



## Effectiveness of using probiotic Batcinel-K® and CEVAC SET-K® vaccine on some blood parameters in chickens

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### Abstract

In the current study, probiotic Batcinel-K® and CEVAC SET-K® vaccine are used to determine their effects on some hematological and biochemical parameters of broiler-chickens. Three hundred broilers chicks "Ross-308" at one-day old were divided into six groups of 50 chicks /each. Blood was taken at 56<sup>th</sup>, 63<sup>rd</sup> and 112<sup>nd</sup> days old. The count of erythrocytes and leukocytes was calculated. Hemoglobin and total serum protein, serum albumin and globulins concentration was determined. Results show that these selected probiotic can improve some of the blood indexes of birds, especially in combination with the vaccine. At the 63<sup>rd</sup> day old of broilers chicken, globulin parameters in 4<sup>th</sup> and 5<sup>th</sup> treatment groups were higher by 2.97% (P<0.01) and 5.19% (P<0.05) respectively, compared to the control group. Moreover, at the 112<sup>nd</sup> day, level of total protein in the 4<sup>th</sup> group at the end of the experiment was higher than in control group at 8.1% (P<0.05), as well as in the 5<sup>th</sup> group by 3.96% (P<0.01). In addition to the concentration of albumin and globulin that were higher relatively concerning the control.

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### Introduction

Probiotics were initially added to the feed for enhancing animal productivity and improve their health, resulting in increased disease resistance. The inhibitory activity of probiotic against gut infections is due to metabolites such as hydrogen peroxide, organic acids and other inhibitory substances such as bacteriocins produced by probiotic bacteria (1,2). Moreover, supplementation of probiotic enhances immune system and inhibit pathogens (3). Probiotic "Vetlactoflorum-M" can use for reducing the infection of birds with pathogenic microflora, including salmonella, by 20.0% (4). The use of probiotics to enhance productivity in poultry is currently of great interest. Probiotics and prebiotics may improve health by stimulating production of antibody (5). Researchers showed that probiotics in feed and water have a therapeutic and prophylactic effect. The growth-stimulating effect was also

noted. In the same way, the feed conversion coefficient when antibiotics were added to the feed and the use of probiotics in feed and water was significantly better in the birds of the experimental groups than in the control group (6). Furthermore, probiotic effect positively on hematological, biochemical and immunological parameters of blood in broiler-chickens (7-9), additionally it improves meat quality (10), natural resistance (11) and productivity of broiler-chickens (12). The number of *E. coli* decreased in chickens treated aerosol with *Enterococcus faecium* also *E. coli* in turkeys decreased when they received the *Enterococcus faecium* strain with drinking water once a week (13). Both monoprotiotics and a combination of probiotics with organic acids, when added to rations, reduce the number of pathogenic bacteria in the ileum and cecum (14).

Our study aimed to estimate the effectiveness of the use of the probiotic Batcinel-K® and vaccine against salmonellosis CEVAC SET K® on broiler chickens. The

following tasks were set for us, to study the effect of the preparation on natural resistance of organism on broiler chickens.

## Materials and methods

In the current experiment have been studied the prophylactic effectiveness of the Batcinel-K®, the effect of this probiotic preparation and CEVAC SET K® were studied, an inactivated avian salmonellosis vaccine made from formalin-inactivated cultures of the strains *Salmonella typhimurium* and *Salmonella enteritidis*, for the natural resistance in broiler chickens.

Batcinel-K® is liquid preparation of spore-forming bacteria *Bacillus subtilis* BIM- B 454 D. Probiotic prepared by the staff of Institute of Experimental Veterinary Medicine named S.N. Vyshel'skovo and Institute of Microbiology of the Belarusian National Academy of Science. We purchased 300 chicks of Ross-308 from Vitebsk Broiler chickens farm in Belarus and divided them into 6 groups of 50 birds each one (M = 40g), chicks reared on litter and fed *ad libitum*.

