Influence of the probiotic preparation Immunoflor on the physiological status of a young chicken of a productive herd of egg cross

V G Semenov¹, V V Boronin¹, N I Kosyaev², S S Kozak³, N G Ivanov², R N Ivanova⁴ and E N Ivanova⁵

¹Department of Morphology, Obstetrics and Therapy, Chuvash State Agrarian University, 29, K. Marx Street, Cheboksary, 428003, Russia
²Department of Parasitology, Epizootology and Veterinary and Sanitary Expertise, Chuvash State Agrarian University, 29, K. Marx Street, Cheboksary, 428003, Russia
³All-Russian Scientific Research Institute of the Poultry Industry – branch of the Federal State Budgetary Scientific Institution of the Federal Scientific Center “All-Russian Research and Technology Institute of Poultry Farming of the Russian Academy of Sciences”, Rzhavki, 1, Solnechnogorsk District, Moscow Region, 141552, Russia
⁴Department of Biotechnology and Processing of Agricultural Products, Chuvash State Agrarian University, 29, K. Marx Street, Cheboksary, 428003, Russia
⁵Department of General Education Disciplines, Chuvash State Agrarian University, 29, K. Marx Street, Cheboksary, 428003, Russia

E-mail: semenov_vg@edu.academy21.ru, https://orcid.org/0000-0002-0349-5825

Abstract. The article presents the results of studies of the effectiveness of the use of the complex probiotic preparation Immunoflor in young hens of egg cross. In the course of the research work, it was found that the use of the probiotic preparation Immunoflor does not affect the clinical and physiological state of the body of young chickens, but at the same time reduces the incidence, mortality and increases the safety of the chickens by enriching and balancing the poultry diet, reducing the conversion feed, optimization of digestion, stimulation of the development of positive microflora in the gastrointestinal tract. The use of the complex probiotic preparation Immunoflor in the diet of young chickens at a dose of 15 g/t of water and 15 g/t of feed contributes to an increase in the number of erythrocytes, leukocytes and hemoglobin concentration, activation of cellular and humoral factors of nonspecific resistance of the body of chickens, providing a normal physiological state and homeostasis.

1. Introduction

World poultry farming is the most science-based and dynamic branch of the agricultural-industrial complex and it makes a major contribution to ensuring food safety. The most important factor in increasing the efficiency of the poultry products production is the organization of adequate, scientifically grounded standardized feeding. An increase of chicken livestock is an integral part of the rapid development of industrial poultry farming, but at the same time the risk of diseases of various etiologies in birds also increases [1].

Under conditions of intensive growth from the first day of young chickens life, the changes
associated to the organism restructuring and adaptation to new feeding patterns are noticeable [2]. During this period digestive disorders occur, natural resistance decreases, as well as the organism resistance to the effect of unfavorable environmental factors [3].

In the industrial poultry farming, vaccinations and dehelmintization are carried out, antibiotics and other chemical agents are often used for the purposes of diseases prevention, ensuring the poultry viability, increasing its productivity [4]. The use of antibiotics in poultry farming has been recognized as an important contributor to the appearance of antimicrobial-resistant pathogens leading to life-threatening human infections worldwide [5]. Most of them have a negative effect on the bird’s organism, often causing dysbacteriosis. The widespread use of antibiotics as growth stimulants in poultry farming has led to the problem of the formation of resistant microflora [6]. In addition, they significantly disturb the intestinal microbalance in young poultry [7]. Therefore, the search for new alternative ways to solve this problem is an urgent matter.

As for now, one of the promising ways to solve the problems of poultry farming is the use of biostimulants such as probiotics. Probiotics are preparations containing microorganisms that facilitate stabilization of the optimal microbial balance in the intestine, that ensures increased resistance of poultry to various diseases and improves growth and development indicators [8]. They are referred to as environmentally safe preparations that have a positive effect on the organism [9]. Probiotics can have positive effects through a variety of mechanisms, including modulation of the intestinal microbiota, which is closely related to the maturation of the immune system [10]. Probiotics can improve growth indicators, promote nutrients metabolism, maintain intestinal integrity, resist colonization by pathogens, and modulate the organism immunity [11].

The purpose of this work is to evaluate the effectiveness of the use of the complex probiotic preparation Immunoflor in young crossbreed laying chickens Dekalb White.

