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Effect of dietary supplementation of probiotics, enzymes and their combination on growth performance, meat yield, intestinal microbiota and plasma analysis of broiler chicks

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Abstract

This study was carried out to evaluate the effect of dietary supplementation of the commercial probiotics (*Bacillus amyloliquefaciens*) (*BA*), an enzyme mixture containing xylanase, amylase and protease (XAP) enzymes, and their combination (*BA*+XAP) on growth performance, carcass characteristics, some blood biochemical parameters, and intestinal microbiota of broiler chicks. A total number of 320 one-day-old unsexed broiler chicks (Ross) with an average body weight of 43.65±0.19 g were assigned to 4 dietary treatments (80 chicks/treatment), each containing 4 replicates (20 chicks/replicate). The dietary treatments were as follows: control) fed basal diet without supplementation, T1) fed basal diet supplemented with *BA* probiotic at level 15 g / kg diet, T2) fed basal diet supplemented with XAP enzymes at level 400mg / kg diet, and T3) fed basal diet supplemented with AB and XAP at levels 15 g and 400mg /kg diet, respectively. The results indicated that dietary supplementation of *BA* probiotic significantly increased body weight, body weight gain, dressing weight and breast weight, and improved feed conversion ratio and performance index of broiler chicks compared with the other treatments. Also, the dietary supplementation of XAP enzymes or the mixture of *AB* and XAP significantly increased body weight and body weight gain of broiler chicks compared with control but did not effect on feed conversion ratio and performance index of broiler chicks. Feed consumption for all treated chicks was significantly higher than control. The highest plasma total proteins level was found in T2 followed by the mixture T3 and control, respectively, and the lowest level was found in T1. Also, T2 had the highest albumin level followed by T1. The highest plasma globulin level was found in T3 compared with T1 and control groups, followed by T2 compared with T1 group. T1 and T2 had the highest Triglyceride level compared with the other treatments. In conclusion, supplementing diets of broiler chickens with BA probiotics, XAP enzymes and their combination displayed positive effects on growth performance, immune status, and intestinal microbiota; the *BA* treatment showed the most pronounced results

Keywords: probiotics, enzymes, broiler chicks, growth performance, meat yield.

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1. Introduction

Antibiotics have been used at sub-therapeutic level as growth promoters in the feed of livestock and poultry for many decades ago. However, the use of antibiotics as growth promoters (AGPs) was associated with some problems such as antibiotic-bacterial resistance and the presence of drug residues in animal products, which led to several European legislation to prohibit the use of antibiotic growth promoters (AGPs) in animal feed (Alloui *et al*., 2013; Castanon, 2007). Many natural and safe alternatives that have similar beneficial effects of AGPs have been proposed as growth promoters to serve as alternatives for synthesized AGPs (Mehdi *et al*., 2018). These alternatives include probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, bacteriophages, clay, and metals (Gadde *et al*., 2017). Probiotics are live cultures of microorganisms that have beneficial effects on the health of animals through favoring the growth of benefic microorganisms and hindering that of pathogenic (Adugna and Belete, 2020). *Bacillus amyloliquefaciens* strain is a spore forming aerobic bacterial strain that has been used as probiotics in poultry production (Bajagai, 2017). It has been reported as an alternative for the AGPs in broilers diets (Lei *et al*., 2015; Ahmed *et al*., 2014; Teng *et al*, 2017). An *et al*. (2008) observed that addition of *B. amyloliquefaciens* in broiler diets improved body weight gain, feed conversion ratio, and immune response of broiler chickens against Newcastle disease virus and increased concentrations of cecal lactic acid bacteria. *Bacillus amyloliquefaciens* have a high potency to produce lipase enzyme (Selvamohan *et al*., 2012), α-amylase (Gangadharan *et al*., 2008; Gracia *et al*., 2003), and cellulase (Lee *et al*., 2008). Exogenous enzymes have been used extensively in poultry diets to enhance productive performance (Slominski, 2011). Also, enzymes improved the feed conversion of broiler chicks and reduced environmental problems via sustaining digestion and limiting output of excreta (Khattak *et al*., 2006). Xylanase, amylase and protease are being used either individually or in combination to breakdown their substrate and to assist other exogenous or endogenous enzymes by releasing their respective substrates from feed matrix (Singh, 2018). In several studies, the use of multi enzyme combination, such as xylanase, amylase and protease, was reported to improve broilers performance and nutrient digestibility (Cowieson and Olukosi *et al*., 2007; Ravindran, 2008; Romero *et al.* 2013, 2014; Tang *et al.*, 2014). Carbohydrases, such as xylanase, exert their mode of action via degrading cell wall components of dietary ingredients, releasing intracellular encapsulated nutrients, and improving access of enzymes and therefore increase exposure to cell contents, which increases nutrient utilization (Cowieson, 2005). Exogenous amylase and protease sustain endogenous digestive enzymes (Gracia *et al*., 2003) and limit losses of endogenous amino acids through modifying the secretion of pancreatic enzymes (Jiang *et al*., 2008) and mucin production (Cowieson and Bedford, 2009).

