

Evaluation of the Administration Effects of Probiotics Against *Campylobacter Jejuni* on the Immune System of Broiler Chickens

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Abstract

The purpose of this research is to determinate the impact of the probiotics administration on serum protein and properdin, fractionation of proteins, lysozyme. Probiotics administration (*Lactobacillus paracasei* J.R., *Lactobacillus rhamnosus* 15b, *Lactobacillus lactis* γ and *Lactobacillus lactis* FO) in different quantities had a positive effect on the organism in increasing the total protein in favor of the serum albumin, maintaining a greater than one ratio between them and serum globulins, furthermore an increase in metabolic rate is indicated as well as the increasing of the nutrients transport capacity and endogenous synthesis. The fodder recipes that contained probiotics led to a rising slope of the alpha and beta electrophoretic fractions between batches, due to the increase of the total protein the differences being statistically not significant. Additionally it was recorded an increasing of the synthesis of gamma electrophoretic fractions, which highlights the increasing adaptability and response to exogenous biotic factors. Adding probiotics (4 strains) in the last period of growth or (2 strains) over the lifetime of rearing chicken, caused a significant increase of lysozyme, although the increase of properdin and phagocytic index is not significant.

Keywords: broiler, hematology, immunity, probiotics

1. Introduction

Poultry farming has become an important source of income in Romania. Digestive tract of broilers found both beneficial bacteria and pathogenic bacteria. Lately the increasing of broilers immunity is tried using different nutritional supplements, one of the most important being with probiotics. One of the pathogenic bacteria that are found in the in the digestive tract of birds is *Campylobacter jejuni*. *Campylobacter jejuni*, was classified as the leading cause of bacterial gastroenteritis in the western world [1].

Lactobacillus spp. and *Bifidobacterium spp.*, *Enterococcus spp.*, *Bacillus spp.* and *Pediococcus spp.* can be used as [2]. Probiotics improving the intestinal balance of the broiler [3].

Immunomodulation is the totality of the mechanisms that determine the achievement of significant changes to components and immune functions, because of that, it changes the immune status. These changes are caused by many factors, called immunomodulators that have the ability to influence immune system function. In this case we are dealing with a range of external factors that occur after contact and interaction with the structures and functions of the immune system, causing changes of these.

Assessment of immunomodulatory effect (immunostimulatory) can be done after dosing specific and nonspecific immune effectors such as

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properdin serum, lysozyme, and WBC counts. A WBC count is a blood test to measure the number of white blood cells (WBCs) in the blood. WBC values provide important data on changes in cellular defense system of the body. Such restructuring reflects responsiveness to various specific and nonspecific microbial aggressions. Other components which may be dosed are serum proteins and protein fractions.

Albumins-generate the osmotic pressure of plasma, are blood transporters for thyroid hormones, bilirubin, fatty acids, plasma calcium. **Alpha 1 globulins** may be tissue hormone (erythropoietin or carriers for thyroxine, steroids, vitamin B. **Alpha 2 globulins** are the blood transporters for copper, regulates the cardiovascular system, increase phagocytic ability of red blood cells used by the reticulohistiocytic system. **Beta 1 globulins** serve to transport blood iron, have role in immunological defense. **Beta 2 globulins** have a defense function of blood, transporters for triglycerides and cholesterol. **Gamma globulins** ensure immunological defense of the body, they are also called immunoglobulins (IgG, IgA, IgM, IgA D E and IgE).

Lysozyme (N-acetyl-muramic-hydrolysis) is a carbohydrase who unbind the glycosidic bonds (1-4) from acid N-acetyl-muramic and N-acetyl-glucosamine, which are part peptidoglycan from wall structure of bacterial cell. Mureinei destruction causes imbalances in osmotic pressure between inside and outside of the cell, which in this way will be destroyed. When pH is equal with 7, it exerts a lytic activity on the saprophytic and pathogenic bacteria, especially on positive Gram. Contributes to complement fixation and facilitates phagocytosis.

Properdine is a basic gamma globulin with a molecular weight of 184,000 Daltons and electrophoretic migration similar to α_2 -macroglobulin. Its structure has five identical subunits (A, B, D, E and P) non-covalently linked.