2. Materials and methods
The working methods consisted in the studying the effectiveness of the use of the complex probiotic preparation Immunoflor (Limited liability company “PK Kros Pharm”, Mytishchi, Russia) in young up to 120 days of age crossbreed laying chickens Dekalb White.

The scientific and economic experiment was carried out under the conditions of ‘Poultry farm Gornomariyskaya’, Agricultural Production Cooperative, of the Mari El Republic. The material obtained in the course of the scientific experiment was processed at the Department of Morphology, Obstetrics and Therapy and in the laboratory of clinical and hematological research at the FSBEI HE (Federal State Budgetary Educational Institution of Higher Education) Chuvash State Agrarian University. In the course of the scientific and economic experiment, three groups of chickens at the age of one day, 500 birds in each group, were selected according to the principle of analogues.

The conditions of keeping, feeding and drinking the chickens were the same. The temperature, relative air humidity were measured with a combined device “TKA-PKM”, model 42 (manufacturer – Limited liability company Scientific and Technical Enterprise “TKA”, St.Petersburg, Russia), air velocity – with a thermal anemometer “TKA-PKM”, model 50 (manufacturer – Limited liability company Scientific and Technical Enterprise “TKA”, St. Petersburg, Russia), CO2 content in the air, concentration of NH3 and H2S – with a universal gas analyzer UG-2 (manufacturer – Limited liability company Promekopribor, St. Petersburg, Russia), microbial count and dust – with the apparatus of Yu A Krotov (manufacturer – Limited liability company NIKI MLT-Povolzhye, Penza, Russia).

In order to establish reasonability of using the complex probiotic preparation Immunoflor, developed by Limited liability company “PK Kros Pharm” (Mytishchi, Russia), it was given to the Dekalb White crossbreed chickens. In the first experimental group Immunoflor was given to chickens
from the first to the twenty-first day of life as a part of the basic diet in accordance with the instructions for use, at the rate of 15 g/t of water. In the second experimental group chickens were fed with Immunoflor as a part of the basic diet at the rate of 15 g/t of feed during the same period. Chickens of the control group did not get the abovementioned preparation.

Immunoflor is a complex probiotic preparation consisting of natural ingredients. This product is intended to enrich and balance the diets of farm animals, including poultry, in order to increase productivity by optimizing digestion, stimulating of positive microflora growth in the gastrointestinal tract, increasing viability, and reducing feed conversion rate.

The composition of the abovementioned preparation includes the following components:
- Bacillus subtilis and Bacillus licheniformis – are the producers of amylases, proteases, amino acids, polypeptide antibiotics and some polysaccharides;
- Bifidobacterium globosum – have significant antagonistic activity against putrefactive bacteria;
- Enterococcus faecium – have high enzymatic activity, due to the synthesis of bacteriocins, suppressing pathogenic microorganisms; activate intestinal immunity, ferment carbohydrates with the formation of lactic acid;
- Saccharomyces cerevisiae – are the yeast cells that absorb oxygen in the course of their vital activity, creating anaerobic conditions, being unfavorable for the pathogenic microflora growth, while these conditions are also conditionally pathogenic;
- Chitosan – decreases the levels of cholesterol and uric acid in the blood, has antibacterial and antifungal properties, improves the calcium absorption from feed; enhances intestinal peristalsis;
- Lactose is a disaccharide that is a nutrient substrate for lactic acid bacteria and for the digestive tract.

Clinical state of birds, food and water consumption were monitored on a daily basis, attention was paid to behavior, mobility, reactions to external stimuli, the following indicators were measured: body temperature (using KD-132-1 thermometer, China), heart rate (calculated by the heart auscultation) and respiratory movements (by observing the movement of the wings and tail), using generally accepted methods in veterinary medicine, with a determination of the general state of health.

Erythrocytes were counted in the Goryaev counting chamber, the total number of leukocytes – in the Goryaev counting chamber by the test tube method, the hemoglobin concentration was determined using the Sahlihemometer.

The phagocytic activity of leukocytes was determined using a daily agar culture of Staphylococcus aureus according to V S Gostev, lysozyme activity of blood plasma – using a daily agar culture of Micrococcus lysodeiticus according to V G Dorofeychuk, bactericidal activity of blood serum – using daily agar culture of Escherichia coli according to O V Smirnova et al.