Furthermore, it has been reported that dietary external enzymes positively modulate intestinal microbiota and improve growth performance (Ptak *et al*., 2015). As mentioned above, *Bacillus amyloliquefaciens* exerts beneficial effects on nutrient utilization and performance of broilers via sustaining the secretion of digestive enzymes. This suggests that using a blend of both *Bacillus amyloliquefaciens*, as probiotics, and an external mixture of digestive enzymes (xylanase, amylase, and protease) is likely show pronounced cumulative beneficial effect on broiler's performance. Therefore, this study aimed to evaluate the effect of dietary supplementation of probiotic (*Bacillus amyloliquefaciens*), enzymes mixture (Xylanase, amylase and protease) and a combination of them on growth performance, meat yield, plasma analysis and intestinal microbiota of broiler chicks.

2. Materials and methods

This study was carried out at the poultry research farm, Department of Animal Production, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt during the period from 16 February to 22 March 2021.

2.1 Birds, diets, housing and experimental design

A total number of 320 one-day-old unsexed Ross (308) broiler chicks, with an average body weight of (43.67±0.19 g), were used in this experiment until 35 day of age. All chicks were wing-banded, individually weighed, and randomly distributed into 4 dietary treatments each containing four replicates of 20 chicks (n= 80/treatment). Each replicate was housed in a floor pen measuring 1.15 length × 2 m width with deep litter from wood shaving (7cm thickness). Starter and grower diets were formulated to meet all nutrient requirements of broiler chicks according to NRC (1994). The ingredients and calculated nutrient content of the diets are presented in table (1). The treatment groups were as follow: control) fed a basal diet without supplementation; T1) fed the basal diet supplemented with *Bacillus amyloliquefaciens* probiotics (*BA*) at level 15 g /kg diet; T2) fed the basal diet supplemented with an enzymes mixture of xylanase, amylase and protease (XAP) at level 0.4 g / kg diet; T3) fed the basal diet supplemented with *BA* and XAP at levels 15 and 0.4 /kg diet, respectively. Feed and fresh water were provided ad libtium. The probiotic (Enviva ®PRO 201 BA) used her is a product of Danisco company, U.S.A. which contains 3× 108 CFU/g. The enzyme mixture (EXTRA ® XAP 101TPT) was purchased from Danisco company, Finland; it contains β-xylanase (20000 U /g), α-amylase (2000 U/g) and protease (40000 U/g). All chicks were raised under the same environmental conditions and received 23 hours light/ day throughout the experimental period. The vaccination program and Hygiene procedure were performed according to the standard managerial procedures.

Table (1): Composition and calculated nutrient content of starter and grower diets of broiler chicks

Ingredients	Starter	Grower
	%	%
Yellow corn (8.8%)	58	58.8
Soybean meal (46%)	28.1	26
Gluten (60%)	8.58	6.28
Wheat bran	-	2
Oil	1.2	3.2
Limestone	1	0.9
Di- Ca phosphate	2.3	2
Lysine	0.15	0.15
Methionine	0.07	0.07
NaCal	0.30	0.30
Premix[1]	0.30	0.30
Total	100	100
calculated value		
ME (kcal /kg feed)	3003.88	3100.29
Crude protein (%)	23.01	21.04
Crude fat (%)	3.84	5.86
Crude fiber (%)	3.44	3.49
Lysine (%)	1.20	1.13
Methionine (%)	0.53	0.48
Calcium	1.05	0.93
Available Phosphors	0.51	0.45

