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IMPACT OF *BACILLUS SUBTILIS* PROBIOTIC ON GROWTH, VISCERAL AND LYMPHATIC ORGAN WEIGHTS, INTESTINAL HISTOMORPHOLOGY, AND PATHOGENIC BACTERIA OF BROILERS

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Abstract

This work aimed to examine the impacts of dietary *Bacillus subtilis* (BS) supplementation on several parameters. Twelve groups of 600 day-old male Ross chicks were created for the study. Each treatment was allotted into five replicates, with ten birds each. Four distinct diet treatments were provided for 42 days: diet A served as the control, whereas diets B, C, and D had 125, 250, and 500 mg of BS per kg of feed. Every diet was fed in three stages: continuous (days 1–42) and starter only (days 1–21), finisher only (days 22–42), and both. The results showed that the D-3 and C-3 groups had significantly higher feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), livability, and European Production Efficiency Factor (EPEF). The D-3 and C-3 groups also showed the largest relative weights of the liver, gizzard, pancreas, bursa, and spleen, as well as the enhanced weights of these organs. Groups D-3 and C-3 had highest villus height (VH), decreased crypt depth (CD), and enhanced VH: CD in the ileum, jejunum, and duodenum. The high levels of BS (D-3 and C-3) eliminated *Salmonella*, *C. perfringens*, and *E. coli* from the ileum, caecum, and colon. From the results above, a conclusion could be drawn that dietary BS supplementation could be a safe substitute for AGPs in broiler diets.

Key words: *Bacillus subtilis*, *E. coli*, *Salmonella*, *C. perfringens*, histomorphology

Natural feed additives have recently replaced artificial growth promoters and antibiotics in farm animals and birds to boost immunity and productivity. This has resulted in improved carcass features and growth performance (Abd El-Hack et al., 2023 a, b; Kamal et al., 2023; El-Ratel et al., 2024; Mohamed et al., 2024). Food safety can be promoted throughout the entire value chain by using probiotics and prebiotics to reduce aflatoxin residues in chicken products. This would lessen aflatoxin's detrimental economic and public health impacts (Abd El-Hack et al., 2023 c; Mahgoub et al., 2024).

Khan et al. (2022) stated that *Bacillus subtilis* is frequently used as a chicken sector probiotic. Higher growth rates and improved weight gain were observed when BS was supplemented, not only in healthy poultry birds but also in those with *Eimeria* (Park et al., 2020) and *Salmonella* (Zaghari et al., 2020) infections (Bai et al., 2017; Gadde et al., 2017; Bahrampour et al., 2020; Ahmad et al., 2024; Mushtaq et al., 2024). Likewise, elevated gross energy retention, apparent dry matter, crude protein, and pathogenic bacteria limitation have all been linked to enhancing FCR (Sen et al., 2012).

Elshaghabee et al. (2017) found that BS could produce various enzymes, secretory proteins, carotenoids, antimicrobial and small extracellular substances with effector functions. This makes them appropriate for possible use in biotherapeutics. Guo et al. (2020) discovered that adding BS to the poultry diet resulted in an increase in villus height (VH), a reduction in crypt depth (CD), and an improvement in the VH: CD ratio in various sections of the intestine. Concerning the addition of BS, hens affected by coccidiosis had more pronounced intestinal lesions and histomorphological alterations. Abdel-Moneim et al. (2020) found that adding BS improved growth performance, specifically through increased VH

and VH: CD. Adding BS to broiler feed led to higher VH and VH: CD ratios and increased beneficial bacteria (Sen et al., 2012). Various strains of BS have been chosen as probiotics because of their *in-vitro* antibacterial and anticancer characteristics (Nhung et al., 2017; Grant et al., 2018).

Including BS promotes the proliferation and regulation of beneficial bacteria by preventing the colonization of harmful *C. perfringens* and *E. coli*. Also, it serves as a defence mechanism against chicken coccidiosis (Lee et al., 2015). BS supplementation has been found to promote the growth of beneficial microorganisms (Mazkour et al., 2019) and have antibacterial effects against *E. coli*, *Salmonella*, and *C. perfringens* (Manhar et al., 2016; Ali et al., 2019). The present study hypothesized that dietary BS supplementation will positively affect broilers' growth performance and other health parameters. This experiment aimed to identify the most effective concentration and timing of supplementing BS in a partially controlled chicken housing system, considering concurrently the sporadic addition of BS at various levels and feeding phases.