The prophylactic and therapeutic efficacy of the probiotic preparation use was determined by monthly registration of sick and culled birds according to the data of veterinary statistical reporting.

The numerical values of the experimental data were processed using the method of variance statistics as for the reliability of differences in the compared data (P<0.05-0.001), using the Microsoft Excel computer program.

3. Results and discussion

The farm adheres to the technological parameters that ensure creation of the normal conditions of keeping, care, feeding, as well as the microclimate according to the special recommendations for farming birds of Dekalb White breed.

It was established that the microclimate indicators in the poultry houses for young chickens corresponded to zoological hygienic requirements (table 1). The main microclimatic indicators in the poultry house had an insignificant range of fluctuations and averaged the following: T, °C – 18±0.40, relative humidity – 65.9±0.6 %, air velocity – 0.20±0.01 m/s, bacterial count – 68.7±1.8 m.b./m³, ammonia concentration – 6.1±0.54 mg/m³, hydrogen sulfide concentration – 3.9±0.22 mg/m³, carbon dioxide concentration – 0.18±0.07 %, dust content – 4.1±0.26 mg/m³.
In the course of the experiment, it was established that the incidence of young birds in the control group was 18%, which is 6% lower than in the 1st and 2nd experimental groups. At the same time mortality in the 1st and 2nd experimental groups was 6 and 8%, which is 6 and 4% lower than in the control group.

Table 1. Microclimate parameters in poultry houses.

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| T, °C              | 34±0.50     | 31±0.30     | 29±0.40     | 28±0.10     | 22±0.2      | 19±0.20     | 19±0.40     |
| R, %               | 64.4±0.70   | 63.1±0.40   | 64.9±0.60   | 66.1±0.50   | 63.3±0.40   | 64.5±0.80   | 65.9±0.60   |
| v, m/s             | 0.1±0.03    | 0.1±0.01    | 0.1±0.01    | 0.1±0.04    | 0.2±0.03    | 0.2±0.05    | 0.2±0.01    |
| NH3, mg/m³         | 5.6±0.21    | 4.7±0.44    | 4.6±0.31    | 5.7±0.46    | 6.7±0.37    | 7.1±0.41    | 6.1±0.54    |
| H2S, mg/m³         | 3.9±0.21    | 3.1±0.27    | 3.4±0.43    | 3.2±0.45    | 4.1±0.11    | 3.7±0.28    | 3.9±0.22    |
| CO₂, %             | 0.15±0.02   | 0.16±0.01   | 0.18±0.01   | 0.17±0.04   | 0.16±0.02   | 0.14±0.05   | 0.18±0.07   |
| Microorganisms, ths. m.b./m³ | 57.2±2.1 | 61.9±1.7 | 67.3±4.1 | 64.2±3.1 | 69.1±2.3 | 72.3±3.3 | 68.7±1.8 |
| Dust, mg/m³        | 2.9±0.41    | 3.5±0.32    | 3.7±0.33    | 3.4±0.23    | 3.1±0.52    | 3.8±0.31    | 4.1±0.26    |

Table 2. Indicators of the physiological state of young chickens.

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Body temperature, °C | 38.8±0.14   | 39.4±0.19   | 39.1±0.22   |             |             |             |             |
| Pulse, BPM         | 177.3±2.1   | 178.5±2.6   | 178.1±2.3   |             |             |             |             |
| Breath, movements/min | 54.2±1.6   | 55.1±1.3    | 54.7±1.1    |             |             |             |             |

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Body temperature, °C | 39.6±0.09   | 40.1±0.11   | 39.9±0.17   |             |             |             |             |
| Pulse, BPM         | 171.3±1.8   | 172.4±2.1   | 171.9±1.8   |             |             |             |             |
| Breath, movements/min | 54.4±1.4   | 55.9±1.1    | 55.1±1.3    |             |             |             |             |

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Body temperature, °C | 40.1±0.14   | 40.3±0.17   | 40.1±0.11   |             |             |             |             |
| Pulse, BPM         | 170.7±1.4   | 171.8±1.9   | 171.2±1.3   |             |             |             |             |
| Breath, movements/min | 30.9±1.2   | 31.7±0.9    | 31.3±0.3    |             |             |             |             |