[1] each kg premix contained: vitamin A (acetate), 6250000 I.U.; vitamin D3 (Cholecalciferol), 2500000 I.U.; vitamin E (α – tocopherol), 25000 mg; vitamin k,1750 mg; vitamin B1, 500 mg; vitamin B2, 2750mg; vitamin B6, 1250 mg; vitamin B12, 10 mg; nicotinic acid (niacin), 20000mg; Calcium pantothenate, 5000mg; folic acid , 500 mg; biotin 50mg; iron sulfate,22000 mg; manganese oxide,31000 mg; copper sulfate,2500 mg ; zinc oxide,37500 mg ; potassium iodide,650 mg; sodium selenite, 113 mg; cobaltous sulfate,50 mg ; Ethoxyquin,250 mg; wheat bran (carrier), 120 gm; limestone (carrier), up to 1kg.

2.2 Data collection

2.2.1 Growth performance

The initial and final live body weights of broiler chicks were individually weighed at 1 and 35 days of age, respectively. Feed consumption (FC) during the experimental period was calculated on per replicate basis as the difference between the added and refused feed amounts at the end of experiment. Body weight gain (BWG), feed conversion ratio (FCR), and the performance index (PI) of broiler chicks during the experimental period were calculated according to the following equations:

$$\text{BWG} = \text{Final body weight(g)} - \text{Initial body weight(g)}$$

$$\text{FCR} = \text{FC (g)} / \text{BWG (g)}$$

$$\text{PI} = (\text{Live Weight (Kg)}) / \text{FCR} \times 100$$

2.2.2 Sampling

At the end of the experimental period at 35 days of age, 32 chicks (2 chicks/replicate) representing the body weight average of their treatments were fasted for 12 hours then slaughtered to determine the carcass traits. During the slaughtering process, one blood sample from each chick was collected in non-heparinized tubes and then centrifuged at 4000 rpms for 15 minutes to obtain plasma which was kept in Eppendorf tubes and stored at -20ºC for later analyses. After slaughtering process, 64 fresh digesta samples were collected from ileum (32 samples) and caeca (32 samples), which were kept in the laboratory in sealed sterile tubes at 4ºC until enumeration of microbial populations.

2.2.3 Carcass traits determination

After scalding, defeathering, evisceration, and removal the head and legs, the weights of dressed carcass, carcass cuts up parts (whole breast, both thighs, both wings), internal organs (liver, gizzard, heart, spleen and bursa) and abdominal fat pad were recorded. The relative weights of dressed carcass, carcass cuts, internal organs and abdominal fat were expressed as percentage of live body weight.

2.2.4 Blood analysis

Plasma total proteins, albumin, triglycerides, cholesterol and glucose were measured by spectrophotometer using commercial kits (spectrum) purchased from Egyptian Company for Biotechnology (S.A.E). Globulin was calculated as the difference between total protein and albumin.

2.2.5 Enumeration of intestinal bacteria

One gram of each digesta sample was mixed homogeneously with 9 ml of peptone water (0.1% w/v). The mixture was then serially diluted in 0.9% sterile saline solution. The dilutions from 10^{-3} to 10^{-5} were used for enumeration of total *Coliforms* and *bacillus amyloqufiones*. While *lactobacilli* were counted using the dilutions from 10^{-5} to 10^{-7}. These dilutions were inoculated in selective agar media and counted following conventional microbiological techniques. All microbiological analyses were performed in duplicates, in which 100 µl from each diluted sample were inoculated on agar plates, and the average values were used for statistical analysis. Results were expressed as the log of colony forming units (CFU) per gram of digesta. MRS agar plates were inoculated by 100 µl from the dilutions (10^{-10}) of each sample and then incubated anaerobically for up to 48 hours to enumerate lactobacilli in the ileal and cecal digesta of broiler chicks. Also, 100 µl from the dilutions (10^{-3} to 10^{-5}) of each sample were inoculated on nutrient agar and Macconky agar plates and incubated aerobically for 24 h at 37 °C to estimate the population of *bacillus amyloqufeinces* and total coliforms in the digesta of ileum and cecal of broiler chicks.

