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Article

Effect of Dietary Inclusion of Gum Arabic (*Acacia senegal*) and *Lactobacillus acidophilus* or their combination on broiler chickens

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Abstract

The present research trial was carried out to explore the effects of supplementation of gum arabic (GA) as natural prebiotic, *Lactobacillus acidophilus* (LA) as probiotic or their combination of prebiotic and probiotic as synbiotic on overall growth performance, and lymphoid organs weights, gut morphology and pathogenic bacteria in broiler chickens. For this reason, day-old 300 Ross unsex chicks were allotted to six groups each subdivided in five replicates with ten birds per replicate. Chicks kept in the control group (T1) were fed the basal diet. Chickens fed groups (T2 and T3) (prebiotic) received gum arabic at a 5 and 7.5 g /kg diet, respectively. Chickens fed (T4) (probiotic) LA received LA at 1 g/kg diet. Chicks of (T5 and T6) were fed a basal diet containing (synbiotic) 5 g GA + 1 g LA/kg diet or (synbiotic) 7.5 g GA + 1 g LA /kg diet, respectively. Results indicated significantly higher feed intake, body weight gain, improved ($P<0.05$) FCR, livability and European Production Efficiency Factor (EPEF) in group T6 followed by T5, T4, T3 and T2. Same pattern of improved weight of heart, liver, gizzard, pancreas, bursa of fabricius, spleen and thymus was recorded for the groups studied. Significantly higher ($P<0.05$) villus height (VH), lower ($P<0.05$) crypt depth (CD) and higher ($P<0.05$) VH:CD ratio in duodenum, jejunum and ileum were recorded in synbiotic group T6 followed by T5, T4, T3 and T2. Similarly, supplementation of synbiotic in group T6 and T4 groups resulted in reduction of *E. coli* and *Salmonella* while, increasing of *Lactobacillus spp* from caecum 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.

Keywords: Broiler chickens, Gum Arabic, *Lactobacillus acidophilus*, Performance, Blood

INTRODUCTION

The future of 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 **(Wang *et al.*, 2020)**, nutrients digestion and absorption **(Zaghari *et al.*, 2020)**, feed conversion ratio (FCR) **(Guo *et al.*, 2020)**, livability **(Abdel-Moneim *et al.*, 2020)** and EPEF **(Abudabos *et al.*, 2020)** have been documented in broiler chickens supplemented with *Lactobacillus acidophilus*. Similarly, *Lactobacillus acidophilus* 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 ratio were documented by **Kridtayopas *et al.* (2019)**. Poultry diets fortification with *Bacillus subtilis* indicated restricted growth of several pathogenic microbes **(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)**. Various types of dietary fiber, such as gum Arabic, are considered a source of natural prebiotics for use in broiler diets that deserve attention **(Khalid *et al.*, 2014)**. Gum Arabic is a soluble dietary fiber found mainly in acacia trees via stem and branch exudates **(Ahmed *et al.*, 2016)** and contains a mixture of oligosaccharides and polysaccharides responsible for their prebiotic properties **(Al-Fadil *et al.*, 2013)**. Gum Arabic is not degraded by intestinal enzymes, but it is readily fermented by the microbiota when it reaches the cecum and promotes the growth of commensal bacteria **(Adil *et al.*, 2010)**. However, promoting the growth of commensal bacteria by soluble fiber such as gum Arabic could improve the health and performance of broiler chickens **(Lockyer and Stanner, 2019)**. 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 **(Khan *et al.*, 2022)**. Keeping in view the above mentioned properties, an experiment was conducted to explore the usefulness of *Lactobacillus acidophilus* and gum arabic alone and in synbiotic combinations in broiler chicks.

MATERIALS AND METHODS

Birds housing, feeding and management

Day-old 300 Ross unsexed chicks were allotted to six groups each subdivided in five replicates with ten birds per replicate. Chicks kept in the control group (T1) were fed the basal diet. Chicks of groups (T2 and T3) fed gum arabic (T2 and T3) (GA, prebiotic) at 5 and 7.5 g/kg diet, respectively. Chicks of (T4) fed probiotic containing *Lactobacillus* acidophilus (5×10^{11} CFU/g) at 1 g/kg diet (Chinobio Trading Co., Ltd., Ningxia, China). Chicks of groups (T5 and T6) fed a basal diet containing (synbiotic) 5 g GA + 1 g probiotic/kg diet or (synbiotic) 7.5 g GA + 1 g LA /kg diet, respectively. The commercial probiotic was used in accordance with the manufacturer instructions.