Material and methods

Following the criteria for the Care and Use of Laboratory Animals developed by the Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, at the University of Agriculture, Peshawar, Pakistan, and the principles set forth by the Committee on Research Ethics, the Ethics Declaration for Animal Care and Maintenance was followed.

Birds, management and diets

A total of 600 day-old male Ross chicks were kept to detect the best BS level and feeding phase. The allocation of all the chicks was done randomly across 12 trial groups. Each group was divided into five replicates, each replicating 10 chicks. Four distinct dietary regimens were employed: diet A acted as the control, while diets B, C, and D were supplemented with BS (GalliPro®, Chr. Hansen Holding A/S, Hoersholm, Denmark, containing 4×10^9 cfu/g BS DSM 17299) at concentrations of 125, 250, and 500 mg per kg of diet, respectively, for the broiler chicks. The diet was administered in three distinct phases: the starter phase-1, which lasted from the 1st day to the 21st day; the finisher phase-2, which lasted from the 22nd day to the 42nd day; and the constant feeding phase-3, which lasted from the 1st day to the 42nd day (Table 1). After a week of consistent 35°C temperature maintenance, we reduced the temperature by 15°C each week until it stabilized at 24°C, which we maintained until the study's conclusion. Throughout the experiment, a light cycle consisting of 23 hours of illumination and 1 hour of darkness was preserved. The chicks got access to clean water and nutrition while maintaining an average relative humidity of 65%.

Experimental design

Growth performance

During the investigation, the variables feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), livability, and European Production Efficiency Factor (EPEF) were assessed following the methodology described by Khan et al. (2022).

Visceral and lymphoid tissue traits

For each replication, five broilers were chosen randomly, weighed, and then killed on day 42. In total, there were 20 broilers in each group. The internal visceral parts, such as the heart, liver, gizzard, pancreas, lymphoid organs, spleen, thymus, and bursa, were swiftly collected and measured to ascertain their respective weights.

Intestinal histomorphology

On the 42nd day, samples of the middle section, the upper part, and the lower part of the small intestine were collected and rinsed with normal saline solution after three birds per group were randomly selected and slaughtered. The intestinal samples were obtained for morphological analysis and microscopy utilizing the methods outlined by Abdelqader et al. (2013). Formalin (10%) was employed for the process of fixation. The sections that were sliced were placed on glass slides for hematoxylin and eosin (H&E) staining. Different concentrations of ethanol were utilized to remove water from the sections. Xylene was used to make the sections clear. The sections were then embedded in paraffin and cut into slices with a thickness of five microns using a microtome. For each sample, ten fully developed and undamaged crypt villi units were selected, and the average heights of the villi and depths of the crypts were determined by taking the means of the recorded measurements. To quantify the VH and CD, intestinal samples were observed using an Olympus CX41 microscope from Japan. The images obtained were then analyzed using a Nikon NIS-Element BR image tester from Nikon Co., Tokyo, Japan. The value of CD was calculated by combining the VH and CD values using the methods described by Khan et al. (2022).

Pathogenic bacterial count

We aseptically collected 1 g of samples from the slaughtered birds' ileum, caecum, and colon. These samples were then homogenized and diluted tenfold with normal saline in sterile mixer bags. Each specimen was aliquoted with 100 microliters and inoculated onto microbiological media for *E. coli*, *Salmonella*, and *C. perfringens*. The cultures were maintained under appropriate conditions, including time, oxygen level, and extra requirements. This experiment was conducted in the laboratory following a sequential tenfold dilution ranging from 10^{-1} to 10^{-7}. The bacterial colonies in the broiler chicken's ileum, caecum, and colon were quantified utilizing a colony counter. The outcomes were expressed as $\log^{10}$ CFU/g of digesta, according to Khan et al. (2022).