2.3 Statistical analysis

Data were statistically analyzed by Analysis of Variance (ANOVA) using the General Linear Model (GLM) of SAS (2009). Significant differences among treatment means were separated by Duncan's multiple range tests (Duncan,

1955) with a 5% level of probability. All data obtained were analyzed by using the following Model:

Yij= μ +Ti+ eij

Where, Yij & yijk = the analyzed measurement, μ= is the overall mean, Ti = is the effect of dietary treatments (i =control, probiotic, enzymes and probiotic-enzymes combination)

3. Results

3.1 Growth performance

The effects of dietary supplementation of probiotic, enzymes and their combination on growth performance of broiler chicks are shown in table (2). The results revealed that addition of *BA* probiotic in broiler diets significantly ($P < 0.01$) increased the final body weight and body weight gain, and improved feed conversion ratio and performance index compared with the other treatments. The live body weight and body weight gain of broiler chicks fed diets supplemented with XAP enzymes or the mixture of *BA* and XAP were significantly ($P < 0.01$) higher than control group. The dietary supplementation of XAP enzymes or the mixture of *BA* and XAP had no effect on feed conversion ratio and performance index of broiler chicks during the experimental period compared with control. In term of feed consumption, the treated groups exhibited the highest value of daily and total feed consumption compared with control group.

Table (2): Effect of dietary supplementation of probiotic, enzymes and their combination on growth performance of broiler chicks aged 1-35 days of age.

Parameters	Treatments				Significance
	C	T1	T2	T3	
Initial BW (g)	43.76±0.42	43.62±0.36	43.61±0.38	43.62±0.36	*Ns*
Final BW(g)	1757±31.17^{c}	2062±25.15^{a}	1887±25.72^{b}	1880±27.19^{b}	**
BWG (g)	1714±31.09^{c}	2018±25.11^{a}	1844±25.61^{b}	1837±27.15^{b}	**
FC (g)	2769±20.07^{b}	3169±20.48^{a}	3091±40.26^{a}	3073±53.46^{a}	**
FCR (g feed/g gain)	1.69±0.033^{a}	1.59±0.02^{b}	1.70±0.024^{a}	1.70±0.026^{a}	**
Performance index	110.8±3.93^{b}	132.9±3.34^{a}	114.2±3.05^{b}	114.1±3.10^{b}	**

^{a,b,c} Means with different superscripts within the same row are significantly different ($p < 0.05$). Ns= non-significant. **= significant ($p < 0.01$), C = basal diet (control). T1= basal diet+ 15 g probiotic/ kg diet. T2= basal diet +0.4 g enzymes/ kg diet T3 = basal diet +15 g probiotic +0.4 g enzymes/kg diet.

3.2 Carcass traits

Results shown in Table (3) indicated that the dressing weight of broiler chicks fed diet supplemented with *BA* probiotic was significantly higher than the other treatment at 35 days of age. The dietary supplementation of XAP enzymes or the mixture of *BA* and XAP significantly increased the dressing weight of broiler chicks at 35 days of age compared with control. The breast weight of broiler chicks fed diet supplemented with *BA* probiotic was significantly higher than those fed diet supplemented with the mixture of *BA* and XAP, or control diet, without significant differences among the other treatments. The dietary supplements had no effect on the weights of thigh, wing and abdominal fat. Also, no significant differences were observed in the relative weights of dressing carcass, breast, thigh and abdominal fat of broiler chicks among all treatments. While the wing percentage of treated broilers were significantly lower than control.

Table (3): Effect of dietary supplementation of probiotic, enzymes and their combination on meat yield and carcass parts of broiler chicks at 35 days of age (means±SE).

