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## Impact of Dietary Supplementation of Probiotic or Enzymes on Carcass Traits and Intestinal Microflora of Broiler

Mohamed A. Elzagheer<sup>1</sup>; Yasein M. Mahdi<sup>1</sup>; Nadi M. Essa<sup>1</sup> and Mohamed El-Sagheer Mohamed<sup>2\*</sup> 

<sup>1</sup> Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.

<sup>2</sup> Department of Poultry Production, Faculty of Agriculture, Assiut University, Assiut, Egypt

\*Corresponding author email: [sagheer68@aun.edu.eg](mailto:sagheer68@aun.edu.eg)

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### Abstract

The present study aimed to determine the effect of supplementing diets of broiler with probiotic or enzymes on carcass traits and intestinal microflora. One hundred thirty five, one-day-old- Ross-308 chicks were randomly distributed into three experimental groups and every group was divided into three replicates of 15 chicks each. The first group received only basal diets (starter for 2 weeks and grower for 3 weeks) as control (C), the 2<sup>nd</sup> group (T1) received the basal diets supplemented with probiotic (Guardizen-M) at the level of 1g/kg diet, while the 3<sup>rd</sup> group (T2) received the basal diets supplemented with enzymes (Fra<sup>®</sup>Multizyme) at the level of 0.5g/kg diet. The trial lasted for 35 days of age. The results indicated that percentages of carcass, breast, thigh, drumsticks were significantly ( $P \leq 0.05$ ) higher for groups T1 and T2 compared with the control one. Using probiotics or enzymes resulted in a considerable ( $P \leq 0.05$ ) lower amount of abdominal fat. The differences in percentages of drip loss, cooking loss, water holding capacity and giblets were not significant among all groups. Intestinal Lactobacillus bacteria was significantly ( $P \leq 0.05$ ) increased and coliform bacteria was remarkably ( $P \leq 0.05$ ) decreased in T1 and T2 groups compared with the control one. According to our findings, it might be concluded that supplementing broiler diets with 1g probiotic (Guardizen-M) or 0.5g enzymes (Fra<sup>®</sup>Multizyme)/kg diets have positive effects on carcass traits and intestine morphology.

**Keywords:** *probiotic, enzyme, carcass traits, intestinal microflora, broilers chickens*

### Introduction

Probiotics and enzymes are produced worldwide, not just to increase production but also to investigate their possibility as effective antibiotic substitutes. Probiotics are live microbial dietary supplements that help the host by supporting intestinal microbial balance (Fuller, 1989). Lactobacillus, Lactococcus, Enterococcus, and Streptococcus are the most popular probiotic bacteria (Rinkinen *et al.*, 2003). In the past 15 years, probiotics have been used as potential antimicrobial growth promoters (AGP) substitutes without any negative clinical

consequences (Furrie *et al.*, 2006). Previous research suggested that adding probiotics to the feed could enhance growth performance (Deniz *et al.*, 2011), meat quality (Mahajan *et al.*, 2000), improve carcass traits of broiler chickens (Shabani *et al.*, 2012), promote intestinal lactobacilli counts (Lee *et al.*, 2010), and reduce the number of coliforms in broilers (Hassan and Ryu, 2012). Multi-strain probiotics have more potential than single strain probiotics, according to research reports, and can improve chicken growth performance, feed efficiency, and gut health by stabilizing the intestinal microbiota (Mountzouris *et al.*, 2010).

Non-starch polysaccharides (NSPs), such as cellulose and hemicellulose, have been found to be present in varying levels in different feed ingredients, which may prevent the absorption and utilization of dietary nutrients (Dhawan and Kaur 2007). Other researchers have noted that the inclusion of NSPs in the diet of chicken can result in increased intestinal viscosity, lower nutritional digestibility, poor feed conversion ratios, and subpar bird performance (Meng and Slominski 2005). Exogenous enzymes can be added to diets as a method to enhance the utilization of NPS-rich nutrients (Montanhini *et al.*, 2013). By altering the intestinal microbiota and minimizing the negative effects of microbial fermentation in the small intestine, these enzymes also reduce the viscosity of the intestinal digestion. The addition of commercial enzymes can improve the nutritional value of feed ingredients and give diet formulation more flexibility. Exogenous enzymes have been reported to improve nutrients utilization and increase the digestibility of fibrous materials (Anjumand, 2010).

The purpose of this study was to determine the impact of adding probiotics (Guardizen-M) and enzymes mixture (Fra<sup>®</sup>Multizyme) in diets for broilers on carcass characteristics and intestinal microbiota.

## Materials and Methods

The current study was conducted at the Poultry Farm, Animal Production Department, Faculty of Agriculture, Al-Azhar University, Assiut branch, Egypt, during the period from February 2020 to March 2020.

