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Efficacy of Gum Arabic and *Bacillus subtilis* Alone and in Synbiotic Form on Overall Performance, Visceral and Lymphoid Organs Along with Intestinal Histomorphology and Selected Pathogenic Bacteria in Broiler Chickens

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The experimental work was performed by SK under the supervision of NC and AH. NA facilitated the work regarding feed formulation and GA, BS and their synbiotic supplementation in broiler feed.

Key words
Gum arabic, *Bacillus subtilis*, Synbiotic, *E. coli, Salmonella, C. perfringens*, Thymus, Bursa

ABSTRACT

The present research trial was carried out to explore the effects of supplementation of gum arabic (GA) and *Bacillus subtilis* (BS) alone and in synbiotic form on overall growth performance, visceral and lymphoid organs weights along with intestinal histomorphology and selected pathogenic bacteria in broiler chickens. Day-old 200 Ross male chicks were allotted to five groups each subdivided in four replicates with ten birds per replicate. Similarly, five different types of feed i.e., diet A as control while B, C, D and E having 1.5% GA, 30mg/kg BS, 1.5% GA+30mg BS and 0.75% GA+15mg/Kg BS respectively, were offered during 42 days of experimental trial. Statistical analysis was performed by SPSS following CRD design and data was expressed as means along with SEM after performing Tukey's test. Results indicated significantly high feed intake, body weight gain, improved (P<0.05) FCR, livability and EPEF in group D followed by C, B and E. Same pattern of improved weight of heart, liver, gizzard, pancreas, bursa, spleen and thymus was recorded for the groups studied. Significantly higher (P<0.05) VH, lower (P<0.05) CD and higher (P<0.05) VH:CD in duodenum, jejunum and ileum were recorded in synbiotic group D followed by C, E and B. Similarly, supplementation of synbiotic in group D and *Bacillus subtilis* group C resulted in complete eradication of *E. coli*, *Salmonella* and *C. perfringens* from ileum, caecum and colon of experimental broiler chickens. It was concluded from the present findings that although prebiotic and probiotic can significantly improve the overall performance alone, the best results can be obtained from their combine synbiotic form.

INTRODUCTION

The future of livestock and poultry production is greatly influenced by the consumer's preferences for antibiotics free products (Khan *et al*., 2022) due to increasing concern regarding antimicrobial resistance (Park *et al*., 2020). Commercial companies have also shifted to more safe and acceptable feed additives (Yadav and Jha, 2019) such as enzymes, diet acidifications, phytochemicals, prebiotics and probiotics (Gadde *et al*., 2017a). A probiotic is a highly selected microbial strain that when fed in sufficient amounts bring beneficial effects on its host's health (Markowiak and Ślizewska, 2018). The most suitable probiotics contain *Bacillus* species due to its spore forming property and high resistance quality against unfavorable long-term storage, high environmental and feed processing temperatures. Improved growth performance (Bahrampour *et al*., 2020; Wang *et al*., 2020), nutrients digestion and absorption (Zaghari *et al*., 2020), FCR (Upadhaya *et al*., 2019; Guo *et al*., 2020), livability (Abdel-Moneim, 2020; Park *et al*., 2020) and EPEF (Abudabos *et al*., 2020) have been documented in broiler chickens supplemented with *Bacillus subtilis*. Similarly, *Bacillus subtilis* resulted in improved relative weight of heart, liver, gizzard, bursa, spleen and thymus (Abudabos *et al*., 2016) in poultry chickens. Better villus height (VH), low crypt depth (CD) along with high VH:CD were documented by Kridtayopas *et al*. (2019). Poultry diets