Table 1. Ingredients and calculated chemical composition of the basal diet

Ingredients	Starter phase 0–21 days	Finisher phase 22–42 days
Corn	49.25	51.66
Wheat	2.00	5.00
Fish meal	2.00	–
Corn gluten (60%)	6.00	6.50
Soybean meal (45%)	34.18	31.07
Animal fat	1.52	1.26
Monocalcium phosphate	1.61	1.57
Limestone	0.60	0.70
Choline-chloride (50%)	0.10	0.10
DL-Methionine (88%)	0.24	0.14
Mineral premix	0.10	0.10
Vitamin premix	0.10	0.10
NaCL	0.30	0.30
Calculated composition (%)		
DM	88.34	88.24
ME (Kcal/Kg)	3000	3200
Moisture	11.66	11.36
CP	22.00	20.00
Available phosphorus	0.45	0.40
Calcium	1.00	0.90
Methionine + Cysteine	0.95	0.80
Lysine	1.25	1.11
Tryptophan	0.28	0.25
Threonine	0.86	0.78
Lab analysis		
DM	88.77	88.64
Moisture	11.23	11.36
CP	21.88	19.70
CF	3.92	4.30
Ash	7.10	6.94

DM = dry matter, ME = metabolic energy, CP = crude protein, CF = crude fat.

Statistical analysis

The data analysis was conducted using the SPSS software, specifically version 21.0. A one-way analysis of variance was employed to ascertain the differences across 12 interactive groups. The data was presented using SEM, and any variations between means were determined using Tukey's tests. A significant variance was reported at $P<0.05$.

Results

Growth performance of broilers

Group D-3 (4030 g), C-3 (4026 g), and B-3 (3977 g) showed significantly higher FI compared to the other groups (Table 2), with less than $P<0.05$. Following closely were D-1 (3876 g) and D-2 (3872 g). The results showed that the groups that were consistently fed high amounts of BS gained significantly more BWG than the others. These groups were D-3 (2409 g) and C-3 (2403 g), followed by B-3 (2337 g) and D-1 (2275 g). Similarly, there was a notable increase in FCR in the group that received continuous high-level feeding and subsequent supplementation with BS (biological substance). Groups D-3 (1.673) and C-3 (1.675) showed the highest improvements in FCR, followed by B-3 (1.702) and D-1 (1.704). Groups D-3 (92.00) and C-3 (90.00) showed the only noticeable improvement in livability, with no significant differences ($P>0.05$) detected. Similarly, groups D-3 (315.42), C-3 (307.28), and B-3 (287.74) showed a statistically significant ($P>0.05$) increase in EPEF compared to all other experimental broiler groups.

Visceral and lymphoid organs

Table 3 shows that the relative weights of the heart (0.512 g and 0.509 g), liver (2.462 g and 2.446 g), gizzard (1.681 g and 1.670 g), and pancreas (0.236 g and 0.234 g) all went up in groups D-3 and C-3. The variation that was observed was statistically significant ($P<0.05$). Groups B-3 and D-1 had the next highest weights, while groups A-1, A-2, and A-3 had the lowest. It was found that the bursa (0.186 g and 0.184 g), spleen (0.130 g and 0.129 g), and thymus (0.617 g and 0.612 g) were bigger in groups D-3 and C-3 compared to the control ($P<0.05$). The results showed that the relative weights of internal visceral and lymphoid organs increased noticeably due to the high levels of feeding BS in groups D and C during continuous phase 3.

Intestinal histomorphology

The findings from Table 4 showed a substantial increase ($P<0.05$) in VH in the D-3 and C-3 groups in the duodenum (1956.00 µm and 1936.67 µm), jejunum (1270.40 µm and 1255.07 µm), and ileum (630.60 µm and 625.80 µm), compared to the control groups, which had the lowest VH. Significant reductions ($P<0.05$) in CD were observed in the duodenum (203.93 µm and 208.47 µm), jejunum (175.80 µm and 178.53 µm), and ileum (161.07 and 163.87 µm) for groups D-3 and C-3, compared to the higher levels found in the control. The VH: CD ratio in the duodenum (9.605 µm and 9.299 µm), jejunum (7.235 µm and 7.037 µm), and ileum (3.920 µm and 3.823 µm) was significantly higher ($P<0.05$) in groups D-3 and C-3 contrasted to all other groups.

