AGRICULTURAL ACADEMY
AGRICULTURAL INSTITUTE - STARA ZAGORA

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INFLUENCE OF PRE- AND PROBIOTIC SUBSTANCES ON THE QUALITATIVE AND QUANTITATIVE INDICATORS OF SHEEP MILK AND MEAT

ABSTRACT

of a dissertation for the acquisition of the educational and scientific degree „DOCTOR"

In the professional field: 6.3. Animal husbandry
Field of higher education:
6. Agricultural sciences and veterinary medicine

Doctoral program: Sheep and goat breeding

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STARA ZAGORA, 2026

I express my heartfelt gratitude to my scientific supervisors prof. dr Stayka Laleva and assoc. prof. Nevyana Stancheva for their help in the implementation of the dissertation work. I also sincerely thank assoc. prof. Nikolay Ivanov for his valuable advice and assistance in the research activity.

I thank my colleagues from the Agricultural institute - Stara Zagora for their moral support.

The dissertation consists of 151 pages, number of figures – 12, number of tables – 39, number of publications on the dissertation – 3, number of literary sources – 210, of which 28 in Cyrillic and 182 in Latin.

The numbering of the tables and figures in the summary does not correspond to that in the dissertation.

The dissertation defense will be held on…….2026 g. at…….time in the conference hall of the Agricultural Institute – Stara Zagora. The materials related to the defense are available from the scientific secretary of the Agricultural Institute – Stara Zagora.

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The reviews and opinions of the members of the scientific jury, as well as the abstract, are published on the website of the Agricultural institute – Stara Zagora.

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ABBREVIATIONS

SM – *Musculus Semimembranosus*

LL – *Musculus Longissimus Lumborum*

IP – *Musculus Iliopsoas*

pH – hydrogen-ion index

C4:0 – Butyric acid

C6:0 – Caproic acid

C8:0 – Caprylic acid

WHC – Moisture holding capacity

NDF – Neutral detergent fiber

IlF – Ile de France

BDSP – Synthetic Bulgarian dairy population

IB – *Immunobeta*

I. INTRODUCTION

Since the ban on the use of antibiotics in the feeding of farm animals, scientists are constantly looking for alternatives to improve the complex digestive processes of productive animals. Thus, the importance of new sources of beneficial bacteria as additives to animal and human food is gaining increasing popularity.

To eliminate resistance to chemical substances, probiotic products are used alone or in combination with prebiotics. Numerous scientists are investigating the effects of the use of pre- and probiotic substances to determine their influence on the gastrointestinal tract, the human immune system and animal productivity. Antimicrobial resistance (AMR) is a problem of particular importance in the field of health.

Recently, the importance of biologically active substances for modulating the productive indicators of sheep is becoming increasingly relevant. To eliminate resistance to chemical substances, various probiotic and prebiotic additives are being used as a subject of scientific research.

II. AIM AND TASKS OF THE STUDY

The aim of the doctoral dissertation is to investigate the influence of the prebiotic *Immunobeta* and its combination with the probiotic *Zoovit* on the growth, slaughter and meat quality of lambs from Ile-de-France, as well as the effect of the prebiotic *Immunobeta* on the quantity and quality of milk from Bulgarian dairy synthetic sheep.

To achieve the study goal, the following tasks were scheduled:

To achieve the goal of the study, the following tasks were planned:

1. Determination of the slaughter age, average daily gain and slaughter yield in lambs with ILF after adding the prebiotic *Immunobeta* and with the combination of the prebiotic *Immunobeta* + probiotic *Zoovit.*

2. Analysis of the technological properties and chemical composition of lamb meat with IF, after adding the prebiotic *Immunobeta* and with the combination of the prebiotic *Immunobeta* + probiotic *Zoovit.*

3. Analysis of the amino acid and fatty acid profiles of lamb meat with IF, after adding the prebiotic *Immunobeta* and with the combination of the prebiotic *Immunobeta* + probiotic *Zoovit.*

4. Analysis of the hematological and biochemical parameters of lambs with IF, after adding the prebiotic *Immunobeta* and with the combination of the prebiotic *Immunobeta* + probiotic *Zoovit*.

5. Development of the digestive system in lambs with IF, after the addition of the prebiotic *Immunobeta* and with the combination of the prebiotic *Immunobeta* + probiotic *Zoovit.*

6. Determination of the daily milk yield in BDSP sheep, after the addition of the prebiotic *Immunobeta*.

7. Determination of the chemical composition and urea content in the milk of BDSP sheep, after the addition of the prebiotic *Immunobeta.*

8. Analysis of the amino acid and fatty acid profile and the content of organic acids in the milk of BDSP sheep, after the addition of the prebiotic *Immunobeta*.

9. Analysis of the hematological and biochemical parameters of BDSP sheep, after the addition of the prebiotic *Immunobeta*.

III. MATERIAL AND METHODS

Experiment I

The study was conducted at the sheep farm at the Agricultural Institute - Stara Zagora. A total of 45 lambs of the Ile de France breed were divided into three groups of 15 animals each – control and two experimental. The three groups were matched by maximum initial live body weight, sex and type of birth. The lambs were fed ad libitum (+ 5 to 10% residue) with a ration consisting of concentrate and alfalfa hay.

Each of the animals in experimental group I received orally once a day in the morning 8 g of the prebiotic *Immunobeta*, while those in experimental group II received the same amount of the prebiotic plus 4 g of the probiotic *Zoovit*.

Table 1. Compound feed composition for Ile-de-France lambs

Ingredients	**% content**
Soybean meal	4.00
Limestone	3.00
Salt	0.50
Wheat	42.00
Premix -16-97-K	0.20
Sunflower meal	20.00
Maize	30.30

The probiotic preparation *Zoovit* contains four strains of lactic acid bacteria and one strain of *Propionibacterium* The supplement *Immunobeta* is a prebiotic with pronounced immunostimulating activity

During the experiment, the following parameters of lamb growth were analyzed: live body weight at the beginning and end of the experiment, average daily gain and age at slaughter in days. The duration of the experiment was until a live weight of 23-25 kg was reached. After reaching the specified live weight, five male lambs were slaughtered from each group. The following parameters were determined: warm carcass weight, left half weight and slaughter yield.

Slaughter yield was calculated according to the equation: Slaughter yield (%) = (a/b) × 100, where: a – weight of the cooled carcass without head, kidneys and perirenal fat; b – live weight before slaughter. The carcass was divided into two parts and the weight of the left half was measured. Meat samples were taken from each slaughtered animal from *Musculus Longissimus Lumborum* (*m. LL*), *Musculus Semimembranosus* (*m. SM*) and *Musculus Iliopsoas* (*m. IP*). The meat samples were transported in a refrigerated bag and examined 24 hours after slaughter, stored at a temperature of 0-4 °C.

The samples from *m. LL* and *m. SM* were analyzed to determine technological properties and chemical composition, and the samples from *m. IP* – for technological

properties (the results for *m. SM* and *m. IP* are reflected in the corresponding tables in the dissertation).

1. Determination of technological properties of meat

The pH of the meat was measured with a pH meter Testo 205. The water holding capacity (WHC) was estimated using the classical pressing method of **Grau and Hamm (1953)**. The cooking loss was determined by roasting a meat sample at 150°C for 20 minutes. The tenderness of the meat was determined with a penetrometer DSD VEB Feinmess (Dresden, Germany) and was reported in degrees of penetrant – °P. The color of the meat was determined in the CIE L* a* b* color space with a Minolta CR400 colorimeter.

2. Determination of the chemical composition of the meat and organic compounds

The protein content was analyzed with an automatic Kjeldahl distillation system UDK 149 VELP Scientifica, Italy. Fat content was determined by Soxhlet extraction according to BSS 8549:1992, while mineral content was determined as described in ISO 936:1998, after ashing of the meat samples in a muffle furnace.

The analysis of amino acids and lipids (g/100 g) in samples of *m. Longissimus Lumborum* was performed using the method for the determination of polar metabolites in meat by hydrolysis and derivatization before gas chromatography. Organic acids and sugars in the meat (g/100 g) were analyzed using the method described for experiment II.

3. Determination of hematological and blood biochemical parameters

Blood samples were taken from 8 lambs from each group (total 24 lambs) in the morning after a 24-hour fast from the *v. jugularis interna* at the beginning and end of the experimental period for the analysis of hematological and biochemical parameters.

4. Determination of the length of the small intestine, large intestine and rumen papillae, rumen volume and total microbial count in the rumen and intestinal contents

After slaughtering 5 lambs from each group, the length of the small intestine, large intestine and rumen papillae, rumen volume and total microbial count in the rumen and intestinal contents were determined. The rumen and intestinal contents samples were analyzed according to BSS standards.