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fortification with *Bacillus subtilis* indicated restricted growth of several pathogenic microbes (Grant *et al*., 2018; Abdel-moneim *et al*., 2020). Probiotics and normal flora suffer great intolerance for low pH, temperature, oxygen and harsh environment of the gut without an essential prebiotic feed substrate (Saiyed *et al*., 2015) to bring certain health related benefits (Markowiak and Ślizewska, 2018). Gum Arabic, one of the best and oldest known of all natural gums, is obtained from stems and branches of *Acacia seyal* and *Acacia senegal* (Abdalla *et al*., 2015a). Basically, it is an edible, odorless, brittle and tasteless exudate that contains many nutritional components such as electrolytes, arabic acids, sugars and minerals including calcium (Abdalla *et al*., 2015a). Gum arabic indicated improved feed intake and weight gain in broiler chickens (Al-fadil *et al*., 2013). Addition of gum arabic in poultry diet indicated improved relative weight of internal organs (Tabidi and Ekram, 2015) lower serum cholesterol, triglycerides, creatinine and glucose levels (Abdalla *et al*., 2015a, b). Gum arabic reduced the mortality in broiler birds, due to its prebiotic property, by promoting the growth of beneficial microbiota and reducing the feed toxins and harmful bacteria through binding (Al-fadil *et al*., 2013). Prebiotic, such as gum arabic, has the ability to selectively modulate the gut bacteria and chicken immunity (Bozkurt *et al*., 2014) and inhibit the growth of many anaerobic bacterial growth through favor of beneficial bacteria and competitive exclusion inside poultry gut (Wang *et al*., 2016; Khan *et al*., 2022). Keeping in view the above mentioned properties, an experiment was conducted to explore the usefulness *Bacillus subtilis* and gum arabic alone and in synbiotic combinations in broiler chicks.

MATERIALS AND METHODS

Birds housing, feeding and management

Day-old 200 Ross chicks were randomly allotted five groups, each subdivided in four replicates and ten birds per replicate (Table I). Five diets, A as control while B, C, D and E having 1.5% GA, 30mg/kg BS (7.5x10^7 CFU/g), 1.5% GA+30mg BS (7.5x10^7 CFU/g) and 0.75% GA+15mg/Kg BS (3.75x10^7 CFU/g) respectively, were fed to broilers. The temperature of semi-controlled house was kept at 95F during the first week that was reduced at 5F per week up to 75F and then kept constant till day-42. Average relative humidity was 70% and the chicks were allowed to *ad-libitum* fresh water and feed.

Experimental plan

Feed intake, body weight gain, FCR, Livability and European Production Efficiency Factor (EPEF) were calculated as per the procedure of Khan *et al*. (2022).

At day-42, five broilers per replicate were randomly chosen and slaughtered. The internal visceral organs including heart, liver, gizzard, pancreas and lymphoid organs including bursa, spleen and thymus were rapidly collected and weighed to find out their relative weights.

Intestinal histomorphology

On day 42, three birds per replicate were randomly selected, slaughtered and specimens of mid duodenum, jejunum and ileum were collected and washed with normal saline. The intestinal specimens were prepared for microscopy and morphological study as per the procedures described by Abdelqader *et al*. (2013). Simply, formalin (10%) was used for fixation, different graded ethanol for dehydration, xylene for clarification, paraffin for embedding, microtome for cutting five micron thickness and finally glass slides were used for mounting the cut sections for hematoxylin and eosin (H and E) staining. For every specimen, ten fine structured and intact crypt villi unite were selected and finally the averages of recorded values were taken as mean villi heights and crypts depths. Intestinal specimens were examined under microscope (Olympus CX41, Japan) and scanned with image analyzer (Nikon NIS-Element BR, Nikon Co., Tokyo Japan) for measuring villi heights (VH) and crypts depths (CD) as per the procedure of Abdelqader *et al*. (2013) while VH:CD was calculated from VH and CD combined values.

Intestinal pathogenic bacteria

On day-42, three broilers from each replicate were randomly selected, slaughtered and one gram content from ileum, caecum and colon were aseptically collected, homogenized and tenfold diluted with normal saline in sterile mixer bags. A serial tenfold dilution from 10^{-1} to 10^{-7} was performed at the laboratory and 100ul of each sample was applied on selective microbial media for *Escherichia coli* (MacConkey-Sorbitol Agar), *Salmonella* (SS Agar) and *C. perfringens* (Reinforced *Clostridial* agar) for appropriate duration, oxygen concentration and other culture requirements. A colony counter was used for counting bacterial colonies and finally the results were shown $\log_{10}$ CFU/g digesta of ileum, caecum and colon of broiler chickens as per the procedure of Khan *et al*. (2022).

