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Potential Impacts of Dietary *Bacillus licheniformis* Inclusion on Growth Performance, Serum Metabolites, Antioxidant Profiles and Immune Status of Broiler Chickens

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ABSTRACT

Background: Probiotics have become increasingly important in poultry industry due to their potential beneficial impacts on improved gut health, enhanced growth performance and boosted immune system.

Methods: One hundred and sixty one day old Ross308 broiler chicks were allocated at random into four groups, with each group having four replicates of ten chicks. The groups were fed a basal diet containing *Bacillus licheniformis* (*B. licheniformis*) probiotic at concentrations of 0, 250, 500 and 1000 mg/kg feed. Feed intake (g), feed conversion (g/g) and body weight gain (g) were recorded. Dressing percentage and goblets parts were recorded upon slaughtering of control and *B. licheniformis* groups. Blood samples were centrifuged for serum collection and further analyses of antioxidant and metabolites profiles. Additionally, the antibody titer of the humral immune response against the Newcastle virus vaccine was evaluated using the ELISA test.

Result: The results of the current study showed that dietary probiotic *B. licheniformis* consumption improved (P<0.001) feed intake, feed conversion ratio and body weight gain. Dressing and goblets percentage remained unaffected by the dietary probiotic *B. licheniformis* incorporation if compared to control. Serum antioxidant and metabolites profiles were improved with the probiotic *B. licheniformis* incorporation. Additionally, significant differences between control, 250 *B. licheniformis*, 500 *B. licheniformis* groups compared to 1000 *B. licheniformis* group on antibody titer (p<0.05) against Newcastle. It could be concluded that adding 250 or 500 mg/kg of *B. licheniformis* probiotic to the feed significantly boosted broiler chick growth, improved their metabolic function and enhanced their antioxidant capabilities.

Key words: Antioxidants, *B. licheniformis*, Carcass, Feed, Growth, Metabolites, Probiotics.

INTRODUCTION

The Earth's population of the world is expected to reach 9.7 billion by the middle of the 21st century and 10.4 billion by 2100 (Gu *et al.,* 2021; Mohammed and Alshaibani 2025; Mohammed *et al.,* 2025). The increasing number of people on earth poses a serious threat to food security, requiring a substantial expansion of food production (Fróna *et al.,* 2019). The need for increased food output requires us to adopt efficient and sustainable practices that minimize our environmental footprint (Wang *et al.,* 2021). Consequently, we need to implement technological innovations in agriculture, minimize food waste and create fair distribution systems to guarantee sufficient food for a growing population (Mok *et al.,* 2020; Khan *et al.,* 2021).

Poultry is a highly efficient and inexpensive source of animal protein (Korver 2023; Alyousef *et al.,* 2025). This makes it crucial for meeting the nutritional needs of a growing global population (Smith *et al.,* 2024). It plays a vital role in combating malnutrition, especially in developing countries, by providing essential nutrients (Gržinić *et al.,* 2023). Therefore, the incorporation of dietary probiotics in broiler chicken diets has gained significant attention due to its potential impact to enhance poultry production and health (Krysiak *et al.,* 2021; Ayana and Kamutambuko, 2024). Studies have demonstrated that dietary probiotics significantly enhance broiler chicken performance, resulting in improved feed conversion, nutrient utilization, weight gain and gut health (Yousaf *et al.,* 2022; Hu *et al.,* 2024; Naeem and Bourassa, 2025).

Bacillus licheniformis (*B. licheniformis*) is gaining significant attention in poultry production due to its potential as a beneficial probiotic (Ramirez-Olea *et al.,* 2022; Qin *et al.,* 2024).