Experiment II

The study was conducted at the sheep farm at the Agricultural Institute – Stara Zagora. The experiment involved 52 sheep of the BDSP breed, divided into two groups - control and experimental, with 26 sheep in each with maximum equalization in live weight, lactation stage and morning milk yield.

The average daily amount of concentrate given to the sheep during the experiment was 0.850 kg. The sheep from the experimental group received a daily supplement of the prebiotic *Immunobeta*, well mixed with the concentrate, at a dose of 0.006 kg/head/day (according to the manufacturer's recommendations).

Table 2. Compound feed composition for BDSP lambs

Ingredients	% content
Wheat	26.00
Sunflower meal	10.00
Alfalfa hay	10.00
Lupine	20.00
Maize	30.70
Limestone	2.50
Salt	0.60
Premix -16-97-K	0.20

During the experiment, the daily milk yield of each sheep, the chemical composition of the milk, the content of urea, amino acids and fatty acids in the milk, cholesterol, organic acids and blood parameters were determined.

During the experimental period, a total of five milk controls were carried out in the milking hall of the sheep farm at the Agricultural Institute – Stara Zagora: two

controls in April and one each in May, June and July. The first milk control was carried out before adding the feed with *Immunobeta*, and all the others – after adding the product. After milking the sheep and determining the milk produced, individual samples of 0.05 l were taken. All sheep were milked twice a day.

5. Determination of chemical composition and organic compounds in milk

The qualitative composition of milk, including urea content, was analyzed after each milk yield control in the Laboratory for Quality of Milk and Dairy Products at the Agricultural Institute – Stara Zagora. Fatty acids, cholesterol and amino acids in milk were analyzed as described in experiment I. Sugars and organic acids in milk were analyzed by GC-MS of metabolites.

6. Determination of hematological and blood biochemical parameters

Blood samples were taken from 10 sheep from each group in the morning after a 24-hour fast from the v. jugularis interna at the beginning and end of the experimental period for analysis of hematological and biochemical parameters.

7. Statistical analysis

Statistical analysis of the data was performed with the statistical software IBM SPSS v. 19. The results were presented as mean±SD, calculated by descriptive statistical methods. The comparison of variables from three independent groups with equal variances and an almost normal distribution was performed by one-way analysis of variance (One-Way ANOVA) based on Fisher's F-criterion. The following Student's t-tests were applied: t-test for independent samples for comparison of two groups and t-test for paired samples for comparison of the same group at the beginning and end of the experiment. In cases where the requirements for the use of parametric statistical tests were not met, the corresponding non-parametric alternatives were used. Differences at $p \leq 0.05$ were considered statistically significant, and a trend towards significance was assumed at $p \leq 0.10$.

IV. RESULTS AND DISCUSSION

Experiment I

1. Growth performance

Table 3 presents the growth results of ILF lambs that consumed a concentrated mixture and a supplement of the prebiotic *Immunobeta* and a combination of the prebiotic *Immunobeta* + probiotic *Zoovit*.

Table 3. Growth performance of ILF lambs after dietary supplementation with the prebiotic *Immunobeta* and with the combination of prebiotic *Immunobeta* + probiotic *Zoovit*

Parameters	Groups of animals						
	Control group-a		Experimental group I-b		Experimental group II-c		Significance
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Initial live body weight, kg	5	8.43±1.37	5	8.50±0.50	5	8.17±1.61	NS
Final live body weight, kg	5	23.27±1.10	5	25.07±0.50	5	26.08±0.88	a:c*
Daily weight gain, kg/day	5	0.23±0.03	5	0.28±0.01	5	0.27±0.01	a:b*, a:c*
Age at slaughter, days	5	97.33±1.53	5	69.00±1.00	5	82.00±1.73	a:b*, a:c*

*** - p≤0.05, NS - Not Significant**

The mean live body weight of lambs in the beginning of the trial was 8.43 kg in the control group, 8.50 kg in the *Immunobeta* group and 8.17 kg in the *Immunobeta* + *Zoovit* group. The data from the table showed that the final body weight was the highest in experimental group II – 26.08 kg, compared to lambs from experimental group I and controls: 25.07 kg and 23.27 kg, respectively. There was a statistically significant difference between control and *Immunobeta* + *Zoovit* supplemented lambs (p≤0.05). **Hussein** (**2014**) and **Antunovic et al.** (**2005**) demonstrated higher final live weight in probiotic-supplemented lambs in relation to controls. The average daily weight gain was the highest in lambs from experimental group I – 0.28 kg/day, followed by animals from experimental group II with 0.27 kg/ден, whereas the lowest daily weight gain was found in controls 0.23 kg/day (p≤0.05). Our data are

comparable to those reported by **Hussein** (**2018**) and **Dimova et al.** (**2013a**). Non-supplemented lambs attained the slaughter weight most slowly – for 97.33 days compared to those from experimental groups I and II with age at slaughter of 69.00 and 82.00 days, respectively. The differences were statistically significant ($p \leq 0.05$).

The data in **Table 4** show that the mass of the warm carcass is highest in animals from experimental groups I and II – 13.12 kg, followed by the control, respectively, 11.89 kg. The highest slaughter yield was found in animals from experimental group I – 52.32%, while in those from the control group and experimental group II it has relatively close values, respectively 51.12% and 50.26%.

Table 4. Slaughter traits in ILF lambs after dietary supplementation with the prebiotic *Immunobeta* and with the combination of prebiotic *Immunobeta* + probiotic *Zoovit*

Parameters	Groups of animals						
	Control group-a		Experimental group I-b		Experimental group II-c		Signifi cance
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Hot carcass Weight, kg	5	11.89±0.90	5	13.12±0.56	5	13.12±0.80	NS
Slaughter yield, %	5	51.12±3.08	5	52.32±1.16	5	50.26±1.54	NS
Left half weight, kg	5	6.05±0.45	5	6.72±0.36	5	6.54±0.39	NS

NS - Not Significant

2. Technological properties and chemical composition of meat

The results of determining the technological properties of meat from *m. LL* are presented in **Table 5.**

In lambs from the first experimental group, the average WHC was 19.25%, in the second experimental group – 17.63%, and in control animals – 12.32%. Therefore, the water loss from meat from *m. LL* of lambs from the first and second experimental groups exceeded that in the control group. Significance was established between the control and both experimental groups at $p \leq 0.05$.

Regarding meat color, the average values of color coordinates (L*, a* and b*) in the groups varied within close limits and did not show significant intergroup

differences. **Maiorano and Bednarczyk** (**2016**) and **Gomes et al.** (**2009**) also did not find reliable differences in meat color after adding probiotics to the diet of broilers and bulls. **Liu et al.** (**2022**) reported that dietary probiotic intake resulted in a reduction in L* values of lamb meat. We find that the animals from the first experimental group have the most tender meat – 322.20 °P, while the animals from the control group and those from the second experimental group have close values, 286.40 °P and 285.89 °P, respectively. The reported differences are unproven.

Table 5. Technological properties of *m. LL* from ILF lambs supplemented with prebiotic *Immunobeta* and with the combination of prebiotic *Immunobeta* + probiotic *Zoovit*

Parameters		Groups of animals						
		Control group-a		Experimental group I-b		Experimental group II-c		Significant
		n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
pH_{24} values		5	5.48±0.05	5	5.50±0.01	5	5.47±0.04	NS
WHC, %		5	12.32±0.83	5	19.25±3.41	5	17.63±3.76	a:b*, a:c*
Colour	L*	5	44.09±1.30	5	44.14±0.87	5	44.06±0.91	NS
	a*	5	16.23±0.64	5	16.46±0.68	5	16.50±2.90	NS
	b*	5	7.17±1.21	5	8.65±1.65	5	7.48±2.71	NS
Tenderness, °P		5	285.89±36.48	5	322.20±39.67	5	286.40±56.35	NS
Cooking loss, %		5	32.12±6.31	5	37.04±3.16	5	35.65±6.19	NS

*** - p≤0.05, NS - Not Significant**

Table 6 presents the results of the chemical composition of meat samples from *m. LL*. We did not find significant differences in the studied indicators.

The water content in *m. LL* in the control group and lambs consuming supplements was 76.92%, 76.99% and 77.05%, respectively. The protein content in the meat also varied within narrow limits: 19.37%, 19.03% and 19.37% for the non-supplemented, first and second experimental groups. The highest fat content in the meat was that of the lambs from experimental group I – 2.91%, followed by the control with 2.51% and finally, the second experimental group with 2.31%. **Gomes et al.** (**2009**) did not report any differences in the fat content in the meat of beef cattle as

a result of yeast supplementation. **Titi et al.** (**2008**) reported that yeast supplementation reduced protein content but increased fat content in carcasses of Awassi lambs**. Raghebian et al.** (**2007**) reported that meat fat and dry matter were slightly higher in the group receiving high levels of *S. cerevisiae* SC47.