Properdine has antibacterial effect against many germs, especially Gram-negative ones, it has a certain antiviral activity (bacteriophages), anticancer action, intervening in lysis of erythrocytes in certain pathological conditions. The amount of properdin, or properdin titer increases following administration of low doses of endotoxin or zimozane and decreases in advanced forms of the disease cancer. The

concentration of properdin can be, as in the case of lysozyme, a parameter that reflects the natural immunological reactivity [4].

2. Materials and methods

At the age of 42 days, 16 broilers hybrid ROSS 308 (4 chicken from each replication) were randomly selected for blood sampling. Blood samples were collected from the wing vein into a centrifuge tube (without heparin).

Serum was separated by centrifugation at 6000 rpm for 10 minutes; it was collected in Eppendorf tubes and stored at -20 degrees Celsius to be used for evaluation of various blood parameters.

In order to determine the effect of probiotics in broilers administration on the immune system of broilers a research was conducted with seven experimental versions as follows:

- **L0 version** the chickens were feed with a fodder, it didn't contain probiotics and aa synthesis except of the requirement values presented in user growth of hybrid Ross 308;
- **L1 version** the chickens were feed with compound fodder, in which was included a premix with probiotics (*Lactobacillus paracasei* J.R., *Lactobacillus rhamnosus* 15b) starting on day 0;
- **L2 version** the chickens were feed with compound feed, in which was included a premix with probiotics (*Lactobacillus paracasei* J.R., 15b *Lactobacillus rhamnosus*, *Lactobacillus lactis* and *Lactobacillus lactis* y FO) starting on day 0;
- **L3 version** the chickens were feed with compound feed in which was included a premix with probiotics (*Lactobacillus paracasei* J.R., *Lactobacillus rhamnosus* 15b) just in the last week of of growth from 35 to 42 days;
- **L4 version** the chickens were feed with a fodder, in which was included a premix with probiotics (*Lactobacillus paracasei* JR, *Lactobacillus rhamnosus* 15b, *Lactobacillus lactis* y and *Lactobacillus lactis* FO) just in the last week of of growth from 35 to 42 days;
- **L5 version** the chickens were feed with a fodder, in which was included a premix with probiotics (*Lactobacillus paracasei* J.R., *Lactobacillus rhamnosus* 15b, *Lactobacillus lactis* y and *Lactobacillus lactis* FO) in which were added two aa L threonine and DL

methionine the values were higher with 25% than required by the user growth, starting on day 0;

- **L6 version** the chickens were feed with a fodder, in which was included a premix with probiotics (*Lactobacillus paracasei* J.R., *Lactobacillus rhamnosus* 15b, *Lactobacillus lactis* y and *Lactobacillus lactis* FO) in which were added two aa L threonine and DL methionine the values were higher with 25% than required by the user growth just in the last week of growth from 35 to 42 days.

Chickens were fed as recommended in the guide ross308 [5].

Collection of blood from chicken

Venous blood was collected in vacutainer system using the following materials:

- tube EDTA (purple cap) for blood count; -tube with coagulation activator (red cap) for blood biochemical examination; 21 G blood collecting holder;

- gloves for examination;
- alcohol and sanitary pads.

Birds were restrained by a trained worker, pointing out the venipuncture site, brachial vein.

Before collecting the blood the skin surface was antiseptic with alcohol, following the ventipuncture. For the dosage of of lysozyme was used diffusion method. Method principle: on a plate culture of *Micrococcus lysodeicticus* is incorporated, apply the serum samples that should be analyzed into Multiwell plates. By diffusion serum lysozyme form inhibition zone around Multiwell plates. Inhibition zone diameter is proportional with the concentration of serum lysozyme which included shing germs in the environment. Work technique: it is prepared the Veronal sodium tampon with veronal sodium 9.78 g.

For the dosage of of properdin, it is used a method whose principle is that isolated properdin by complexing the inulin is treated with reactive biuret. The intensity of the color reaction is determined colorimetrically [6, 7].

3. Results and discussion

The results obtained from the data processing are shown in the table 1.

