



Effects of a probiotic containing the spore-forming bacterium *Bacillus subtilis* on the productivity, egg quality, and hematological parameters in quails

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Introduction of probiotics to poultry feeding practices significantly reduces the risk of infections of the gastrointestinal tract, thus preventing the potential emergence of antibiotic resistance. To conduct the experiment, we selected Japanese quails aged 45 days. Two groups were formed, consisting of 20 individuals each, according to the analogue principle. The intake of the probiotic feed supplement by the experimental-group quails led to notable improvements in an array of parameters, compared with the control. Thus, it produced increases of 15.8% in the total yield of eggs, 15.6% in the laying capacity per one average laying quail, and 10.3% in the laying intensity. The parameters of eggs were also enhanced: we noted a 15.0% increase in the mass of eggs, a 9.8% increase in the mass of egg shell, a 21.0% increase in the yolk mass, a 12.1% increase in the albumen mass, and a 10.7% increase in the yolk/egg mass ratio. The egg width, egg height, egg volume were higher by 11.4%, 7.6%, and 7.0%, respectively. The probiotic promoted rises in the albumen parameters: 20.0% in height of albumen, 9.2% in lesser diameter, and 8.3% in larger diameter. At the same time, the increases in yolk measured 20.4% in height, 10.0% in lesser diameter, 13.1% in larger diameter, and 7.8% in the yolk index. Moreover, the quails that received the probiotic consumed 12.8% less fodder per 10 eggs than the control. The mass parameters were also observed to have been elevated. Thus, we observed increases of 7.5% in live mass, 38.8% in mean daily mass gain, and 37.8% in absolute mass. Accordingly, we registered a 8.5% greater pre-slaughter live mass, a 13.0% higher mass of non-eviscerated carcass, a 7.8% greater mass of semi-eviscerated carcass, a 12.0% heavier eviscerated carcass, and a 25.6% larger mass of the thoracic muscles. Furthermore, the experimental-group quails had improved blood parameters, in particular increases in the hemoglobin level by 7.2%, erythrocytes by 24.7%, total protein by 16.1%, albumin by 13.5%, globulins by 18.5%, glucose by 13.8%, and calcium by 21.8%. Thus, the intake of the probiotic containing *B. subtilis* increased the productivity, stimulated the immunity, and enhanced metabolism in the quails, allowing obtaining high-quality and safe egg products.

Keywords: quails; probiotic feed supplement; nutrition; laying capacity; live mass; body mass gains; fodder consumption; blood; quality indicators of eggs.

Introduction

Rapid development of poultry farming forces specialists into a rigid framework to maintain poultry productivity at a high level. Feeds and feed supplements must correspond to rigorous criteria, which could require additional costs. At the same time, the industry faces the challenge such as the development and spread of resistant microorganisms that pose threat to both the health of consumers and the environment, a problem that stems from the sphere's long history of extensive use of antibiotics and other drugs (Mörschbacher et al., 2024; Lan et al., 2025).

Numerous feed supplements were developed using biotechnologies and are used to improve the health of poultry. Long use of antibiotics as growth stimulators in poultry farming prevented the spread of both pathogenic and beneficial microorganisms in the digestive tract. Meanwhile, pathogenic bacteria have acquired resistance. In addition, a health risk for people is also posed by the residues of antibiotics in the products of animal origin (Lokapimasari et al., 2017; Yu et al., 2021).

Bacteria that adapt to antibiotics and become unresponsive to them have become a substantial global problem. To address this growing threat, both the European Union and the United States took measures to restrict the use of antibiotics in animal farming. Thus, the EU prohibited the application of antibiotics for growth stimulation in 2006. In the US, a gradual refuse from the use of growth stimulation antibiotics began in 2017.

Against the background of banning antibiotics and other chemical compounds that are growth factors, researchers have started to seek alternative feed supplements. As of now, the market offers myriad

drugs that positively affect animal organisms (Gupta et al., 2016; Ranzanova et al., 2017). Among a high number of feed supplements of natural origin, a special attention should be paid to probiotics and their properties (Poberezhets et al., 2021; Darboe et al., 2023).

Probiotic feed supplements have a potential to be an alternative to antibiotics, because they can be added directly to fodder, and they exert beneficial effects and improve the balance of the gut microflora. An important characteristic of a probiotic is production of the high- and low-molecular-weight compounds bacteriocins, which display a broad spectrum of action toward competitive microflora (Khan et al., 2020; Chudak et al., 2021; Poberezhets et al., 2023).

The fight against antibiotic resistance and the prohibition of feed antibiotics open great opportunities for the market of spore-based probiotics. A promising direction in poultry farming is the use of bacteria of the *Bacillus* genus. Those strains of microorganisms are common in nature, are resistant to digestive enzymes, and can retain their vitality in the gastrointestinal tract for a long period. The mechanisms of probiotic action of the bacterium *Bacillus subtilis* are related to the synthesis of antimicrobial compounds, enhancement of non-specific and specific immunities, stimulation of the growth of normal gut microflora, and synthesis of digestive enzymes (Jazi et al., 2020; Zhao et al., 2020; Wang et al., 2021).