Items		Treatments				Significance
		C	T1	T2	T3	
Dressing	Weight (g)	1264.7±25.40[c]	1431.8±20.08[a]	1353.5±38.89[b]	1349.5±13.90[b]	**
	(%)	72.97±0.54	73.77±0.21	73.89±0.53	73.52±0.47	*Ns*
Breast	Weight (g)	423.20±7.50[b]	490.00±8.17[a]	475.25±20.38[ab]	452.00±12.02[b]	*
	(%)	24.00±0.50	25.30±0.61	25.91±0.67	24.64±0.68	*Ns*
Thigh and drumstick	Weight (g)	401.60±13.47	437.75±13.83	395.00±13.03	411.00±7.93	*Ns*
	(%)	22.75±0.57	22.55±0.58	21.59±0.53	22.39±0.37	*Ns*
Wing	Weight (g)	135.50±6.42	140.43±3.53	128.86±3.14	129.50±1.40	*Ns*
	(%)	7.80±0.26[a]	7.20±0.10[b]	7.13±0.12[b]	7.06±0.09[b]	**
Abdominal fat	Weight (g)	30.38±2.45	33.27±3.44	27.55±2.48	32.16±2.67	*Ns*
	(%)	1.75±0.13	1.71±0.17	1.50±0.11	1.75±0.14	*Ns*

[a,b,c] Means with different superscripts within the same row are significantly different ($p < 0.05$). Ns= non-significant, *= significant ($p < 0.05$), **= significant ($p < 0.01$), C = basal diet (control). T1= basal diet+ 15 g probiotic/ kg diet. T2= basal diet +0.4 g enzymes/ kg diet T3 = basal diet +15 g probiotic +0.4 g enzymes/kg diet.

3.3 Internal organs

The weights and relative weights of liver, heart, gizzard, spleen and bursa of broiler chicks at 35 day of age are illustrated in Table (4). The dietary treatments had no effect on the weights and percentages of the internal organs except the weight of bursa. Broiler chicks fed diet supplemented with *BA* probiotic (T1) and those fed *BA* and enzyme mixture (T3) showed higher bursa weight than the control value, and those fed XAP showed an intermediate value.

3.4 Blood biochemical parameters

The dietary treatments exerted a significant effect on all blood biochemical parameters measured (Table 5) except plasma cholesterol level. The highest total proteins level was found in XAP enzymes group (T2) followed by the mixture of *BA* and XAP group (T3) and control, respectively, and the lowest total proteins level was found in *BA* probiotic group (T1). Also, T2 had the highest albumin level followed by T1. The highest plasma globulin level was found in T3 compared with T1 and control groups, followed by T2 compared with T1 group. The dietary supplementation of *BA* probiotics (T1) and XAP enzymes (T2) significantly increased triglyceride level compared with the treatments.

Table (4): Effect of dietary supplementation of probiotic, enzymes and their combination on the internal organ weights of broiler chicks at 35 days of age.

Organs		Treatments				Significance
		C	T1	T2	T3	
Liver	Weight (g)	41.71±2.61	48.89±1.78	46.23±2.56	42.51±3.15	*Ns*
	(%)	2.40±0.12	2.52±0.08	2.53±0.15	2.31±0.15	*Ns*
Gizzard	Weight (g)	32.51±0.42	33.81±2.27	32.62±1.28	33.91±1.64	*Ns*
	(%)	1.88±0.04	1.75±0.12	1.80±0.10	1.84±0.07	*Ns*
Heart	Weight (g)	8.53±0.44	9.40±0.53	9.83±0.45	8.72±0.41	*Ns*
	(%)	0.49±0.02	0.48±0.02	0.54±0.01	0.47±0.02	*Ns*
Spleen	Weight (g)	3.07±0.30	2.51±0.24	2.88±0.31	2.79±0.29	*Ns*
	(%)	0.18±0.02	0.13±0.01	0.16±0.02	0.15±0.02	*Ns*
Bursa	Weight (g)	$2.00±0.16^{c}$	$2.70±0.23^{a}$	$2.09±0.22^{bc}$	$2.66±0.15^{ab}$	*
	(%)	0.11±0.01	0.14±0.01	0.11±0.01	0.15±0.01	*Ns*

^{a,b,c} Means with different superscripts within the same row are significantly different (p < 0.05). Ns= non-significant, *= significant (p < 0.05), C = basal diet (control). T1= basal diet+ 15 g probiotic/ kg diet. T2= basal diet +0.4 g enzymes/ kg diet T3 = basal diet +15 g probiotic +0.4 g enzymes/kg diet.