Table 1. The values of chicken immunological indicators from experimental variants (Average±SD)

Specification	a/g	Total protein (g/dL)	Albumins (g/dL)	Alfa 1 globulins (g/dL)	Alfa 2 globulins (g/ g/dL)	Beta 1 globulins (g/ g/dL)	Beta 2 globulins (g/ g/dL)	Gama globulins (g/ g/dL)
n	4	4	4	4	4	4	4	4
L0	1.402±0.238	2.800±0.424	1.600±0.163	0.167±0.047	0.237±0.047	0.167±0.047	0.135±0.047	0.532±0.205
L1	1.832±0.047	2.900±0.374	1.900±0.141	0.200±0.081	0.30±0.081	0.200±0.081	0.110±0.020	0.200±0.081
L2	1.500±0.081	2.900±0.141	1.700±0.081	0.112±0.025	0.300±0.041	0.200±0.070	0.100±0.000	0.400±0.081
L3	1.330±0.060	3.000±0.336	1.700±0.182	0.175±0.050	0.250±0.057	0.200±0.000	0.175±0.050	0.475±0.095
L4	1.612±0.083	3.100±0.163	1.900±0.070	0.200±0.081	0.300±0.040	0.200±0.040	0.100±0.000	0.400±0.081
L5	1.172±0.256	3.250±0.288	1.725±0.206	0.200±0.000	0.250±0.057	0.175±0.050	0.175±0.050	0.750±0.311
L6	1.532±0.258	3.000±0.454	1.800±0.336	0.200±0.000	0.250±0.058	0.200±0.000	0.125±0.050	0.450±0.192
P-value (Tukey test)								
L0 vs L1	0.028*	0.999	0.309	0.970	0.704	0.968	0.963	0.134
L0 vs L2	0.983	0.999	0.987	0.739	0.704	0.968	0.844	0.922
L0 vs L3	0.996	0.976	0.987	1.000	1.000	0.968	0.749	0.999
L0 vs L4	0.611	0.854	0.309	0.970	0.704	0.968	0.844	0.922
L0 vs L5	0.511	0.491	0.961	0.970	1.000	1.000	0.749	0.563
L0 vs L6	0.932	0.976	0.739	0.970	1.000	0.968	1.000	0.992
L1 vs L3	0.008**	0.999	0.739	0.992	0.865	1.000	0.241	0.301
L1 vs L5	0.000***	0.746	0.837	1.000	0.865	0.991	0.241	0.003**
L2 vs L4	0.965	0.976	0.739	0.249	1.000	1.000	1.000	1.000
L2 vs L6	1.000	0.999	0.987	0.249	0.865	1.000	0.963	1.000

From the table above can be note following data:
The albumin - globulins - ratio is between 1.172 to T5 at the chickens that were feed with a fodder which included a premix with probiotics (*Lactobacillus paracasei* JR, *Lactobacillus*

rhamnosus 15b, *Lactobacillus lactis* y and *Lactobacillus lactis* FO) to which were added two aa L threonine and DL methionine, the values were 25% more than the required by the user, starting with day 0; and 1832 to L1 at the chickens

that were feed with compound fodder premix which introduced a probiotic (*Lactobacillus paracasei* JR, *Lactobacillus rhamnosus* 15b) starting with day 0.

After statistical interpretation was found that between the ratio recorded at chickens from T0 where the chickens were feed with a fodder that were not included probiotics and aa synthesis more than the required values presented in user growth of hybrid Ross 308, and chickens from L1 differences are statistically significant ($p \leq 0.05$). Also significant differences are noticed between recorded reports of chicken from L1 and L3, where the chickens were feed with a fodder which included a premix with probiotics (*Lactobacillus paracasei* JR, *Lactobacillus rhamnosus* 15b) only in the last week of growth from 35-42 days ($p \leq 0.01$); also between the chicken from L1 and L5 there were registered differences between albumine-globulin report, that are statistically significant ($p \leq 0.001$).

Therefore the highest report is recorded at chickens from L5.

Total proteins

Total proteins values are between 2.8 at L0 and 3.25 to L5. The differences between total protein values in the 6 variants are statistically insignificant.

Albumins

The proteins from the serum of slaughtered broilers had values between 1.6 at L0 and 1.9 to L1 and L4. Even in this case the differences were not statistically significant ($p \geq 0.05$).

Alfa 1 globulins

Alpha 1 globulins had values ranging from 0.112 at L2 to 0.2 L1, L4, L5 and L6, differences recorded being statistically not significant.

Alfa 2 globulins

Alfa 2 globulins had values ranging from 0.237 at L0 and 0.2 at L2 and L4. The differences were statistically not significant.

Beta 1 globulins

They had values between 0.167 at L0 and 0.2 at L1-L4 and L6. Differences recorded were statistically not significant.

Beta 2 globulins

the differences being statistically insignificant ($p \leq 0.05$). Phagocytic index was between 20.073 at T0 and 22.89 at T4 and T5, the differences being statistically not significant ($p \leq 0.05$).