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The bacterium *B. licheniformis* contributes to a balanced gut environment by encouraging the proliferation of beneficial bacteria while suppressing harmful ones (Jin *et al.,* 1997; Dumitru *et al.,* 2024; Wongsamart *et al.,* 2025). It can enhance the intestinal barrier function, reducing the risk of pathogen invasion (Kan *et al.,* 2021; Han *et al.,* 2023; Huang *et al.,* 2023). *B. licheniformis* has been found to alleviate intestinal damage caused by conditions like necrotic enteritis (Xu *et al.,* 2021; Xiao *et al.,* 2024). By improving digestion and nutrient absorption, *B. licheniformis* can lead to increased body weight gain and improved feed conversion ratios (Qin *et al.,* 2024). *B. licheniformis* produces various digestive enzymes, such as protease, amylase and lipase, which aid in the breakdown of feed (Shleeva *et al.,* 2023). Consequently, *B. licheniformis* might offer a valuable tool for enhancing growth performance, careful consideration is essential for optimal results. Therefore, this study sought to determine the effects of the dietary probiotics (*B. licheniformis*) supplementation (0, 250, 500, or 1000 mg/kg feed) on growth performances, carcass traits, serum metabolites and biochemical profiles and Newcastle disease antibody titer of growing broilers.

MATERIALS AND METHODS

All experimental procedures were received ethical committee clearance of King Faisal University [KFU-REC-2025-MAR-EA252670]. The procedure of experiment was executed in the experimental animal research station of King Faisal University. *B. licheniformis*® is a probiotic feed additive containing of live spores of a distinct *Bacillus licheniformis* strain (Strain Identification Number DSM 28710) available commercially.

Site of study and experimental design

This study was conducted from November 2024 to January 2025. A total of one hundred sixty one-day old Ross308 broiler chicks were classified via random assignment over four groups (Fig 1). The basal diet's composition adhered to the nutritional guidelines for the strain as recommended by Aviagen (2018). These birds were received the basal diet with added supplements at levels of 0, 250, 500, or 1000 mg/kg of *Bacillus licheniformis*, which were commercially available (*B. licheniformis*®) (control, 250 *B. licheniformis*, 500 *B. licheniformis* and 1000 *B. licheniformis*, respectively). The doses of *B. licheniformis*® were incorporated directly into the feed during mixing to guarantee homogeneity. The birds were raised in 16-square-foot floor pens. For the initial three days, the chicks were maintained at 33.0°C. Subsequently, the temperature was slowly lowered to 31.0°C over the following 48 hours. The birds were raised subsequently at the averaging 31.0 °C natural ambient temperature and 60.0% relative humidity during the study. The lighting program was maintained at 23 h light to 1h dark for the first 7 days, followed by 18 h light to 6 dark for the remaining of the experiment. The chicks had *ad libitum* access to water and diets.

Growth and carcass traits

Body weight gain (Kg) and feed intake (Kg) were measured at 7, 14, 21 and 28 days old on a pen basis and feed conversion ratio were calculated (Abdel-Moneim *et al.,* 2025). On day 28 of age, eight chicks per group representing the groups' average weight were chosen at random for carcass assessment to guarantee impartiality in the sample. The selected chicks were fasted for 12:0 h and weighed, then slaughtered, defeathered and their internal organs removed. The eviscerated carcasses and giblets were weighed of all groups. The eviscerated carcasses and giblets were recorded using digital balance to ensure accuracy and expressed as a percentage of live body weight (Abdel-Moneim *et al.,* 2025).

Blood sample collection and analyses

Blood samples of control and *B. licheniformis*® groups were collected from the brachial vein (10 bird/group). The obtained blood samples were centrifuged to obtain serum

