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Influence of the prebiotic Immunobeta and the combination Immunobeta + Zoovit probiotic on haematological parameters in breed Ile-de-France lambs

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Abstract. *The aim of the study was to evaluate the effect from supplementation of the prebiotic Immunobeta and the probiotic Zoovit on haematological parameters in Ile-de-France lambs. The experiment was conducted in the experimental farm of the Agricultural Institute, Stara Zagora. A total of 45 lambs divided into 3 groups of 15 animals each with similar initial live body weight, sex and birth type were included – one control and two experimental. Each animal from experimental group I was supplemented once daily with 8 g prebiotic Immunobeta and those from experimental group II received the same amount of the prebiotic plus 4 g probiotic Zoovit. Blood samples for analysis were collected from 8 animals per group in the beginning and the end of the trial. The group supplemented with the combination Immunobeta + Zoovit had statistically significantly higher haemoglobin content (P<0.05) and haematocrit (P=0.013) that the group fed a diet with Immunobeta prebiotic. The mean corpuscular volume was lower (P<0.05) in experimental group I vs. controls. The addition of the Immunobeta prebiotic resulted in reduced haemoglobin (P=0.012), haematocrit (P=0.012), mean corpuscular volume (P<0.001), mean corpuscular haemoglobin concentration (P<0.001), mean platelet volume (P=0.002), platelet distribution width (P=0.001) and volume of platelet participation (P=0.002) at the end of the trial period. After the supplementation with synbiotic, there was a trend towards lower haemoglobin content (P=0.088). The Immunobeta + Zoovit addition to the diet resulted in statistically significantly reduced haematocrit (P<0.05), reduced mean corpuscular volume, mean corpuscular haemoglobin concentration, mean platelet volume (P<0.001), platelet distribution width (P<0.01) and volume of platelet participation levels (P<0.05).*

Keywords: Ile-de-France, lambs, prebiotics, probiotics, *Immunobeta*, *Zoovit*, blood

Introduction

The spread of antibiotic resistance in animals and humans is increasingly accompanied with more possibilities for seeking and applying new biological sources, such as probiotics, prebiotics, plant extracts, enzymes and others, influencing digestive processes and health status in general.

The effects from the application of prebiotic, probiotic and synbiotic additives in lambs, sheep and goats were reported in numerous studies (Milewski et al., 2013; Otzule and Ilgaza, 2015; El-Sayed and Mousa, 2020; Mahmoud et al., 2020; Santana et al., 2023).

El-Mehanna et al. (2017), Gujar et al. (2018) and Raksasiri et al. (2019) found no substantial effect on red blood cell counts, haemoglobin and haematocrit in lambs, goats and cattle supplemented with

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synbiotic in relation to non-supplemented controls.

Raghebian et al. (2016), Ayala-Monter et al. (2019), Devyatkin et al. (2021) demonstrated that the addition of probiotic to feed had no influence on the haematology in lambs and sheep. By the end of the experimental period however, the haemoglobin content, the haematocrit and red blood cells were increased compared to the trial beginning in lambs, calves and cattle (Sarwar et al., 2011; Hussein, 2014; Agazzi et al., 2014; Raghebian et al., 2016; Ozeredova and Vasilev, 2017). Significant effects ($P<0.01$) in red and white blood cell counts, lymphocytes, and haemoglobin were reported in lambs supplemented with probiotic and Saccharomyces cerevisiae + *Lactobacillus acidophilus* probiotic by Sheikh et al. (2019) and Mousa et al. (2022). Insignificant differences in haemoglobin content, red and white blood cell counts were found out by Dimova et al. (2013) and Dimova et al. (2013a) in calves and lambs from the Bulgarian Dairy Synthetic Population supplemented with the probiotic Zoovit.

The addition of prebiotic to the diet of lambs and sheep had no impact on red blood cells, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, red blood cell distribution width and volumetric contribution of platelets to the total volume of blood (Ayala-Monter et al., 2019; Zheng et al., 2021). In prebiotic-supplemented weaned pigs, Valpotić et al. (2017) also reported no effect on red blood cell counts, haemoglobin, haematocrit, and mean corpuscular haemoglobin concentration.

Klebaniuk and Czech (2007), Król (2011), Ilgaza and Arne (2021), however, found out that the addition of prebiotics increased red blood cell counts, haemoglobin and haematocrit in calves and lambs.