The basal diet was formulated on form mash in two feeding stages, starter (1–14 days) and grower (15 – 35 days), to meet the nutrient requirements of Ross 308 (Aviagen, 2019, New York, USA) as indicated in Table 1. The study was conducted under similar management and optimal environmental conditions according to the Modern Broiler Production Guide. All broilers were provided with feed and water *ad libitum* for 35 days. The temperature was maintained at 33◦C for the first week of rearing and then gradually decreased by 3 ◦C each week until it reached 22 ◦C for the remainder of the period. Relative humidity in the room chamber was maintained between 50% and 65% (1 to 24 days) and 50% (25 to 35 days). The lighting duration was 24 h during brooding and 23 h during the rest of the period. Feed and water were available to all birds throughout the duration of the study. All broilers were vaccinated against Newcastle Disease (NDV), Infectious Bronchitis (IBV), and Infectious Bursal Disease (IBDV) (Fort Dodge Animal Health, Fort Dodge, IA, USA).

Indicators of Growth Performance

During the study period, body weight and feed intake were recorded, and then average body weight gain, and feed conversion ratio were calculated. In addition, Livability (%) = (number of birds at the end / number of birds at the beginning) x 100 European Production Efficiency Factor (EPEF) = (Live weight of bird (Kg) x Livability %) / (Age of bird (days) x FCR).

Serum Biochemical Indices

Blood samples (3 ml) were collected at the end of the experimental period (35 days) from 5 chickens per treatment during slaughtering in tubes without heparin. All samples were immediately separated into serum by centrifugation at 2500× g for 20 min and then stored at −20◦ C until analysis of blood biochemical indicators. Blood biochemical indicators include total protein, albumin, glucose, triglycerides, cholesterol, uric acid, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were analyzed with colorimetric kits according to the manufacturer's instructions using an automated spectrophotometric analyzer (Chem Well, Awareness Technology, Palm City, FL, USA).

Organs weight

At the end of the study (at 35 days of age), 5 birds were randomly selected from each dietary treatment 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 35, during slaughtering 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 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 ratio was calculated from VH and CD combined values.

Table 1. Composition and calculated analysis of the experimental starter and grower diets

Ingredient	Starter (%)	Grower (%)
Corn grains	53.71	61.92
Soybean meal (44%)	33.42	28.05
Corn gluten meal (60%)	5.22	3.20
Soybean oil	3.32	2.94
Limestone	1.28	1.15
Dicalcium phosphate	1.84	1.68
DL-methionine	0.39	0.22
Vitamins and minerals premix*	0.30	0.30
L- lysine HCl	0.12	0.14
Salt (NaCl)	0.40	0.40
Total	100	100
Analyzed and calculated composition (NRC, 1994)		
Crude protein %	23	20
Metabolizable energy (Kcal/kg diet)	3094	3142
Methionine %	0.80	0.58
Calcium %	1.00	0.90
Available phosphorous %	0.49	0.45
Lysine %	1.25	1.11

* Composition (per 3 kg): vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Cecal Microbiota

On day 35, during slaughtering one gram from Cecal digesta samples were collected from 10 chickens per treatment group in a sterile 1.5-ml Eppendorf tube and stored (−20° C) until analysis to count microbiota colonies according to the method of **Azzam *et al.* (2020)**. Approximately one gram of the cecal content was serially diluted in 9 ml of buffered peptone water (1:10) until the desired dilution was achieved. The colonies were clear and easy to count (50 to 300 colonies). From each dilution, 0.1 ml was cultured on selective media for the bacterial species studied. Selective agar media were used for the enumeration of bacterial target groups such as *Lactobacillus spp*. on de Man, Rogosa, and Sharpe agar (MRS, Himedia, Mumbai, India), while *Salmonella spp*. and *Escherichia coli* by eosin methylene blue agar (EMB, Hardy Diagnostics, Santa Maria, CA, USA) according to the method of **Qaid *et al.* (2021)**. Colonies were counted using a colony counter, and results were expressed as log10 colony forming units per gram.

Statistical data analysis:

The trial was operated using a completely random design. Duncan's multiple range tests **(Duncan,1955)** were employed to evaluate the differences in means (P< 0.05) and were used in conjunction with The General Linear Model (GLM) approach of **SAS (2003)**.

RESULTS

Growth performance

Overall growth performance of broiler birds (Table 2) shows the effect of gum arabic, *Lactobacillus acidophilus* 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 35 days.