Table (5): Effect of dietary supplementation of probiotic, enzymes and their combination on blood metabolites of broiler chicks.

Parameters	Treatments				Significance
	C	T1	T2	T3	
Total protein (g/dl)	$6.49±0.14^{c}$	$5.93±0.12^{d}$	$8.04±0.19^{a}$	$7.44±0.17^{b}$	**
Albumin (g/dl) (A)	$3.07±0.17^{c}$	$3.73±0.13^{b}$	$4.21±0.12^{a}$	$3.07±0.03^{c}$	**
Globulin (g/dl) (G)	$3.42±0.20^{b}$	$2.20±0.20^{c}$	$3.83±0.19^{ab}$	$4.37±0.16^{a}$	**
A:G ratio	$0.92±0.09^{bc}$	$1.77±0.21^{a}$	$1.11±0.08^{b}$	$0.71±0.02^{c}$	**
Cholesterol (mg/dl)	221.12±23.54	170.90±15.58	167.27±19.59	178.53±7.37	*Ns*
Triglyceride (mg/dl)	$179.80±15.11^{b}$	$234.25±19.16^{a}$	$241.57±11.68^{a}$	$182.16±3.03^{b}$	**
Glucose (mg/dl)	105.56±5.46	95.51±6.20	96.56±5.59	114.64±9.08	*Ns*

^{a,b,c} Means with different superscripts within the same row are significantly different (p < 0.05). Ns= non-significant, **= significant (p < 0.01), C = basal diet (control). T1= basal diet+ 15 g probiotic/ kg diet. T2= basal diet +0.4 g enzymes/ kg diet T3 = basal diet +15 g probiotic +0.4 g enzymes/kg diet.

3.5. Intestinal bacterial count

Statistical analysis of intestinal microbiota is shown in Table (6). The results indicated that the highest value of lactobacillus count in ileum of broiler chicks was found in T1 compared with T2 and control, followed by T3 compared with control. Also, T1 had the highest values of *bacillus amyloliquefaciens* count in the ileum of broiler chicks compared with the other treatments, which was followed by T3 compared with T2 and control. Interestingly, the total coliform count in the ileum and caeca of broiler chicks in treated groups T1, T2 and T3 were significantly lower than control. On the other hand, the dietary supplements had no effect on cecal lactobacillus count of broiler chicks at 35 days old. While the cecal count of *Bacillus amyloliquefaciens* of broiler chicks in T1 and T3 were significantly higher than those in T2 and control.

Table 6: Effect of dietary supplementation of probiotic, enzymes and their combination on the intestinal microbiota (log CFU/g) of broiler chicks aged 35 days.

Type of microbiota	Treatments				Significance
	C	T1	T2	T3	
Ileal microbiota (log_{10} CFU/g)					
Lactobacillus spp.	8.45±0.12[c]	9.51±0.15[a]	8.79±0.21[bc]	9.17±0.06[ab]	**
B. amyloliquefaciens	4.39±0.15[c]	5.45±0.16[a]	4.30±0.11[c]	4.83±0.11[b]	**
Total coliforms	6.19±0.11[a]	5.48±0.17[b]	5.37±0.13[b]	5.32±0.12[b]	**
Cecal microbiota (log_{10} CFU/g)					
Lactobacillus spp.	8.28±0.07	8.32±0.09	8.20±0.11	8.41±0.15	*Ns*
B. amyloliquefaciens	4.37±0.08[b]	5.59±0.10[a]	4.34±0.27[b]	5.54±0.12[a]	**
Total coliforms	6.75±0.15[a]	6.15±0.11[b]	6.04±0.13[b]	6.06±0.19[b]	*

[a,b,c] means without mutual superscripts within the same row are significant difference ($p < 0.05$). NS = Non-significant ($p > 0.05$), * = significant ($P < 0.05$), **= significant ($P < 0.01$). C= bird fed basal diet without addition (control), T1= bird fed basal diet +probiotic, T2= birds fed basal diet + enzymes, T3 = birds fed basal diet +probiotic +enzymes.