Table 6. Chemical composition of *m. LL* from ILF lambs supplemented with prebiotic *Immunobeta* and the combination of prebiotic *Immunobeta* + probiotic *Zoovit*

Parameters	Groups of animals						Significance
	Control group		Experimental group I		Experimental group II		
	n	x̄±SD	n	x̄±SD	n	x̄±SD	
Water content, %	5	76.92±1.11	5	76.99±0.28	5	77.05±0.12	NS
Dry matter, %	5	23.08±1.10	5	23.01±0.28	5	22.95±0.12	NS
Protein content, %	5	19.37±0.82	5	19.03±0.19	5	9.37±0.31	NS
Fat content, %	5	2.51±0.33	5	2.91±0.37	5	2.31±0.54	NS
Ash content, %	5	1.20±0.20	5	1.07±0.10	5	1.27±0.15	NS

NS - Not Significant

The following **table 7** presents the results of the analysis of the amino acid profile of *musculus Longissimus Lumborum* in lambs of the ILF breed.

The relatively narrow range of variation of individual amino acids in relation to the average determined the lack of significant differences.

The highest content of essential amino acids in the meat of the first experimental group compared to the control group was leucine (2.44 g/ 100g), valine (1.80 g/ 100g), lysine (1.75 g/ 100g), threonine (1.72 g/ 100g), phenylalanine (1.68 g/ 100g), and of the conditionally essential amino acids arginine was 2.02 g/ 100g, glycine 1.61 g/ 100g and tyrosine 1.40 g/ 100g.

Table 7. Amino acid profile of *m. LL* in control, first experimental and second experimental groups of ILF lambs, g/ 100g

Parameters	Groups of animals						
	Control group		Experimental group I		Experimental group II		Significance
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Alanine	5	1.64±0.23	5	1.97±0.23	5	2.24±0.42	NS
Valine	5	1.51±0.21	5	1.80±0.22	5	2.05±0.38	NS
Leucine	5	2.04±0.29	5	2.44±0.30	5	2.77±0.52	NS
Isoleucine	5	1.30±0.19	5	1.56±0.19	5	1.77±0.33	NS
Glycine	5	1.34±0.19	5	1.61±0.19	5	1.83±0.34	NS
Proline	5	1.16±0.16	5	1.39±0.17	5	1.57±0.29	NS
Serine	5	1.27±0.18	5	1.52±0.18	5	1.73±0.32	NS
Threonine	5	1.44±0.21	5	1.72±0.21	5	1.96±0.36	NS
Methionine	5	0.70±0.10	5	0.84±0.10	5	0.96±0.18	NS
Aspartic acid	5	2.58±0.36	5	3.09±0.37	5	3.52±0.66	NS
Glutamic acid	5	3.20±0.45	5	3.84±0.46	5	4.36±0.82	NS
Phenylalanine	5	1.40±0.20	5	1.68±0.20	5	1.90±0.36	NS
Tyrosine	5	1.17±0.16	5	1.40±0.17	5	1.59±0.30	NS
Agrinine	5	1.68±0.24	5	2.02±0.24	5	2.29±0.43	NS
Histidine	5	0.60±0.08	5	0.72±0.09	5	0.82±0.15	NS
Lysine	5	1.46±0.21	5	1.75±0.21	5	1.99±0.37	NS
Cysteine	5	0.25±0.04	5	0.30±0.04	5	0.34±0.07	NS
Tryptophan	5	0.31±0.05	5	0.38±0.04	5	0.43±0.08	NS
Creatine	5	0.40±0.06	5	0.48±0.06	5	0.55±0.10	NS
Hypoxanthine*	5	0.32±0.05	5	0.39±0.05	5	0.44±0.08	NS
Total amino acids	5	25.79±3.59	5	30.88±3.70	5	35.11±6.56	NS

NS - Not Significant, * Purine derivative

In the group that received the prebiotic, glutamic acid was 3.84 g/ 100g, aspartic acid 3.09 g/ 100g.

In the lambs of the second experimental group, which consumed a combination of prebiotic + probiotic, and in those that consumed only prebiotic, all amino acids registered higher levels compared to the control group, with statistically insignificant differences ($p>0.05$). In the analysis of fatty acids in meat *m. LL,* no statistically significant differences were found between the three groups of lambs. We report an unreliable higher concentration in the total content of the indicators in the group that took the synbiotic compared to the other groups (see Table 15 of the dissertation).

3. Haematological and blood biochemical parameters

Table 8 present the haematological parameters of ILF lambs at the end of the trial.

Table 8. Haematological parameters in control, first experimental and second experimental groups of ILF lambs at the end of the trial period

Parameters	Groups of animals						p-value
	Control group		Experimental group I		Experimental group II		
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Red blood cells (RBC, $\times 10^{12}$/L)	8	11.32±1.37	8	11.24±0.65	8	12.45±2.07	0.387
Haemoglobin (HGB, g/L)	8	104.88±11.33 **ab**	8	99.50±2.67 **b**	8	111.63±16.76 **a**	0.037
Haematocrit (HCT, L/L)	8	0.35±0.03 **ab**	8	0.33±0.01 **b**	8	0.37±0.05 **a**	0.010
Mean corpuscular volume (MCV, fL)	8	31.38±1.71	8	29.36±1.44	8	29.76±1.53	0.073
Mean corpuscular haemoglobin (MCH, pg)	8	9.23±0.44	8	8.80±0.44	8	8.93±0.27	0.108
Mean corpuscular haemoglobin concentration (MCHC, g/L)	8	296.00±6.07	8	302.00±6.80	8	302.13±14.87	0.396
Red cell distribution width (RDW, %)	8	17.73±1.34	8	17.83±1.21	8	18.28±0.88	0.410
Mean platelet volume (MPV, fL)	8	4.79±0.20	8	4.63±0.18	8	4.65±0.15	0.169
Platelet distribution width (PDW)	8	13.44±0.39	8	13.30±0.26	8	13.40±0.21	0.640
Plateletcrit (PCT, %)	8	0.15±0.05	8	0.17±0.05	8	0.18±0.03	0.287

a; b – different letters within a row indicate significant differences at $p<0.05$

With the exception of haemoglobin ($p<0.05$) and haematocrit ($p=0.010$), there were no statistically significant differences in the values of the other indicators.

Haemoglobin is influenced by factors such as gender, age, etc. (**Angelov et al., 1999**). The haemoglobin content in our study was within the physiological range. It

recorded the highest value in animals from experimental group II – 111.63 g/L, followed by the control group with 104.88 g/L and experimental group I with 99.50 g/L. Some authors found that the addition of prebiotics and synbiotics to the feed of calves resulted in significantly higher haemoglobin levels than those in the control group (**Ilgaza and Arne, 2021**). **Mallaki et al.** (**2021**), **Hamdon et al.** (**2022**), **Al-Musodi and Al-Zwean** (**2023**) and **Sen et al.** (**2024**) reported a significant increase in haemoglobin in lambs fed probiotic bacterial strains.

We report a higher hematocrit concentration in the group receiving the prebiotic + probiotic combination: 0.37 L/L, followed by the control group – 0.35 L/L and the first experimental group – 0.33 L/L, with a statistically significant difference between the two experimental groups (p=0.010). **Król** (**2011**) reports hematocrit values very close to ours in calves receiving prebiotics – 0.301 L/L.

In terms of the mean corpuscular haemoglobin, the highest value was established in control lambs – 9.23 pg, followed by those from the second experimental group – 8.93 pg and the first experimental group – 8.80 pg. The difference between the control and the first experimental group was low – 4.66%. The parameter was within the reference limits.

No statistical difference was found in the indicator mean hemoglobin concentration in erythrocytes, and the difference between its values in the three groups was minimal. In the control group, the indicator averaged 296.00 g/L, in the first experimental group – 302.00 g/L, and in the lambs that received the synbiotic supplement – 302.13 g/L. **Dar et al.** (**2017**) found significantly higher values for the mean corpuscular haemoglobin concentration in calves that received a prebiotic and synbiotic compared to those in the control group.

We report a statistically significant decrease at the end of the experiment in hemoglobin and hematocrit (p=0.008) in the prebiotic group of lambs (**Table 9**). **Ilgaza and Arne** (**2021**) reported an effect of inulin supplementation on hemoglobin and hematocrit levels in calves. We report a statistically significant decrease in MCV, MCH, MPV and PDW at the end of the experimental period.