Synbiotic group T6 showed significantly the highest (P<0.05) FI and BWG followed by T5, T4, T3 and T2 , respectively, when compared with T1 group. Dietary addition of GA and LA in synbiotic form indicated insignificant (P<0.05) improvement in FCR, high livability and EPEF in T6 group followed by T5, T4, T3 and T2, respectively, in comparison with control group (T1). The mixture of GA and LA in synbiotic form indicated synergistic effects on growth performances of broiler chickens. During the entire trial, the mortality rate was very low (only three broilers died), and birds appeared in a good satisfactory health conditions.

Table 2. Effect of experimental treatments on growth performance in broiler chickens uring the whole experimental period.

Treatments	Growth performance				
	Daily weight gain, g/bird	Daily feed intake (g/bird/day)	FCR (g feed/g gain)	LI (%)	EPEF
T1	57.65^{b}	102.86	1.784	98.0^{b}	323.12^{d}
T2	59.94^{ab}	103.00	1.718	98.0^{b}	348.52^{c}
T3	60.51^{a}	102.57	1.695	100.0^{a}	363.89^{a}
T4	60.08^{a}	103.57	1.724	100.0^{a}	355.33^{b}
T5	60.51^{a}	103.71	1.714	100.0^{a}	359.90^{ab}
T6	60.82^{a}	102.86	1.691	100.0^{a}	366.63^{a}
SEM	0.146	0.325	0.003	1.25	7.25
p-value	0.0001	0.056	0.075	0.071	0.004

$^{a–e}$Means with different superscripts in a column differ significantly (P < 0.05). *SEM =Standard Error of the mean; Where, T1= control group; T2, gum arabic (GA) at 5gm gm/kg diet; T3= gum arabic (GA) at 7.5 gm/kg diet; T4= L. acidophilus (5×10^{11} CFU/g) at 1 g/kg diet; T5= 5 gum Arabic + 1g/kg diet L. acidophilus; T6= 7.5gm gum Arabic + 1g/Kg diet L. acidophilus; FCR = feed conversion ratio, LI = livability, EPEP = European Production Efficiency Factor.

Blood Biochemical Indices

Data for biochemical constituents of blood serum of 35 days-old broiler chicks are shown in Table 3. The results revealed no significant effect of using different supplementations on most of the biochemical parameters of blood serum except for plasma cholesterol. In such case, all the dietary treatments showed lower values of blood cholesterol than the control group ($p \leq 0.05$).

Table 3. Effect of experimental treatments on Some blood constituents in broiler chickens at the end of the experimental period.

Treat.	Some blood constituents								
	TP g/dl	Alb. g/dl	Glob. g/dl	Gluco mg/dl	Choles mg/dl	Creat. mg/dl	Ca mg/dl	AST U/L	ALT U/L
T1	3.15	1.72	1.43	250	182^{a}	0.48	8.51	45.8	7.43
T2	3.60	1.80	1.80	239	126^{bc}	0.47	8.26	45.9	7.50
T3	3.96	2.00	1.96	230	120^{bc}	0.47	8.44	44.8	7.28
T4	3.80	1.83	1.97	240	130^{b}	0.43	8.35	45.3	7.44
T5	3.88	1.90	1.98	230	106^{c}	0.40	8.48	44.8	7.35
T6	3.97	1.95	2.02	236	102^{c}	0.42	8.56	44.4	7.25
SEM	0.223	0.028	0.154	1.768	3.576	0.023	1.345	2.435	0.234
p-value	0.235	0.063	0.056	0.231	0.002	0.187	0.545	0.265	0.163

$^{a-e}$Means with different superscripts in a column differ significantly ($P < 0.05$). *SEM =Standard Error of the mean; Where, T1= control group; T2, gum arabic (GA) at 5 gm/kg diet; T3= gum arabic (GA) at 7.5 gm/ kg diet; T4= L. acidophilus (5×10^{11} CFU/g) at 1 g/kg diet; T5= 5gm gum Arabic + 1g/Kg diet L. acidophilus; T6= 7.5gm gum Arabic + 1g/Kg diet L. acidophilus; TP =Total protein; Alb = Albumin; Glob = Globulin; Gluco = Glucose; Choles = Cholesterol; Creat = Creatinine; Ca = Calcium; AST= aspartate aminotransferase and ALT = alanine aminotransferase.