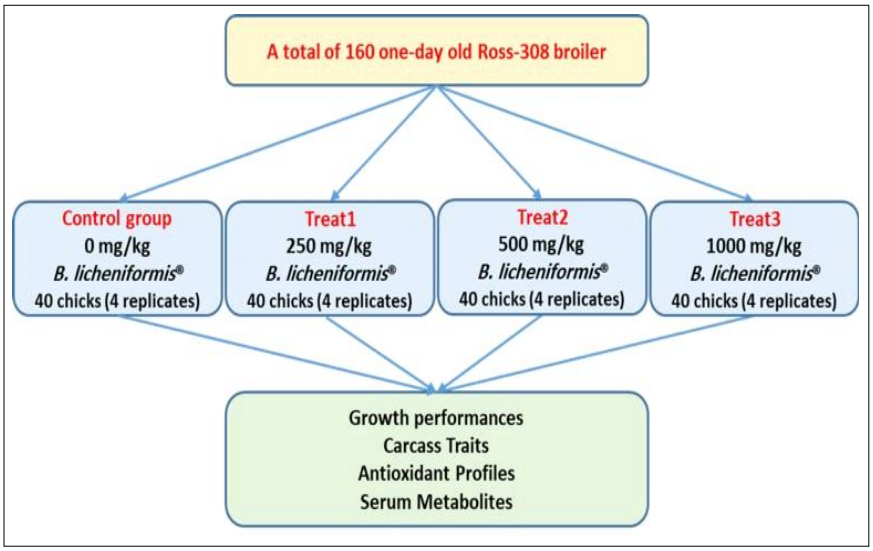

Fig 1: Experimental design of study for *B. licheniformis* ® additives to chicks.

for biochemical and antioxidant analyses (Analyzer Skyla VB1). The biochemical analysis encompassed total proteins, liver enzyme levels, urea, creatinine and mineral concentrations. The antioxidant enzymatic activities of serum malondialdehyde (MDA) was measured using a colorimetric method that involves its interaction with 2-thiobarbituric acid at a wavelength of 532 nm (Mihara and Uchiyama, 1978). The ferric reducing ability (FRAP) test was conducted as described by Benzie and Strain with modification (Benzie and Strain, 1996). The ABTS radical cation decolorization test was conducted as the recommendation of Re *et al*. (1999). The free radical scavenging activity (FRSA) was assessed as outlined by Blois with modification (Blois, 1958). Total thiol (sulfhydryl group, -SH) concentrations (TTL) were quantified as described by Hu with modification (Hu, 1994).

Blood samples and serological test of antibody titer against Newcastle disease

Blood samples (10 bird/group) were obtained by puncturing the *Vena ulnaris* and then transferred into 2 ml Micro-centrifuge Tubes. The clot blood samples were centrifuged for 3 minutes at 12,000 rpm for obtaining serum and stored at -20°C until use. The antibody level for Newcastle disease was detected using ELISA method on serum samples. The ELISA kits (ID Screen® Newcastle Disease REF NDVS-CV-5P LOT J36, France) were used to measure the antibody titer against Newcastle (Oberländer *et al.,* 2020).

Statistical analysis

Body weight gain (g), feed conversion (kg/kg), dressing and giblets percentage (%), serum antioxidant and metabolites values of control and *B. licheniformis*® treated groups were statistically analyzed using SAS program (SAS 2008) through General Linear Model of one way ANOVA procedure according to the model:

$$Yij = \mu + Ti + eij$$

Where,

μ = Mean.

Ti = Effects of *B. licheniformis* ® concentrations (250 *B. licheniformis*, 500 *B. licheniformis* and 1000 *B. licheniformis*).

Eij = Standard error.