The overview of the available literature made clear that, on one hand, a lot of various types of biologically active preparations with pre- and probiotic properties are offered, which probably act on haematological indicators through a different mechanism. On the other hand, there are certain discrepancies regarding the action of probiotics, prebiotics and synbiotics on haematological parameters in lambs and sheep. By conducting this experiment, we will either confirm or reject some of the controversies and establish the influence of specific probiotics and prebiotics. The aim of the study was to evaluate the effect from supplementation of the prebiotic *Immunobeta* and the probiotic *Zoovit* on haematological parameters in Ile-de-France lambs.

Material and methods

The experiment was conducted in the experimental farm of the Agricultural Institute, Stara Zagora. A total of 45 Ile-de-France (IF) lambs divided into 3 groups of 15 animals each with similar initial live body weight, sex and birth type were included – one control and two experimental. The lambs were reared in group boxes supplied with feeders for hay and concentrate and drinkers with constant access to fresh tap water according to Ordinance No. 40 on the conditions for raising agricultural animals, considering their physiological and behavioural characteristics. The animals received an ad *libitum* diet (+ 5 to 10% residue) corresponding to their age that met all requirements for nutrients and biologically active substances. The diet included concentrate and alfalfa hay (Table 1 and Table 2).

Table 1. Composition of compound feed of Ile-de-France lambs

Component	%
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

Table 2. Nutritional content of compound feed of Ile-de-France lambs

Component	%
Protein	15.90
Fat	2.40
Fibre	5.43
Moisture	11.40
Ca	1.20
P	0.50
Salt	0.570

The compound feed contained 1.12 units for growth, 2778.25 kcal/kg and TDN 0.174. Each animal from experimental group I was supplemented once daily with 8 g prebiotic *Immunobeta* and those from experimental group II received the same amount of the prebiotic plus 4 g probiotic *Zoovit*. The probiotic preparation *Zoovit* contains four strains lactic acid bacteria: *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus salivarius subsp. termophilus, Lactobacillus acidophilus, Lactobacilus lactis* and one strain *Propinibacterium*.

Table 3. Chemical composition of the Zoovit probiotic

Component	%
Protein	29.29
Lactose	52.14
Fat	0.95
Dry matter	94
Lactic acid	2.75
Propionic acid	3.10
Minerals	8.76
Active cells count	not less than 2.5×10^7 cfu/g
Colibacteria	not detected
Moulds and yeasts	not detected
Salmonellae in 25 g	not detected
Coagulase-positive staphylococci in 1 g	not detected

The additive *Immunobeta* is a prebiotic with marked immunostimulatory effect. It is produced from *Saccharomyces cerevisiae* strains via enzymatic autolysis. It contains 30% β-glucans, 25% mannan oligosaccharides and 7% nucleotides.

Blood samples for analysis were collected from the jugular vein (*v. jugularis interna)* of 8 animals from each group (total of 24 lambs) in the morning after 12-hour fasting at the beginning and at the end of the trial in vacutainers with K_2EDTA as anticoagulant. After the collection, the samples were assayed on a haematological analyser Mindray BC-20800.

Statistical analysis

Data were processed with one-way analysis of variance ANOVA, Student's t-test for dependent variables to compare the haematological parameters with licensed statistical software IBM SPSS Statistics 19 and MS Excel 2016.

The homogeneity of data variance and the normal distribution of individual variables were tested. Normally distributed variables with equal variances were tested by one-way analysis of variance ANOVA, whereas parameters with normal distribution – by Student's t-test.

In case of statistically significant F-value ($P \leq 0.05$), the LSD post hoc test was used for between-group comparisons. In case of zero hypothesis rejection, the non-parametric analogue of ANOVA (Kruskal-Wallis and Bonferroni-corrected post hoc Mann-Whitney test) were applied. The comparison of dependent samples was done with the Wilcoxon rank sum test.

The differences were considered statistically significant at $P<0.05$, and values $P<0.10$ were interpreted as showing a tendency towards statistical significance.

Results and discussion

Table 4 presents the hematological parameters in IF lambs at the end of the experimental period. Except for haemoglobin ($P<0.05$), haematocrit ($P=0.013$) and mean corpuscular volume ($P<0.05$), there were no statistically significant differences in the values of the other haematological parameters.

