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INFLUENCE OF PREBIOTIC IMMUNOBETA AND THE COMBINATION IMMUNOBETA + ZOOVIT PROBIOTIC ON BLOOD BIOCHEMICAL PARAMETERS IN ILE-DE-FRANCE LAMBS

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Abstract: The aim of the present study was to determine the effect of the addition of prebiotic Immunobeta and probiotic Zoovit on the biochemical parameters of blood in Ile-de-France lambs. The research was carried out at the Agricultural Institute - Stara Zagora. It includes a total of 45 Ile de France lambs, divided into three groups of 15 - one control and two experimental. The groups were formed by the method of analogues, equalized by live weight at the beginning of the experiment, type of birth and sex. The animals of the I experimental group received 8 g of the prebiotic Immunobeta individually once a day, and those of the II experimental group the same amount of prebiotic with the addition of 4 g of the Zoovit probiotic. Blood for the study of 8 animals from each group was taken at the beginning and at the end of the experiment. In the indicators of albumin, urea, glucose, cholesterol, creatinine and bilirubin, significant differences were reported after the addition of the prebiotic Immunobeta compared to those at the beginning of the trial. A significant decrease in albumin ($P<0.05$), glucose ($P<0.001$), cholesterol ($P=0.001$), bilirubin ($P<0.001$) and increase in urea and creatinine levels ($P<0.01$) were found after administration of prebiotic Immunobeta and a tendency to decrease total protein ($P<0.10$). The addition of prebiotic Immunobeta + prebiotic Zoovit to the ration of lambs leads to a significant decrease in the values of albumin ($P<0.05$), glucose ($P=0.012$), cholesterol ($P<0.001$), bilirubin ($P<0.001$), alanine aminotransferase ($P<0.05$), increase in urea ($P<0.001$), urea/creatinine ratio ($P<0.05$) and tendency to increase in creatinine ($P<0.10$) after taking the combination prebiotic Immunobeta + probiotic Zoovit. A trend towards a decrease in cholesterol levels was found in the group receiving the prebiotic Immunobeta. An unproven tendency to increase the level of alkaline phosphatase was found in lambs receiving Immunobeta + Zoovit. A trend towards a higher globulin level was found in the control group of lambs.

Keywords: Ile-de-France, Lambs, Prebiotics, Probiotics, Blood, Cholesterol

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1. Introduction

The use of subtherapeutic doses of antibiotics in farm animals, as growth promoters and therapeutic doses for the control and treatment of infectious diseases, is the reason for the development of antimicrobial resistant microorganisms (Abreu et al., 2023; Caneschi et al., 2023). One of the possibilities to limit AML is preventive strategies with the use of probiotics, prebiotics and other biologically active substances. Probiotic therapies are at this stage an alternative to address the problems associated with increasing resistance due to the ability of probiotics to modulate the gut microbiota and the host's immune system, as well as their ability to act as a growth factor.

Regardless of numerous studies to clarify the effect of probiotics on strengthening the immune system, improving the intestinal microflora and the health status of animals (Vosooghi-Poostindoz et al., 2014; Mousa et al., 2019; Elaref et al., 2020; Chashnidel et al., 2020; Abdullah et al., 2022; Kholif et al., 2022; Reuben et al., 2022; Al-Shaar et al., 2023; Fenta et al., 2023; Mao et al., 2023; Santana et al., 2023; Zhang et al., 2023; Zhou et al., 2023) new multidisciplinary studies are needed to determine which therapies are effective in a given animal species and how to apply them to overcome AMR.

Administration of probiotics helps improve the overall health status of the body by accelerating protein and carbohydrate-fat metabolism with an increase in the concentration of serum total protein, albumin and globulin (Devyatkin et al., 2021).

El-Katcha et al. (2016), Moarrab et al. (2016) and Hussein (2018) reported no significant effect on blood plasma parameters except for cholesterol, which was lower in animals receiving probiotic and synbiotic compared to the control group. It is likely that the reduction in the level of cholesterol in the blood caused by the action of probiotics and synbiotics is the result of inhibition of its synthesis through assimilation processes. The decrease in cholesterol concentration is due to the hypocholesterolemic effect of inulin and the deconjugation of bile fatty acids, in the process of which lactic acid bacteria take part. This leads to reduced absorption of fatty acids in the intestine and, accordingly, to their lower concentration in the blood (Yoo and Kim, 2016; Moarrab et al., 2016).