Table 9. Hematological indicators in IlF lambs with the participation of the prebiotic *Immunobeta* in the first experimental group at the beginning and at the end of the experimental period

Parameters	Experimental group I				
	At the beginning of the trial		At the end of the trial		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Red blood cells (RBC, $\times 10^{12}$/L)	8	11.79±1.87	8	11.24±0.65	0.402
Haemoglobin (HGB, g/L)	8	123.38±16.19	8	99.50±2.67	0.008**
Haematocrit (HCT, L/L)	8	0.41±0.05	8	0.33±0.01	0.008**
Mean corpuscular volume (MCV, fL)	8	34.98±2.84	8	29.36±1.44	0.000***
Mean corpuscular haemoglobin (MCH, pg)	8	10.50±0.85	8	8.80±0.44	0.000***
Mean corpuscular haemoglobin concentration (MCHC, g/L)	8	301.13±4.19	8	302.00±6.80	0.777
Red cell distribution width (RDW, %)	8	17.56±1.30	8	17.83±1.21	0.734
Mean platelet volume (MPV, fL)	8	5.21±0.34	8	4.63±0.18	0.008**
PDW (platelet distribution width)	8	14.11±0.42	8	13.30±0.26	0.001***
Plateletcrit (PCT, %)	8	0.27±0.08	8	0.17±0.05	0.070

**** - p<0.01, *** - p≤0.001**

Table 10 presents the results of haematological parameters at the beginning and at the end of the experimental period in ILF lambs from the II experimental group that received the prebiotic *Immunobeta* + probiotic *Zoovit*.

We report a decrease in the concentration of mean corpuscular volume (p=0.008), mean corpuscular hemoglobin (p<0.001) and mean platelet volume (p=0.001). **Ilgaza and Arne** (**2021**) found higher erythrocyte counts in calves that consumed an inulin supplement (p<0.01) on the 28^{-th} and 56^{-th} days of the study.

Table 10. Hematological indicators in lambs from ILF with the participation of prebiotic *Immunobeta* + probiotic *Zoovit* in the second experimental group at the beginning and at the end of the experimental period

Parameters	Experimental group II				
	At the beginning of the trial		At the end of the trial		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Red blood cells (RBC, $\times 10^{12}$/L)	8	13.15±4.27	8	12.45±2.07	0.727
Haemoglobin (HGB, g/L)	8	144.88±49.26	8	111.63±16.76	0.125
Haematocrit (HCT, L/L)	8	0.48±0.15	8	0.37±0.05	0.070
Mean corpuscular volume (MCV, fL)	8	36.24±1.43	8	29.76±1.53	0.008**
Mean corpuscular haemoglobin (MCH, pg)	8	10.93±0.53	8	8.93±0.27	0.000***
Mean corpuscular haemoglobin concentration (MCHC, g/L)	8	303.00±8.57	8	302.13±14.87	0.783
Red cell distribution width (RDW, %)	8	18.28±1.65	8	18.28±0.88	1.000
Mean platelet volume (MPV, fL)	8	5.28±0.31	8	4.65±0.15	0.001***
PDW (platelet distribution width)	8	14.14±0.52	8	13.40±0.21	0.070
Plateletcrit (PCT, %)	8	0.33±0.16	8	0.18±0.03	0.070

**** - p<0.01, *** - p≤0.001**

The following **table 11** presents the results of the biochemical analysis of blood serum in ILF lambs at the end of the experiment. With the exception of alkaline phosphatase, there were no significant differences in the studied serum biochemical parameters at the end of the experimental period.

Alkaline phosphatase in the blood serum had the highest values in the animals of the II experimental group – 68.13 U/L, followed by the animals of the I experimental group with 52.00 U/L, and the animals of the control group had the lowest values – 35.13 U/L, respectively. Our study shows a significantly higher concentration of the indicator in the second experimental group compared to the lower one in the control at $p<0.05$. The higher level of serum alkaline phosphatase in experimental groups I and II compared to the control, but within the reference values, was probably due to more intensive growth (**Devyatkin et al., 2021**).

Table 11. Biochemical indicators in ILF lambs in the control group, first and second experimental groups at the end of the experimental period

Parameters	Groups of animals						p-value
	Control group		Experimental group I		Experimental group II		
	n	x̄±SD	n	x̄±SD	n	x̄±SD	
Albumin (ALB, g/L)	8	30.45±1.94	8	29.89±2.73	8	29.69±2.09	0.643
Total protein (TP, g/L)	8	58.79±2.45	8	55.46±5.54	8	56.09±2.76	0.186
Globulin (GLO, g/L)	8	28.34±1.89	8	25.58±3.16	8	26.40±2.49	0.099
Albumin/globulin ratio (A/G)	8	1.08±0.10	8	1.19±0.10	8	1.16±0.15	0.177
Glucose (GLU, mmol/L)	8	4.79±0.70	8	5.18±0.64	8	5.36±0.60	0.214
Urea (BUN, mmol/L)	8	9.65±1.20	8	9.29±1.74	8	9.13±1.98	0.820
Phophorus (P, mmol/L)	8	2.14±0.44	8	2.43±0.74	8	2.54±0.19	0.118
Cholesterol (CHOL, mmol/L)	8	2.22±0.39	8	1.73±0.27	8	2.18±0.58	0.062
Alanine amino-transferase (ALT, U/L)	8	14.25±8.56	6	14.83±10.01	8	15.25±7.59	0.973
Bilirubin (TBIL, µmol/L)	8	5.12±2.53	8	3.32±0.94	8	3.98±1.22	0.158
Alkaline phosphatase (ALP, U/L)	8	35.13±25.54 **b**	8	52.00±27.00 **ab**	8	68.13±26.27 **a**	0.049
Creatinine (CRE, µmol/L)	8	79.00±6.97	8	76.38±9.09	8	76.00±5.78	0.682
Urea/creatinine ratio	8	30.50±3.42	8	30.13±4.82	8	30.13±7.43	0.962

a; b – different letters within a row indicate significant differences at $p<0.05$

The results of biochemical blood tests from the first experimental group at the beginning and end of the experiment are shown in **Table 12.**

Table 12. Biochemical blood parameters in ILF lambs with the participation of the prebiotic *Immunobeta* in the first experimental group at the beginning and end of the experiment

Parameters	Experimental group I				
	At the beginning of the trial		At the end of the trial		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Albumin (ALB, g/L)	8	33.53±2.35	8	29.89±2.73	0.070
Total protein (TP, g/L)	8	59.76±2.55	8	55.46±5.54	0.116
Globulin (GLO, g/L)	8	26.24±3.16	8	25.58±3.16	0.712
Albumin/globulin ratio (A/G)	8	1.30±0.24	8	1.19±0.10	0.687
Glucose (GLU, mmol/L)	8	8.55±1.53	8	5.18±0.64	0.000***
Urea (BUN, mmol/L)	8	6.48±1.33	8	9.29±1.74	0.004**
Phophorus (P, mmol/L)	8	2.33±1.03	8	2.43±0.74	0.828
Cholesterol (CHOL, mmol/L)	8	4.67±1.56	8	1.73±0.27	0.001***
Alanine aminotransferase (ALT, U/L)	6	28.33±16.48	6	14.83±10.01	0.183
Bilirubin (TBIL. μmol/L)	8	8.66±2.09	8	3.32±0.94	0.000***
Alkaline phosphatase (ALP, U/L)	8	48.63±33.11	8	52.00±27.00	0.851
Creatinine (CRE, μmol/L)	8	58.50±10.65	8	76.38±9.09	0.070
Urea/creatinine ratio	8	27.75±5.15	8	30.13±4.82	0.470

**** - p<0.01, *** - p≤0.001**

In our study, we report a significant decrease in bilirubin at the end of the experiment (3.32 μmol/L) compared to the beginning (8.66 μmol/L) at p<0.001 and an unproven trend of increasing creatinine values at the end of the experimental period (76.38 μmol/L) compared to the beginning (58.50 μmol/L) at p=0.070. We found a statistically significant difference in the lower concentration after taking the prebiotic *Immunobeta* in glucose (p<0.001) and cholesterol (p=0.001). We reported an increase in urea (p<0.01).

Table 13 presents the results of the biochemical analysis of blood serum in animals from the second experimental group at the beginning and at the end of the study.

We report a statistically significant decrease (p=0.008) in serum blood sugar from 8.74 mmol/L at the beginning to 5.36 at the end. The serum urea concentration at the end of the experiment increased significantly to 9.13 (p<0.001) from 5.01 mmol/L at the beginning. A significant decrease in cholesterol levels (p<0.001) was

also observed - from 5.04 mmol/L at the beginning to 2.18 mmol/L at the end of the experimental period. Serum levels of the bile pigment bilirubin decreased significantly ($p<0.001$) from 10.40 μmol/L to 3.98 μmol/L at the end of the study, corresponding to a reduction of 61.73%.