Bacterial count

The outcomes from Table 5 indicate that adding BS at various dosages and stages considerably impacts the community of gut bacteria in the experimental broilers. Administering high levels of BS addition (250 and 500 mg/kg) and continuously feeding it to broilers for 42 days reduced the populations of selected harmful bacteria in various sections of the lower gut. The samples collected from the ileum and colon of broiler birds in groups D-3 and C-3 did not contain any *E. coli* (log10 0.000). Also, giving the broilers a lot of BS during the continuous feeding phase (groups C-3 and D-3) eliminated all the *C. perfringens* and *Salmonella* in their colon, ileum, and caecum.

Table 2. Effect of BS at different levels and phases on the overall performance of broiler chickens

Group	FI (g)	BWG (g)	FCR	LI (%)	EPEF
A-1	3784.00 d	2179.00 f	1.737 a	86.00	257.06 cd
A-2	3775.00 d	2173.00 f	1.737 a	82.00	244.27 d
A-3	3791.00 cd	2183.00 f	1.737 a	84.00	251.42 cd
B-1	3835.00 bcd	2211.00 def	1.734 a	88.00	267.09 cd
B-2	3802.00 bcd	2191.00 ef	1.735 a	86.00	258.58 cd
B-3	3977.00 a	2337.00 b	1.702 d	88.00	287.74 abc
C-1	3856.00 bcd	2242.00 cd	1.720 bc	86.00	266.85 cd
C-2	3847.00 bcd	2232.00 cde	1.724 b	84.00	258.90 cd
C-3	4026.00 a	2403.00 a	1.675 e	90.00	307.28 ab
D-1	3876.00 b	2275.00 c	1.704 d	86.00	273.46 bcd
D-2	3872.00 bc	2254.00 cd	1.718 c	86.00	268.66 cd
D-3	4030.00 a	2409.00 a	1.673 e	92.00	315.42 a
SEM	12.12	10.74	0.003	0.71	3.36
P value	< 0.05	< 0.05	< 0.05	0.71	0.03

Where BS = *Bacillus subtilis*, FI = Feed intake, BWG = Body weight gain, FCR = Feed conversion ratio, LI = Livability, and EPEF = European Production Efficiency Factor. A = BS 0 mg/kg, B = BS 125 mg/kg, C = BS 250 mg/kg, D = BS 500 mg/kg, 1 = Starter phase, 2 = Finisher phase, 3 = Continuous phase.

This means the same column with different letters significantly differs (P≤0.05).

Table 3. Effect of BS at different levels and phases on relative weights* of internal visceral and lymphoid organs of broiler chickens

Group	Heart	Liver	Gizzard	Pancreas	Bursa	Spleen	Thymus
A-1	0.459 cd	2.205 d	1.506 d	0.209 d	0.164 d	0.115 d	0.545 d
A-2	0.457 d	2.201 d	1.503 d	0.209 d	0.163 d	0.115 d	0.545 d
A-3	0.461 cd	2.217 cd	1.514 cd	0.209 d	0.163 d	0.114 d	0.543 d
B-1	0.466 c	2.242 c	1.530 c	0.214 bc	0.169 bc	0.118 bc	0.562 bc
B-2	0.463 cd	2.226 cd	1.520 cd	0.213 c	0.168 c	0.117 c	0.558 c
B-3	0.490 b	2.359 b	1.611 b	0.216 b	0.171 b	0.120 b	0.568 b
C-1	0.489 b	2.353 b	1.607 b	0.215 bc	0.169 bc	0.119 bc	0.564 bc
C-2	0.464 cd	2.232 cd	1.524 cd	0.213 c	0.168 c	0.118 c	0.559 c
C-3	0.509 a	2.446 a	1.670 a	0.234 a	0.184 a	0.129 a	0.612 a
D-1	0.492 b	2.365 b	1.615 b	0.216 b	0.170 b	0.120 b	0.568 b
D-2	0.486 b	2.337 b	1.596 b	0.215 bc	0.169 bc	0.119 bc	0.564 bc
D-3	0.512 a	2.462 a	1.681 a	0.236 a	0.186 a	0.130 a	0.617 a
SEM	0.002	0.012	0.008	0.001	0.001	0.001	0.003
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Where BS = *Bacillus subtilis*, A = BS 0 mg/kg, B = BS 125 mg/kg, C = BS 250 mg/kg, D = BS 500 mg/kg, 1 = Starter phase, 2 = Finisher phase, 3 = Continuous phase of feeding BS.