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Body temperature, °C | 40.2±0.17   | 40.7±0.14   | 40.4±0.11   |             |             |             |             |
| Pulse, BPM         | 168.4±2.4   | 169.1±1.9   | 169.2±1.4   |             |             |             |             |
| Breath, movements/min | 26.4±1.4   | 28.1±1.1    | 27.4±1.6    |             |             |             |             |

| Indicator          | 1-2         | 3-7         | 8-14        | 15-21       | 22-28       | 29-35       | 36 and over |
|--------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Body temperature, °C | 40.3±0.18   | 40.6±0.14   | 40.3±0.09   |             |             |             |             |
| Pulse, BPM         | 146.9±2.3   | 148.1±2.1   | 147.9±2.4   |             |             |             |             |
| Breath, movements/min | 27.1±0.7   | 28.4±0.9    | 27.8±0.7    |             |             |             |             |

T – air temperature, °C; R – relative air humidity, %; v – air velocity, m/s.

The results of the studies on the clinical and physiological state of young chickens of the experimental group, given in table 2, demonstrate that in association with the use of the complex probiotic preparation Immunoflor at the dose of 15 g/t of water and 15 g/t of feed for 21 days, body temperature, heart rate and respiratory rate during the experiment stayed within normal physiological values, and the difference in the corresponding values as compared to the control group was insignificant (P>0.05).
It was found that viability of young laying hens in the first and second experimental groups was higher than in the control by 6% and 4% respectively, but at the same time, when the tested preparation was included in the basic diet with water, this indicator turned out to be 2% higher than when included in the diet with feed (table 3).

| Indicator                          | Group                        | Control | The 1st experimental | The 2nd experimental |
|-----------------------------------|------------------------------|---------|----------------------|---------------------|
| Experiment duration, days         |                              | 120     | 50                   | 50                  |
| Number of birds                   |                              |         | 50                   | 50                  |
| Number of sick birds              |                              | 9       | 4                    | 6                   |
| Percentage of sick birds          |                              | 18      | 10                   | 12                  |
| Mortality, number of birds        |                              | 6       | 3                    | 4                   |
| Mortality, %                      |                              | 12      | 6                    | 8                   |
| Viability, number of birds        |                              | 44      | 47                   | 46                  |
| Viability, %                      |                              | 88      | 94                   | 92                  |

It was found that the hematological indicators, such as the count of erythrocytes and leukocytes, as well as the hemoglobin concentration in young chickens of the first and second experimental groups at the end of the experiment had higher values than in the control group, however they rested within normal physiological values (table 4).

| Age, days | Indicator               | Control     | The 1st experimental | The 2nd experimental |
|-----------|-------------------------|-------------|----------------------|----------------------|
| 30        | Erythrocytes, $\times 10^{12}/l$ | 1.87±0.06   | 2.01±0.08            | 1.96±0.09            |
|           | Leucocytes, $\times 10^9/l$    | 28.40±1.17  | 29.10±1.14           | 28.80±1.66           |
|           | Hemoglobin, g/l           | 74.81±1.48  | 78.63±1.37           | 76.37±1.74           |
|           | Erythrocytes, $\times 10^{12}/l$ | 1.96±0.09   | 2.11±0.07            | 2.08±0.04            |
| 60        | Leucocytes, $\times 10^9/l$    | 34.60±1.90  | 37.50±2.14           | 36.90±1.91           |
|           | Hemoglobin, g/l           | 77.49±1.81  | 81.69±1.39           | 78.91±1.12           |
|           | Erythrocytes, $\times 10^{12}/l$ | 1.94±0.07   | 2.21±0.04*           | 2.18±0.06*           |
| 90        | Leucocytes, $\times 10^9/l$    | 31.60±1.37  | 35.40±0.91*          | 34.90±0.87           |
|           | Hemoglobin, g/l           | 78.77±1.19  | 81.19±1.21           | 80.73±1.06           |

*P<0.05

The number of erythrocytes in the blood of chickens increased at all physiological phases of young birds development. Thus, during the period from the first to the third physiological phases (1-60 days), the count of erythrocytes in the control, 1st and 2nd experimental groups increased from 1.87±0.06 to 1.96±0.09×10^{12}/l, from 2.01±0.08 to 2.11±0.07×10^{12}/l and from 1.96±0.09 to 2.08±0.04×10^{12}/l, respectively. The difference in the analyzed hematological indicator in young birds of the control and experimental groups was statistically significant at certain periods of research. During the periods of the end of the third physiological phase (60 days) and growing up to 90 days, the count of erythrocytes in the blood of young chickens also increased and amounted to 1.94±0.07×10^{12}/l, 2.21±0.04×10^{12}/l and 2.18±0.06×10^{12}/l, respectively (P<0.05).