Relative weights of visceral and lymphoid organs

Table 4 shows the effect of supplementation of gum arabic and Lactobacillus acidophilus alone and in synbiotic combination on relative weights (%) of visceral and lymphoid organs of broiler chickens. Insignificantly high ($P<0.05$) weight of heart and liver was recorded in synbiotic group T6 followed by T5, T4, T3 and T2, respectively, as compared to the control group (T1). Moreover, insignificantly ($P<0.05$) high gizzard and pancreas weights were also recorded in group T6 and T4 followed by group T5 and control group T1. An insignificantly high ($P<0.05$) relative weight of bursa, spleen and thymus was recorded in group T6 followed by T5, T4 and T3, while the least weight was recorded for the control group (T1).

Table 4. Effect of experimental treatments on relative weights of visceral and lymphoid organs in broiler chickens at the end of the experimental period.

Treatments	Visceral organs %				Lymphoid organs %		
	Liver	Heart	Gizzard	Pancreas	Bursa	Thymus	Spleen
T1	2.25	0.465	1.510	0.210	0.165	0.546	0.116
T2	2.30	0.468	1.536	0.212	0.167	0.550	0.115
T3	2.42	0.480	1.551	0.218	0.170	0.552	0.118
T4	2.35	0.478	1.552	0.211	0.168	0.548	0.117
T5	2.40	0.481	1.561	0.220	0.170	0.550	0.118
T6	2.48	0.500	1.636	0.225	0.173	0.560	0.121
SEM	0.115	0.251	0.145	0.025	0.014	0.118	0.025
p-value	0.254	0.115	0.233	0.452	0.625	0.335	0.422

[a–e]Means with different superscripts in a column differ significantly (P < 0.05). *SEM =Standard Error of the mean; Where, T1= control group; T2, gum arabic (GA) at 5 gm/kg diet; T3= gum arabic (GA) at 7.5 gm/kg diet; T4= L. acidophilus (5×10^{11} CFU/g) at 1 g/kg diet; T5= 5 gm gum Arabic + 1g/Kg diet L. acidophilus; T6= 7.5 gm gum Arabic + 1g/Kg diet L. acidophilus

Intestinal histomorphology

Table 5 shows the effect of supplementation of gum arabic and *Lactobacillus acidophilus* alone and in synbiotic combination on intestinal histomorphology of broiler chickens. A significant improvement (P<0.05) was documented in villus height in synbiotic group T6 in duodenum, jejunum and ileum followed by synbiotic fed group (T5) and gum arabic fed group (T3).

The least villus height was recorded for the control group (T1) in different parts of small intestine. Significantly low (P<0.05) crypt depth (CD) was also recorded for group T6 in duodenum, jejunum and ileum followed by group T5, T4 and T3, while high crypt depth in was noted in the control group (T1). Present findings indicated a significantly improved (P<0.05) VH:CD ratio in symbiotic group T6 in duodenum, jejunum and ileum of broilers followed by group T5, T4 and T3, while the lowest was recorded for control group (T1).

Bacterial count

Table 6 shows the effects of supplementation of gum arabic, *Lactobacillus acidophilus* and their symbiotic combination on selected bacterial count of broiler chickens. Addition of gum arabic in broiler feed (groups T2 and T3) and probiotic (group 4) caused significantly (P<0.05) restricted *E. coli* and *Salmonella* counts in caecum of experimental broiler chickens when compared with control group. Similarly, a significantly high (P<0.05) count of *Lactobacillus* in cecum was recorded in group-T3 when compared with control group. Addition of symbiotic (group T5 and T6) also restricted the growth of *E. coli* and *Salmonella* in cecal while increasing of *Lactobacillus* count from caecum of tested broiler birds compared with the other groups.

Table 5. Effect of experimental treatments on intestinal histomorphology in broiler chickens at the end of the experimental period.

Items	Dietary treatments						SEM	p-value
	T1	T2	T3	T4	T5	T6		
Doudenum (µm)								
VH	1810^{c}	1830^{bc}	1852^{b}	1840^{b}	1900^{a}	1950^{a}	7.25	0.001
CD	240^{a}	236^{a}	210^{b}	206^{b}	200^{b}	196^{b}	2.16	0.025
VH/CD	7.54^{c}	7.75^{bc}	8.82^{b}	8.93^{b}	9.50^{a}	9.95^{a}	0.16	0.001
Jejunum (µm)								
VH	1150^{b}	1152^{b}	1176^{b}	1169^{b}	1200^{a}	1210^{a}	5.96	0.002
CD	210^{a}	206^{a}	196^{b}	192^{b}	187^{bc}	178^{c}	1.81	0.001
VH/CD	5.47^{b}	5.60^{b}	6.00^{ab}	6.09^{ab}	6.42^{a}	6.80^{a}	0.08	0.014
Ileum (µm)								
VH	570^{c}	578^{c}	605^{b}	596^{b}	615^{a}	622^{a}	3.16	0.002
CD	193^{a}	185^{ab}	173^{b}	165^{c}	160^{c}	159^{c}	1.86	0.001
VH/CD	2.95^{c}	3.12^{c}	3.50^{b}	3.61^{ab}	3.64^{ab}	3.91^{a}	0.06	0.030