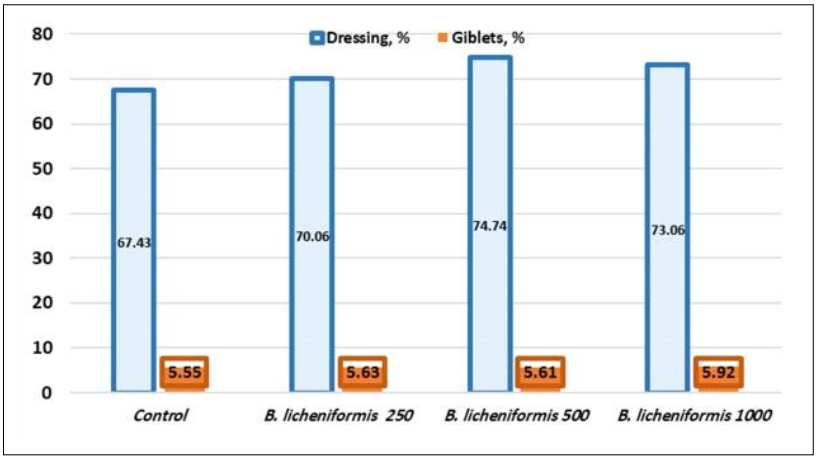

Fig 2: Effects of *B. licheniformis* (250, 500 and 1000) on dressing and giblets percentage of broiler chicks at 28 d of age.

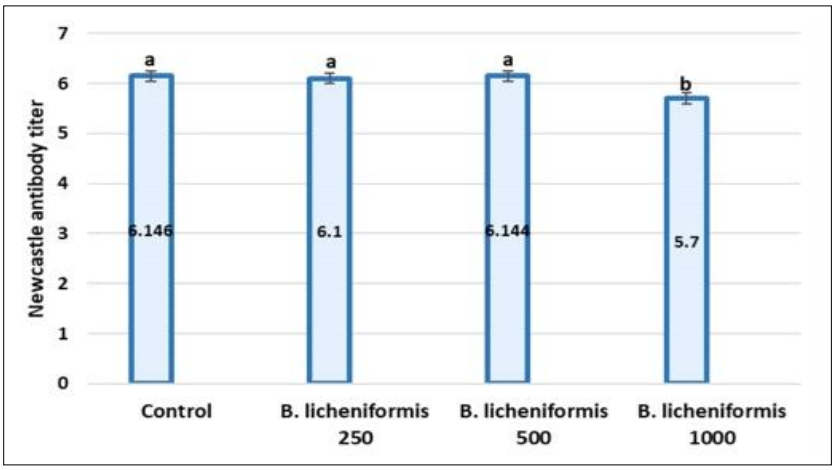

Fig 3: Effects of *B. licheniformis* (250, 500 and 1000) on newcastle disease antibody of broiler chicks at 28 d of age.

To compare the significant among means of control and *B. licheniformis* groups, Duncan's multiple range test (1955) was used.

RESULTS AND DISCUSSION

The effects of *B. licheniformis* ® dietary inclusions (250, 500 and 1000 *B. licheniformis*®) to Ross-308 broiler chicks on feed intake, body weight, feed conversion, carcass traits, serum metabolites and antioxidant capacity were shown in (Fig 2,3) and (Table 1,2).

Growth performances

Changes of weekly body weight (g), weekly feed intake (g) and weekly feed conversion (kg/kg) are presented in (Table 1). Values of finally body weight (week 4) was significantly increased in 250, 500, 1000 *B. licheniformis*® groups compared to that of control group. Control group had the lowest values of body weight across all weeks compared to *B. licheniformis*® groups. *B. licheniformis* (500) group consistently had the highest values across all weeks compared to control and other *B. licheniformis* groups. Regarding to weekly feed intake, there is a clear trend of increasing feed intake across all treatments from week 1 to week 4. The control group showed the lowest feed intake in weeks 2, 3 and 4 compared to *B. licheniformis* groups. In addition, from week 2 to week 4, *B. licheniformis* (500) group consistently showed a significantly ($P<0.003$) higher feed intake compared to control and other *B. licheniformis* groups (250 and 1000). Concerning to feed conversion (kg/kg), a lower feed conversion ratio generally indicates better efficiency. In weeks 1, 2 and 3, there were no significant differences between any of the groups. In week 4, *B. licheniformis* groups (250 and 500) showed significantly better feed conversion ratios than control and *B. licheniformis* group (1000) was between them. Dressing and goblets percentage none significantly increased ($P>0.05$) by the dietary probiotic *B. licheniformis* incorporation if compared to control (Fig 2).