In laboratory conditions, we studied the effect of the biologically active supplement probiotic Batcinel-K® on some physiological indexes on the organism of birds with an optimal dose of the drug. Randomly the chicks (of both sexes) were divided into six groups; first group broilers of the 1st control group were fed only standard full feed. Second group broiler chickens of the 2<sup>nd</sup> experimental group were supplied only a probiotic Batcinel-K® with drinking water at a dose of 0.1-0.2 cm<sup>3</sup>/bird starting at one-day-old for 5 days daily, in 9 cycles with an interval of 7-10 days until the end of the experiment. Third experimental group vaccinated only by inactivated CEVAC SET K® vaccine against avian salmonellosis at the 7<sup>th</sup> week once subcutaneously in the upper third region of the neck at the dose of 0.5 cm<sup>3</sup> and revaccinating on 14<sup>th</sup> week of age subcutaneously in the region of the upper third of the neck at 0.5 cm<sup>3</sup>. Fourth experimental group starting from a day old, supplemented with probiotic Batcinel-K® for 5 days daily, until the 5<sup>th</sup> week of age with drinking water in a dose of 0.1-0.2 cm<sup>3</sup>/bird in 4 cycles with an interval of 7-10 days. Probiotic Batcinel-K® was added to drinking water from the 6<sup>th</sup> week in a dose of 0.2 cm<sup>3</sup> /bird once daily for 5 days, (a week before the 1st vaccination). In the 7<sup>th</sup> week, birds were vaccinated subcutaneously in the upper third of the neck at a dose of 0.5 cm<sup>3</sup> by the first vaccination. From 13 until 14 weeks of age, a probiotic Batcinel-K® was added to drinking water at a dose of 0.3 cm<sup>3</sup>/bird within 5 days daily (5 days before revaccination). At 14<sup>th</sup> week of age 2<sup>nd</sup> vaccination (revaccination) subcutaneously in the upper third of the neck at a dose of 0.5 cm<sup>3</sup>. Fifth experimental group, starting from one-day old until 5 weeks of age, were supplied with a probiotic Batcinel-K® with drinking water at a dose of 0.1-0.2 cm<sup>3</sup>/bird/day during 5 days daily in 4 cycles with an interval of 7-10 days. From 6<sup>th</sup> week of age, probiotic Batcinel-K® was added in a dose of 0.2 cm<sup>3</sup>/bird/day for 3

days (a week before the 1st vaccination). On the 7<sup>th</sup> week, 1-st vaccination is applied in the upper third region of the neck subcutaneously, at a dose of 0.5 cm<sup>3</sup>. From the 7<sup>th</sup> until the 13<sup>th</sup> week of age, probiotic Batcinel-K® was added at a dose of 0.3 cm<sup>3</sup>/bird/day for 3 days with an interval of 7-10 days. 2<sup>nd</sup> vaccination (revaccination) was applied at the 14<sup>th</sup> week, subcutaneously in the upper third region of the neck at a dose of 0.5 cm<sup>3</sup>. Sixth experimental group, starting from a day old to 3 weeks' old supplied a probiotic Batcinel-K® daily with drinking water at a dose of 0.1-0.2 cm<sup>3</sup>/bird during 5 days in 2 cycles with an interval of 7 days. On the 4<sup>th</sup> week, probiotic was added in a dose of 0.2 cm<sup>3</sup>/bird/day for 2 days (a week before the 1st vaccination). On the 7<sup>th</sup> week, 1-st vaccination was applied subcutaneously in the upper third region of the neck at a dose of 0.5 cm<sup>3</sup>. From 7<sup>th</sup> until the 13<sup>th</sup> week, probiotic was added at a dose of 0.3 cm<sup>3</sup>/bird/day for 2 days with an interval of 7-10 days. At 14<sup>th</sup> week, 2-nd vaccination was applied subcutaneously in the upper third region of the neck at a dose of 0.5 cm<sup>3</sup>.

To study hematological parameters, blood was taken at 56<sup>th</sup>, 63<sup>rd</sup> and 112<sup>nd</sup> days old. Day-old broiler chickens had standard biochemical parameters. Blood was carried out according to (15). The blood was obtained by decapitation and from the heart and axillary vein in compliance with aseptic and antiseptic rules in two sterile tubes at 56<sup>th</sup>, 63<sup>rd</sup> and 112<sup>nd</sup> days old (15). In one of them, the blood was stabilized with trilon B anticoagulant (disodium EDTA - ethylenediaminetetraacetic acid) at the rate of 0.1-0.2 ml of a 10% solution per 10 ml of blood, the other was used to obtain serum. Blood serum was obtained after blood coagulation at a temperature from 18-20°C, followed by centrifugation at 1500 rpm for 10-15 min. To accelerate the separation of serum, coagulated blood was separated from the walls of the tube with stainless steel spoke and placed in a thermostat at a temperature of from 37°C to 38°C for 45-60 min (16).