Table 13. Biochemical blood parameters in lambs of the ILF breed with the participation of the prebiotic *Immunobeta* + probiotic *Zoovit* in the second experimental group at the beginning and end of the experiment

Parameters	Experimental group II				
	At the beginning of the trial		At the end of the trial		p-value
	n	x̄±SD	n	x̄±SD	
Albumin (ALB, g/L)	8	32.05±2.33	8	29.69±2.09	0.289
Total protein (TP, g/L)	8	58.16±4.39	8	56.09±2.76	0.195
Globulin (GLO, g/L)	8	26.11±3.99	8	26.40±2.49	0.855
Albumin/globulin ratio (A/G)	8	1.24±0.21	8	1.16±0.15	0.378
Glucose (GLU, mmol/L)	8	8.74±1.45	8	5.36±0.60	0.008**
Urea (BUN, mmol/L)	8	5.01±0.92	8	9.13±1.98	0.000***
Phophorus (P, mmol/L)	8	2.72±0.20	8	2.54±0.19	0.070
Cholesterol (CHOL, mmol/L)	8	5.04±0.96	8	2.18±0.58	0.000***
Alanine aminotransferase (ALT, U/L)	8	32.13±19.95	8	15.25±7.59	0.070
Bilirubin (TBIL, μmol/L)	8	10.40±2.30	8	3.98±1.22	0.000***
Alkaline phosphatase (ALP, U/L)	8	46.38±24.08	8	68.13±26.27	0.114
Creatinine (CRE, μmol/L)	8	63.50±18.99	8	76.00±5.78	0.082
Urea/creatinine ratio	8	21.50±8.93	8	30.13±7.43	0.070

**** - p<0.01, *** - p<0.001**

4. Development of the lambs' digestive system and microbiological analysis

Analysis of the digestive organs showed a brighter color of the rumen and rumen papillae in the group consuming the synbiotic compared to the first experimental and control groups (photos **1**, **2** and **3**).

Photo 1 – Control group

Photo 2 – First experimental group (*Immunobeta*)

Photo 3 – Second experimental group (*Immunobeta* + *Zoovit*)

We report significantly greater rumen papillae length in both groups of lambs that consumed supplements compared to the control group ($p>0.05$) (see Table 25 of the dissertation).

Regarding rumen lactic acid bacteria (CFU/1 g), we report a decrease in the group receiving the synbiotic supplement compared to the first experimental and control groups (see Table 26 of the dissertation). **Park et al. (2024)** reported changes

in microbial composition after administration of a probiotic in combination with a biosubstance in dairy cows.

Experiment II

5. Daily milk yield and milk quality

Table 14 provides data about the daily milk yield and the chemical composition of milk in BDSP sheep at the beginning of the experiment (before supplementation with *Immunobeta* prebiotic).

Table 14. Daily milk yield (l) and milk composition of BDSP sheep during the first milk yield control, before supplementation with the prebiotic *Immunobeta*

Parameters	Groups of animals		
	Control group	Experimental group	p-value
	$\bar{x}$±SD	$\bar{x}$±SD	
First milk yield control - April (without *IB*)			
Milk (daily), l, n=26	1.46±0.35	1.45±0.35	0.945
Fat, %, n=20	7.16±1.76	7.61±1.38	0.367
Nonfat milk solids, %, n=20	10.49±0.57	10.60±0.40	0.194
Density, ºA, n=20	33.60±2.25	33.64±1.86	0.695
Protein, %, n=20	3.82±0.20	3.86±0.15	0.239
Lactose, %, n=20	5.76±0.31	5.82±0.22	0.189
Salts, %, n=20	0.85±0.05	0.86±0.03	0.165

We report that the sheep from both groups were characterized by very similar daily milk yields at the beginning of the experiment – 1.46 l in the control and 1.45 l in the experimental group.

Table 15 presents data on the daily milk yield and the chemical composition of milk in sheep of the BDSP breed in the control and experimental (*Immunobeta*) groups during the months of April, May, June and at the end of lactation - July. No statistically significant difference ($p>0.05$) was found between the groups during the lactation period, neither in terms of daily milk yield nor in terms of the chemical composition of the milk.

Table 15. Daily milk yield (l) and milk composition in BDSP sheep during the milk controls in April, May, June and July, during supplementation with the prebiotic *Immunobeta*

Parameters	Groups of animals		
	Control group	Experimental group	p-value
	x̄±SD	x̄±SD	
Second milk yield control - April (with *IB*)			
Milk, l (daily), n=26	1.06±0.42	1.09±0.48	0.752
Fat, %, n=25	8.45±1.54	7.95±1.65	0.148
Nonfat milk solids, %, n=25	10.79±1.27	10.92±1.83	0.318
Density, ºA, n=25	33.64±3.85	34.55±5.95	0.138
Protein, %, n=25	3.92±0.46	3.97±6.67	0.304
Lactose, %, n=25	5.93±0.70	6.00±1.01	0.313
Salts, %, n=25	0.88±0.10	0.89±0.15	0.223
Third milk yield control - May (with *IB*)			
Milk, l (daily), n=26	0.78±0.37	0.85±0.39	0.544
Fat, %, n=24	8.02±1.54	7.95±1.22	0.942
Nonfat milk solids, %, n=24	12.70±1.55	12.52±1.38	0.951
Density, ºA, n=24	41.23±4.81	40.61±4.67	0.726
Protein, %, n=24	4.63±0.56	4.56±0.50	0.984
Lactose, %, n=24	6.98±0.85	6.88±0.76	0.959
Salts, %, n=24	1.03±0.13	1.02±0.11	0.992
Fourth milk yield control – June (with *IB*)			
Milk, l (daily), n=26	0.89±0.35	0.90±0.38	0.946
Fat, %, n=26	8.11±1.42	8.30±1.47	0.437
Nonfat milk solids, %, n=26	11.23±1.80	11.93±1.82	0.141
Density, ºA, n=26	35.61±6.16	38.08±6.12	0.098
Protein, %, n=26	4.09±0.66	4.34±0.67	0.136
Lactose, %, n=26	6.17±0.99	6.55±1.00	0.143
Salts, %, n=26	0.91±0.15	0.97±0.15	0.130
Fifth milk yield control – July (with *IB*)			
Milk, l (daily), n=26	0.43±0.24	0.51±0.27	0.230
Fat, %, n=26	7.98±1.30	8.30±1.58	0.210
Nonfat milk solids, %, n=26	12.46±1.53	12.59±1.62	0.415
Density, ºA, n=26	40.37±5.35	40.58±5.21	0.742
Protein, %, n=26	4.54±0.56	4.59±0.59	0.410
Lactose, %, n=26	6.85±0.84	6.92±0.89	0.410
Salts, %, n=26	1.02±0.12	1.03±0.13	0.398

Many herbal products act as prebiotics, containing oligosaccharides, inulin and glucans. In a study, **Boris and Yarzhinovska** (**2016**) reported an increase in milk yield between 8.20% and 16.40% in sheep fed herbal products. Apart from the reduced milk fat content, no other significant changes in the chemical composition of the milk were found.

Table 16 presents data on milk urea (mg/dl) in control sheep and those that received the prebiotic *Immunobeta* during the control milking in April, May, June and July.

Table 16. Urea content (mg/dl) in BDSP milk during April, May, June and July

Parameters	Groups of animals				
	Control group		Experimental group		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
First milk yield control - April (without *IB*)					
Urea, mg/dl	20	25.05±4.46	20	24.45±4.95	0.690
Second milk yield control - April (with *IB*))					
Urea, mg/dl	25	29.65±4.31	25	29.52±5.38	0.925
Third milk yield control - May (with *IB*)					
Urea, mg/dl	24	27.14±4.74	24	28.58±5.80	0.464
Fourth milk yield control - June (with *IB*)					
Urea, mg/dl	24	23.14±6.19	23	23.99±7.92	0.683
Fifth milk yield control – July (with *IB*)					
Urea, mg/dl	18	17.68±4.01	18	18.68±4.51	0.486

Cannas (**2002**), cited by **Mihaylova** (**2008**) states that normal levels of urea in milk in sheep range between 25-40 mg/dl. According to the author, levels of this metabolite above 40-50 mg/dl are associated with excess protein in the diet and low reproductive performance. Low concentrations of urea in milk may indicate protein deficiency and, consequently, low milk yield.

Although urea levels in milk of ewes from both groups during the milk yield controls conducted in April-June were characterized by normal values, at the end of lactation (in July) a slight decrease in the concentration of the metabolite was observed. At this time, the amount of milk produced naturally decreases. During the grazing period, when NDF increases compared to easily digestible carbohydrates, **Bedo et al.** (**1998**), cited by **Mihaylova** (**2008**) reported a decrease in milk yield, as well as in the level of urea in milk. The urea content in milk in the group receiving the prebiotic *Immunobeta* was 5.66% higher than in the control group, although the level of the metabolite at the end of lactation in both groups was slightly below the established norms. This is probably due to the fact that grazing during this period was limited, associated with a change in the composition of pasture vegetation, and reduced protein intake through grazing. Based on the results of the analysis of urea in

milk at the end of the experiment (July) in the control group and the group receiving the prebiotic *Immunobeta*, it can be assumed that fermentation in the rumen in the group of sheep fed the prebiotic was more intense.