Serum metabolites and antioxidant capacity

The effects of *B. licheniformis* dietary inclusions (250, 500 and 1000 mg/kg diet) on serum metabolites and antioxidant capacity to broiler chickens were shown in (Table 1). The *B. licheniformis* dietary inclusions (250, 500 and 1000) resulted in higher total serum protein values compared to the control group. In 250 *B. licheniformis* group, albumin and creatinine showed the highest values ($P<0.01$) compared to the control and other *B. licheniformis* groups. Glucose values were not differed among groups ($P=0.50$). The *B. licheniformis* inclusions of all groups were decreased bilirubin values compared to control ($P<0.0001$). Regrading to AST and ALP, there were no significant differences in AST ($P=0.74$) and ALP ($P=0.30$) levels among the control and *B. licheniformis* groups. Regrading to ALT and GGT, there were significant decreased in ALT ($P= 0.001$) and GGT ($P=0.03$) in *B. licheniformis* 500 and *B. licheniformis* 1000 compared to control and *B. licheniformis* 250 groups. Regarding to antioxidant capacity, the FRAP, FRSA, ABTS and TTL values were not differed among control and *B. licheniformis* groups whereas MDA values were significantly ($P = 0.01$) decreased in *B. licheniformis* groups if compared to control group.

Table 1: Effects of *B. licheniformis* (250, 500, 1000 mg/kg) on feed intake, feed conversion and body weight gain of broiler chicks.

Parameters	W	Control	*B. licheniformis* 250	*B. licheniformis* 500	*B. licheniformis* 1000	SEM	P value
Weekly body weight, g	W1	145.92^{b}	162.90^{a}	155.32^{ab}	151.100^{b}	3.58	0.015
	W2	392.28^{b}	425.90^{a}	408.18^{ab}	403.18^{b}	7.00	0.030
	W3	743.98^{b}	813.73^{a}	799.10^{ab}	820.63^{a}	17.38	0.050
	W4	1097.33^{b}	1428.53^{a}	1417.85^{a}	1303.45^{a}	76.88	0.003
Weekly body weight gain, g	W1	100.25^{c}	118.00^{a}	109.75^{ab}	106.00^{bc}	3.72	0.006
	W2	246.25	263.25	252.75	251.75	3.55	0.188
	W3	351.75	387.75	391.00	417.50	13.50	0.230
	W4	353.35^{b}	615.00^{a}	618.75^{a}	482.75^{ab}	63.20	0.016
Weekly feed intake, g	W1	118.38	128.08	120.28	124.72	2.18	0.210
	W2	431.40^{b}	460.88^{a}	449.02^{ab}	432.99^{b}	7.00	0.008
	W3	910.42^{c}	1031.91^{a}	995.59^{ab}	960.85^{b}	25.87	0.001
	W4	1550.19^{b}	1753.22^{a}	1737.64^{a}	1721.14^{a}	47.24	0.011
Weekly feed conversion, kg/kg	W1	0.811	0.786	0.774	0.827	0.025	0.2797
	W2	1.102	1.082	1.100	1.076	0.016	0.8465
	W3	1.269	1.269	1.247	1.286	0.040	0.2631
	W4	1.426^{a}	1.226^{b}	1.235^{b}	1.322^{ab}	0.134	0.772

^{a,b,} Values between groups at the same row with different superscripts differ at $P<0.05$.

W- Week; SEM- Standard error.

Newcastle disease antibody

Regarding to Newcastle antibody titer, the results showed significant differences between control, 250 *B. licheniformis* and 500 *B. licheniformis* groups compared to 1000 *B. licheniformis* group ($p<0.05$) on antibody titer against Newcastle (Fig 3).