This means the same column with different letters significantly differs (P≤0.05).

*Relative weights of visceral and lymphoid organs expressed in percent.

Table 4. Effect of BS at different levels and phases on intestinal histomorphology of broiler chickens

Group	Villus height (µm)			Crypt depth (µm)			VH: CD		
	duodenum	jejunum	ileum	duodenum	jejunum	ileum	duodenum	jejunum	ileum
A-1	1830.00 d	1189.07 e	591.40 e	246.53 a	210.60 a	192.87 a	7.426 d	5.649 d	3.068 d
A-2	1822.93 d	1183.60 e	589.60 e	248.87 a	213.27 a	196.07 a	7.331 d	5.555 d	3.009 d
A-3	1822.33 d	1185.93 e	590.20 e	246.13 a	209.20 a	193.00 a	7.420 d	5.675 d	3.061 d
B-1	1862.33 c	1209.27 d	601.33 d	234.40 b	199.93 b	183.73 b	7.951 c	6.053 c	3.275 c
B-2	1855.67 c	1207.07 d	599.33 d	236.33 b	201.93 b	185.07 b	7.857 c	5.982 c	3.241 c
B-3	1893.67 b	1228.67 c	612.00 bc	222.47 c	190.27 c	175.00 c	8.517 b	6.462 b	3.500 b
C-1	1901.33 b	1229.87 c	612.20 bc	223.53 c	191.33 c	175.87 c	8.509 b	6.430 b	3.482 b
C-2	1861.67 c	1207.67 d	600.40 d	235.33 b	200.87 b	184.07 b	7.916 c	6.017 c	3.264 c
C-3	1936.67 a	1255.07 b	625.80 a	208.47 d	178.53 d	163.87 d	9.299 a	7.037 a	3.823 a
D-1	1904.00 b	1236.13 c	617.20 b	221.47 c	188.13 c	172.93 c	8.605 b	6.576 b	3.572 b
D-2	1891.33 b	1228.80 c	609.80 c	224.40 c	191.00 c	175.53 c	8.443 b	6.439 b	3.477 b
D-3	1956.00 a	1270.40 a	630.60 a	203.93 d	175.80 d	161.07 d	9.605 a	7.235 a	3.920 a
SEM	3.368	2.127	1.067	1.143	0.960	0.889	0.055	0.041	0.022
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Where BS = *Bacillus subtilis*, A = BS 0 mg/kg, B = BS 125 mg/kg, C = BS 250 mg/kg, D = BS 500 mg/kg, 1 = Starter phase, 2 = Finisher phase, 3 = Continuous phase of feeding BS. VH = Villus height, CD = Crypt depth, VH: CD = Villus height to Crypt depth ratio. This means the same column with different letters significantly differs (P≤0.05).

Table 5. Effect of BS at different levels and phases on intestinal bacterial count of broiler chickens