In an experiment with dairy goats receiving a probiotic in the ration Mohammed et al. (2013) found an unreliable increase in the blood serum levels of urea, creatinine and cholesterol and a decrease in the transaminases acetaminotransferase, alanine aminotransferase and glucose. Milewski and Sobiech (2009), conducting an experiment with sheep supplemented with a probiotic with the participation of Saccharomyces cerevisiae found a reliable increase in the concentration of glucose, Na^+ and Cl^- ions, a decrease in the level of creatinine and a change in the acid-base balance. The authors indicate that changes in blood biochemical indicators suggest that the probiotic supplement has a stimulating effect on energy metabolism, as well as a protective effect on kidney function, but also helps prevent acidosis in experimental animals.

Despite a number of data, the beneficial effect of probiotics in animals is not consistent, necessitating the development of probiotic therapies for the relevant species and categories of animals, as prevention, which can delay and influence the development of resistance

The aim of the present study is to determine the effect of the addition of prebiotic Immunobeta and a combination of prebiotic Immunobeta + probiotic Zoovit on blood biochemical parameters in Ile-de-France lambs.

2. Materials and Methods

The experiment was conducted in the experimental base at the Agricultural Institute - Stara Zagora. The experiment involved 45 lambs of the Ile de France breed (ILF), divided into three groups - control and two experimental - 15 in each. The groups were formed by the method of analogues, equalized by live weight at the beginning of the experiment, type of birth and sex. The lambs were raised in groups in pens, equipped with feeders for hay and concentrated feed and with drinkers with constant access to clean drinking water, according to the requirements of Ordinance No. 40 (URL1), for the conditions for raising farm animals, taking into account their physiological and ethological characteristics. The animals were fed ad libitum (+ 5 to 10% residue) in a ratio corresponding to their age and meeting the requirements for nutrients and biologically active substances. The ration included concentrate and alfalfa hay (Table 1 and Table 2).

The combined feed contains 1.12 feed units, 2778.25 Kcal/kg and TDN 0.174.

The animals of the I experimental group received 8 g of the prebiotic Immunobeta individually once a day, and those of the II experimental group the same amount of prebiotic with the addition of 4 g of the Zoovit probiotic.

Table 1. Composition of the combined fodder for feeding IlF lambs

Component	% of investment
Soy meal	4.00
Chalk	3.00
Salt	0.50
Wheat	42.00
Premix-16-97-K	0.20
Sunflower meal	20.00
Corn	30.30

Table 2. Nutritional composition of the combined feed

Component	% of investment
Component	15.90
Protein	2.40
Fats	5.43
Fibers	11.40
Moisture	1.20
Approx	0.50
P	0.570

The probiotic preparation Zoovit includes four strains of lactic acid bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus lactis* and one strain of *Propinibacterium*.

Immunobeta is a prebiotic preparation with a pronounced immunostimulating effect, obtained from certain strains of Saccharomyces cerevisiae yeast through a process of enzymatic autolysis. Its composition includes 30% β-glucans, 25% mannanoligosaccharides and 7% nucleotides.

Chemical composition of Zoovit probiotic was given in Table 3.

Table 3. Chemical composition of Zoovit probiotic

Component	% of investment
Protein	29.29
Lactose	52.14
Fats	0.95
Dry substance	94
Lactic acid	2.75
Propionic acid	3.10
Mineral substances	8.76
Number of active cells	no less from 2,5 x 10^7 cfu/g
Coliforms	are not established
Molds and yeasts	are not established
Salmonella in 25 g	are not established
Coagulase-positive staphylococci in 1 g	are not established

Blood for the study of 8 animals from each group (total 24) was taken in the morning after a 12-hour fast from the external jugular vein (v. jugularis interna) at the beginning and at the end of the experiment. After collection, the blood samples were analyzed on a MN Chip biochemical analyzer.

Data were statistically processed by one-way analysis of variance ANOVA using IBM SPSS software (ver. 19) and Student's t-test for dependent samples was performed to compare the values of biochemical indicators.