The count of erythrocytes and leukocytes was calculated in the Goryaev chamber, the hemoglobin content was determined by a unified colorimetric method using a KFK-ZOM photoelectrocolorimeter, Russia. The leukogram was displayed based on counting 100 cells in blood smears stained according to Pappenheim (17).

The total serum protein was determined by the biuret method on an Eurolyser automatic biochemical analyzer (spectrophotometer- SF 2000-M) using reagents from the Cormany kit (Poland), the protein content was expressed in g/l. according to (18). The determination of serum albumin concentration was determined by a unified method by reaction with bromocresol green. The content of albumin and globulins was expressed in g/l. Studies of the indicated parameters of blood serum were carried out on a Eurolyser biochemical analyzer using diagnostic kits from Cormany (Poland) at laboratories of Vitebsk State academy of Veterinary Medicine (17,18).

The digital material of experimental studies is subjected to mathematical and statistical processing on a personal

computer by methods of variation statistics. The arithmetic means of each variational series, standard deviations of the average, degree of probability of the null hypothesis are determined in comparison with the control by calculating the Student-Fisher criterion. At  $P < 0.05$ , the difference in the arithmetic mean of the compared variation series was considered significant (19,20).

### Results

The results of general biochemical and hematological blood parameters of broiler chickens (Table 1-3). The data in Table 1 shows that during the technological cycle of growing chickens, there was a general tendency to increase hemoglobin, erythrocytes and leukocytes levels. The hemoglobin level at 56 day of old in the 4<sup>th</sup> experimental group was 22.35% higher than the control group. By the 63 day of old, in the 4<sup>th</sup> group in comparison to the control group higher by 17.40%, in the 5<sup>th</sup> - by 15.95% and the 6<sup>th</sup> - by 16.78%. By the 112 day of old in the 4<sup>th</sup> group, compared to the control group, this indicator was higher by 12.18% in the 5<sup>th</sup> - by 11.21% and the 6<sup>th</sup> - by 11.27%.

The level of red blood cells on 56<sup>th</sup> day of old in the 4<sup>th</sup> experimental group was higher than in the control group by 33.49%, at 63 day of old in the 4<sup>th</sup> - by 29.60%; in the 5<sup>th</sup> - by 24.00% and the 6<sup>th</sup> - by 21.60%. At 112 day of old in the 2<sup>nd</sup> experimental group, the content of erythrocytes was higher - by 9.03%; in the 3<sup>rd</sup> - by 9.69%, in the 4<sup>th</sup> - by 31.94%, in the 5<sup>th</sup> - by 15.20% and the 6<sup>th</sup> - by 10.57%.

The leukocyte level at 56 day of old in the 4<sup>th</sup> group was higher - by 8.14%; in the 5<sup>th</sup> - by 7.13% and the 6<sup>th</sup> - by

6.01%, at 63 day of old in the 4<sup>th</sup> - by 24.47, in the 5<sup>th</sup> - by 16.04 and the 6<sup>th</sup> - by 12.42%. At 112 day of old, the leukocyte count in the 2<sup>nd</sup> experimental group was higher by 10.66, in the 3<sup>rd</sup> - by 12.71, in the 4<sup>th</sup> - by 25, 28, in the 5<sup>th</sup> - by 17.47 and the 6<sup>th</sup> - by 16.62%.

The data in table 2 indicate a significant increase in the content of protein metabolism throughout the trail in the experimental groups 2, 4, 5 and 6. So, on the 56 day of old, the level of total protein in the 2<sup>nd</sup> experimental group was higher than in the serum of the blood of chickens of the control group by 15.85, in the 4<sup>th</sup> by 24.52%; in the 5<sup>th</sup> group - by 19.70%; in the 6<sup>th</sup> - by 18.09%.