6. Amino acid and fatty acid profiles of milk

Table 17 presents the data on the amino acid profiles of milk from the control and *Immunobeta*-supplemented groups of sheep at the end of the study. The between-group differences were statistically insignificant ($p>0.05$). Milk alanine content was higher in the supplemented than in the control group (4.43% vs 3.29%). Valine content in the group fed the prebiotic also exceeded the respective level in the controls (9.67% vs 9.34%).

The amino acid leucine in the control group was 10.01% compared to a higher value of 10.77% in the experimental group. The amino acid proline showed a statistically insignificant trend ($p=0.080$) of increase in the group consuming the prebiotic supplement *Immunobeta*.

Table 17. Amino acids in milk from BDSP from the control and experimental groups at the end of the experimental period, %

Parameters	Groups of animals				
	Control group		Experimental group		p-value
	n	x̄±SD	n	x̄±SD	
Alanine	10	4.43±0.73	10	3.29±1.43	0.112
Valine	10	9.34±1.42	10	9.67±1.81	0.656
Leucine	10	10.01±1.27	10	10.77±1.26	0.197
Isoleucine	10	3.72±0.71	10	3.90±0.43	0.491
Glycine	10	2.41±0.46	10	2.55±0.29	0.421
Proline	10	8.67±0.71	10	9.72±1.59	0.080
Serine	10	5.40±0.70	10	5.91±0.68	0.117
Threonine	10	4.94±0.95	10	4.97±0.85	0.762
Methionine	10	2.93±0.56	10	3.11±0.33	0.226
Aspartic acid	10	2.56±0.46	10	2.70±0.27	0.400
Glutamic acid	10	15.07±4.00	10	14.15±2.58	0.545
Phenylalanine	10	5.37±0.87	10	5.26±0.98	0.793
Tyrosine	10	9.12±1.17	10	8.91±1.70	0.747
Agrinine	10	3.06±0.61	10	3.35±0.58	0.105
Histidine	10	1.64±0.34	10	1.85±0.20	0.111
Lysine	10	7.14±1.42	10	6.03±1.40	0.093
Cysteine	10	3.54±0.63	10	3.20±0.65	0.450
Tryptophan	10	0.63±0.12	10	0.66±0.07	0.226
Total amino acids.g/100g	10	6.08±1.12	10	6.66±0.75	0.105

Table 18 shows the results of the analysis of fatty acids and cholesterol in milk from the control group of sheep and the *Immunobeta* group at the end of the experimental period. For the fatty acid C4:0, we found a decrease in the experimental group of 2.17% compared to the higher concentration of 2.25% in the control group. A similar trend is observed for the fatty acids C6:0 and C8:0.

Some herbs have prebiotic properties. In a study with herbal supplements to sheep fed with herbs, **Jarzynowska and Peter** (**2017**) reported more favorable changes in the ratio of unsaturated/saturated, polyunsaturated/saturated and cholesterol-beneficial and cholesterol-increasing fatty acids. The authors also observed a reduction in saturated fatty acids in sheep's milk to which herbs were added.

Table 18. Fatty acid profile (%) and cholesterol content in BDSP milk from the control and experimental groups at the end of the experimental period

Parameters	Groups of animals				
	Control group		Experimental group		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Butyric acid (C4:0)	10	2.25±0.43	10	2.17±0.46	0.668
Caproic acid (C6:0)	10	2.09±0.40	10	1.95±0.48	0.912
Caprylic acid (C8:0)	10	2.53±0.49	10	2.19±0.56	0.159
Capric acid (C10:0)	10	5.36±1.28	10	6.03±1.27	0.393
Lauric acid (C12:0)	10	3.56±0.86	10	4.20±0.43	0.023*
Myristic acid (C14:0)	10	12.77±1.00	10	11.64±2.45	0.201
Myristoleic acid (C14:1)	10	0.44±0.08	10	0.46±0.05	0.479
Palmitic acid (C16:0)	10	26.67±4.93	10	24.16±4.14	0.075
Palmitoleic acid (C16:1)	10	1.39±0.23	10	1.51±0.17	0.205
Margaric acid (C17:0)	10	0.75±0.15	10	0.80±0.09	0.199
Stearic acid (C18:0)	10	10.04±2.04	10	11.52±1.61	0.063
Oleic acid (C18:1n-9)	10	24.89±3.93	10	25.57±1.95	0.632
Linoleic acid (C18:2n-6)	10	3.18±0.61	10	3.41±0.33	0.308
α-linolenic acid (C18:3n-3)	10	1.60±0.30	10	1.72±0.16	0.291
Conjugated linoleic acid (CLA c9t11)	10	1.44±0.28	10	1.57±0.16	0.231
Arachidic acid (C20:0)	10	0.61±0.10	10	0.64±0.07	0.370
Arachidonic acid (C20:4n-6)	10	0.17±0.03	10	0.19±0.03	0.075
Eicosapentaenoic acid (C20:5n-3)	10	0.15±0.03	10	0.17±0.02	0.164
Docosahexaenoic acid DHA (C22:6n-3)	10	0.10±0.02	10	0.11±0.02	0.208
Cholesterol. mg/dL	10	11.95±1.56	10	12.04±1.25	0.883
Total fatty acids. g/100 g	10	7.59±1.52	10	8.04±0.88	0.433

*** - p<0.05**

Regarding cholesterol in milk, we did not find statistically significant differences between the control and experimental groups. However, in the experimental group of sheep we observed a slight increase in the parameter – 12.04 mg/dL compared to the value in the control group of 11.95 mg/dL. The increase in cholesterol in the experimental group compared to the control group was 0.75%.

Figures 1-4 show the content of organic acids in milk.

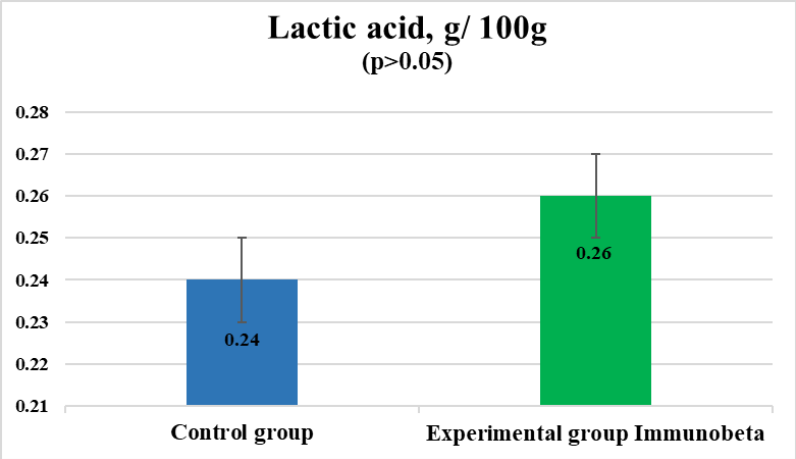

Figure 1. Milk lactic acid content from the control and experimental group at the end of the trial period, (n=10)

Figure 1 presents the results from the analysis of milk lactic acid in the control and experimental groups of BDSP sheep. From the results obtained, no statistically significant difference was found at p>0.05 for the research indicator.

Lactic acid concentration was 0.26 g/100 g in the experimental group compared to a lower level in the controls of 0.24 g/100 g. The difference was 8.33%.

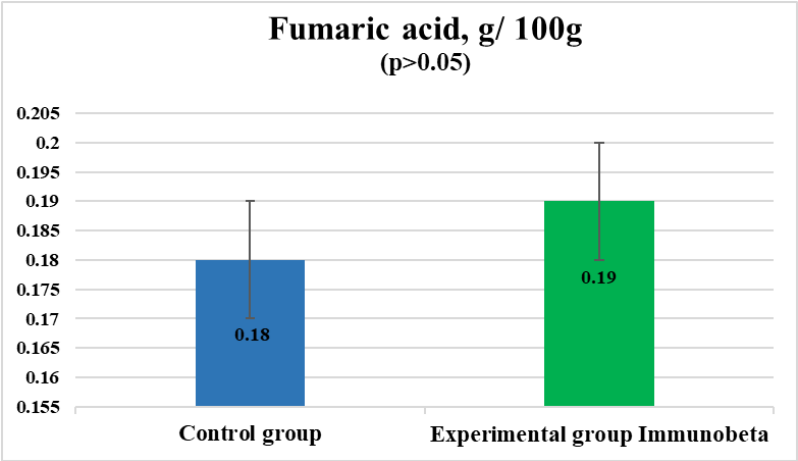

Figure 2. Milk fumaric acid content from the control and experimental group at the end of the trial period, (n=10)

Milk fumaric acid (**Figure 2**) content was greater in the supplemented group of sheep: 0.19 g/100 g vs the almost identical average value of 0.18 g/100 g in the control group.