Probiotics that contain *B. subtilis* can positively affect the laying capacity and quality of eggs in poultry and promote decline in the fodder conversion coefficient through the synthesis of digestive enzymes, such as amylase, lipase, and protease, which helps to digest feed more effectively, thus contributing to a high absorption of feed nutrients. In this way, poultry obtains more energy, organic, and mineral compounds for the formation of eggs. Introduction of probiotics to

poultry feeding substantially reduces the risks of infections of the gastrointestinal tract, allowing rarer use of expensive drugs and rarer prophylaxis of the emergence of antibiotic resistance (Manafi et al., 2020; Jiang et al., 2022). Moreover, a *Bacillus subtilis*-containing probiotic can positively affect the hematological parameters in poultry, leading to strengthened organism's resistance, boosted productivity, and safe poultry products (Cirilo et al., 2023; Yefimov et al., 2024).

The objective of the study was to assess the effects of the probiotic feed supplement with *B. subtilis* on the productivity, quality of eggs, and hematological parameters in quails.

Materials and methods

The program of the scientific experiment was approved by the commission of the Faculty of Technologies of Production, Processing, and Robotic Technology in Poultry Farming of Vinnytsia National Agrarian University (Protocol of the Session of Ethic Committee No. 4 as of September 23, 2024). The protocol and procedures used in this experiment ethically corresponded to the Law of Ukraine On the Protection of Animals from Abuse and the Directive 2010/63/EU of European Parliament on the protection of animals. The quails were held in single-tier cages, which allowed creating optimal parameters of microclimate for the birds, particularly: the air temperature within 18–25 °C, 60–70% humidity, and 18 hour lighting every day. At the end of the experiment, we conducted a control slaughter of the poultry, four individuals from each group. To study the morphological and biochemical parameters in blood, we selected four quails from each group, from which we collected blood in the morning, prior to feeding, after decapitation under a light ether narcosis.

The experiment was carried out under the conditions of vivarium at Vinnytsia National Agrarian University according to the generally accepted methods (Ibatullin et al., 2017). We selected Japanese quails aged 45 days, which were divided according to the analogue principle into two groups consisting of 20 individuals each.

The experimental period lasted 90 days. The control group consumed the main diet (MD) – complete-diet mixed feed. During the experiment, the quails were given fodder and water. The experimental quails, in addition to the diet, received the Klostat probiotic feed supplement in a dose of 50 g/tonne of mixed feed.

The Klostat probiotic provides a targeted probiotic action because of the strain of *B. subtilis* PB6, therefore supporting the health and resistance of the gut microflora of the animals by stimulating the growth of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*. One kilogram of the probiotic supplement contained no less than 2×10^9 CFU (colony-forming units) of the spore-forming bacterium *B. subtilis* PB6.

During the study, we determined the growth intensity by individually weighing 20 birds of each group at the beginning and the end of the experiment (Ibatullin et al., 2017). According to the results of weighing, we determined the live mass of the quails and the gains in absolute and mean daily mass during the experiment. At the same time, we made an account of survival of the poultry according to the records of dead birds, and also calculated the consumption of fodder per 10 eggs.

To determine the quality parameters of the eggs, we selected 10 eggs from each group, according to the methods described in the study by Ibatullin et al. (2017). During the experiment, we measured the morphological parameters of eggs and mass of eggs and egg's components (Hamilton, 2022) such as shell, yolk, and albumen using electronic scales Radwag WLC 2/C/1 (Radom, Poland, 2015) with an accuracy up to 0.01 g. We studied the changes in the shape and size of the quail eggs: larger and lesser diameters of the eggs, diameter and height of the air cell, height and diameter of albumen and yolk using a Mitutoyo digital caliper (Kawasaki, Japan, 2019) and measured the thickness of the shell using a Mitutoyo 103-137 micrometer (Kawasaki, Japan, 2018).

During the control slaughter, we determined the pre-slaughter parameters: pre-slaughter live mass, mass of non-eviscerated, semi-eviscerated, and eviscerated carcass, and also the mass of the muscular tissue.

To identify the morphological composition of carcasses of the quails, we determined body weight prior to slaughter, weight of non-eviscerated, semi-eviscerated (i.e. those with removed intestines and cloaca, filled crop, oviduct and ovary), eviscerated carcasses (the internal organs and abdominal-cavity fat and esophagus of which were removed, without head, legs and wing metacarpals), slaughter yield, and weight of the muscles. During the control slaughter, we weighed the internal organs using the electronic scales Radwag WLC 2/C/1.