4. Discussion

4.1. Growth performance

The positive effects of probiotic or enzymes supplementation on growth performance of broiler chicks have been documented in many previous studies. For example, An *et al.* (2008) found that supplementation of *BA* probiotic at levels 0.1 or 0.2% of broiler diets significantly increased average daily gain. Ahmed *et al*. (2014) reported that addition of *BA* probiotic to broiler diet at level 20g/kg feed had positive linear effect on average daily gain. Lei *et al*. (2015) stated that supplementation of *BA* probiotics at levels 30 and 60 mg/kg of broiler diet increased the total body weight gain during the entire experimental period (0 - 42 days of age) compared with control group. Singh (2018) observed that addition of XAP enzymes in broiler diets at level 100g/ton of diets increased average daily gain by 12% relative to un-supplemented diets. In the present study (Table 2), the improvement of body weight, body weight gain and feed conversion ratio of broiler chicks fed diets supplemented with probiotic could be attributed to the ability of *BA* probiotic to produce lipase (Selvamohan *et al*., 2012), α-amylase (Gracia *et al*., 2003; Gangadharan *et al*., 2008), cellulase (Lee *et al*., 2008) and proteases (Gould *et al*., 1975), which increase nutrient availability. Moreover, *BA* supplementation has been reported to increase activities of trypsin, amylase, lipase, chymotrypsin and lipase in the gut of broilers at 42 days of age (Wang *et al*., 2021; Sun *et al*., 2022). Also, The *BA* supplementation could improve nutrients digestibility; this was confirmed by Lei *et al*. (2015) who indicated that dietary *BA* supplementation significantly increased the total apparent digestibility of crude protein (CP), dry matter (DM) and gross energy during both starter and finisher phases of broiler chicks. Similarly, Gharib- Naseri *et al.* (2021) exhibited that addition of *BA* probiotic to broiler diets significantly improved the apparent ileal digestibility of cystine, valine and lysine compared with control.

4.2 Carcass traits

Regarding of meat yield, treated groups had the highest carcass weight compared with control, with superiority of *BA* probiotics treatment over all treatments. Also, *BA* probiotics treatment had the highest breast weight compared with XAP treatment and control. The increase of carcass weight and breast weight of treated broilers compared with control due to the increase of live body weight of treated broiler compared with control as shown in table (2). However, the dietary supplements had no effect on the weights of thigh, wing and abdominal fat. Also, no significant differences were observed in the relative weights of dressing carcass, breast, thigh and abdominal fat of broiler chicks among all treatments. Similar results were obtained by Al-Harth, (2017) and Amerah *et al*. (2017). Hussain *et al*. (2019) who found that the supplementation of exogenous enzymes (protease, mannanase and Xylanase) did not affect carcass, breast and thigh yield of broiler chicks. Also, Ciurescu *et al*. (2020) illustrated that addition of *Bacillus subtilis* probiotics in broiler diets had no effect on the carcass, breast, and legs' yield. In contrast to the present study, some authors reported that probiotics supplementation reduced the abdominal fat. Ahmat *et al*. (2021) reported that dietary *B. amyloliquefaciens* supplements significantly improved the carcass yield and reduced broilers chicken abdominal fat.

4.3 Internal organs

In the present study, the dietary treatments had no effect on weight and relative weight of liver, heart and gizzard. Our findings on dietary enzymes supplementation are comparable to those of Hajati, (2010), Al-Harth, (2017) and Hussain *et al*. (2019) who reported that the supplementation of exogenous enzymes in broiler diets had no effect on relative weights of gizzard, liver, and heart. Also, the obtained results related dietary probiotics supplementation are in agreement with Zhang *et al.* (2012) and Ciurescu *et al*. (2020) who reported that probiotics supplementation had no effect on the internal organ size (liver, heart and gizzard). Kırkpınar *et al*. (2018) observed that dietary supplements of probiotics, enzymes and their combination had no effect on relative weights of gizzard, liver, and heart. In terms of lymphoid organs, the bursa of Fabricius functions as a central lymphoid organ, required for development of the antigen-specific B cell repertoire. It is necessary for the differentiation of prebursal stem cells into bursal stem cells present in the bursa until the 5th week after hatching (Yvernogeau, *et al*., 2022). Thus, it could be evaluated the immune status of chickens by measuring the weight of immune organs such as the bursa of Fabricius, spleen, and thymus (Ahmat *et al*., 2021). The current results indicated that addition of *BA* probiotic only or combined with XAP enzymes to broiler diets significantly increased bursa weight compared with control group. The relative bursa weight of broiler chicks fed diets supplemented with *BA* probiotics or the mixture of *BA* probiotics and XAP enzymes numerically increased compared with other treatments. Our findings agreed with those of Park and Kim (2014) who found that broiler chicks fed diets supplemented with *B. subtilis* probiotics had the highest relative bursa weight compared with control. Kırkpınar *et al*. (2018) stated that dietary supplements of probiotics, enzymes and their combination tended to slightly increase the relative weight of bursa of Fabricius. Luan *et al*. (2019) found that broiler chicks treated with *BA* probiotics had the highest relative weights of bursa and spleen compared with control group at 21 and 42 day-olds, respectively.