A similar pattern was determined in the dynamics of the hemoglobin concentration in the blood of young chickens from the experimental groups. It was found that the level of this hematological indicator in young birds of the 1st and 2nd experimental groups was higher than in the control group. However, the difference in the hemoglobin concentration in the blood of young poultry in the
experimental groups was insignificant (P>0.05). In young chickens of the 1st and 2nd experimental groups, as compared to the control group at the age of 30 days, the hemoglobin concentration in the blood was higher by 3.82 and 1.56 g/l, at the age of 60 days – by 4.2 and 1.42 g/l, at the age of 90 days – by 2.42 and 1.96 g/l, respectively.

The total count of leukocytes in the blood of young chickens of the control, 1st and 2nd experimental groups increased during the period from the first to the third physiological phases of development and, vice versa, decreased during the periods of the end of the third phase and growing up to 90 days, respectively. It was established that the count of white blood cells in the blood of young birds of the 1st and 2nd experimental groups was higher as compared to the control group: at day 30 - by 0.7 and 0.4×10^9/l, at day 60 - by 2.9 and 2.3×10^9/l and at day 90 - by 3.8 (P<0.05) and 3.3×10^9/l, respectively, while the difference in the count of leukocytes between the experimental groups in certain periods of growing was insignificant and increased only in the period from the third phase of growing up young chickens.

Table 5. Indicators of leukocytesphagocytic activity, %.

| Indicator      | Age        | Control       | The 1st experimental | The 2nd experimental |
|----------------|------------|---------------|----------------------|----------------------|
| Phagocytic     | 30 days    | 31.64±0.75    | 36.72±0.49***        | 35.51±0.74***        |
| activity       | 60 days    | 35.42±0.66    | 38.59±0.82*          | 37.71±0.69*          |
|                | 90 days    | 37.59±0.92    | 41.83±0.37**         | 40.56±0.61*          |

* P<0.05, ** P<0.01, ***P<0.001

It was established that the blood phagocytic activity in young chickens of the control, 1st and 2nd experimental groups increased sequentially during the growing period (table 5). The phagocytic activity of blood leukocytes in young chickens of the 1st and 2nd experimental groups was higher than the control values at all periods of the study. Thus, the level of the specified cellular factor of nonspecific resistance of the organism in the blood of chickens of the 1st and 2nd experimental groups was higher than the control value on day 30 – by 13.8 and 10.9%, on day 60 – by 8.2 and 6.1%, on day 90 – by 10.1 and 7.3%, respectively (P<0.05-0.001).

Table 6. Indicators of blood plasma lysozyme activity, %.

| Indicator      | Age        | Control       | The 1st experimental | The 2nd experimental |
|----------------|------------|---------------|----------------------|----------------------|
| Lysozyme activity | 30 days    | 17.64±0.85    | 19.83±0.56           | 18.57±0.31           |
|                 | 60 days    | 19.34±1.69    | 21.67±1.12           | 20.86±1.33           |
|                 | 90 days    | 20.29±0.51    | 24.62±0.84**         | 23.58±0.78**         |

** P<0.01

The dynamics of the blood plasma lysozyme activity in young chickens of the compared groups, given in Table 6, demonstrate a similar pattern. Thus, the activity of lysozyme in the blood plasma of the control, 1st and 2nd experimental groups increased throughout the experiment. The activity of the specified humoral factor of the organism nonspecific resistance in the blood plasma of chickens of the 1st and 2nd experimental groups was higher than the control values at all periods of growing, however, the statistically relevant difference in the values of the compared indicators was observed only on day 90 of growing and amounted to 4.33 and 3.29%, respectively (P<0.01).

The established pattern in the dynamics of plasma lysozyme activity is also confirmed by a change in the blood serum bactericidal activity in young chickens (table 7). Thus, the specified blood serumactivity in young chickens of the control, 1st and 2nd experimental groups increased sequentially. It was found that the blood serum bactericidal activity in young chickens of the 1st and 2nd experimental groups was higher than the control values at all periods of growing. Thus, the level of the
studied indicator of the humoral component of the organism resistance in chickens of the 1st and 2nd experimental groups was higher than in the control group on day 30 – by 12.4 and 6.6%, on day 60 – by 11.96 and 9.02%, on day 90 – by 10.5 and 8.5%, respectively (P<0.05-0.001).