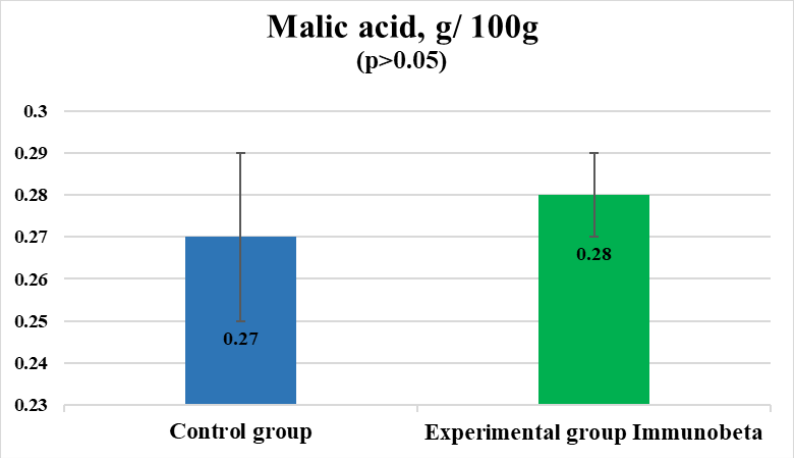

Figure 3. Milk malic acid content from the control and experimental group at the end of the trial period, (n=10)

Similarly to the other assayed organic acids in milk, malic acid content (**Figure 3**) exhibited a similar trend with very close average values in both groups (0.28 g/100 g in the experimental and 0.27 g/100 g in the control).

Figure 4 presents the results from the analysis of milk sugars and alcohols in BDSP sheep.

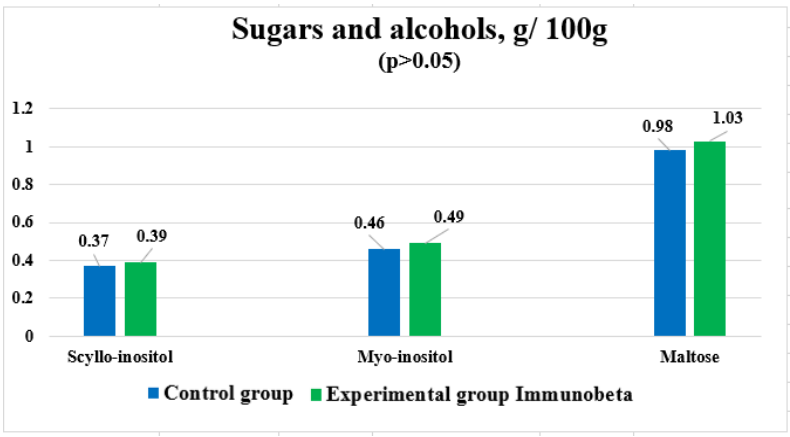

Figure 4. Milk sugars and alcohols' content from the control and experimental group at the end of the trial period, (n=10)

The data on the figure showed no significant between-group differences (p>0.05). Similar to the results for organic acids, the studied indicators had higher average values in the group that received the prebiotic supplement *Immunobeta*. The carbohydrate scyllo-inositol content was higher in the experimental group of sheep (0.39 g/100 g) compared to the level in controls (0.37 g/100 g) – a level higher by 5.41% in the prebiotic group. A higher concentration was also reported for the other studied carbohydrate myo-inositol in the experimental group: 0.49 g/100 g compared

to 0.46 g/100 g in the control. Milk maltose in the experimental group was higher – 1.03 g/100 g compared to a lower level of 0.98 g/100 g in the milk of control sheep.

7. Haematological and blood biochemical parameters

Table 19. Hematological parameters in BDSP sheep in the experimental group at the beginning and end of the experiment

Parameters	Groups of animals				
	Experimental group at the beginning		Experimental group at the end		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Red blood cells (RBC, $\times 10^{12}$/L)	7	9.38±1.44	7	8.12±1.82	0.453
Haemoglobin (HGB, g/L)	7	91.71±10.45	7	73.86±17.72	0.016*
Haematocrit (HCT, L/L)	7	0.31±0.04	7	0.26±0.06	0.125
Mean corpuscular volume (MCV, fL)	7	33.77±2.06	7	32.24±2.29	0.016*
Red cell distribution width (RDW, %)	7	15.19±0.68	7	16.04±0.73	0.125
Platelets (PLT, $x10^{9}$/L)	7	200.00±117.69	7	221.00±155.85	1.000
Mean platelet volume (MPV, fL)	7	5.27±0.50	7	5.03±0.45	0.375
Platelet distribution width (PDW)	7	14.06±0.81	7	13.69±0.76	1.000
Plateletcrit (PCT, %)	7	0.11±0.07	7	0.12±0.09	1.000

*** - p<0.05**

Table 19 presents the results of the analysis of hematological parameters in BDSP sheep that consumed the prebiotic supplement *Immunobeta*. The comparative haematological analysis in the experimental group of BDSP sheep performed at the beginning and at the end of the experiment demonstrated a significantly decreased haemoglobin at the trial's end of 73.86 g/L compared to the higher initial level of 91.71 g/L (p=0.016). In a study by **Osita et al.** (**2019**) haemoglobin concentration was reported to be higher (p<0.05) in sheep that consumed leguminous plants and *S. cerevisiae*.

Regarding the mean corpuscular volume, a statistically significant reduction (p=0.016) of 32.24 fL occurred at the end of the experimental period compared to the

higher value at the beginning of 33.77 fL. The difference is 4.53% lower concentration at the end compared to the beginning of the experiment.

Table 20 presents the data from the blood biochemical analysis in BDSP sheep before and after supplementation with the prebiotic *Immunobeta.*

Table 20. Serum biochemical parameters in BDSP sheep in the experimental group at the beginning and end of the experiment

Parameters	Groups of animals				
	Experimental group at the beginning		Experimental group at the end		p-value
	n	$\bar{x}$±SD	n	$\bar{x}$±SD	
Albumin (ALB, g/L)	10	31.09±3.25	10	28.51±2.22	0.021*
Total protein (TP, g/L)	10	73.53±11.60	10	70.29±6.26	0.021*
Globulin (GLO, g/L)	10	42.44±10.14	10	41.78±5.87	0.754
Albumin/globulin ratio (A/G)	10	0.78±0.22	10	0.70±0.12	0.063
Glucose (GLU, mmol/L)	10	2.31±0.38	10	3.43±0.49	0.002**
Urea (BUN, mmol/L)	10	9.19±2.68	10	7.92±1.10	0.161
Total cholesterol (CHOL, mmol/L)	10	2.03±0.27	10	2.30±0.47	0.126
Alanine aminotransferase (ALT. U/L)	10	19.50±4.25	10	20.20±4.98	0.539
Bilirubin (TBIL, µmol/L)	10	8.15±2.60	10	9.53±3.83	0.124
Creatinine (CRE, µmol/L))	10	52.50±8.40	10	67.70±8.84	0.002**
Urea/creatinine ratio	10	43.80±13.31	10	29.20±5.27	0.010**
Total carbon dioxide (tCO_2, mmol/L)	10	24.80±1.69	10	15.00±1.15	0.678

*** - p<0.05 , ** - p≤0.01**

The table shows that some of the parameters (globulin, globulin/globulin ratio, urea and carbon dioxide) decreased at the end of the experimental period. The small differences in the arithmetic mean values of the research parameters did not lead to significant differences between the two groups.

We report a statistically significant decrease in albumin, total protein ($p<0.05$) and urea/creatinine ratio ($p=0.010$) at the end of the experiment, while we detect an increase in glucose ($p=0.002$) and creatinine ($p=0.002$).

Table 21 presents the results from serum electrolytes' assay at baseline in the experimental group at the beginning and the end of the experiment.

Table 21. Serum electrolytes in BDSP sheep in the experimental group at the beginning and end of the experiment

Parameters	Groups of animals				
	Experimental group at the beginning		Experimental group at the end		p-value
	n	x̄±SD	n	x̄±SD	
Potassium (K+, mmol/L)	10	6.08±1.14	10	4.87±0.42	0.001***
Sodium (Na+K, mmol/L)	10	139.90±2.13	10	141.20±2.49	0.318
Na+/K+ ratio	10	23.60±3.98	10	29.10±2.08	0.000***
Chlorides (C-, mmol/L)	10	106.00±3.53	10	109.10±6.59	0.229
Calcium (Ca, mmol/L)	10	2.40±0.27	10	2.43±0.15	0.644
Magnesium (Mg, mmol/L)	10	1.09±0.09	10	0.89±0.05	0.000***
Phosphorus (P, mmol/L)	10	2.28±0.54	10	1.75±0.33	0.109

***** - p≤0,001**

In the case of potassium, a statistically significant decrease (p=0.001) to 4.87 mmol/L at the end of the experiment compared to its higher initial level of 6,08 mmol/L was reported. The decrease in the indicator at the end of the experimental period after supplementation with the prebiotic *Immunobeta* was by 19.90%. In terms of blood sodium, a slight increase of 0.93% occurred at the end of the period compared to its beginning with values of the indicator 141.20 mmol/L at the end and 139.90 mmol/L at the beginning and minimal deviation below the lower reference range for both measurements. The difference is statistically unproven (p>0.05).