$^{a–e}$Means with different superscripts in a row differ significantly ($P < 0.05$). *SEM =Standard Error of the mean; Where, T1= control group; T2, gum arabic (GA) at 5 gm/kg diet; T3= gum arabic (GA) at 7.5 gm/kg diet; T4= L. acidophilus (5×10^{11} CFU/g) at 1 g/kg diet; T5= 5 gm gum Arabic + 1g/Kg diet L. acidophilus; T6= 7.5 gm gum Arabic + 1g/Kg diet L. acidophilus; VH= Villus height and CD= Crypt depth.

DISCUSSION

Overall growth performance of broiler chickens

Antibiotics growth promoters can be safely replaced by diet fortification with *Lactobacillus acidophilus* (LA) alone and in symbiotic 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)**. Studies have shown that LA as a dietary additive can significantly promote the growth performance of broilers (Nguyen et al., 2015). Consistent with previous studies, our results showed that LA can significantly increase BW and ADG and improve FCR during the first 35 d of production. The EPEF in the LA increased as compared with the control group, indicating that economic benefits were improved. However, some studies have reported that LA does not affect growth performance of broiler chickens **(Lee *et al.*, 2014)**, which may be related to the type and additive amount of LA. The current results showed a positive effect on the overall performance parameters of broilers by the addition of gum Arabic during the period of 1 to 35 days, resulting in an increase in BW, BWG and EPEF, as well as an improvement in FCR, whereas FI was lower compared with control group. These results may indicate that gum Arabic has the ability to ferment through the activity of commensal bacteria in the intestine, reflecting its positive effect on the overall performance parameters of broiler chickens. These results are consistent with those of **Siham *et al.* (2015)**, with broiler chickens, and **Amber *et al.* (2017)**, with rabbits. Both found that administration of gum Arabic (0.25–0.75%) improved overall performance (BWG and FCR).

Table 6. Effect of experimental treatments on cecal microbiota in broiler chickens at the end of the experimental period.

Items	Dietary treatments						SEM	p-value
	T1	T2	T3	T4	T5	T6		
Cecal (log10)								
Escherichia coli	6.10^{a}	5.0^{ab}	3.2^{b}	3.05^{b}	2.5^{c}	2.3^{c}	0.21	0.001
Salmonella spp.	7.31^{a}	5.86^{b}	3.20^{c}	4.26^{bc}	2.10^{d}	2.00^{d}	0.26	0.025
Lactobacillus spp.	9.8^{c}	10.0^{b}	11.0^{a}	10.6^{b}	11.8^{a}	11.9^{a}	0.18	0.004
Lactobacillus / Escherichia coli	1.61^{c}	2.0^{c}	3.44^{b}	3.47^{b}	4.72^{ab}	5.17^{a}	0.04	0.001

$^{a–e}$Means with different superscripts in a row differ significantly (P < 0.05). *SEM =Standard Error of the mean; Where, T1= control group; T2, gum arabic (GA) at 5 gm/kg diet; T3= gum arabic (GA) at 7.5 gm/kg diet; T4= L. acidophilus (5×10^{11} CFU/g) at 1 g/kg diet; T5= 5 gm gum Arabic + 1g/Kg diet L. acidophilus; T6= 7.5 gm gum Arabic + 1g/Kg diet L. acidophilus.

Similarly, **Khan *et al.* (2022)** and **Al-Baadani *et al.* (2023)** concluded that administration of up to 0.5% and 0.75% gum Arabic, respectively, improved overall performance indicators in broilers. In contrast to our findings, gum Arabic did not affect the BWG, FI, or FCR of broilers **(Tabidi *et al.*, 2015)**. **Ahmed *et al.* (2021)** reported that gum Arabic decreased FI in mice due to increased satiety. However, the physical properties of gum Arabic might contribute to the lower FI in some levels of gum Arabic by influencing the rate of passage through the digestive tract and thus improving nutrient utilization, which is reflected in the improved FCR in this study. **Adil *et al.* (2010)** reported that gum Arabic supplementation has the ability to improve nutrient utilization of broilers. In this study, the increase of EPEF in chickens fed on gum Arabic may indicate an optimal economic status of production. According to **Rehman *et al.* (2020)** synbiotics have significant effects on growth performance and FCR of supplemented poultry birds. On the other hand, **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, **Wang *et al.***