At 63 day of old, the level of total protein in the 2<sup>nd</sup> experimental group was higher in blood serum of birds than in the control group by 14.73, in the 3<sup>rd</sup> group - by 22.26, in the 4<sup>th</sup> group - by 29.55%; in the 5<sup>th</sup> group - by 26.29%; in the 6<sup>th</sup> group - by 25.92%. On the 112 day of old, the level of total protein in the 2<sup>nd</sup> group was higher in blood serum of birds than in the control group by 12.44%; in the 3<sup>rd</sup> group - by 14.85, in the 4<sup>th</sup> group - by 25.83, in the 5<sup>th</sup> group - by 20.44%; in the 6<sup>th</sup> group - by 20.75% (Table 2). Albumin content in the chickens of the treatment groups was higher concerning the control group. At 63 day of old, the albumin level in the 2<sup>nd</sup> experimental group was higher in blood serum of chickens than of the control group by 12.78% 4 in the 4<sup>th</sup> - by 16.93% 4 in the 5<sup>th</sup> - by 15.39 and in the 6<sup>th</sup> - by 16.83%. At 112 day of old, in the 2<sup>nd</sup> experimental group, the albumin content was higher in the blood serum of chickens than of the control group by 19.79%; in the third - by 24.35%; in the 4<sup>th</sup>, by 20.89%; in the 5<sup>th</sup>, by 22.80; in the 6<sup>th</sup>, by 25.55% (Table 2).

Table 1: Hematological parameters of broiler (Mean± SE)

Age	Group	Hemoglobin (G/L)	Erythrocyte ( $\times 10^{12}/L$ )	Leukocyte ( $\times 10^9/L$ )
56 Days (8 weeks)	1	46.53±3.18	2.12±0.26	16.65±0.87
	2	49.77±2.69*	2.62±0.33*	15.98±0.36*
	3	46.67±3.50	2.1±0.12	16.94±0.58*
	4	56.93±6.49***	2.83±0.11**	16.01±0.66*
	5	56.62±6.29***	2.57±0.32*	16.32±1.03
	6	56.96±5.57***	2.63±0.24*	16.29±0.59
63 Days (9 weeks)	1	69.08±2.10	2.50±0.25	16.93±0.58
	2	72.76±2.37*	2.97±0.33*	16.26±0.36*
	3	69.63±3.87	2.51±0.12	17.19±0.45
	4	81.10±5.54***	3.24±0.11***	16.22±0.70*
	5	80.10±5.35***	2.94±0.32*	16.6±0.54
	6	80.67±5.38	3.04±0.25**	16.61±0.52
112 Days (16 weeks)	1	99.06±2.55***	4.54±0.25	23.7±0.83
	2	103.11±2.92***	4.95±0.34**	20.60±0.31
	3	99.64±0.75***	4.51±0.10	21.38±0.45*
	4	111.15±5.01***	5.23±0.13***	21.22±0.70*
	5	110.03±5.43***	4.93±0.30**	21.61±0.54**
	6	110.73±5.55***	5.02±0.30***	21.62±0.52**

\* Significant with control at  $P < 0.05$ ; \*\* Significant with control at  $P < 0.01$ ; \*\*\* Significant with control at  $P < 0.001$ .

Table 2: Biochemical parameters of blood serum broiler (Mean± SE)

Age	Group	Total Protein (G/L)	Albumin (G/L)	Globulin (G/L)	A/G
56 Days (8 weeks)	1	29.08±0.99	20.17±0.79	8.96±1.035	2.31±0.31
	2	30.51±0.68	20.35±0.49	12.32±2.34***	1.80±0.39**
	3	34.34±0.73***	21.22±0.67**	14.54±1.59***	1.49±0.16***
	4	36.21±2.07***	19.01±0.51**	16.47±1.02***	1.26±0.13***
	5	34.81±2.12***	19.36±1.80**	15.70±0.84***	1.26±0.12***
	6	33.69±0.93**	18.16±1.10**	15.29±0.67***	1.18±0.12***
63 Days (9 weeks)	1	35.75±2.51	18.76±0.43	10.60±0.33	1.77±0.089
	2	37.62±1.361**	21.32±0.47***	15.64±1.64***	1.58±0.09
	3	40.09±1.86***	22.80±0.86***	17.63±0.57***	1.30±0.08**
	4	42.48±1.63***	22.10±0.56***	19.69±0.54***	1.13±0.03***
	5	41.41±1.27***	21.89±0.69***	18.92±0.46***	1.16±0.07***
	6	41.29±0.76***	21.61±0.63***	18.52±0.44***	1.17±0.04***
112 Days (16 weeks)	1	38.75±2.51	20.16±0.30	12.26±0.24	1.26±0.07**
	2	40.66±1.38**	21.75±0.29**	18.38±0.68***	1.19±0.03***
	3	43.29±1.86***	21.33±0.79*	17.96±0.55***	1.19±0.03***
	4	45.98±1.63***	26.52±0.64***	21.89±0.12***	1.21±0.03**
	5	44.84±1.30***	23.32±1.099***	21.58±0.88***	1.08±0.08***
	6	44.66±0.82***	22.89±0.57***	20.52±0.43***	1.12±0.02***

\* Significant with control at P<0.05; \*\* Significant with control at P<0.01; \*\*\* Significant with control at P<0.001.