Table 7. Indicators of blood serum bactericidal activity, %.

| Indicator          | Age  | Control          | The 1st experimental | The 2nd experimental |
|--------------------|------|------------------|----------------------|----------------------|
| Bactericidal activity | 30 days | 46.29±0.41       | 52.83±0.49***        | 49.57±0.82**         |
|                    | 60 days | 49.84±1.83       | 56.61±1.12*          | 54.78±0.93*          |
|                    | 90 days | 52.48±0.51       | 58.62±0.74***        | 57.38±0.67***        |

* P<0.05, *** P<0.001

4. Conclusion

The use of the complex probiotic preparation Immunoflor in the diet of young chickens at the dose of 15 g/t of water and 15 g/t of feed results in an increase in the number of erythrocytes, leukocytes and hemoglobin concentration, activation of cellular and humoral factors of nonspecific resistance of chicken organisms, ensuring normal physiological state and homeostasis

References

[1] Tang R Y, Wu Z L, Wang G Z and Liu W C 2018 The effect of Bacillus amyloliquefaciens on productive performance of laying hens. Italian Journal of Animal Science 17(2) 436 doi: 10.1080/1828051X.2017.1394169
[2] Islam M and Yang Chul-Ju 2017 Efficacy of mealworm and super mealworm larvae probiotics as an alternative to antibiotics challenged orally with Salmonella and E. coli infection in broiler chicks. Poultry Science 96(1) 27 doi: 10.3382/ps/pew220
[3] Xiang Q, Wang C, Zhang H, Lai W, Wei H and Peng J 2019 Effects of different probiotics on laying performance, egg quality, oxidative status, and gut health in laying hens. Animals 9(12) 1110 doi: 10.1093/jas/skz258.708
[4] Al-Khalafiah H S 2018 Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. Poultry Science 97(11) 3807 doi: 10.3382/ps/pey160
[5] Mandal A, Mandal R K, Yang Y, Khatri B, Kong B W and Kwon Y M 2021 In vitro characterization of chicken gut bacterial isolates for probiotic potentials. Poultry Science 100(2) 1083 doi: 10.1016/j.psj.2020.11.025
[6] Hajiaghapour M and Rezaeipour V 2018 Comparison of two herbal essential oils, probiotic, and mannan-oligosaccharides on egg production, hatchability, serum metabolites, intestinal morphology, and microbiota activity of quail breeders. Livestock Science 210 93 doi: 10.1016/j.livsci.2018.02.007
[7] Xie Z, Zhao Q, Wang H, Wen L, Li W, Zhang X, Lin W, Li H, Xie Q and Wang Y 2020 Effects of antibacterial peptide combinations on growth performance, intestinal health, and immune function of broiler chickens. Poultry Science 99(12) 6481 doi: 10.1016/j.psj.2020.08.068
[8] Sugiharto S 2016 Role of nutraceuticals in gut health and growth performance of poultry. Journal of the Saudi of Agricultural Sciences 15(2) 99 doi: 10.1016/j.jssas.2014.06.001
[9] Ramlucken U, Laloo R, Roets Y, Moonsamy G, Jansen van Rensburg C and Thantsha M S 2020 Advantages of Bacillus-based probiotics in poultry production. Livestock Science 241 104215 doi: 10.1016/j.livsci.2020.104215
[10] Bial M, Barbe F, Chevaux E, Sienkiewicz O and Zhao X 2021 Effects of novel probiotic strains of Bacillus pumilus and Bacillus subtilis on production, gut health, and immunity of broiler chickens raised under suboptimal conditions. Poultry Science 100(3) 100871 doi: 10.1016/j.psj.2020.11.048
[11] Liang W, Li H, Zhou H, Wang M, Zhao X, Sun X, Li C and Zhang X Effects of Taraxacum and
Astragalus extracts combined with probiotic Bacillus subtilis and Lactobacillus on Escherichia coli–infected broiler chickens. *Poultry Science* **100**(4) 101007
doi: 10.1016/j.psj.2021.01.030