The laboratory hematological parameters were measured at the Management of the Food Safety Service of Vinnytsia Oblast, according to the generally accepted methods (Vlizlo et al., 2012). To determine the morphological blood parameters, we used a Heska Element HT5 veterinary hematological analyzer (Barrie, Canada, 2016). We employed the colorimetric method to measure hemoglobin, electric impedance method to analyze erythrocytes, and laser flow cytometry to determine leukocytes. The total protein, albumins, globulins, glucose, the activities of aspartate (AST) and alanine aminotransferase (ALT), alkaline phosphatase, urea, and total calcium in blood serum were studied using an automatic biochemical analyzer (Barcelona, Spain, 2018) with necessary reagents.

The biometric processing of the experiment results was performed using the ANOVA disperse analysis. The data in the tables are presented as mean value (\bar{x}) and standard deviation (SD). The differences between the samplings were considered significant at $P < 0.05$.

Results

In the experimental group, the use of the *Bacillus subtilis*-based probiotic supplement in feeding the laying quails promoted increases of 15.8% ($F = 11.303$) in the total yield of eggs, 15.6% ($F = 4.836$) in the laying capacity per one average laying quail, and 10.3% ($F = 5.086$) in the laying intensity, compared with the control (Table 1).

Table 1

Egg productivity of the quails following the intake of the probiotic (*B. subtilis*), ($\bar{x} \pm SD$, $n = 20$)

Parameter	Group	
	control	experimental
Total yield, eggs	1,168 ± 12	1,352 ± 11***
Laying capacity per one average laying quail, eggs	61.6 ± 1.5	71.2 ± 1.3***
Laying intensity, %	64.8 ± 1.7	75.1 ± 1.1***

Note: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ differences between the control and experimental groups.

The obtained results revealed increases of 15.0% ($F = 5.202$) in the mass of eggs, 9.8% ($F = 2.743$) in shell, 21.0% ($F = 3.832$) in yolk, 12.1% ($F = 3.042$) in albumen, and 10.7% ($F = 4.412$) in the yolk/egg mass ratio (Table 2).

Table 2

Morphological composition of the quail eggs following the intake of the *B. subtilis*-based probiotic ($\bar{x} \pm SD$, $n = 10$)

Parameters	Group	
	control	experimental
Egg mass, g	10.80 ± 0.21	12.42 ± 0.23***
Mass of the main components of the egg, g:		
Shell	1.64 ± 0.05	1.80 ± 0.03**
Yolk	3.77 ± 0.16	4.56 ± 0.13**
Albumen	5.39 ± 0.17	6.06 ± 0.14**
Ratio of the mass of egg components to egg mass, %:		
Shell	15.20 ± 0.39	14.52 ± 0.24
Yolk	34.90 ± 0.34	37.25 ± 0.41***
Albumen	49.90 ± 0.83	48.79 ± 0.61

Note: see Table 1.

We registered increases of 11.4% ($F = 2.476$) in the lesser diameter of eggs, 7.6% ($F = 3.152$) in larger diameter, 7.0% ($F = 4.308$) in egg volume, and 10.3% ($F = 4.288$) in the shape index (Table 3).

Table 3
Shapes and sizes of the eggs following the intake of the probiotic supplement ($x \pm SD$, $n = 10$)

Parameter	Group	
	control	experimental
Lesser egg diameter, mm	22.45 ± 0.63	25.01 ± 0.82*
Larger egg diameter, mm	30.79 ± 0.64	33.12 ± 0.37**
Egg volume, mL	17.30 ± 0.21	18.52 ± 0.19***
Ratio of diameters	1.371 ± 0.030	1.325 ± 0.026
Shape index, %	72.91 ± 0.53	75.48 ± 0.28***

Note: see Table 1.

The studied probiotic containing strain of *B. subtilis* also notably improved the quality parameters of the quails' eggs. The consumed supplement led to increases of 20.0% ($F = 2.913$) in the height of albumen, 9.2% ($F = 2.334$) in lesser diameter, and 8.3% ($F = 2.585$) in larger diameter, compared with the control (Table 4).

Table 4
Qualitative parameters of the quail eggs following the intake of the *B. subtilis*-based probiotic ($x \pm SD$, $n = 10$)

Parameter	Group		
	control	experimental	
Dense layer of albumen	Height, mm	4.05 ± 0.17	4.86 ± 0.22**
	Lesser diameter, mm	41.4 ± 1.1	45.2 ± 1.2*
	Larger diameter, mm	76.8 ± 1.8	83.2 ± 1.7*
	Albumen index	0.070 ± 0.014	0.082 ± 0.012
Yolk	Height, mm	9.50 ± 0.37	11.44 ± 0.28***
	Lesser diameter, mm	21.02 ± 0.48	23.13 ± 0.39***
	Larger diameter, mm	25.23 ± 0.61	28.54 ± 0.56***
	Yolk index	41.08 ± 0.59	44.27 ± 0.45***

Note: see Table 1.