Table 3: Parameters of enzymes metabolism in the serum of broiler (Mean± SE)

Age	Group	AST (microkat/L)	ALT (microkat/L)
56 Days (8 weeks)	1	184.11±6.42	9.11±0.51
	2	167.78±6.80	8.3±0.64**
	3	186.41±4.23	8.43±0.67**
	4	182.11±4.58	8.14±0.49**
	5	189.05±20.85	8.39±0.19**
	6	163.42±22.51*	8.44±0.23**
63 Days (9 weeks)	1	175.05±5.65	6.14±0.37
	2	155.79±6.98*	6.12±0.57
	3	174.68±4.48	6.13±0.18
	4	170.87±4.47	5.95±0.25
	5	177.26±21.04	6.03±0.58
	6	151.65±22.30*	6.03±0.55
112 Days (16 weeks)	1	137.89±3.43	6.14±0.37
	2	118.37±4.36*	6.12±0.57
	3	126.89±4.24	6.13±0.18
	4	121.65±4.69	5.95±0.25
	5	123.99±8.50	6.03±0.58
	6	122.67±4.30	6.03±0.55

\* Significant with control at P<0.05; \*\* Significant with control at P<0.01; \*\*\* Significant with control at P<0.001.

At 56, 63 and 112 day of old in broiler chickens, the globulin levels in the experimental groups were higher than the control value. So, on the 7<sup>th</sup> day after the 1<sup>st</sup> vaccination, the level of globulins in the 2<sup>nd</sup> experimental group was higher by 24.49% (P<0.05), on the 4<sup>th</sup> - by 46.66%; in the 5<sup>th</sup> - by 39.80%; in the 6<sup>th</sup> - by 36.15% (P<0.001). At 63 day of old, the globulin levels in the 4<sup>th</sup> experimental group were higher 48.05%; in the 5<sup>th</sup> - by 42.26%; in the 6<sup>th</sup> - by 39.25% (P<0.01). At 112 day of old, in the 4<sup>th</sup> experimental group,

globulin content was higher by 34.21% (P<0.001), in the 2<sup>nd</sup> - by 5.01%; in the 3<sup>rd</sup> - by 5.26% (P<0.05), in the 5<sup>th</sup> - by 18.02%; in the 6<sup>th</sup> - by 15.84% (P<0.01).

At the 112th day, total protein levels in the 4<sup>th</sup> group to the end of the experiment was higher than in control group to 8.1% (P<0.05) in the 5<sup>th</sup> group by 3.96% (P<0.01). The concentration of albumin was higher relative to the control group. Similarly, results of globulins were higher than the control group. In the 63<sup>rd</sup> day of broilers chicken, globulin

content was as high by 2.97% ( $P < 0.01$ ) and 5.19% ( $P < 0.05$ ) in 5<sup>th</sup> and 4<sup>th</sup> treatment groups compared to the control group, respectively (Table 2).

In chickens of all groups observed a decrease of alanine aminotransferase (ALT) activity. In the 14<sup>th</sup> day after the 1<sup>st</sup> vaccination of broiler chickens, the aspartate aminotransferase (AST) level in the 2<sup>nd</sup> group was lower than the control group - by 11.90% ( $P < 0.05$ ), also there was a decrease on the aminotransferase enzymes level AST, ALT in the serum at 112 day of old of experimental chickens (Table 3), the content of AST in the 2<sup>nd</sup> group was lower than in the control group - by 10.08%; 3<sup>rd</sup> - by 7.98% ( $P < 0.05$ ), 4<sup>th</sup> - by 13.67% ( $P < 0.01$ ), 5<sup>th</sup> - by 11.78%; 6<sup>th</sup> - by 11.04% ( $P < 0.05$ ) (Table 3).

Throughout the observation period, there is increasing activity of aminotransferase enzymes in the blood serum of experimental chickens was noted (Table 3).