Statistical analysis

Statistical package SPSS version 21.0 software (SPSS Inc., Chicago, IL) was used during statistical analysis. Analysis was performed by using completely randomized design while statistical model included effect of five different diets. Data were expressed as means along with SEM and differences among means were tested through Tukey's test. Difference was considered as significant where P < 0.05 as per the procedure of Khan *et al*. (2022).

Statistical model; $Y_{ij}=\mu+\tau_j+\varepsilon_{ij}$

Table I. Feed ingredients, calculated and proximate composition of control feed.

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

RESULTS

Overall growth performance of broiler birds

Table II shows the effect of gum arabic, *Bacillus subtilis* and their synbiotic combination on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), livability (LI) and European production efficiency factor (EPEF) on broiler fed for 42 days. Synbiotic group D showed significantly highest (P<0.05) FI and BWG followed by C, B and E when compared with A group. Dietary addition of GA and BS in synbiotic form indicated a significant (P<0.05) improvement in FCR, high livability and EPEF in D group, C, B and E in comparison with control A. The mixture of GA and BS in synbiotic form indicated synergistic effects on growth performances of broiler chickens.

Table II. Effect of gum arabic, *Bacillus subtilis* and their synbiotic combination on FI, BWG, FCR, livability and EPEF of broiler birds fed for 42 days.

Group	FI (g)	BWG (g)	FCR	LI (%)	EPEF
A	3776.25[c]	2175.00[d]	1.736[a]	85.00[b]	253.67[c]
B	3970.00[b]	2351.25[bc]	1.688[c]	90.00[ab]	298.40[b]
C	4007.50[ab]	2393.75[ab]	1.674[d]	92.50[ab]	314.80[ab]
D	4056.25[a]	2435.00[a]	1.666[e]	97.50[a]	339.29[a]
E	3937.50[b]	2320.00[c]	1.697[b]	90.00[ab]	292.92[b]
SEM	23.10	20.89	0.01	1.23	6.99
P-Value	< 0.05	< 0.05	< 0.05	0.01	< 0.05

Where A, control group; B, gum arabic (GA) @ 15%; C, *Bacillus subtilis* (BS) @ 30mg/Kg feed; D, 15% GA+ 30mg/Kg BS; E, 0.75% GA+15mg/Kg BS. FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio; LI, livability; EPEP, European Production Efficiency Factor. Means having different superscripts in same column are vary significantly (P<0.05).

Table III. Effect of gum arabic, *Bacillus subtilis* and their synbiotic combination on relative weight (%) visceral and lymphoid organs of broilers fed for 42 days.

Group	Heart	Liver	Gizzard	Pancreas	Bursa	Spleen	Thymus
A	0.467[d]	2.258[d]	1.540[c]	0.212[d]	0.166[d]	0.117[c]	0.552[c]
B	0.477[c]	2.289[c]	1.553[b]	0.217[cd]	0.167[d]	0.118[c]	0.554[c]
C	0.519[b]	2.495[a]	1.703[a]	0.238[b]	0.188[b]	0.130[a]	0.623[a]
D	0.531[a]	2.513[a]	1.711[a]	0.247[a]	0.194[a]	0.133[a]	0.626[a]
E	0.479[c]	2.369[b]	1.557[b]	0.222[c]	0.175[c]	0.126[b]	0.560[b]
SEM	0.006	0.024	0.018	0.003	0.003	0.001	0.008
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

For details of groups, see Table II. Organs weight is relative to live body weight at day 42. Means in same column with different superscripts differ significantly (P<0.05).

Relative weights of visceral and lymphoid organs

Table III shows the effect of supplementation of gum arabic and *Bacillus subtilis* alone and in synbiotic combination on relative weights (%) of visceral and lymphoid organs of broiler chickens. A significantly high

(P<0.05) weight of heart and liver was recorded in synbiotic group D followed by C, E and B as compared to the control A. Significantly (P<0.05) high gizzard and pancreas weights were also recorded in group D and C followed by group E and control group A. A significantly high (P<0.05) relative weight of bursa, spleen and thymus was recorded in group D followed by C and E while the least weight was recorded for group B and the control group A.