### Experimental design

One hundred and thirty-five unsexed, healthy one-day old broiler chicks (Ross-308) were obtained from commercial hatchery, and randomly distributed into three groups of 45 chicks, in three replicates of 15 birds each. The first group received only basal diets (starter for 2 weeks and grower for 3 weeks) as control (C), the 2<sup>nd</sup> group (T1) received the basal diets supplemented with probiotic (Guardizen-M) at level of 1g/kg diet, while the 3<sup>rd</sup> group (T2) received the basal diets supplemented with enzymes (Fra<sup>®</sup>Multizyme) at level of 0.5g/kg diet. The trial lasted till 5 weeks of age. The basal diets were formulated according to NRC (1994), as shown in Table (1).

Probiotic (Guardizen-M) consists of mixed probiotics concentrates 5.6 g (a minimum  $1 \times 10^{10}$  CFU) *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae*, *Candida*

*pintolopesii* and Lactose or Dextrose 994.4g. The enzyme complex preparation (Fra<sup>®</sup>Multizyme) contains Xylanase 16,000 BXU/g, 1,3(4)  $\beta$ -glucanase 2,400 BU/g, Pectinase 210 U/g, Alpha-amylase 2,100 IU/g, Mannanase 3,000 MNU/g, Protease 7 mg and Phytase 1,000 FTU/g.

### Birds' husbandry

The chicks were kept in floor pens at the same area (200 × 150 × 250 cm) in an open house at similar hygienic and normal environmental conditions, using the sawdust as litter at 7 cm deepness. All birds reared on similar managerial conditions and fed the experimental diets *ad libitum* and given free access to water during the whole experimental period.

From one to three days of age, continuous light was used for chicks, then the lighting program of 23 hours of light and one hour of darkness per day was used from the fourth day of life until the end of the experiment. The brooding temperature (indoor) was set initially at 34°C and gradually declined until reach 22°C at the 4<sup>th</sup> and 5<sup>th</sup> weeks, as described previously by Ghareeb *et al.*, (2014). All birds were vaccinated against Newcastle Disease (ND), Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB).

**Table 1. The basal diets and its calculated nutrient content**

Ingredients	Starter (%) ( 1-14 days)	Grower (%) (15-35 days)
Yellow corn (8.8%CP)	58.00	58.80
Corn Gluten (60%CP)	8.58	6.28
Soybean meal (46% CP)	28.10	26.00
Limestone	1.00	0.90
Wheat bran	0	2.00
Soya Oil	1.20	3.20
Di-calcium phosphate	2.30	2.00
DL – Methionine	0.07	0.07
L-Lysine	0.15	0.15
NaCl	0.30	0.30
Vitamins minerals mixture <sup>1</sup>	0.30	0.30
<b>Total</b>	<b>100</b>	<b>100</b>
<b>Chemical composition<sup>2</sup></b>		
Metabolizable energy (kcal/kg diet)	3003.88	3100.29
Crude protein, (%)	23.01	21.04
Crude fiber, (%)	3.44	3.49
Ether extract, (%)	3.84	5.86
Calcium, (%)	1.05	0.93
Available phosphorus, (%)	0.51	0.45
Methionine, (%)	0.53	0.48
Lysine, (%)	1.20	1.13

Vitamins and minerals premix each kg consist of: Vit A, 10000 IU; D3 3000 ICU; Vit E, 10 mg; B1, 5 mg; B6, 1500 mg; B12, 10mg B5, 10 mg; Niacin, 30 mg; Folic acid, 50 mcg; Chloride, 500 mg; copper, 10 mg; iron, 50 mg; Manganese, 60 mg; Zinc, 50mg, and selenium, 0.1 mg according to NRC (1994).

## Data collection

### Carcass Traits

At 35 days of age (end of experiment), 5 birds were taken at random from each replicate, slaughtered; plucked and eviscerated then the dressed weight was obtained. Dressed carcasses were weighed and calculated as relative to live body weight. Breast, back, thigh, drumsticks, internal organs (liver, heart, empty gizzard, pancreas, proventriculus, spleen and bursa), the abdominal fat, blood, feather, (feet and sank) and head were separated, weighed and calculated as relative to live body weight.

Approximately 100g sample of breast muscle from three birds (one per replicate) from each group was stored in freezer -20°C for evaluation of chemical and physical characteristics of meat. After thawing, the moisture, crude protein, ether extract and ash contents were determined according to the procedure described by AOAC (2004). The measurements of drip loss from 0 to 24 hour were determined according to Zhou *et al.*, (2010). Cooking loss was conducted as described by Cai *et al.*, (2018). Water holding capacity (WHC%) was evaluated 5 hours after slaughter, using the methodology described by Hamm (1960).