A check was made for homogeneity of variances and for normal distribution of individual variables. When rejecting the null hypothesis, a non-parametric analogue of the one-factor analysis of variance - the Kruskal-Wallis test - was applied, and for the non-parametric alternative of the t-test for related samples - the Wilcoxon test.

One-way parametric analysis of variance (ANOVA) was performed on variables with equal variances and a normal distribution. Differences at $P<0.05$ were considered statistically significant, and a trend toward significance at $P<0.10$.

3. Results

Table 4 presents the results of the biochemical analysis of blood serum in ILF lambs.

The albumin values in the three groups of lambs were close: 30.45 g/L, 29.89 g/L and 29.69 g/L for the control, I and II experimental groups, respectively. The values of the globulin are close in the I and II experimental groups - 25.58 and 26.40 g/L, respectively, and the differences between them are unreliable. In the control group, we report a slight tendency towards an increase in the globulin level at $P<0.10$.

Table 4. Biochemical indicators of blood in Ile de France lambs in control group, I experimental group with the participation of prebiotic Immunobeta and II experimental group with prebiotic Immunobeta + probiotic Zoovit

Parameters	Groups of animals			
	Control group (n=8)	I experimental group (n=8)	II experimental group (n=8)	P-value
	x̄±SD	x̄±SD	x̄±SD	
Albumin (G/L)	30.45±1.94	29.89±2.73	29.69±2.09	0.788
Total protein (g/L)	58.79±2.45	55.46±5.54	56.09±2.76	0.182
Globulin (g/L)	28.34±1.89	25.58±3.16	26.40±2.49	0.098
Ratio albumin/globulin	1.08±0.10	1.19±0.10	1.16±0.15	0.166
Glucose (mmol/L)	4.79±0.70	5.18±0.64	5.36±0.60	0.214
Urea (mmol/L)	9.65±1.20	9.29±1.74	9.13±1.98	0.820
Phosphorus (mmol/L)	2.14±0.44	2.43±0.74	2.54±0.19	0.146
Cholesterol (mmol/L)	2.22±0.39	1.73±0.27	2.18±0.58	0.062
Alanine aminotransferase (U/L)	14.25±8.56	31.00±48.45	15.25±7.59	0.893
Bilirubin (umol/L)	5.12±2.53	3.32±0.94	3.98±1.22	0.129
Alkaline phosphatase (U/L)	35.13±25.5	52.00±27.00	68.13±26.27	0.051
Creatinine (umol/L)	79.00±6.97	76.38±9.09	76.00±5.78	0.682
Ratio urea/creatinine	30.50±3.42	30.13±4.82	30.13±7.43	0.988

Table 5. Biochemical indicators of blood in Ile de France lambs with the participation of prebiotic Immunobeta in the I experimental group at the beginning and at the end of the experiment

Parameters	I experimental group		
	In the beginning (n=8)	At the end (n=8)	P-value
	x̄±SD	x̄±SD	
Albumin (G/L)	33.53±2.35	29.89±2.73	0.027*
Total protein (g/L)	59.76±2.55	55.46±5.54	0.093
Globulin (g/L)	26.24±3.16	25.58±3.16	0.712
Ratio albumin/globulin	1.30±0.24	1.19±0.10	0.288
Glucose (mmol/L)	8.55±1.53	5.18±0.64	0.000***
Urea (mmol/L)	6.48±1.33	9.29±1.74	0.004**
Phosphorus (mmol/L)	2.33±1.03	2.43±0.74	0.889
Cholesterol (mmol/L)	4.67±1.56	1.73±0.27	0.001***
Alanine aminotransferase (U/L)	23.63±16.45	31.00±48.45	0.674
Bilirubin (umol/L)	8.66±2.09	3.32±0.94	0.000***
Alkaline phosphatase (U/L)	48.63±33.11	52.00±27.00	0.851
Creatinine (umol/L)	58.50±10.65	76.38±9.09	0.007**
Ratio urea/creatinine	27.75±5.15	30.13±4.82	0.470

*= P<0.05, **= P<0.01, ***= P<0.001.

The lowest levels of total protein were found in the serum of animals from experimental group I - 55.46 g/L, followed by lambs from experimental group II - 56.09 g/L and the control group - 58.79 g/L. No mathematical differences were found.

The concentration of glucose recorded the highest value in the II experimental group - 5.36 mmol/L, followed by the I group with 5.18 mmol/L and the control group with 4.79 mmol/L, the differences being mathematically unreliable.