(2020) stated that chickens grow faster when supplemented with *Lactobacillus acidophilus*. 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.*, 2017**a and 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 of **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 *Lactobacillus acidophilus* and GA was also documented by **(Khan *et al.*, 2022)**. However, other studies reported positive effects of synbiotics on the growth performance of broilers. **Pelicano *et al.* (2004)** found that growth and FCR were significantly improved when MOS was added with probiotics such as Bacillus subtilis or *L. acidophilus* and *casei, Streptococci lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae*. Similarly, a mixture of Lactobacillus and Aspergillus fermentative products increased broilers' BWG **(Falaki *et al.*, 2011)**. This result was confirmed by Ghahri et al. (2013), who observed an increase in feed intake and growth of broilers using a similar synbiotic in diets.

Blood

Selected biochemical indicators in blood are among the tests performed to evaluate the body metabolism of broiler chickens **(Raju *et al.*, 2019)**. Therefore, selected blood biochemical indicators were determined to investigate the effects of feed additives on the health and nutritional status of broiler chickens. The obtained results cleared that there were nonsignificant ($P<0.05$) differences in all blood biochemical indices except the cholesterol. However, insignificant increase in total protein and globulin had recorded to symbiotic followed by probiotic groups. Similar trend was observed by **(Panda *et al.*, 2006)**. **Abdel-Fattah** and **Farah**, **(2009)** found that activity of enzymes such as AST, ALT and ALP in serum were not influenced due to probiotic, prebiotic or synbiotic supplementation. The findings of our study and also from previous studies indicated that probiotic feeding has a cholesterol depressing effect in broiler chickens. However, the mechanism(s) of action for producing hypocholesterolemic effect is not yet fully understood. **Nelson** and **Gilliand (1984)** reported that some of the microorganisms present in the probiotic preparation could utilize the cholesterol present in the gastrointestinal tract for their own metabolism, thus reducing the amount absorbed. **Mohan *et al.* (1995)** reported that the reduction in serum cholesterol could be attributable to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation. *Lactobacillus acidophillus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis **(Abdulrahim *et al.*, 1996)**. *Lactobacillus* has a high bile salt hydrolytic activity, which is responsible for deconjugation of bile salts **(Surono, 2003)**. Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to excrete in faces **(Klaver** and **van der Meer, 1993)**. Probiotic microorganism inhibits hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in the cholesterol synthesis pathway there by decrease cholesterol synthesis **(Fukushima** and **Nakano, 1995)**.

In the available pieces of literature, the cholesterol-lowering effect of probiotics has been quoted by several investigators **(Dabiri *et at.,* 2009; Lee *et al.,* 2010)**. However, serum cholesterol was not affected when chickens were fed a probiotics-containing diet for three weeks of age, showing a time-dependent effect **(Tollba *et al.,* 2004)**. Similarly, probiotics supplementation (Lacto Sacc and Yea Sacc) significantly reduced total plasma cholesterol and lipids **(Abdel-Azeem *et al.,* 2005)**. Supplementation of the diet with 1 and 2 g of Bio-Buds (dried SC fermentation product) decreased yolk and serum cholesterol and increased antibody production significantly **(Abou El-Soud *et al.,* 2006)**. This reduction may be due to the ability of bacteria to assimilate or degrade the cholesterol to bile acids, followed by deconjugation to prevent re-synthesis. Additionally, **Kalavathy *et al.* (2008)** found a significant decrease in serum triglycerides and lipids using Lactobacillus cultures.

The absence of significant changes in most of the plasma biochemical constituents due to probiotics, or synbiotics are in partial agreement with the results of **Ashaverizadeh *et al.* (2009)**; they reported that antibiotics and probiotics did not affect total protein, albumin, globulin, AST, ALT, triglycerides, cholesterol, HDL, LDL, and VLDL. However, **Tollba *et al.* (2004)** showed that probiotics (Lactobacillus, Pediococuss) significantly increased plasma protein, albumin, and globulin fractions in poultry reared under natural and heat-stress conditions. In addition, **Abou El-Soud (2006)** found that Natural yeast (SC) significantly increased the total serum protein and globulin levels. However, **El-Ghamry** and **Fadel (2004)** found that *S. cerevisiae* and *Trichodermo reesei* did not affect the plasma total protein, albumin, and globulin. On the other hand, **Abdel-Azeem *et al.* (2005**) found that probiotics supplementation increased total plasma protein, plasma albumin, and Ca; **Shareef** and **Al-Dabbagh (2009)** stated that SC at 1%, 1.5%, and 2% increased total serum protein. The contradiction in response to probiotic supplementation among the above-mentioned investigations and that found herein could be elucidated based on the strain of bacteria, feed composition, and environmental and hygienic conditions **(Obianwuna *et al.,* 2023)**.