The effects of *B. licheniformis* dietary inclusions (250, 500 and 1000 mg/kg diet) to Ross-308 broiler chicks on feed intake, body weight gain, feed conversion, carcass traits, serum metabolites and antioxidant capacity and Newcastle disease antibody are showed in (Fig 2,3) and (Tables 1-2). The results of the current study indicated the highest significant improvement of *B. licheniformis@* dietary inclusions to Ross-308 broiler chicks on growth performance, serum metabolites and antioxidant capacity at level of 250 and 500 mg/kg. The effects of *B. licheniformis* could be attributed to several factors including improved feed utilization, gut health, boosted immune system and reduction of pathogens (Kan *et al.,* 2021; Ramirez-Olea *et al.,* 2022).

Growth performances

The effects of *B. licheniformis* dietary inclusions to broiler chicks on growth performances have been explored in several studies (Dumitru *et al.,* 2024; Wongsamart *et al.,* 2025). Our results indicated that dietary 250 mg/kg *B. licheniformis* inclusion gave the highest body weight gain and feed conversion compared with 500 mg/kg and 1000 mg/kg *B. licheniformis* and control groups. *B. licheniformis* acts as a multi-faceted probiotic in broiler chicks, improving growth performance by enhancing digestion and nutrient absorption, promoting a healthy gut environment and balanced microbiota, stimulating the immune system and positively influencing metabolic functions and stress responses (Kan *et al*., 2021; Pan *et al.,* 2022). These combined effects of *B. licheniformis* contribute to better feed conversion ratios, increased body weight gain and improved health and productivity in broiler chickens (Ramirez-Olea *et al.,* 2022; Qin *et al.,* 2024).

B. licheniformis produces various digestive enzymes like amylase, pectinase and cellulose, protease and lipase, which aid in breaking down complex feed components including carbohydrates, proteins and fats. This enhances nutrient digestibility and makes them more available for absorption by the chicks (Su *et al.,* 2020, Shleeva *et al.,* 2023; Sun *et al.,* 2023). *B. licheniformis* consumes oxygen in the gut, creating an anaerobic environment that favors the growth of beneficial bacteria like Lactobacilli and Bifidobacteria (Ramirez-Olea *et al.,* 2022; Dumitru *et al.,* 2024). By consuming oxygen and competing for nutrients and colonization sites, *B. licheniformis* helps to inhibit the growth of harmful aerobic bacteria such as *E. coli* and *C. perfringens* (Abd El Hack *et al.,* 2020). *B. licheniformis* supplementation can lead to a more balanced and diverse gut microbiota, which is crucial for overall health and nutrient absorption (Han *et al.,* 2023).

Serum metabolites, antioxidant capacity and newcastle disease antibody

The values of serum metabolites and antioxidant capacity were improved in *B. licheniformis* groups compared to control group as in other studies (Xu *et al*., 2021; Yu *et al*., 2022). Total protein and albumin (g/dl) were significantly increased in *B. licheniformis* groups compared to control one. This could be attributed to the significant improvement of feed intake and feed conversion obtained in those

Table 2: Effects of *B. licheniformis* on body weight gain, feed intake and feed conversion of broiler chicks on plasma biochemistry profiles of broiler chickens at 28 d of age.