The highest cholesterol levels of 2.22 mmol/L were recorded in the animals of the control group, followed by the II experimental group with 2.18 mmol/L and 1.73 mmol/L in those of the I experimental group. There is a tendency to decrease the levels of the indicator in the I experimental group at P=0.062.

Regarding the level of urea, the highest values are the control group - 9.65 mmol/L, followed by the animals of the I experimental group - 9.29 mmol/L and those of the II - 9.13 mmol/L, as the differences are statistical unproven. In the lambs of I and II experimental groups, which received as supplements, the serum concentration of urea was lower compared to the control group. No statistical significance between groups was reported.

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 group - respectively 35.13 U/L. Our study showed a trend of elevated serum alkaline phosphatase level (P=0.051) in the second experimental group.

Regarding the phosphorus in the serum of the lambs from the three groups, we find close values, and the differences are statistically unproven.

The results of the analysis of the biochemical indicators in the I experimental group at the beginning and at the end of the experiment are presented in table 5. The data show a decrease in the albumin values from the beginning of the experiment - 33.53 G/L to 29.89 G/L at its end, with differences proven at P<0.05 (Table 4).

We report a tendency to decrease the concentration of serum total protein - 59.76 g/L at the beginning to 55.46 g/L at the end of the experimental period.

A significant reliable decrease was found in the glucose values - from 8.55 mmol/L at the beginning to 5.18 mmol/L at the end of the experiment (P<0.001), as expressed in percentage this decrease is 39.42%.

For the urea indicator, we found higher values (9.29 mmol/L) at the end of the experiment compared to the beginning of the experimental period (6.48 mmol/L), and these differences were proven at P<0.01.

The difference in phosphorus concentration at the beginning (2.33 mmol/L) and at the end (2.43 mmol/L) of the experimental period was minimal (4.12%) and statistically unproven.

The serum cholesterol level decreased significantly from 4.67 mmol/L at the beginning to 1.73 mmol/L at the end, the difference being significant at P=0.001.

Alanine aminotransferase is an indicator of normal liver function. Of note, the serum alanine aminotransferase level increased undetectably from 23.63 U/L at baseline to 31.00 U/L at the end of the experiment.

Serum bilirubin values showed a highly significant decrease at the end of the experiment (3.32 umol/L) compared to the beginning (8.66 umol/L) at P<0.001.

Creatinine showed an increase at the end of the experimental period (76.38 umol/L) compared to the beginning (58.50) at P<0.01.

Table 6 presents the results of the biochemical analysis of the blood serum of the animals of the II experimental group at the beginning and at the end of the experiment. The obtained results show similar trends as in experimental group I (Table 5).

When adding prebiotic + probiotic, analogously to the addition of only the prebiotic supplement in the feed of the lambs, the albumin values decreased reliably at the end of the experiment compared to its beginning - from 32.05 g/L to 29.69 g/L at P<0.05.

The serum level of globulin at the beginning of the experimental period was 26.11 g/L, and at the end 26.40 g/L. The difference is minimal and is statistically unproven.

About glucose in the blood serum is a significant decrease (P=0.012) was reported from 8.74 mmol/L at baseline to 5.36 at baseline.

The concentration of urea in the serum increased significantly from 5.01 mmol/L at the beginning to 9.13 at the end of the experiment (P<0.001).

In the case of phosphorus, no statistically proven difference was found, as the concentration of the indicator at the beginning of the experimental period was higher - 2.72 mmol/L, and at the end it decreased to 2.54 mmol/L. The percentage difference is 6.62%.

There was a significant decrease in cholesterol level from 5.04 mmol/L at the beginning to 2.18 mmol/L at the end of the experimental period at P<0.001.

We found a significant decrease (P<0.05) in the level of alanine aminotransferase from 32.13 U/L at the beginning of the experimental period to 15.25 U/L at the end.

Serum levels of the bile pigment bilirubin decreased significantly (P<0.001) from 10.40 umol/L at baseline to 3.98 umol/L at the end of the trial. Expressed as a percentage, the reduction is 61.73%.

The research shows a trend towards a slight increase in serum creatinine concentration from 63.50 umol/L at the beginning of the experiment to 76.00 umol/L at the end at P<0.10.