The probiotic increased the height of egg yolk by 20.4% ($F = 4.818$), lesser diameter by 10.0% ($F = 3.411$), larger diameter by 13.1% ($F = 3.997$), and the yolk index by 7.8% ($F = 4.299$).

The experimental-group quails were observed to consume 12.8% fodder per 10 eggs, compared with the control.

Also, the intake of the probiotic positively affected the live mass and body-mass gains of the quails (Table 5).

Table 5
Effects of the *B. subtilis*-based probiotic on live mass and mass gains in the quails ($x \pm SD$, $n = 20$)

Parameter	Group	
	control	experimental
Live mass at the beginning of the experimental period, g	161.5 ± 1.1	162.8 ± 1.6
Live mass at the end of the experimental period, g	197.4 ± 3.5	212.3 ± 3.6**
Mean daily gain, g	0.400 ± 0.023	0.555 ± 0.041**
Absolute gain in live mass, g	35.9 ± 1.7	49.5 ± 1.9***

Note: see Table 1.

At the end of the experiment, we determined that the probiotic supplement produced 7.5% ($F = 2.967$) higher live mass, 38.8% ($F = 3.297$) higher average daily mass gain, and 37.8% ($F = 5.334$) greater absolute mass, compared with the control. Furthermore, the probiotic increased the survival of the quails by 2.0%.

According to the results of the control slaughter, the supplement promoted increases of 8.5% ($F = 3.092$) in the pre-slaughter live mass, 13.0% ($F = 4.405$) in the mass of non-eviscerated carcass, 7.8% ($F = 2.562$) in the mass of semi-eviscerated carcass, 12.0% ($F = 3.199$) in the mass of eviscerated carcass, and 25.6% ($F = 2.787$) in the mass of the thoracic muscles (Table 6).

Other important parameters that we measured are blood parameters. The circulatory system provides stable homeostasis of the entire organism. Hematological parameters are those on which metabolism depends. In turn, metabolism of nutrients from the feed determines the productivity and organism's resistance.

According to the results of the experiment, the probiotic positively affected the morphological blood parameters, supporting them

within the range of physiological norm. Thus, in the experimental-group quails, the hemoglobin level was higher by 7.2% ($F = 3.139$) and that of erythrocytes greater by 24.7% ($F = 4.999$) than in the control (Table 7).

Table 6
Slaughter parameters of the quails following the intake of the *B. subtilis*-based probiotic ($x \pm SD$, $n = 4$)

Parameter	Group	
	control	experimental
Pre-slaughter live mass, g	186.8 ± 3.9	202.6 ± 3.3*
Mass of non-eviscerated carcass, g	165.4 ± 3.8	187.0 ± 3.1**
Mass of semi-eviscerated carcass, g	148.6 ± 3.1	160.2 ± 3.3*
Mass of eviscerated carcass, g	125.4 ± 3.8	140.5 ± 2.8*
Mass of the thoracic muscles	35.6 ± 2.5	44.7 ± 2.1*
Mass of the thigh muscles	16.8 ± 1.5	21.2 ± 1.4

Note: see Table 1.

Table 7
Morphological blood parameters of the quails following the intake of the *B. subtilis*-based probiotic ($x \pm SD$, $n = 4$)

Parameter	Group	
	control	experimental
Hemoglobin, g/L	112.3 ± 2.1	120.4 ± 1.5*
Erythrocytes, 10 ¹² /L	2.31 ± 0.07	2.88 ± 0.09**
Leukocytes, 10 ⁹ /L	28.68 ± 0.84	30.15 ± 0.65
ESR, mm/h	1.76 ± 0.37	1.93 ± 0.31

Note: see Table 1.

The intake of the probiotic by the experimental group increased the level of the total protein in blood by 16.1% ($F = 10.221$), albumins by 13.5% ($F = 4.629$), and globulins by 18.5% ($F = 4.361$, Table 8).

Table 8
Biochemical blood parameters of the quails following the intake of the probiotic supplement containing the spore-forming bacterium *B. subtilis* ($x \pm SD$, $n = 4$)

Parameter	Group	
	control	experimental
Total protein, g/L	36.56 ± 0.36	42.45 ± 0.45***
Albumins, g/L	17.30 ± 0.38	19.63 ± 0.33**
Globulins, g/L	19.23 ± 0.49	22.78 ± 0.65**
ALT, U/L	4.27 ± 0.24	4.52 ± 0.25
AST, U/L	209 ± 12	226 ± 10
Alkaline phosphatase, U/L	1107 ± 48	1121 ± 56
Total bilirubin, μmol/L	3.50 ± 0.18	3.25 ± 0.25
Cholesterol, mmol/L	3.75 ± 0.17	3.10 ± 0.15*
Triglycerids, mmol/L	3.43 ± 0.30	3.13 ± 0.18
Glucose, mmol/L	5.45 ± 0.19	6.20 ± 0.16*
Creatinine, μmol/L	6.23 ± 0.13	5.55 ± 0.18*
Urea, mmol/L	1.90 ± 0.20	1.63 ± 0.15
Calcium, mmol/L	2.40 ± 0.11	2.90 ± 0.16*

Note: see Table 1.