The sodium/potassium ratio registered a statistically significant difference at p<0.001, with a value of 29.10 at the end of the experiment compared to 23.60 at the beginning. Chlorine also recorded an increase at the end of 109.10 mmol/L compared to 106.00 mmol/L at the beginning, with the difference mathematically unproven at the given level of statistical significance.

We report a calcium value at the beginning of the experiment from 2.40 mmol/L to 2.43 mmol/L at the end of the experiment (change of 1.25%). We report a decrease in magnesium to 0.89 mmol/L at the end of the experiment compared to the beginning – 1.09 mmol/L. (p<0.001). We report a phosphorus value at the end of the

study of 1.75 mmol/L compared to a higher level of 2.28 mmol/L at the beginning, but the difference was not significant. The decrease at the end of the experimental period was 23.25%. The blood electrolytes tested were within the reference limits.

V. SYNOPSIS

Based on the conducted experiment on the use of the prebiotic preparation *Immunobeta* and its combination with the probiotic *Zoovit*, we report reliable differences in lambs of the ILF breed in terms of average daily gain, age at slaughter, as well as in terms of the moisture-holding capacity of the meat. We report significantly higher live weight at the end of the experiment in the group that consumed the combination of the supplement *Immunobeta* + *Zoovit*, compared to the control. We do not report statistically significant differences in terms of meat quality indicators, as well as those in the amino acid and fatty acid composition. We report that the meat of lambs from the group that consumed a synbiotic supplement was characterized by a higher concentration ($p>0.05$) of the studied fatty and amino acids, as well as organic compounds. A hypocholesterolemic effect was observed in lambs that consumed food supplements, compared to the control group, as well as some other blood parameters that are important for the health of the animals.

The greater length of the rumen papillae ($p>0.05$) in the supplemented group provides grounds for continuing and expanding the analyses to determine the influence of prebiotic and synbiotic supplements on the rumen and intestinal microflora in lambs. In many farms, digestive disorders occur during the growth period of lambs, which can sometimes be difficult to prevent. Our study showed that the use of supplements clearly limits the problems associated with digestive disorders in lambs during their growth, which is an incentive for future scientific research in this direction.

Regarding the daily milk yield of BDSP sheep, no significant statistically significant differences were found between the control group and the group supplemented with the prebiotic *Immunobeta.* However, the daily milk yield in the experimental group of animals was higher. We establish reliable differences in some

biochemical serum blood parameters in sheep that used the *Immunobeta* supplement, which have important implications for the health status of the animals. No statistically significant differences were demonstrated in the amino acid and fatty acid profile, as well as in the organic acids in the milk of the experimental BDSP sheep.

Based on the results obtained with the addition of the prebiotic *Immunobeta* and its combination with the probiotic *Zoovit*, it can be concluded that the inclusion of these products in the diet of ILF lambs and BDSP sheep may contribute to the improvement of the studied parameters.

VI. CONCLUSIONS AND RECOMMENDATIONS

Based on the results obtained, the following conclusions can be drawn:

1. Lambs consuming the combination of supplements *Immunobeta* + *Zoovit* are characterized by a significantly higher live weight before slaughter compared to those in the control group.

2. Lambs receiving the prebiotic Immunobeta and those receiving the supplement *Immunobeta* + *Zoovit* are characterized by a significantly higher average daily gain compared to lambs in the control group. Animals receiving the prebiotic and its combination with a probiotic reach a certain live weight before slaughter in a significantly shorter period compared to the control group.

3. Significantly higher moisture retention capacity of the *m. LL* was reported in lambs from the 1^{-st} and 2^{-nd} experimental groups compared to the control.

4. A negligible effect on the total concentration of amino acids, fatty acids and organic compounds in *m. LL* in lambs receiving the prebiotic *Immunobeta* and synbiotic.

5. Significant decrease in hemoglobin and hematocrit in the group receiving the prebiotic *Immunobeta* at the end of the experiment compared to the beginning, as well as a decrease compared to the group receiving the synbiotic supplement. Significant decrease in the values of some of the erythrocyte and platelet indices in the blood after receiving the prebiotic *Immunobeta* compared to the beginning.

6. Significant decrease in some of the erythrocyte and platelet indices in the blood after receiving the combination of the prebiotic *Immunobeta* + probiotic *Zoovit* compared to the beginning.

7. Significant decrease in serum levels of glucose, cholesterol, bilirubin and increase in urea compared to the beginning after receiving the prebiotic supplement *Immunobeta* and the combination with the probiotic *Zoovit*. Significant increase in alkaline phosphatase in the synbiotic group compared to the control at the end of the experiment.

8. The use of the prebiotic *Immunobeta* and synbiotic in lambs with ILF did not affect the length of the small intestine, large intestine and rumen papillae.

9. Higher daily milk yield in ewes receiving *Immunobeta* ($p>0.05$) compared to the control group.

10. The prebiotic *Immunobeta* did not affect the concentration of urea in the milk of BDSP sheep.

11. Lack of effect on the total concentration of amino acids, fatty acids and organic acid composition in the milk of BDSP sheep after administration of *Immunobeta*.

12. In the group of BDSP sheep, after receiving the prebiotic *Immunobeta*, a significant decrease in hemoglobin and mean erythrocyte volume was recorded compared to the beginning.

13. In BDSP sheep treated with *Immunobeta*, a significant decrease in serum albumin, total protein, urea/creatinine ratio, an increase in glucose and creatinine was found at the end of the experiment compared to the beginning. A significant decrease in potassium and magnesium and an increase in the Na+/K+ ratio were recorded.

• As a result of the conducted studies for evaluation of the influence of the prebiotic supplement *Immunobeta* and the combination of *Immunobeta* + *Zoovit* probiotic in ILF lambs, we allow recommending the preparations to improve growth performance and technological qualities of lamb meat.

• Based on the results obtained with the prebiotic supplement *Immunobeta* in BDSP sheep, we recommend the use of the preparation, taking into account the tendency towards increased daily milk yield.

VII. CONTRIBUTIONS

1. The effect of the addition of the prebiotic Immunobeta and its combination with the probiotic *Zoovit* on the growth, slaughter parameters and meat quality of lambs of the Ile de France breed was studied.

2. The technological properties and chemical composition of *m. LL* and *m. SM* and the technological properties of *m. IP* in lambs of the ILF breed were analyzed when consuming the prebiotic supplement *Immunobeta* and its combination with the probiotic *Zoovit.*

3. The development of individual digestive system compartments, the rumen and intestinal microbiome and blood parameters in ILF lambs supplemented with the prebiotic *Immunobeta* or its combination with the probiotic *Zoovit* were studied.

4. The effect of the prebiotic *Immunobeta* on daily milk yield, milk chemical composition and milk urea in BDSP sheep was established.

5. The haematological and biochemical blood parameters of BDSP sheep supplemented with the prebiotic *Immunobeta* were analysed.

VIII. LIST OF PUBLICATIONS IN RELATION TO THE PHD THESIS

1. Slavov, I., N. Ivanov, S. Laleva, (2024). Influence of the prebiotic Immunobeta and the combination Immunobeta + Zoovit probiotic on haematological parameters in breed Ile-de-France. Agricultural Science and Technology, 16, 4, 58-66.

https://agriscitech.eu/influence-of-the-prebiotic-immunobeta-and-the-combination-immunobeta-zoovit-probiotic-on-haematological-parameters-in-breed-ile-de-france-lambs/

https://www.webofscience.com/wos/cabi/full-record/CABI:20240548052

2. Ivanov, N., I. Slavov, S. Laleva, (2024). Influence of prebiotic Immunobeta and the combination Immunobeta + Zoovit probiotic on blood biochemical parameters in Ile-de-France lambs. Black Sea Journal of Agriculture, 7, 6, 758-765.

https://dergipark.org.tr/en/pub/bsagriculture/issue/86776/1567145

https://www.webofscience.com/wos/alldb/full-record/CABI:20250186140

3. Slavov, I., N. Ivanov, S. Laleva, (2025). Influence of the addition of the prebiotic Immunobeta and its combination with the probiotic Zoovit on the development of the digestive system and the microbiological analysis of rumen and intestinal contents in Ile de France lambs. Bulgarian Journal of Animal Husbandry, 62, 2, 11-20.

https://agriacad.eu/ojs/index.php/bjah/article/view/2851

https://www.webofscience.com/wos/cabi/summary/468360a2-5eda-47e2-b8dd-423328045f84-01635005b1/relevance/1