Haemoglobin is the main component of erythrocytes in animals, and its main function is transportation – to carry oxygen to the tissues in the animal body. The parameter is influenced by some factors such as sex, age, etc. (Angelov et al., 1999). The content of haemoglobin recorded the highest value in the animals of 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. The mean levels of the parameter differed significantly between the experimental groups I and II ($P<0.05$). Dar et al. (2017) found the highest haemoglobin in calves receiving a synbiotic compared to those from the control group. Other authors also demonstrated that the addition of prebiotics and synbiotics to calf feed resulted in significantly higher haemoglobin content than in the control group (Ilgaza and Arne, 2021). Mallaki et al. (2021), Hamdon et al. (2022), Al-Musodi and Al-Zwean (2023) and Sen and Pu-Hui (2024) reported a significant increase in haemoglobin of lambs fed probiotic bacterial strains.

Regarding the mean corpuscular volume, it recorded the highest levels in the control lambs - 31.38 fl, followed by the second experimental group - 29.76 fl and the first experimental group - 29.36 fl. MCV values differed statistically significantly (P<0.05) between the animals of the experimental group I and the control group. El-Sayed and Mousa (2020) reported results similar to ours for MCV in lambs receiving a probiotic on days 15 and 30 - 32.4 and 31.7, respectively.

Haematocrit reflects the volume of blood cells as a percentage of the total amount of blood. The haematocrit value determines the mean corpuscular volume and the mean corpuscular haemoglobin concentration (Angelov et al., 1999). In this study, haematocrit recorded the highest level in the group that received the combination of prebiotic + probiotic - 0.37 L/L, followed by the controls – 0.35 L/L and experimental group I – 0.33 L/L, with statistically significant differences between the experimental groups I and II (P=0.013). Król (2011) reported haematocrit values in calves in the prebiotic group of 0.301 L/L which were very close to ours.

The mean corpuscular haemoglobin was the highest in the lambs from the control group (9.23 pg), followed by lambs from the experimental group II - 8.93 pg and experimental group I - 8.80 pg. The difference in MCH values between the control and the 1st experimental group was 4.66%, e.g. insignificant.

There were no statistical differences in MCHC as the differences among the three groups were minimal. Average MCH was 296 g/L in the control group, 302 g/L in experimental group I and 302 g/L in the lambs supplemented with synbiotic. Dar et al. (2017) found significantly higher mean corpuscular haemoglobin concentration (MCHC) in calves receiving prebiotic and synbiotic compared to control group calves. For the rest of the studied blood parameters– red blood cell distribution width, mean platelet volume, platelet distribution width and volumetric contribution of platelets to the total volume of blood, the differences in average levels were statistically inconsistent.

Table 4. Haematological parameters in Ile-de-France lambs from the control group, experimental groups I and II by 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, ×1012/L)	8	11.32±1.37	8	11.24±0.65	8	12.45±2.07	0.213
Haemoglobin (HGB, g/L)	8	104.88±11.33ab	8	99.50±2.67b	8	111.63±16.76a	0.041
Haematocrit (HCT, L/L)	8	0.35±0.03ab	8	0.33±0.01b	8	0.37±0.05a	0.013
Mean corpuscular volume (MCV, fL)	8	31.38±1.71a	8	29.36±1.44b	8	29.76±1.53ab	0.042
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	0.608
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	0.617
Volume involvement of platelets (PCT, %)	8	0.15±0.05	8	0.17±0.05	8	0.18±0.03	0.287

a; b – different letters indicate statistically significant differences within a row at P<0.05

Thrombocytes are part of the blood components. Also called platelets, they are anucleate cells formed in the bone marrow of animals and humans.

Figure 1 shows the arithmetic means, the standard errors of the mean and the p-value of platelet counts for the three groups of lambs at the end of the experimental period.

The highest platelet counts were registered in the animals from experimental group II - 385.5 $\times 10^9$/L, followed by the experimental group I with 374 $\times 10^9$/L and the control group - 305.38 $\times 10^9$/L. Between-group differences were not statistically significant ($P>0.05$). Saleem et al. (2024) reported higher platelet counts in Saidi rams after supplementation with multi-strain probiotics (MSP) in combination with *Saccharomyces cerevisiae*. Wu et al. (2022) also found significantly increased ($P=0.03$) platelet counts in the experimental group receiving an oligosaccharide supplement with marked prebiotic properties compared to the control group.