Group	*E. coli* (log_{10}/g)			*Salmonella* (log_{10}/g)			*C. perfringens* (log_{10}/g)		
	ileum	caecum	colon	ileum	caecum	colon	ileum	caecum	colon
A-1	4.066 a	6.836 a	5.405 a	2.109 a	2.360 a	2.326 a	2.354 a	2.329 a	2.274 a
A-2	4.066 a	6.837 a	5.405 a	2.115 a	2.363 a	2.326 a	2.365 a	2.330 a	2.274 a
A-3	4.065 a	6.836 a	5.404 a	2.097 a	2.354 a	2.315 a	2.350 a	2.319 a	2.263 a
B-1	3.995 b	6.729 b	5.335 b	1.979 b	2.233 b	2.203 b	2.257 b	2.224 b	2.173 b
B-2	4.065 a	6.836 a	5.404 a	2.015 b	2.269 b	2.231 b	2.334 a	2.304 a	2.249 a
B-3	3.561 d	6.156 e	5.000 d	1.872 cd	2.121 cd	2.086 cd	2.122 d	2.090 e	2.035 e
C-1	3.885 c	6.401 d	5.224 c	1.891 cd	2.146 cd	2.109 cd	2.173 c	2.140 d	2.086 d
C-2	3.996 b	6.729 b	5.335 b	2.005 b	2.259 b	2.222 b	2.285 b	2.254 b	2.200 b
C-3	0.000 f	5.270 g	0.000 f	0.000 e	0.000 e	0.000 e	0.000 f	0.000 g	0.000 g
D-1	3.194 e	5.789 f	4.533 e	1.851 d	2.105 d	2.068 d	2.078 e	2.050 f	1.996 f
D-2	3.886 c	6.543 c	5.225 c	1.903 c	2.157 c	2.120 c	2.208 c	2.177 c	2.123 c
D-3	0.000 f	4.839 h	0.000 f	0.000 e	0.000 e	0.000 e	0.000 f	0.000 g	0.000 g
SEM	0.109	0.048	0.146	0.056	0.063	0.062	0.063	0.062	0.061
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Where BS = *Bacillus subtilis*, A = BS 0 mg/kg (Control), B = BS 125 mg/kg, C = BS 250 mg/kg, D = BS 500 mg/kg, 1 = Starter phase, 2 = Finisher phase, 3 = Continuous phase of feeding BS. EC = *E. coli*, SA = *Salmonella*, CP = *C. perfringens*.

This means the same column with different letters significantly differs (P≤0.05).

Discussion

Bacillus subtilis is a superior growth promoter in young chickens (Bahrampour et al., 2020; Park et al., 2020). Consistent with our results, Dersjant-Li et al. (2014), Jayaraman et al. (2017) and Wang et al. (2018) also reported improved growth performance in broilers added with BS. Wealleans et al. (2017 a, b) found that including BS in chickens increased apparent ME and boosted ileal digestibility, enhancing growth efficiency. The rationale for constant nutrition is that BS is not a typical resident of the gastrointestinal tract of broiler chickens. Instead, it is a facultative anaerobe that can thrive within the digestive system (Clements et al., 2002). On the other hand, Abudabos et al. (2017), Ali et al. (2023) and Ullah et al. (2023) reported no significant effects on weight gain. Various housing conditions could cause these disparities in outcomes, probiotic and broiler strains, livability, and probiotic dosage rates (Guo et al., 2020; Zaghari et al., 2020).

The results of Abdel-Moneim (2020) align with our results, indicating that higher levels of BS lead to a linear increase in BWG. Guo et al. (2020) attribute this to enhanced proteolytic, lipolytic, and amylolytic activities in the duodenum and improved nutritional digestion in broiler chickens. Park et al. (2020) also noted a rise in the FCR in broilers supplemented with BS due to improved feed digestion and nutrient absorption. The studies of Abdel-Moneim (2020) and Bahrampour et al. (2020) strongly support the recent findings. They observed a linear FCR improvement in broiler chickens, which was attributed to enhanced nutrient use. Park et al. (2020) conducted a study that found that the enhanced immune response in the intestines and the improved integrity of the protective lining led to lower mortality, prevented microbial infections, and ultimately increased survival rates. BS can also activate toll-like receptor-4, which creates proinflammatory cytokines (Il-1β, Il6, and Il8) that help chickens' natural immune system work. This, in turn, enhances immunity, reduces the likelihood of infection, and results in a 66.67% increase in livability (Guo et al., 2020). Additionally, recent discoveries concur with the outcomes of Awad et al. (2010) and Abudabos et al. (2020), who indicated similar improvements in the production efficiency factor in broilers.

In contrast, Śliżewska et al. (2020) found that the EPEF decreased in BS-supplemented broiler birds. Guo et al. (2020) suggest variations in the reaction to supplementing with probiotics in broiler chickens can be attributed to environmental temperature, the specific strain of probiotics employed, the animal type involved, and the dosage of probiotics administered. Adding BS to the broiler diet from the first day until slaughter improved the EPEF, increased livability, decreased mortality, and enhanced growth efficiency in chickens (Sokale et al., 2019).