The probiotic supplement, consumed with the mixed feed, led to increases of 13.8% ($F = 3.019$) in the glucose level and 21.8% ($F = 2.575$) in calcium. At the same time, we observed 17.3% ($F = 2.867$) and 10.9% ($F = 3.063$) decreases in cholesterol and creatinine, respectively. Thus, the probiotic with the spore-forming bacterium *B. subtilis* promotes the production of hemoglobin, total protein, and immune cells in the organism, thereby enhancing metabolism, increasing the productivity, improving the egg quality, reducing the level of cholesterol, and increasing the birds' resistance.

Discussion

Probiotics stimulate the growth of beneficial microorganisms and decrease the number of harmful bacteria, consequently normalizing the balance of gut microflora in the organism. Consumption of probiotics can reduce the risk of gastrointestinal diseases through cleansing of the intestines from pathogens and strengthening of the immune system (Duma et al., 2022).

According to the results of the study, consumption of the probiotic supplement with the spore-forming bacterium *B. subtilis* caused increases in the total yield of eggs from the experimental-group quails, laying capacity, and laying intensity, compared with the control. Similar experiments were conducted by other researchers (Khan et al., 2020; Lan et al., 2025), who yielded positive results in terms of probiotics, which enhanced the productivity and egg quality in quails. Our results are also consistent with the studies by Wang et al. (2023), who observed increase in the laying rate and egg mass in the quails that consumed the probiotic.

During the experiment, we studied the effects of the probiotic supplement on the qualitative parameters of quail eggs. This study has demonstrated that the probiotic containing strain of *B. subtilis*, consumed by the experimental-group quails, promoted increases of 15.0% ($F = 5.202$) in the egg mass, 9.8% ($F = 2.744$) in shell, 21.0% ($F = 3.832$) in yolk, and 12.1% ($F = 3.042$) in albumen, compared with the control. Furthermore, we observed increased lesser and larger egg diameters, volume of egg, and shape index. The results we yielded are consistent with numerous other studies (Gupta et al., 2016; Lv et al., 2021). In particular, the data obtained by Mörschbacher et al. (2025) revealed that intake of the probiotic by the poultry increased the mass and improved the egg quality.

In the experimental-group quails, the probiotic increased the lesser egg diameter by 11.4% ($F = 2.476$), larger diameter by 7.6% ($F = 3.152$), and egg volume by 7.0% ($F = 4.308$). In addition, the probiotic increased the lesser and larger yolk diameter. Similar experiments were conducted by other researchers (Darboe et al., 2023), who also observed improved quality parameters of the eggs resulting from probiotic supplementation. Moreover, some studies indicate potential benefits of using probiotics for improving the quality of shell and yolk, and also gut health. According to Cufadar et al. (2024), addition of *B. megaterium* and *B. amyloliquefaciens* to the diet of laying quails caused no significant changes in the productivity parameters, but positively affected the quality parameters of egg yolk.

The intake of the probiotic supplement based on the spore-forming bacterium *B. subtilis* by the experimental-group quails reduced fodder consumption per 10 eggs by 12.8%, compared with the control group. According to Herlinae (2016), probiotic supplementation increases the effectiveness of using feed while raising quails. Those results also align with the studies by Sathya & Muragian (2015), who found that probiotics in poultry feeding can boost metabolism and significantly reduce feed conversion.

In our experiment, consumption of the probiotic feed supplement with the spore-forming bacterium *B. subtilis* promoted increases and gains of live mass in the experimental-group quail. Furthermore, the probiotic led to the increases in the slaughter parameters, measuring 12.0% ($F = 3.199$) in the mass of eviscerated carcass and 25.6% ($F = 2.787$) in the mass of thoracic muscles. The results of the experiment are consistent with the studies by Patel et al. (2015), who found that probiotic feed supplements in a dose of 100 g/t of feed significantly increased the gain in body mass and improved the coefficient of feed conversion. In addition, Jazi et al. (2020) reported that using *B. subtilis* in quails' diet improved the growth and body-mass gains in the poultry.

Positive effects of probiotics on the poultry productivity have been underscored by results of studies by many researchers (Siadati et al., 2012; Poberezhets et al., 2023). For example, Zhao et al. (2020) noted increased performance in poultry following supplementation of the diet with a *Bacillus icheniformis* H2-containing probiotic. In the study where probiotics and enzymes were given together in the diet of broilers, they also promoted body-mass gains (Wang et al., 2021). Similarly, Duma et al. (2022) reported that probiotics improved the growth and development parameters in the poultry.