Intestinal histomorphology

A significant improvement (P<0.05) was documented in villus height in synbiotic group D in duodenum, jejunum and ileum followed by *Bacillus subtilis* fed group C, synbiotic fed group E and gum arabic fed group B (Table IV). The least villus height was recorded for the control A group in different parts of small intestine. Significantly low (P<0.05) crypt depth (CD) was also recorded for group D in duodenum, jejunum and ileum followed by group C, E and B, while high crypt depth in was noted in the control group A. Present findings indicated a significantly improved (P<0.05) VH:CD in synbiotic group D in duodenum, jejunum and ileum of broilers followed by group C, E and B, while the lowest was recorded for control A group.

Bacterial count

Table V shows the effects of supplementation of gum arabic, *Bacillus subtilis* and their synbiotic combination on selected bacterial count of broiler chickens. Supplementation of symbiotic (group D) and *Bacillus subtilis* (group C) resulted in complete eradication of *Salmonella* and *C. perfringens* from ileum, caecum and colon of experimental broiler chickens. Supplementation of symbiotic and *Bacillus subtilis* also resulted in complete eradication of *E. coli* from ilium and colon, while least number of *E. coli* in the caecum was noted for D-group followed by C, E, B and control-A group respectively. Addition of half dose of symbiotic (group E) also restricted the growth of *E. coli* and *Salmonella* in ileum while complete eradication of *Salmonella* and *C. perfringens* from caecum and colon of tested broiler birds. Addition of gum arabic in broiler feed (group B) also significantly (P<0.05) restricted *E. coli* and *Salmonella* counts in ileum, caecum and colon of experimental broiler chickens when compared with control-A group. Similarly, a significantly low (P<0.05) count of *C. perfringens* in cecum and colon was recorded in group-B when compared with control A group.

Table IV. Effect of supplementations of gum arabic, *Bacillus subtilis* and their synbiotic combination on intestinal histomorphology fed for 42 days.

Group	Duodenum (µm)			Jejunum (µm)			Ileum (µm)		
	VH	CD	VH:CD	VH	CD	VH:CD	VH	CD	VH:CD
A	1822.92^{d}	245.92^{a}	7.416^{d}	1160.17^{d}	208.00^{a}	5.580^{d}	573.92^{d}	194.332^{a}	2.957^{d}
B	1842.50^{c}	225.67^{b}	8.168^{c}	1172.58^{c}	190.67^{b}	6.153^{c}	579.92^{c}	178.17^{b}	3.256^{c}
C	1932.92^{b}	208.58^{c}	9.283^{b}	1230.17^{b}	176.42^{c}	6.985^{b}	608.83^{b}	164.83^{c}	3.700^{b}
D	1976.92^{a}	198.75^{d}	9.960^{a}	1258.25^{a}	168.08^{d}	7.496^{a}	622.50^{a}	157.08^{d}	3.968^{a}
E	1855.00^{c}	223.25^{b}	8.314^{c}	1180.58^{c}	188.67^{b}	6.261^{c}	584.00^{c}	176.42^{b}	3.312^{c}
SEM	7.81	2.26	0.12	4.97	1.91	0.09	2.47	1.78	0.05
P-value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

For details of groups, see Table II. VH, Villus height; CD, Crypt depth; VH:CD, Villus height vs. crypt depth. Means in same column under different superscripts differ significantly (P<0.05)

Table V. Effect of supplementation of gum arabic, *Bacillus subtilis* and their synbiotic combination on selected microbial population fed for 42 days.

Group	Ileum (log_{10})			Caecum (log_{10})			Colon (log_{10})		
	EC	SA	CP	EC	SA	CP	EC	SA	CP
A	4.092^{a}	2.2314^{a}	2.302^{a}	6.843^{a}	2.338^{a}	2.382^{a}	5.368^{a}	2.289^{a}	2.252^{a}
B	4.076^{b}	1.974^{b}	2.271^{b}	6.826^{b}	2.247^{b}	2.350^{b}	5.350^{b}	2.197^{b}	2.220^{b}
C	0^{d}	0^{d}	0^{c}	5.257^{d}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
D	0^{d}	0^{d}	0^{c}	3.298^{e}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
E	3.668^{c}	1.532^{c}	0^{c}	5.434^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
SEM	0.252	0.127	0.146	0.169	0.146	0.151	0.342	0.143	0.143
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

For details of groups, see Table II. *EC, E. coli*; *SA, Salmonella*; *CP, C. perfringens.* Values with 0 values means no bacteria seen (bacteria free). Means with different superscripts in the same column are significantly different (P<0.05).