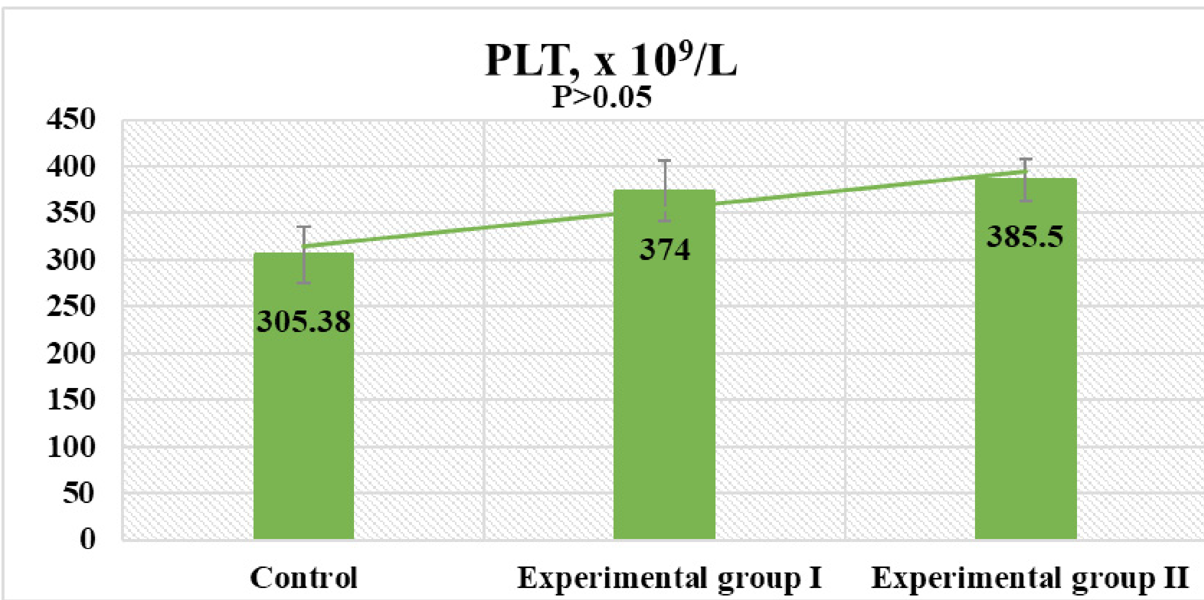

Figure 1. Platelet counts in lambs from the control group, experimental groups I and II by the end of the trial period

The values of haematological parameters in lambs from experimental group I at the beginning and the end of the trial are presented in Table 5. There was a slight insignificant reduction of red blood cell counts from 11.79 $\times 10^{12}$/L at the beginning to 11.24 $\times 10^{12}$/L by the end of the supplementation with Immunobeta prebiotic.

There was a consistent decrease ($P=0.012$) in the haemoglobin by 19.35% between the beginning (123.38 g/L) and the end (99.50 g/L) of the experimental period. Haematocrit showed a significant decrease from 0.41 L/L at the beginning to 0.33 L/L at the end of the trial ($P=0.012$). The statistically significant reduction in haemoglobin and haematocrit values reported by us were in line with the results of other authors in calves (Ilgaza and Arne, 2021).

For the mean corpuscular volume, a decrease from 34.98 fL at the beginning to 29.36 fL at the end of the addition of the prebiotic product Immunobeta was reported. The differences in this haematological parameter values were 16.07% ($P<0.001$). The mean corpuscular haemoglobin decreased from 10.50 at the beginning of the experiment to 8.80 pg at the end of the supplementation ($P<0.001$). The mean platelet volume level registered a significant decrease ($P=0.002$) from 5.21 fL at the beginning to 4.63 fL at the end of the experimental period. The difference between the initial and final values was 11.13%.

There was a highly significant reduction ($P=0.001$) in the platelet distribution width from 14.11 at the beginning to 13.30 at the end of the experimental period. With volume involvement of platelets, a significant decrease in values was also registered - from 0.27% to 0.17% ($P=0.002$).

Table 5. Haematological parameters in lambs from experimental group I supplemented with *Immunobeta* prebiotic at the beginning and at the end of the trial 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, ×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	0.012*
Haematocrit (HCT, L/L)	8	0.41±0.05	8	0.33±0.01	0.012*
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	0.002**
Platelet distribution width (PDW)	8	14.11±0.42	8	13.30±0.26	0.001***
Volume involvement of platelets (PCT, %)	8	0.27±0.08	8	0.17±0.05	0.002**

* - P≤0.05, ** - P≤0.01, *** - P≤0.001

Table 6 presents the haematological parameters in IF lambs from experimental group II supplemented with prebiotic *Immunobeta* + probiotic *Zoovit* at the beginning and at the end of the trial.

