quality indicators of the meat of lambs for slaughter.

3. Achieving an optimal ratio of Omega-6 to Omega-3 fatty acids leads to an increase in the biologically active components of the produced meat products, and this will sustainably increase the benefits for farmers from raising the low-productive Middle Rhodope and Karakachan breeds and satisfy the increased expectations of consumers for healthy food.

## VII. CONTRIBUTIONS:

1. The main productive traits in sheep of the Middle Rhodope and Karakachan breeds were studied. The live weight at birth, on the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age of lambs of the two studied breeds was established. The average daily gain of the lambs of both breeds up to the 10<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of age and the consumption of feed per 1 kg of weight gain were determined. The obtained results would be useful for the preparation of future guidelines for the selection activity and maintenance, preservation and improvement of the breeds – **A contribution of scientific and applied character.**

2. The influence of the biologically active supplement All G – Rich on the fattening and meat qualities, and the technological properties of the meat was studied - **An original contribution of scientific and practical significance.**

3. The influence of the biologically active supplement All G – Rich on  $\Sigma n-6/\Sigma n-3$  fatty acids of *m. Longissimus Dorsi* was studied, as a response to modern trends in healthy nutrition. A significantly lower ratio of  $\Sigma n-6/\Sigma n-3$  was found in the supplemented groups of both breeds, respectively for the Karakachan breed it was 16,37% and for the Middle Rhodope breed – 7,62% - **An original contribution of scientific and practical significance.**

4. The influence of the biologically active supplement All G - Rich on the ratio of  $\Sigma n-6/\Sigma n-3$  fatty acids in *m. Semimembranosus* was determined. It was found to decrease in *m. Semimembranosus* with 15,7% for the Karakachan breed and with 38,78% for the Middle Rhodope breed in both supplemented groups. The conclusions drawn are related to the modern requirements for biologically active components in meat for healthy nutrition - **An original contribution of scientific and practical significance.**

### **VIII. PUBLICATIONS RELATED TO THE DISSERTATION:**

**1. Todorov, P., 2022.** Influence of the biologically active supplement All G - Rich on the fatty acid composition of meat in lambs of the aboriginal Middle Rhodope and Karakachan breeds. Bulgarian Journal Of Animal Husbandry, 59 (1), 59-66.