Our results showed that the concentrations of TP, GLU, TG, CHO, and ALT were not affected by the treatments. The addition of gum arabic had no effect on the ALT and AST activity of rabbits **(El-Ratel *et al.,* 2019)**.

Internal visceral and lymphoid organs

The effect of dietary treatments on lymphoid organs' relative weight at day 35 are shown in Table 4. Dietary supplements had a numerical increase on the thymus and spleen relative weight, while the bursa of Fabricius relative weight increased ($P<0.05$) in the gum arabic, LA, and symbiotic groups compared to control. These results agree with those of **Sato *et al.* (2009)**, who found that the relative weights of lymphoid organs were higher in broiler chickens fed gum arabic. Dietary supplementation with prebiotics (β-glucan and mannooligosaccharide) resulted in higher relative weights of lymphoid organs **(Usama *et al.,* 2018)**. In contrast, **Houshmand *et al.* (2012)** found that mannooligosaccharide as a prebiotic supplement at rate of 0.10% had no effect on the relative weight of lymphoid organs. Parallel to our findings, **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. 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 insignificantly in all of the supplemented groups compared to control. 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. Dietary addition of synbiotics result in improved metabolism, intestinal architecture, short chain fatty acids, ketone bodies, methyl acetate and carbon disulfide in broiler chickens **(Alloui *et al.*, 2013)**.

Intestinal histomorphology

The most commonly used standards to assess nutrient absorption and gut health are villus length, crypt depth, and villus length to crypt ratio **(Ducatelle *et al.*, 2018)**. Moreover, broiler chickens have a strong relationship with increased villus height, gut health, and absorption efficiency **(Alfaro *et al.*, 2007)**. High crypt height may indicate increased proliferative activity to compensate for villus height loss **(Chiang *et al.*, 2010).** The ratio of villus height to crypt height is a useful measurement for estimating the absorptive capacity of the small intestine, which correlates with increased epithelial cell turnover, and longer villi are associated with activated cell mitosis **(Awad *et al.*, 2009)**. Similar to our findings, **Guo *et al.* (2020)** and **Khan *et al.* (2022)** also reported enhanced VH, VH:CD ratio 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 an 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 **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)**. In the current study, villus length and villus length to crypt depth ratio were higher in the duodenum, while the crypt depth of villi decreased when chickens were fed GA powder (0.5 or 1.0%) compared to the control group. In agreement with **Macari** and **Maiorka (2000)**, it was shown that the use of fermented prebiotics by bacteria in the cecum increases the height of the duodenal villi of chickens aged 1 to 7 days after hatching. Moreover, the increase in absorbent surface area of villi in the duodenum occurs more rapidly with the age of chickens up to 7 days. The use of GA in chicken diets (1 to 10 days old) is an effective strategy to improve the early growth and development of the gastrointestinal tract. Moreover, the early development of duodenal morphological and functional characteristics of broiler chickens in the initial stage leads to an improvement in early growth performance **(Jha *et al.*, 2021)**. GA could be altering the gut microbiota and improving the integrity of intestinal epithelial cells, leading to better absorption of nutrients and hence better growth performance. From these results, we suggest that significantly improved FCR in high level gum arabic, *Lactobacillus acidophillus* and their synbiotic supplemented group may be due to improved villus height and high VH:CD ratio in different parts of broiler chickens.