The red blood cell counts demonstrated a negligible decrease from 13.15 ×10^{12}/L at the beginning to 12.45 ×10^{12}/L at the end of the experimental period (P>0.05). Ilgaza and Arne (2021) found no variation in RBC counts in calves after synbiotic intake. They reported RBC counts before taking synbiotics of 7.4 ± 1.44, and 28 days after treatment with 6 g synbiotic - 7.4 ± 0.83. The haemoglobin content was higher at the beginning of the experiment – 144.88 g/L and decreased to 111.63 g/L by its end. The difference was by 22.95%, taking into account an insignificant trend towards a reduction of the indicator at the end of the trial (P=0.088).

There was a significant decrease in haematocrit (P<0.05) from 0.48 L/L to 0.37 L/L after co-administration of the prebiotic *Immunobeta* and the probiotic *Zoovit*. Unlike us, other authors reported the same haematocrit values before and after administration of 6 g of synbiotic in calves for 28 days. However, a negligible decrease was present after intake of 12 g of synbiotic for 28 days - 23.9 ± 3.63 before its intake and 23.2 ± 5.98 after synbiotic intake (Ilgaza and Arne, 2021).

The mean corpuscular volume decreased statistically significantly from 36.24 fL at the beginning of the experiment to 29.76 fL at the end (P<0.001). A highly significant decrease (P<0.001) in mean corpuscular hemoglobin from 10.93 pg at the beginning of the trial to 8.93 pg at its end has occurred.

The mean corpuscular haemoglobin concentration was 303.00 g/L at the beginning of the experiment and 302.13 g/L at its end. The difference between the values of the indicator was insignificant (P>0.05) and below 1%.

Regarding the red blood cell distribution width, differences were not present either. There was a significant decrease (P≤0.001) in the mean platelet volume, 5.28 fL at the beginning and 4.65 fL at the end of synbiotic addition.

The platelet distribution width recorded statistically significantly higher levels at P<0.01 at the beginning of the experiment (14.14) than its end (13.40).

Regarding volume involvement of platelets concentration, initial and final levels were 0.33% and 0.18% (P<0.05), and the difference between the two periods was 45.45%.

Table 6. Haematological parameters in lambs from experimental group II supplemented with *Immunobeta* prebiotic + *Zoovit* probiotic at the beginning and at the end of the trial 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, ×10^{12}/L)	8	13.15±4.27	8	12.45±2.07	0.642
Haemoglobin (HGB, g/L)	8	144.88±49.26	8	111.63±16.76	0.088
Haematocrit (HCT, L/L)	8	0.48±0.15	8	0.37±0.05	0.025*
Mean corpuscular volume (MCV, fL)	8	36.24±1.43	8	29.76±1.53	0.000***
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***
Platelet distribution width (PDW)	8	14.14±0.52	8	13.40±0.21	0.006**
Volume involvement of platelets (PCT, %)	8	0.33±0.16	8	0.18±0.03	0.032*

* - P≤0.05, ** - P≤0.01, *** - P≤0.001

Conclusion

The performed experiment demonstrated:

• Statistically significantly higher haemoglobin content (P<0.05) and haematocrit (P=0.013) in the group supplemented with the combination *Immunobeta* + *Zoovit* compared to the group fed a diet with *Immunobeta* prebiotic. The mean corpuscular volume was lower (P<0.05) in experimental group I vs. controls.

• Reduced haemoglobin (P=0.012), haematocrit (P=0.012), mean corpuscular volume (P<0.001), mean corpuscular haemoglobin concentration (P<0.001), mean platelet volume (P=0.002), platelet distribution width (P=0.001) and volume involvement of platelets (P=0.002) at the end of the trial period in lambs after addition of the *Immunobeta* prebiotic

• Trend towards lower haemoglobin content (P=0.088) after the supplementation with synbiotic

• Statistically significantly reduced haematocrit (P<0.05), reduced mean corpuscular volume, mean corpuscular haemoglobin concentration, mean platelet volume (P<0.001), platelet distribution width (P<0.01) and volume involvement of platelets levels (P<0.05) as a result from *Immunobeta* + *Zoovit* addition to the diet

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