## Discussion

Increased hemoglobin and mean MCHb values might be due to the probiotics which might have enhanced hematopoiesis. Onifade *et al.* observed improved Hb and MCHb concentration in the animals fed diets containing probiotics (21). Enhanced leukocyte might be due to the production of more immune cells (22) that play a vital role in protecting against infectious agents (23). Furthermore, Paryad and Mahmoudi reported higher level of leukocyte in animals fed variant concentrations of probiotics than those fed diets without probiotics (24).

The importance of using probiotics includes increasing survival ratio in birds, enhancing the immune response, reducing gastro-intestinal upsets, increasing growth rates, and improving feeding efficiency. The use of probiotics from *Lactobacillus* spp., Yeast, etc. a lot of attention goes away. Adding these substances to feed improves feed utilization (25,26). Nahanshon *et al.* found that the addition of *Lactobacillus* in laying hens increases the saturation with cells of Peyer's plaques in the ileum, indicating a stimulation of the mucosal immune system that responds to antigenic excitation and releases immunoglobulin A (15). According to many scientists, probiotics improve the immune response in birds, increase the effectiveness of vaccinations as well as digestion (27). The liver plays an important role in metabolic processes, so, AST and ALT are indicators for normal liver function. Metabolic activity of the liver is essential for normal cell functioning.

The results show that the probiotic Batcinel-K® throughout the entire growing cycle has a positive effect on the natural resistance of the blood serum of broiler chickens. However, results of another studies show that probiotic supplementation to fish ration under heat stress significantly improve some of hematological indexes of experimental groups compared to control (28). Moreover, feed additives can decrease effect of heat stress in broiler-chickens (29). A high level of aspartate aminotransferase indicates an increase

in the synthesis of oxalacetic acid, which is necessary for energy production. The activity of alanine aminotransferase in the blood serum of chickens of all groups did not exceed the normal values.

Probiotic Bacteria showed an inhibitory effect against *Salmonella* sp. (30). However, using of probiotics possess positive effect against infection with hydatid disease in mice, so may be applied as an alternative method against various parasitic infestation (31). Addition of organic acid and essential oil to bird ration with different doses enhanced growth performance and intestinal histomorphology as well as hypocholesterolemia effects and could be used as growth promoters (32).

## Conclusion

Blood test results in chickens show that the probiotic Batcinel-K® during the entire rearing cycle has a positive effect on the biochemical parameters of blood serum. However, the most pronounced changes were observed in the experimental groups, supplemented with probiotic Batcinel-K® with the inactivated vaccine CEVAC SET K® against avian salmonellosis.

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## Conflict of interest

No conflict.

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## فعالية استخدام المعزز الحيوي Batcinel-K® واللقاح CEVAC SET-K® على بعض المؤشرات الدموية في الدجاج

عامر رسام العقابي<sup>1</sup>، الفتينا ابلوكاسيفنا كلاسكج<sup>2</sup> و بيوتر البينوفج كراسجكا<sup>3</sup>

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### الخلاصة

استخدم باتسنيل-ك وسيفاك سبت-ك في هذه الدراسة لمعرفة تأثيرها على بعض المعايير الدموية والكيميائية الحيوية في دجاج اللحم. أخذت ثلاثمائة فرخ من سلالة (روس- ٣٠٨) في عمر يوم واحد وقسمت إلى ست مجموعات من ٥٠ كتاكيت/لكل منها. أخذ الدم في أيام ٥٦ و ٦٣ و ١١٢. تم حساب عدد كريات الدم الحمراء والكريات البيض. تم تحديد الهيموجلوبين وبروتينات المصل الكلية والبروتين المصل وتركيز الكلوبولين. أظهرت النتائج أن البروبيوتيك المستخدم يمكن أن يحسن بعض مؤشرات الدم للطيور، خاصة في حال الإعطاء سوية مع اللقاح. في اليوم الثالث والستين من العمر كان تركيز الكلوبولين في مجموعات المعالجة الرابعة والخامسة أعلى بنسبة ٢,٩٧ و ٥,١٩٪ على التوالي، مقارنة بمجموعة السيطرة. كان مستوى البروتين الكلي فيعمر ١١٢ يوم في المجموعة الرابعة أعلى منه في مجموعة السيطرة عند ٨,١٪، وكذلك في المجموعة الخامسة بنسبة ٣,٩٦٪ بالإضافة إلى تركيز الألبومين والكلوبولين التي كانت أعلى نسبياً مقارنة بالسيطرة.