4.4 Blood biochemical parameters

Blood analysis is an established means of assessing clinical and health status of animals on feeding trials since ingestion of dietary components would have measurable effect on blood composition and may be considered as an appropriate measure of long-term nutritional status (Church *et al*., 1984; Akinmutimi, 2004). Concerning the effect of enzymes supplementation on plasma proteins, the obtained results are agreement with the findings of Oguntoye *et al*. (2018) who reported that addition of multi-enzymes (xylaniase, amylase and protease) to broiler diets increased plasma total proteins and globulin compared with control. Regarding the effects of probiotics supplementation on plasma proteins, the current results are contrasted with Oguntoye *et al*. (2018) who demonstrated that addition of the Gro up™ Probiotics (commercial product) at 500ppm of broiler diets significantly increased the levels of total protein, albumin and glucose, and decreased the levels of globulins, total cholesterol and triglycerides compared with the control group. Biswas *et al*. (2018) found that addition of the probiotics *Lactobacillus acidophilus* at 10^6 and 10^7 cfu/g of broiler diets significant decreased the cholesterol compared with the control group, but had no significant effect on total protein, albumin and alkaline phosphatase**.** Saleh *et al*. (2020) indicated that the dietary supplementation of probiotics *Bacillus licheniformis* to broiler diets at level 100 g/ton diet significantly increased plasma albumin level compared with control but had no effect on plasma total protein, total cholesterol and HDL – cholesterol.

4.5 Intestinal bacterial count

Regarding the intestinal microbiota, the obtained results are in agreement with Sen *et al*. (2012) who observed that addition of *Bacillus subtilis* probiotics at level 0.15%, 0.30% and 0.45% of broiler diets decreased the clostridium and coliform counts in the cecum of broiler chicks at 35 days of age compared with control group. Lei *et al.* (2015) documented that *the* dietary supplementation of *Bacillus amyloliquefaciens* significantly reduced the *Escherichia coli* count, while increased the counts of *Lactobacillus* in the cecum of broilers at 21 and 42 days of age compared with control group. Manafi *et al*. (2018) observed a significant reduction the coliform bacteria count in caecal contents of broilers chicks fed diets supplemented multispecies probiotic containing four Bacillus species and Saccharomyces boulardii (Microguard®) compared with control. Gao *et al*. (2017) stated that addition of probiotics *Bacillus subtilis* at level 100, 150, 200 and 250 mg/kg of broiler diets increased the of Lactobacillus count in the cecum, and significant decreased the total aerobes, Salmonella, and E. coli counts in the cecum compared with control group. Biswas *et al*. (2018) reported that dietary supplements of *Lactobacillus acidophilus* probiotics reduced the coliforms counts in the ileum and caecal of broiler chicks. The decrease of pathogenic bacteria in broilers treated with *BA* probiotics is due to the potential of *bacillus amyloliquefaciens* to produce lactic acid, bacteriocin and bacteriocin like substances that can inhibit the growth of pathogenic bacteria (Ahmed *et al*., 2014).

5. Conclusion

Finally, the dietary supplementation of *BA* probiotics and XAP enzymes in single form or in combination could be promoting the growth performance of broiler chicks. *BA* probiotics were more effective than XAP enzymes in improving the immunity status and modulating the intestinal ecosystem of broiler chicks.

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