DISCUSSION

Overall growth performance of broiler chickens

Antibiotics growth promoters can be safely replaced by diet fortification with BS alone and in synbiotic form (Park *et al*., 2020; Zaghari *et al*., 2020; Khan *et al*., 2022). The combination of prebiotic and probiotic is called synbiotic which favors the growth of normal gut flora (Alloui *et al*. 2013), liberates high nutrients which leads to synergistically improved growth performance (Saiyed *et al*., 2015) and livability (Abdel-moneim, 2020; Guo *et al*., 2020). Similar to our findings, Upadhaya *et al*. (2019) and Bahrampour *et al*. (2020) also documented improved growth performance, FCR and EPEF (Abdel-moneim, 2020) in BS supplemented chickens. According to Ahmed *et al*. (2015), Wang *et al*. (2016) and Rehman *et al*. (2020) synbiotics have significant effects on growth performance and FCR of supplemented poultry birds. On the other hand, Wang *et al*. (2018) and Śliżewska *et al*. (2020) documented no significant effects on poultry birds in response to prebiotic and/or synbiotics. These inconsistencies in response may be due to different housing conditions, broiler and probiotic strain, livability and dose rate of probiotics (Guo *et al*., 2020; Zaghari *et al*., 2020). Similar to our findings, Gadde *et al*. (2017a) and Wang *et al*. (2020) stated that chickens grow faster when supplemented with BS. An improved average weight gain was also documented by Bahrampour *et al*. (2020) in Japanese quails, healthy and *Salmonella* infected broilers (Zaghari *et al*., 2020), respectively. This improved growth performance may be due to enhanced ileal digestibility and improved apparent metabolizable energy (Wealleans *et al*., 2017a; b). Likewise, Abdel-moneim (2020) documented that improved body weight was due to enhanced lipolytic, proteolytic and amylolytic activities in duodenum along with increased nutrients digestibility. According to Wang *et al*. (2016) supplementation of synbiotics resulted in high growth of beneficial bacteria and restricted growth of pathogenic microbes, thus livability of the broiler chickens was improved. Similarly, supplementation of gum arabic reduced the mortality due to its prebiotic property by promoting the growth of beneficial microbiota and eradicating the feed toxins through binding and reducing the harmful bacteria (Khan *et al*., 2022). Decreased mortality due to enhanced intestinal immunity and epithetical barrier integrity results in high livability (Park *et al*., 2020). Present findings are in agreement with the results Saiyed *et al*. (2015) who also documented an improvement in EPEF of synbiotic supplemented broiler chickens. Improved growth performance, decreased mortality, improved livability and higher EPEF in broiler chickens due to BS and GA was also documented by Sokale *et al*. (2019) and (Khan *et al*., 2022).

Internal visceral and lymphoid organs

Parallel to our findings, Saiyed *et al*. (2015) also reported better effects on visceral and lymphoid organs weight in broilers fed with synbiotics. It was suggested that the increase in heart weight may be due to compensatory hypertrophy in response to high body weight gain and to efficiently pump the blood to high body mass (Khan *et al*., 2022). An improvement in liver weight was reported by Tabidi and Ekram (2015). This increase may be due to hyperplasia and hypertrophy of hepatocytes in response to high feed intake and high weight gain. High body weight gain due to high feed intake triggers the metabolic processes of the liver hepatocytes to work harder and efficiently to meet the demands of fast growing body mass of broiler birds (Khan *et al*., 2022). The relative weight of gizzard was also improved in all of the supplemented groups. It was suggested that this improvement in gizzard weight may be due to compensatory hyperplasia and/or hypertrophy of gizzard's muscles in response to accumulating and compensating the high feed intake by broiler chickens. We also suggest that improvement in weight of pancreas may be due to increased work load for the high level production of insulin and glucagon to meet the energy and carbohydrates demands of fast growing broiler chickens. Parallel to our findings, Abudabos *et al*. (2016) and Khan *et al*. (2022) also reported a numerical increase in relative weights of liver, bursa, spleen and thymus due to BS supplementation without any significant difference in *Salmonella* challenged broiler chickens. Dietary addition of synbiotics result improved metabolism, intestinal architecture, short chain fatty acids, ketone bodies, methyl acetate and carbon disulfide in broiler chickens (Alloui *et al*., 2013).