We found a difference in the urea/creatinine ratio between the beginning and the end of the experiment at P<0.05 (table 6).

Table 6. Biochemical indicators of blood in Ile de France lambs with the participation of prebiotic Immunobeta + probiotic Zoovit in the II experimental group at the beginning and at the end of the experiment

Parameters	II experimental group		
	In the beginning (n=8) x̄±SD	At the end (n=8) x̄±SD	P-value
Albumin (G/L)	32.05±2.33	29.69±2.09	0.029*
Total protein (g/L)	58.16±4.39	56.09±2.76	0.195
Globulin (g/L)	26.11±3.99	26.40±2.49	0.855
Ratio albumin/globulin	1.24±0.21	1.16±0.15	0.378
Glucose (mmol/L)	8.74±1.45	5.36±0.60	0.012**
Urea (mmol/L)	5.01±0.92	9.13±1.98	0.000***
Phosphorus (mmol/L)	2.72±0.20	2.54±0.19	0.166
Cholesterol (mmol/L)	5.04±0.96	2.18±0.58	0.000***
Alanine aminotransferase (U/L)	32.13±19.95	15.25±7.59	0.035*
Bilirubin (umol/L)	10.40±2.30	3.98±1.22	0.000***
Alkaline phosphatase (U/L)	46.38±24.08	68.13±26.27	0.114
Creatinine (umol/L)	63.50±18.99	76.00±5.78	0.082
Ratio urea/creatinine	21.50±8.93	30.13±7.43	0.035*

*= P<0.05, **= P<0.01, ***= P<0.001.

4. Discussion

In view of the increasing antimicrobial resistance in humans and animals, a thorough search is needed for new alternative sources of biological basis such as prebiotics, probiotics and synbiotics, with a pronounced cumulative effect on the complex physiological, microbiological and biochemical processes in animals and humans.

The results of the effect of probiotic substances on hematological and biochemical parameters in small ruminants are relatively few and contradictory. According to Ayala-Monter et al. (2019) the effect of similar substances depends on the selected microbial strain or combination of strains, dose, time and frequency of intake, diet, breed of animal, physiological stage, production system.

Elliethy et al. (2022) cited significant differences in serum levels of urea, creatinine and minor differences in cholesterol and triglycerides in lambs fed diets supplemented with prebiotics and probiotics.

Albumin is the most abundant protein in the body. It accounts for half of the total plasma protein content in healthy humans and animals.

In the conducted research, we found close values of albumin in all three studied groups, and the differences are unreliable. Similar to our results for albumin and globulin in probiotic-supplemented lambs were reported by Chen et al. (2021). No differences were found between the control group and the synbiotic-supplemented group. Similar results cited by Sheikh et al. (2019) in an experiment with Corydell lambs.

Dar et al. (2022) found an increase in total protein and globulin concentration when consuming a synbiotic in calves. Increased levels of total protein following probiotic intake in lambs were reported by Abed et al. (2018), of albumin and globulin Abdel- Salam et al. (2014), Hussein (2014), Ismaeel et al. (2010), and in goats El-Ella and Kommonna (2013) and Singer et al. (2023).

Serum glucose concentration is affected by many factors, including nutrition, stress, and age. We found an unproven higher content of the metabolite in the synbiotic group compared to the other groups (P<0.05). Chen et al. (2021) found a decrease in serum glucose concentration in lambs supplemented with a probiotic feed.

Cholesterol plays an important role in the composition of the animal organism and some hormones. We found the highest cholesterol levels in animals from the control group and the lowest in lambs from the group receiving the prebiotic Immunobeta. Similar results to ours using a synbiotic to reduce serum cholesterol concentration in lambs were reported by Moarrab et al. (2016). Chen et al. (2021) reported lower cholesterol levels of 2.32 mmol\L in the probiotic-supplemented group compared to the control group 2.49 mmol\L. Research on cholesterol content should continue in this direction.

Urea and creatinine are interrelated. Creatinine is a waste product of muscle activity that is formed in the liver. The serum urea test provides important information about kidney and liver function, helping to diagnose various kidney diseases. Its importance is great in connection with the protein nutrition of animals. The highest values of urea were found in the control group, and the lowest in the lambs that received the prebiotic + probiotic combination. The differences are statistically unproven. Higher levels of urea in lambs receiving a probiotic were reported by Chen et al. (2021). Dimova et al. (2013) reported an increase in magnesium (P<0.05) and a decrease in urea and calcium in lambs supplemented with the probiotic Zoovit.