In the experiments conducted by Chudak et al. (2021), probiotics in the diet of laying hens enhanced body-mass gains, laying capacity, and egg quality, and reduced fodder consumption.

According to Abdel-Moneim et al. (2020), *B. subtilis*-containing probiotic positively affected the egg productivity of poultry, improving metabolism, boosting growth and development, and also strengthening the resistance to diseases. It normalized the gut micro-

flora, inhibited the growth of harmful bacteria, and promoted the synthesis of beneficial compounds such as vitamins and amino acids.

There is a close relationship between blood and the cells and organs, which helps in diagnostics of physiological changes in the organism and allows determining the effectiveness of feed supplements in animal nutrition. The results obtained in this experiment indicate that the studied probiotic stimulates the formation of hemoglobin, erythrocytes, and total protein in blood. Furthermore, the increase in the content of globulin fractions of protein indicates a strengthened immune response. The simultaneous increase in the level of total calcium is associated with a better breakdown of mineral elements in the digestive tract. For example, Cirilo et al. (2023) reported positive effects of probiotic on the hematological parameters of broiler chickens and noted that probiotics increased the number of immune cells, such as lymphocytes and monocytes, which play a key role in combating infections.

In addition, Yefimov et al. (2024) believe that using *Bacillus*-based probiotics stimulates the resistance of the poultry and increases the intensity of metabolism of minerals and vitamins from the fodder.

The yielded results are explained by the positive effects of probiotic feed supplements on the gut microflora and synthesis of biologically active compounds, which improve the breakdown of nutrients, strengthen immunity, and augment egg productivity, body mass gains, and quality of poultry products.

Conclusions

The intake of the probiotic supplement with the *B. subtilis* bacterium led to increases of 15.8% in the total yield of eggs, 15.6% in laying capacity, and 10.3% in laying intensity, compared with the control. Furthermore, it increased the mass of egg components, namely: mass of eggs, shell, yolk, albumen, and yolk/egg mass ratio. The probiotic promoted improvements in the quality parameters of eggs, increasing the diameter, volume, and shape index. In the experimental-group quails, we observed increases in live mass, body mass gains, and slaughter parameters. According to the results of the experiment, the *B. subtilis*-containing probiotic positively affected the hematological parameters in the poultry, in particular increased the levels of hemoglobin, erythrocytes, total protein in blood serum, albumins, globulins, glucose, and calcium, suggesting improved metabolism and strengthened immunity. Therefore, the *B. subtilis*-based probiotic stimulated immune response in quails and enhanced metabolic processes, thus promoting improved general health, increased productivity, and higher egg quality.

The authors declare no conflict of interests.

References

- Abdel-Moneim, A. E., Selim, D. A., Basuony, H. A., Sabic, E. M., Saleh, A. A., & Ebeid, T. A. (2020). Effect of dietary supplementation of *Bacillus subtilis* spores on growth performance, oxidative status, and digestive enzyme activities in Japanese quail birds. *Tropical Animal Health and Production*, 52, 671–680.
- Chudak, R. A., Poberezhets, Y. M., Lotka, H. I., & Kupchuk, I. M. (2021). Suchasni kormovi dobavky u hodivlyi ptytsi [Modern feed additives in poultry feeding]. Vynnytsia National Agrarian University, Vynnytsia (in Ukrainian).
- Cirilo, E. H., Rohloff Jr., N., Andrade, T. S., Souza, C., Kaufmann, C., Kohler, T. L., Datsch, L. L., Vieira, B. S., Vargas Junior, J. G., Carvalho, P. L. O., Eyang, C., & Nunes, R. V. (2023). Effects of probiotics on blood metabolites, enterocytes, growth, and carcass characteristics of broilers challenged with *Salmonella*. *Serovar Heidelberg*, *Livestock Science*, 270, 105–188.
- Cufadar, Y., Beyari, E. A., Hassoubah, S. A., Curabay, B., Sevim, B., Aksoy, A., Alfassam, H. E., Rudayni, H. A., Allam, A. A., Youssef, I. M., Moustafa, M., Al-Shahrani, M. O., Algopishi, U., Hesham, H., & Abd El-Hack, M. E. (2024). Impacts of *Bacillus* probiotics on productive performance and egg quality criteria in laying Japanese quails. *Journal of Applied Poultry Research*, 33(3), 100445.
- Darboe, A. K., Ambula, M. K., & King'ori, A. M. (2023). Effect of probiotics-treated *Moringa oleifera* leaf meal in exotic layer diets on egg characteristics and consumer acceptability. *World Journal of Food Science and Technology*, 7(2), 35–40.