Selected pathogenic bacteria in different parts of intestine

The composition of intestinal microflora is significant for maintaining intestinal homeostasis and health of host **(Zhang *et al.*, 2018)**. Cecal microflora plays an important role in chicken health and growth performance, affecting food transformation, disease resistance, and pathogen colonization **(Awad *et al.*, 2016)**. *Lactobacillus acidophillus* is a kind of aerobic bacterium, which can grow in the intestinal tract and consume oxygen to maintain anaerobic environment and inhibit the growth of harmful aerobic bacteria **(Hong *et al.*, 2005)**. Many studies have shown that *Lactobacillus acidophillus* supplementation caused a significant decrease in the numbers of *Escherichia coli* and *Salmonella*, whereas the numbers of *Lactobacillus* and *Bifidobacterium* increased in the cecum **(Yang *et al.*, 2016)**. 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)**. The microbiota of the cecum is more abundant than the gastrointestinal tract segments and plays a role in the fermentation of indigestible fibers **(Pourabedin** and **Zhao, 2015)**. **Teng** and **Kim (2018)** indicated that GA improves gut health by promoting lactobacilli spp. in young chickens. In our study, chickens that received GA levels (0.50 or 1.0%) had higher *Lactobacillus spp*. and lower *Salmonella spp*. content. These results agree with **Al-Baadani *et al.* (2022)** who found that chickens fed GA (0.25 to 1.0%) had higher *Lactobacillus spp*. and lower *Salmonella spp*. and *Escherichia coli*. These results agree with the study's findings that prebiotics increase lactic acid through fermentation and growth of bacterial populations, especially lactobacilli, in the cecum, thereby lowering pH values. Lactic acid is a major byproduct of Lactobacillus bacteria **(Alvarez-Sieiro *et al.*, 2016)**. Our results confirmed this, which showed a strong negative correlation between *Lactobacillus* count and cecal pH. **Pelicano *et al.* (2005)** found that the low pH of the lumen inhibited acid-sensitive pathogenic bacteria, such as *Salmonella typhimurium*, *Clostridium perfringens*, and *Escherichia coli*. 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 **(Khan *et al.*, 2022)**.

CONCLUSION

Gum arabic and *Lactobacillus acidophilus* 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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تأثير إضافة الصمغ العربي و *Lactobacillus acidophilus* أو مزيجهما إلى علائق دجاج التسمين

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الملخص العربي

ملخص البحث:

تم إجراء التجربة الحالية لاستكشاف آثار مكملات الصمغ العربي (GA) كبريبايوتيك طبيعي، و *Lactobacillus acidophilus* (LA) كبروبيوتيك أو مزيج من البريبايوتيك والبروبيوتيك كسينبيوتيك على أداء النمو العام، وأوزان الأعضاء اللمفاوية، وتشكل الأمعاء. والبكتيريا المسببة للأمراض في دجاج التسمين. لهذا السبب، تم توزيع 300 كتكوت روس غير مجنس بعمر يوم على ست مجموعات، تم تقسيم كل مجموعه إلى خمس مكررات بواقع عشرة كتاكيت لكل مكرره. تم تغذية الكتاكيت الموجودة في المجموعة الضابطة (T1) على النظام الغذائي الأساسي. غذيت مجموعات الكتاكيت (T2 وT3) (البريبيوتيك) على الصمغ العربي بجرعة 5 و7.5 جرام/كجم علف على التوالي. تم تغذية الكتاكيت الذي تم تغذيته على (T4) (بروبيوتيك) بـ *Lactobacillus* acidophilus بمعدل 1 جم/كجم علف. تمت تغذية الكتاكيت (T5 وT6) بنظام غذائي اساسي يحتوي على (سينبيوتيك) 5 جرام صمغ عربي + 1جرام *Lactobacillus* acidophilus /كجم علف أو (سينبيوتيك) 7.5 جرام صمغ عربي + 1جرام *Lactobacillus* acidophilus / كجم علف، على التوالي. أشارت النتائج إلى زياده معنويه في كميه الغذاء المأكول، والزيادة في وزن الجسم، وتحسين ($P<0.05$) الكفاءه الغذائيه، والحيويه ، وعامل كفاءة الإنتاج الأوروبي (EPEF) في المجموعة T6 تليها T5 ثم T4 ثم T3 وأخيرا T2 . تم تسجيل نفس النمط من تحسن وزن القلب والكبد والقوانص والبنكرياس وغده فابريسي والطحال والغدة الصعترية (الثيموسيه) للمجموعات التي تمت دراستها. تم تسجيل ارتفاع ملحوظ في طول الخملات ($P<0.05$) (VH) وعمق الخملات أقل (CD) ($P<0.05$) وأعلى VH:CD ($P<0.05$) في الاثني عشر والصائم واللفائفي في المجموعة التكافلية T6 تليها T5، T4، T3 وT2. وبالمثل، أدت إضافة المكملات الغذائية في مجموعات T6 و T4 إلى تقليل بكتيريا القولون والسالمونيلا بينما أدت زيادة بكتيريا *Lactobacillus spp* من الأعور في دجاج التسمين التجريبي. تم الاستنتاج من النتائج الحالية أنه على الرغم من أن البريبايوتك والبروبيوتيك يمكنهما تحسين الأداء العام بشكل كبير كلا على حده ، إلا أنه يمكن الحصول على أفضل النتائج من شكلهما التكافلي المدمج.