Abudabos et al. (2016) found that supplementation with BS increased the relative weight of broiler chickens' liver, bursa, spleen, and thymus challenged with *Salmonella*. Extensive research has demonstrated that compensatory hypertrophy efficiently pumps blood to a larger body mass, resulting in increased heart weight. According to Khan et al. (2022), the increase in gizzard weight observed in broiler chickens is a compensatory response to their increased feed intake, resulting in hyperplasia of the gizzard muscles. Teo and Tan (2007) observed a similar increase in the weight of the spleen and bursa in broiler chickens fed with BS. Soomro et al. (2019) observed that adding BS had a negligible impact on the weight of the heart, liver, and gizzard in broiler chicks. In broiler chicks, administering a significant amount of bovine excrement consistently also resulted in elevated

liver weight. We assumed that the hepatocytes' hyperplasia and hypertrophy due to high FI and high BWG may cause this increase in liver weight. High BWG, which results from consuming a lot of feed, increases the hepatocytes' workload in the liver to satisfy the needs of the broiler bird's quick BWG (Khan et al., 2022).

Our research showed that higher levels of butyric acid in the duodenum and ileum of broiler chickens caused the VH to rise significantly and the CD to fall. The VH: CD ratio also went up. The findings of Ma et al. (2018) and Guo et al. (2020) strongly support the conclusions. According to Park et al. (2020), poultry contracted with coccidiosis showed enhanced intestinal ulcers and histomorphology when given BS dietary supplements. In addition, according to Hoerr and Schrader (2016), the depth of the crypts in the small intestine remains constant, while the length of the villi decreases as you move along the length of the small intestine. The control groups' current outcomes have validated these initial discoveries. According to Fathi et al. (2017), probiotics can improve poultry productivity, intestinal health, and morphology. Adding BS may improve growth efficiency by enhancing the structure of the intestines and, finally, enhancing the utilization of nutrients from feed to facilitate optimal growth in test birds (Abdel-Moneim et al., 2020; Bahrampour et al., 2020).

Pathogenic bacteria decreased linearly and significantly with increasing BS levels (Lee et al., 2015; Manhar et al., 2016; Mazkour et al., 2019), while the guts of poultry birds contained a high concentration of beneficial bacteria (Bahrampour et al., 2020). Numerous antimicrobial compounds are produced by *Bacillus* species (Urdaci et al., 2004); these include bacteriocins, bacteriocin-like compounds, and substantial quantities of peptides and polypeptides (Belih et al., 2015). Teo and Tan (2007) and Ma et al. (2018) found that enhancing broiler diets with BS decreased the presence of broiler intestinal *E. coli* and *C. perfringens*. The increased expression of TJ proteins is likely responsible for the improved intestinal barrier function and healthy gut of chickens given more BS (Gadde et al., 2017). Antimicrobial tests conducted in a laboratory setting have led to the selection of multiple strains of BS as probiotics (Nhung et al., 2017; Grant et al., 2018). The same happened when Manhar et al. (2016) found that BS can stop food-borne pathogens from sticking to the Caco-2 epithelial cell line. This prevents bacteria-caused enteric illnesses.

Different types of bacteria can make biotherapeutics utilizing enzymes, secretory proteins, vitamins, carotenoids, antimicrobial peptides, and extracellular effects (Elshaghabee et al., 2017). Studies have demonstrated that *Bacillus* supplementation boosts phagocytosis and interferon production while elevating IgM levels (Paul et al., 2013). Adding BS to the diet led to enhance performance and preserving a healthy gut microbiota. It also promoted the rapid growth and spread of helpful microorganisms, impeded the proliferation of detrimental bacteria, and thwarted infections (Guo et al., 2020; Park et al., 2020).

Conclusions

Administering BS at a dose of 250 mg/kg to 500 mg/kg in a constant feeding diet has a positive impact on the structure of the intestines, the weight of internal organs related to digestion and immunity, general efficiency, and the number of harmful bacteria that affect chickens. Therefore, we can infer that this supplementation improves chicken health and performance.

Conflicts of interest

The authors declare no conflict of interest.

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