Alkaline phosphatase is an enzyme that occurs in several forms in the animal body. It is formed in the liver, bones and kidneys, as well as in the placenta of pregnant animals. It is responsible for the removal of phosphate groups from various molecules (dephosphorylation). Our study showed a trend of increased serum alkaline phosphatase level (P=0.051) in the prebiotic + probiotic group. An increased serum alkaline phosphatase concentration in sheep receiving a synbiotic supplement was reported by Fenta et al. (2023).

We found a slight tendency to lower total serum protein at the beginning and at the end of the experiment in the group receiving the prebiotic Immunobeta. Hussein (2018) cited a significant increase in total protein, glucose, urea nitrogen, and aspartate aminotransferase levels compared to the control group. Alanine aminotransferase and cholesterol levels can be determined as indicators of normal liver function. In our study, the level of serum alanine aminotransferase increased unprovably at the end of the experiment after taking the prebiotic Immunobeta compared to the beginning. Contrary to our results, a decrease in serum alanine aminotransferase concentration in goats supplemented with prebiotics was reported by Yuan et al. (2023).

Total protein values in the group receiving the prebiotic + probiotic combination showed a decrease from 58.16 g/L at the beginning of the trial to 56.09 at the end, the differences being insignificant. According to Wang, et al. (2022) administration of probiotics improves immunity and increases serum total protein levels in dairy calves.

We found a proven decrease (P=0.012) in the concentration of glucose at the end of the experiment compared to the beginning in the group of lambs that received the supplement prebiotic Immunobeta + probiotic Zoovit. A similar conclusion was reached by Toghdory et al. (2022) who reported a reliable decrease in serum glucose in lambs fed a probiotic + prebiotic supplement. Mansilla et al. (2024) reported reductions in serum glucose, changes in lipid profile and C-reactive protein values in calves receiving a lactic acid bacteria-based probiotic. Yasmin et al. (2021) and El-Sayed and Mousa (2019) found that after administration of probiotics serum glucose levels in cattle and lambs decreased.

We note an increase in the concentration of urea in the serum at P<0.001 after taking the synbiotic. A trend of increased urea levels after probiotic intake was cited by Azzaz et al. (2015) in goats. We report a decrease in the level of cholesterol after taking the synbiotic, the differences being significant at P<0.001. Abdel-Salam et al. (2014) also cited the lowering of cholesterol values in lambs after supplementation with synbiotics. Other authors also found a reduction in cholesterol values after administration of probiotics in cattle and lambs (Saleem et al., 2017; Yasmin et al., 2021).

5. Conclusion

1. Addition of prebiotic Immunobeta significantly reduced albumin (P<0.05), glucose (P<0.001), cholesterol (P=0.001), bilirubin (P<0.001), increased urea and creatinine levels (P<0.01) and resulted in a trend toward a decrease in total serum protein (P<0.10).
2. The addition of prebiotic Immunobeta + probiotic Zoovit to the ration of lambs leads to a significant decrease in the values of albumin (P<0.05), glucose (P=0.012), cholesterol (P<0.001), bilirubin (P<0.001), alanine aminotransferase (P<0.05), increase in urea (P<0.001), urea/creatinine ratio (P<0.05) and tendency to increase in creatinine (P<0.10).
3. A trend towards a higher level of globulin was found in the control group of lambs and a trend towards lower cholesterol levels in the group receiving the prebiotic Immunobeta (P<0.10).
4. An unproven tendency to increase the level of alkaline phosphatase was found in lambs receiving Immunobeta + Zoovit (P<0.10).

Author Contributions

The percentages of the author’s contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	N.I.	I.S.	S.L.
C	35	35	30
D			100
S	100		
DCP	50	50	
DAI			100
L	45	45	10
W	35	35	30
CR	40	40	20
SR	40	40	20
PM	20	20	60
FA	10	10	80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Permission to conduct the study was obtained with the decision of the Agricultural Academy - Sofia Research Ethics Committee (approval date: 20 November, 2021, protocol code: 17/189).

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