- Duma, Ö., Yildiz, G., & Merhan, O. (2021). Effects of probiotic (*Lactobacillus farciminis*) supplementation in quail (*Coturnix coturnix japonica*) rations on growth performance, blood antioxidant capacity and cecal some short-chain fatty acid concentrations. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 27(1), 15–20.
- Duma, Ö., Yildiz, G., Merhan, O., Ulufer, S., Önk, K., Baran, M. S., & Kaplan, O. (2022). Feeding Japanese quail diets supplemented with probiotics and enzymes. *South African Journal of Animal Science*, 52(3), 383–391.
- Gupta, N. K., Shrivastava, A. K., Praveen, P. K., & Ganguly, S. (2016). Effect of feed supplement and probiotics on growth performance of Japanese quails. *Indian Journal of Animal Nutrition*, 33(4), 486.
- Hamilton, R. M. G. (2022). A comparison of the various equations published for the estimation of characteristics of hen's eggs, the importance of reporting the compression rate for shell strength measurements, and distinction between egg specific gravity and density. *World's Poultry Science Journal*, 78(2), 447–466.
- Herlinae, Y. (2016). Effectiveness of different packaging probiotics on growth and production of quail (*Coturnix coturnix japonica*). *Jurnal Ilmu Hewani Tropika*, 5(2), 95–100.
- Ibatullin, I. I., Zhukorskyi, O. M., & Bashchenko, I. (2017). Metodolohiia ta orhanizatsiia naukovykh doslidzhen' u tvarynnytstvi [Methodology and organization of scientific research in animal husbandry]. *Ahrarna Nauka: Kyiv* (in Ukrainian).
- Jazi, V., Farahi, M., Khajali, F., Abouasaad, S., Ferket, P., & Assadi Soume, E. (2020). Effect of dietary supplementation of whey powder and *Bacillus subtilis* on growth performance, gut and hepatic function, and muscle antioxidant capacity of Japanese quail. *Journal of Animal Physiology and Animal Nutrition*, 104, 886–897.
- Jiang, S., Hu, J. Y., & Cheng, H. W. (2022). The impact of probiotic *Bacillus subtilis* on injurious behavior in laying hens. *Animals*, 12(7), 870.
- Khan, S., Moore, R. J., Stanley, D., & Chousalkar, K. K. (2020). The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. *Applied and Environmental Microbiology*, 86(13), e00600-20.
- Lan, L., Changcai, L., Huixiang, W., Hao, T., Zhe, C., Xufeng, D., Jiaxin, C., Zhengxing, L., Zhichao, W., Yuxia, M., & Min R.. (2025). Probiotic potential of *Enterococcus lactis* in improving egg production and quality in quails during late egg-laying period. *Poultry Science*, 104(2), 765.
- Lokapimasari, W. P., Dewi, A. R., Fathinah, A., Hidanah, S., Harijani, N., Soeharsono, Karimah, B., & Andriani, A. D. (2017). Effect of probiotic supplementation on organic feed to alternative antibiotic growth promoter on production performance and economics analysis of quail. *Veterinary World*, 10(12), 1508–1514.
- Lv, J., Guo, L., Chen, B., Hao, K., Ma, H., Liu, Y., & Min, Y. (2021). Effects of different probiotic fermented feeds on production performance and intestinal health of laying hens. *Poultry Science*, 98(7), 2866–2879.
- Manafi, M., Khalaji, S., & Hedayati, M. (2016). Assessment of a probiotic containing *Bacillus subtilis* on the performance and gut health of laying Japanese quails (*Coturnix coturnix japonica*). *Revista Brasileira de Ciência Avícola*, 18, 599–606.
- Mörschbacher, A. P., Berghahn, E., Shibuya, F. Y., Cardoso, M. L., Ulguim, G. K., Michelon, N. F., Torgeski, N., Vivian, T. P., Wissmann, D. C., Andrade, G. M., Sturza, D. A. F., Santos, H. F., Dilkin, P., Macedo, L. F. S., & Granada, C. E. (2024). Feeding laying hens with lactobacilli improves internal egg quality and animal health. *World Journal of Microbiology and Biotechnology*, 40(5), 42–46.
- Patel, S. G., Raval, A. P., Bhagwat, S. R., Sadrasaniya, D. A., Patel, A. P., & Joshi, S. S. (2015). Effects of probiotics supplementation on growth performance, feed conversion ratio and economics of broilers. *Journal of Animal Research*, 5(1), 155.
- Poberezhets, J. M., Chudak, R. A., Razanova, O. P., Skoromna, O. I., Faronik, T. V., Ohorodnichuk, G. M., Holubenko, T. L., & Glavatchuk V. A. (2023). Effect of dry extract from *Saccharomyces cerevisiae* culture with selenium-containing amino acids on the productivity and chemical composition of meat of broiler chickens. *Regulatory Mechanisms in Biosystems*, 14(2), 161–164.