Beta 2 globulins had values between 0.1 at L2 and L4. Differences recorded were statistically not significant.

Gama globulins

The values of the Gama globulins at the 6 variants were between 0.2 at L1 and 0.75 at L5. Between these variants there were recorded statistically significant differences ($p \leq 0.01$).

Reference values [8]

Total proteins=3.36 g/dL

Albumins=37.4% or 1.63 g/dL

Alfa globulins=18.9% or 0.89 g/dL

Beta globulins=23.9% or 1.12 g/dL,

Gama globulins=20.8% or 1 g/dL

Prebiotics supplementation significantly reduced heterophil percent, heterophil/ lymphocyte ratio (H/L ratio), albumin/globulin ratio, aspartate and alanine aminotransferase (AST and ALT), uric acid and creatinine compared to the AGP-supplemented and control group [9].

Probiotic supplementation did not affect the blood constituents, hemoglobin concentrations [10].

Alkhalaf A *et al.*, 2010 [11], shows that use of selected commercial probiotic resulted in improved performance parameters and reduced serum cholesterol in broiler chickens.

Moradi *et al.*, 2015 [12], show that pro+acid had no significant effect on carcass traits, blood metabolites and white blood cell number ($p > 0.05$).

Use of pro+acid had significantly decreased secum bacterial count than control group ($p > 0.05$). Probiotic and acidifier could improve secum microbial count and can be used as an alternative to antibiotic growth promoters.

Lysozyme, properdin and phagocytic index values of experimental variants at broiler chickens are presented in table 2.

From the table 2 can be note following data:

Lysozyme. Lysozyme values from the 6 experimental variants were between 9.7 at T0 and 15.066 mg/dL at T4 and T5. The differences between these experimental variants were statistically significant ($p \leq 0.05$), we can say that adding probiotics in combined fodder causes growth of the immune indicators.

Properdin. Its values were between 17.873 mg/dL serum at T0 and 20.703 mg at T4 and T5,

WBC counts setpoints for broilers are: lymphocytes-63%±10%, segmented neutrophils 27(%)±6%, eosinophils±2.21%, basophils-1.3%±0.8%, monocytes 4.1%±1%.

Table 2. Lysozyme, properdin and phagocytic index values of experimental variants at broiler chickens

Specification	Lysozyme (mg/dL)	Properdin (mg/dL)	Phagocytic index (%)
L0	9.700±0.726	17.873±0.355	20.073±0.683
L1	12.816±1.697	19.236±1.365	22.220±0.829
L2	14.583±2.318	19.953±2.034	23.500±1.423
L3	13.483±2.098	19.556±1.876	22.350±1.572
L4	15.066±2.476	20.703±2.101	22.890±1.436
L5	15.066±2.476	20.703±2.101	22.890±1.436
L6	14.233±1.155	20.590±1.742	22.83301.105±
P-value(Tukey test)			
L0 vs L1	0.385	0.944	0.311
L0 vs L2	0.057	0.721	0.032
L0 vs L3	0.200	0.866	0.255
L0 vs L4	0.031*	0.539	0.083
L0 vs L5	0.031*	0.408	0.101
L0 vs L6	0.086	0.452	0.112
L1 vs L3	0.999	1.000	1.000
L1 vs L5	0.718	0.923	0.989
L2 vs L4	1.000	1.000	0.998
L2 vs L6	1.000	0.999	0.989

4. Conclusions

1. Administration of probiotics and amino acids in the feed of broilers had an influence on the organism in increasing the total protein for serum albumin levels, maintaining a ratio greater than one, between themselves and serum globulins, furthermore an increase in metabolic rate is indicated, as well as the increasing of the nutrients transport capacity and endogenous synthesis, only by providing an optimal intake of water.

2. The fodder recipes that contained probiotics led to a rising slope of the alpha and beta electrophoretic fractions between batches, due to the increase of the total protein the differences being statistically not significant.

3. Administration of probiotics and amino acids in the feed of broilers had an influence an increasing of the synthesis of gamma electrophoretic fractions, which highlights the increasing adaptability and response to exogenous biotic factors.

4. Adding probiotics (4 strains) in the last period of growth or (2 strains) over the lifetime of rearing chicken, caused a significant increase of lysozyme, although the increase of properdin and phagocytic index is not significant.

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