Parameters	Control	*B.licheniformis* 250	*B.licheniformis* 500	*B. licheniformis* 1000	SEM	P value
Biochemical parameters						
Total protein, g/dl	4.10^{b}	4.60^{a}	4.40^{a}	4. 30^{a}	0.08	0.02
Albumin, g/dl	2.50^{b}	2.80^{a}	2.76^{a}	2.74^{a}	0.060	0.01
Creatinine, mg/dl	0.72^{b}	0.91^{a}	0.75^{b}	0.76^{b}	0.05	0.001
Glucose, mg/dl	146.50	147.0	150.0	148.0	3.52	0.50
Total bilirubin, µmol/l	13.65^{a}	11.30^{b}	11.60^{b}	11.15^{b}	0.62	<0.0001
Aspartate Aminotransferase, U/l	66.45	64.62	63.73	63.07	0.25	0.74
Alanine Aminotransferase, U/l	322.25^{a}	317.6 ^{a}	274.0^{b}	252.5^{b}	13.41	0.001
ã-glutamyl transferase, U/l	45.5^{a}	42.60^{a}	34.66^{b}	29.50^{c}	1.52	0.03
Alkaline Phosphatase, U/l	11.66	11.49	11.54	11.61	0.08	0.30
Antioxidant capacity						
FRAP, µM	821.0	827.0	831	833	7.22	0.68
FRSA, Inh.%	86.20	86.22	86.20	86.16	0.64	0.33
ABTS, µM	1.73	1.71	1.71	1.70	0.003	0.94
TTL, µM	143.32	141.28	140.39	136.57	1.313	0.08
MDA, mMol/dl	1.63^{a}	1.48^{b}	1.45^{b}	1.44^{b}	0.040	0.01

^{a,b,} Values between groups at the same row with different superscripts differ at $P<0.05$.

SEM: Standard error. FRAP: Ferric reducing antioxidant power. FRSA: Free radical scavenging activity. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). TTL: Total thiol levels. MDA: Malondialdehyde.

groups, which subsequently resulting in increased of glucose and creatinine values. This could be attributed to the effect of *B. licheniformis* on gut microbiota. Total bilirubin values were decreased (P<0.0001) in *B. licheniformis* groups compared to control groups. *B. licheniformis* supplementation has shown potential to improve liver health in poultry. By maintaining healthy liver function, these additives may contribute to efficient bilirubin processing and excretion in addition to lower liver enzymes (Table 1). This could be attributed to the effect of *B. licheniformis* on modulation of gut microbiota and the gut-liver axis. The antioxidant capacity were decreased in *B. licheniformis* groups with increasing dose if compared to control group. *B. licheniformis* can produce various bioactive compounds with antioxidant properties as exopolysaccharides, certain amino acids, organic acids and phenols (Shleeva *et al*., 2023).

Immune response against Newcastle disease virus vaccine showing that 250 and 500 *B. licheniformis* groups similar to control group whereas1000 *B. licheniformis* group showed lower immune response. Additionally, all control and *B. licheniformis* groups were in protective level against Newcastle virulent virus. *B. licheniformis* can upregulate the gene expression of tight junction proteins and mucins, strengthening the intestinal barrier and reducing intestinal permeability.

This helps prevent the leakage of harmful substances and pathogens into the bloodstream (Obianwuna *et al.,* 2023; Sun *et al.,* 2023). Additionally, components of *B. licheniformis* such as cell wall polysaccharides and peptidoglycans, can act as antigens, stimulating the gut-associated lymphoid tissue and enhancing the immune response. Furthermore, studies have shown that *B. licheniformis* can increase the levels of immunoglobulins like IgA in the serum and intestinal mucosa, which are essential for defense against pathogens. It can activate macrophages and promote the production of T and B lymphocytes, enhancing the overall immune ability of the chicks (Yu *et al.,* 2022; Qin *et al.,* 2024).

CONCLUSION

It could be concluded that *Bacillus licheniformis* dietary inclusions modulates growth performances, serum metabolites and antioxidant capacity. The best dietary supplement levels were 250 *B. licheniformis*, followed by 500 and 1000 *B. licheniformis* levels, referring to the optimal amount of this additive to achieve maximum benefits and economic efficiency for poultry production. Determining the best dietary additive depends on poultry species and age, dietary composition and environmental conditions.

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Disclaimers

The views expressed in this article are the authors' alone and do not represent the opinions of their institutions.

Informed consent

Ethical approval for all experimental animal procedures and care methods was granted by the University's Animal Care Committee.

Conflict of interest

No conflict of interest for authors to declare.

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