- Poberezhets, J. M., Yaropud, V. M., Kupchuk, I. M., Kolechko, A. V., Rutkevych, V. S., Hraniak, V. F., Burlaka, S. A., & Voitsitskyi, O. V. (2023). Effectiveness of using a feed additive from *Saccharomyces cerevisiae* culture in feeding broiler chickens. *Regulatory Mechanisms in Biosystems*, 14(3), 354–357.
- Poberezhets, J., Chudak, R., Kupchuk, I., Yaropud, V., & Rutkevych, V. (2021). Effect of probiotic supplement on nutrient digestibility and production traits on broiler chicken. *Aggraarteadus*, 32(2), 296–302.
- Razanova, O., Skoromna, O., Chudak, R., Poberezhets, Y., & Ohorodnichuk, H. (2023). Growth rate, indicators of slaughter and quality of pork with the additional introduction of a chelated copper complex into the diet of pigs. *Scientific Horizons*, 26(11), 9–18.
- Sathya, C., & Muragian, P. (2015). Effect of dietary supplementation of probiotic and *Curculigo orchioides* rhizome powder on egg quality parameters and biochemical composition of Japanese quail (*Coturnix coturnix japonica*). *Journal of Advance Research in Pharmacy and Biological Science*, 4(1), 162–170.
- Siadati, T. L., Yuan, D., & Chen, Y. B. (2012). Differ *Bacillus* probiotics affect growth perform ammon emission excreta broilers. *Chinese Journal of Animal Science*, 24(5), 877–885.
- Vlizlo, V. V., Fedoruk, R. S., Ratykh, I. B., Vishchur, O. I., Sharan, M. M., Vudmaska, I. V., Fedorovych, Y. I., Ostapiv, D. D., Stapai, P. V., Buchko, O. M., Hunchak, A. V., Salyha, Y. T., Stefanyshyn, O. M., Hevkan, I. I., Lesyk, Y. V., Simonov, M. R., Nevostruieva, I. V., Khomyn, M. M., Smolianinov, K. B., Havryliak, V. V., Kolisnyk, H. V., Petrukh, I. M., Broda, N. A., Luchka, I. V., Kovalchuk, I. I., Kropyvka, S. Y., Paraniak, N. M., Tkachuk, V. M., Khrabko, M. I., Shtapenko, O. V., Dzen, Y. O., Maksymovych, I. Y., Fedorovych, V. V., Yuskiv, L. L., Dolaichuk, O. P., Ivanytska, L. A., Cirko, Y. M., Kystsiv, V. O., Zahrebelnyi, O. V., Simonov, R. P., Stoianovska, H. M., Kyrlyiv B. Y., Kuziv, M. I., Maior, K. Y., Kuzmina, N. V., Talokha, N. I., Lisna, B. B., Klymyshyn, D. O., Chokan, T. V., Kaminska, M. V., Kozak, M. R., Oliinyk, A. V., Holova, N. V., Dubynskyi, V. V., Iskra, R. Y., Rivis, Y. F., Tsepko, N. L., Kyshko, V. I., Oleksiuk, N. P., Denys, H. H., Slychuk, Y. I., & Martyn, Y. V. (2012). Laboratorni metody doslidzhen' u biolohiyi, tvarynnytstvi ta veterynarniy medytsyni [Laboratory research methods in biology, animal husbandry and veterinary medicine]. *Spalom, Lviv* (in Ukrainian).
- Wang, X., Jian, H., Zhao, W., Li, J., Zou, X., & Dong, X. (2023). Effects of dietary *Bacillus coagulans* on the productive performance, egg quality, serum parameters, and intestinal morphology of laying hens during the late laying period. *Italian Journal of Animal Science*, 22, 95–105.
- Wang, Y., Heng, C., Zhou, X., Cao, G., Jiang, L., Wang, J., Li, K., Wang, D., & Zhan, X. (2021). Supplemental *Bacillus subtilis* DSM 29784 and enzymes, alone or in combination, as alternatives for antibiotics to improve growth performance, digestive enzyme activity, antioxidative status, immune response and the intestinal barrier of broiler chickens. *British Journal of Nutrition*, 125(5), 494–507.
- Yefimov, V., Bilyi, D., Lieshchova, M., & Gufrij, D. (2024). Biochemical blood profile and retinol levels in liver broiler chickens with the use of probiotics. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences*, 26(115), 175–180.
- Yu, W., Hao, X., Zhiyue, W., Haiming, Y., & Lei, X. (2020). Evaluation of the effect of *Bacillus subtilis* and *Pediococcus acidilactici* mix on serum biochemistry, growth promotion of body and visceral organs in Lohmann Brown chicks. *Brazilian Journal of Poultry Science*, 22, 1–9.
- Zhao, Y., Zeng, D., Wang, H., Qing, X., Sun, N., Xin, J., Luo, M., Khalique, A., Pan, K., Shu, G., Jing, B., & Ni, X. (2020). Dietary probiotic *Bacillus licheniformis* H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against *Clostridium perfringens*-induced subclinical necrotic enteritis. *Probiotics and Antimicrobial Proteins*, 12(3), 883–895.