Intestinal histomorphology

Similar to our findings, Guo *et al*. (2020) and Khan *et al*. (2022) also reported enhanced VH, VH:CD and reduced CD in different parts of small intestine of the supplemented chickens. Several species of *Bacillus* genus produce enzymes and effector molecules (Elshaghabee *et al*., 2017) that can stimulate the villus stem cells located at the crypt junction and thus villi height may be improved (Wang *et al*., 2018). According to Abdelqader *et al*. (2013) and Wang *et al*. (2016) dietary supplementation of synbiotics synergistically affected the VH and CD of poultry birds. Brufau *et al*. (2015) reported that supplementation of Duraio gum (0.1%) and cassia gum (0.1%) for 23 days resulted in increase in villus height and villus surface area, thus providing more area for nutrients absorption. Most of the *Bacillus* species produce amylase, protease, proteins, vitamins and also favor the growth of bacteria involved in production of lactic acid that reduces intestinal PH and improves nutrients digestion and absorption (Zaghari *et al*., 2020). This lowered PH in probiotic supplemented broilers also restricts the growth of pathogenic bacteria and promotes the intestinal histomorphology by avoiding mucosal inflammations (Bahrampour *et al*., 2020). According to Hoerr and Schrader (2016) and Khan *et al*. (2022) the villus height decreases along the length of small intestine whereas crypt depth remains relatively constant. Park *et al*. (2020) reported that chickens infected with coccidiosis indicated improved intestinal lesions and histomorphology in response to BS supplementation. Some probiotics can convert lactic acid and acetic acid into butyric acid that is involved in the promotion of intestinal villus growth and overall histomorphology through villus cell gene regulation (Kridtayopas *et al*., 2019). Probiotics, such as *Bacillus subtilis,* can bring about improvements in growth performance, proper gut health and histomorphology of poultry birds (Abdel-moneim *et al*., 2020). From these results, we suggest that significantly improved FCR in high level gum arabic, *Bacillus subtilis* and their synbiotic supplemented group may be due to improved villus height and high VH:CD in different parts of broiler chickens.

Selected pathogenic bacteria in different parts of intestine

Dietary supplementation of BS not only balanced gut microbiota but also facilitated the growth of beneficial bacteria and restricted the pathogenic bacteria (Guo *et al*., 2020). A probiotic can significantly boost the overall growth and gastrointestinal health status along with improved gut microflora and immune responses (Abdel-moneim *et al*., 2020). Similarly, *Bacillus* species can produce several antimicrobial enzymes, effector molecules, vitamins (Elshaghabee *et al*., 2017), bacteriocin, peptides and polypeptides which ultimately reduces the growth of pathogenic bacteria (Belih *et al*., 2015). Gadde *et al*. (2017b) proposed that expression of high TJ proteins in BS supplemented broilers enhanced the intestinal mucosal barrier functions and provided healthy gut. Similar to our findings, Wang *et al*. (2016) also reported that supplementation of prebiotic, probiotic and synbiotic resulted in decreased count of intestinal *E. coli*, *C. perfringens* and coccidiosis in broilers. Addition of prebiotics such as gum arabic also results in high growth of *Bifidobacteria* and *Lactobacilli* and goblet cells discharge which avoids the attachment of pathogenic bacteria to the intestinal epithelium (Brufau *et al*., 2015; Khan *et al*., 2022). A need for further basic knowledge is required as how gut microbes and immune system can be adjusted by different feed additives as substitute to AGPs against gastrointestinal diseases (Gadde *et al*., 2017a; Khan *et al*., 2022).

CONCLUSION

Gum arabic and *Bacillus subtilis* alone or in synbiotic combinations have beneficial effects on overall performance, visceral and lymphoid organs weight along with positive effects on intestinal histomorphology and pathogenic bacteria. Furthermore, synbiotic possess an improved and synergistic effect as compared to prebiotic and/or probiotic alone.

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IRB approval

The experimental work was approved by the Advanced Studies and Research Board (ASRB) of The University of Agriculture Peshawar, Peshawar, KP, Pakistan.

Ethical statement

The Departmental Ethical Committee approved the experiment before practical execution of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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