### Intestine microflora

Excreta samples were obtained directly from bird's intestines after slaughtering in sterile bags and stored on ice for transfer to the lab, where they were prepared for microbiological examination. Each bird's composite excreta sample (One gram) was homogenized after being diluted with 9 ml of 1 percent peptone broth. According to (Difco, 1998), viable counts of bacteria in the excreta samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) in duplicate onto plate count agar medium to count total aerobes, MacConkey agar medium to count *coliform* bacteria and MRS agar medium to count *lactobacillus* colonies. For plate count agar plates were incubated for 48 hours at 32 degrees Celsius. At 37 degrees Celsius, the MacConkey agar plates were incubated for 24 hours. MRS agar plates were incubated at 39°C for 48 hours. The following calculation was used to compute the number of bacteria in the initial volume: Number of bacteria = Number of colonies × (1/Dilution factor) × Cultured volume. Then, the logarithms to base 10 of the obtained values were used in CFU/g for later analyses.

### Statistical analysis

The obtained data were analyzed by the General Linear Model (GLM) procedure of SAS (1998) using one-way ANOVA according to the following model:  $Y_{ij} = \mu + T_i + E_{ij}$

Where,  $Y_{ij}$  = the dependent variable;  $\mu$  = the general mean;  $T_i$  = effect of experimental treatments; ( $i= 1,2;$ )  $E_{ij}$  = the experimental random error. Before analysis, all percentages were subjected to arcsine transformation ( $\log_{10} x + 1$ ) to normalize data distribution. Significant differences of obtained means were determined using Duncan's multiple range tests at the level of  $P < 0.05$  (Duncan,

1955). A level of probability (P. value) of  $\leq 0.05$  was considered significant.

## Results and Discussion

### Carcass traits

#### Edible Parts

Our results presented in Table (2) indicate that broilers of T1 and T2 had the highest ( $P \leq 0.05$ ) percentages of proportional weights of dressed carcass, breast, thigh and drumsticks as compared to control. Also, abdominal fat % was significantly ( $P \leq 0.05$ ) decreased by using probiotic or enzymes as compared to the control. However, the differences in proportional weights of dressing carcass, breast, thigh, drumsticks and abdominal fat were not significant between T1 and T2 groups. On the other hand, there were no significant ( $P > 0.05$ ) differences among all groups in proportional weights of back, heart, liver, gizzard and giblets.

**Table 2. The effect of probiotics and enzymes supplementation on weights of carcass characteristics and abdominal fat as percentage of live body weight**

Items	Treatment			SEM	Sig
	C	T1	T2		
Dressed carcass,	74.08 <sup>b</sup>	77.67 <sup>a</sup>	77.44 <sup>a</sup>	1.38	*
Breast,	25.42 <sup>b</sup>	27.72 <sup>a</sup>	27.50 <sup>a</sup>	1.07	*
Back,	11.28	11.58	11.44	1.07	NS
Thigh,	16.71 <sup>b</sup>	18.12 <sup>a</sup>	17.98 <sup>a</sup>	2.10	*
Gizzard,	2.20	2.21	2.21	0.07	NS
Giblets,	5.09	5.18	5.19	0.10	NS
Abdominal fat,	1.26 <sup>a</sup>	1.09 <sup>b</sup>	1.13 <sup>b</sup>	0.05	*

a and b: Means with different superscripts in the same row are significantly different ( $P \leq 0.05$ ). C= Control, T1= Probiotic, T2= Enzymes, SEM= Standard error of means, Sig= significance, NS= not significant, \*= significant

Our findings agree with the findings of Midilli and Tuncer (2001), who reported that the best carcass and cut up yields were recorded for the enzymes and probiotics supplemented groups of broilers compared with their control. Similarly, Yaqoob *et al.*, (2022) found that enzymes supplementation did not significantly affect liver, gizzard, heart and giblets percentages of broilers. Moreover, Hajati (2010) showed that enzyme supplementation to broiler's diet increased carcass, thighs drumsticks percentage significantly ( $P < 0.05$ ). Also, Algedawy *et al.*, (2011) indicated that addition of probiotic or enzymes in diets for broilers had non-significant effects on the average weights of the giblets (gizzard, heart, liver). Similar results observed by Sarangi *et al.*, (2016), who indicated that addition of probiotic in diets for broilers had no significant effect on heart, gizzard, liver and back weights. According to Ali *et al.*, (2018), abdominal fat% was significantly ( $P < 0.05$ ) decreased by using of probiotic or multi-enzymes in diets of broilers. They also added that using of probiotic or multi-enzymes had insignificant effects on relative weights of heart, liver and gizzard. In addition, Kaushal *et al.*, (2019), stated that probiotic and enzymes supplementation to broiler diets significantly ( $P < 0.05$ ) increased dressed carcass and breast% compared with control, however, they had insignificant effect on relative weight of back. Moreover, Rehman *et al.*, (2020) stated that probiotic did not prove any

significant effect on weights of liver, heart and gizzard for broilers at 1-35 d of age. Nevertheless, Tang *et al.*, (2021) found that probiotic remarkably ( $P < 0.05$ ) increased breast muscle% of broilers compared to control.

On the other hand, the obtained results are in disagreement with Viveros *et al.* (2002), who observed that enzyme supplementation to diets of broiler chicks reduce weight of liver. Moreover, Mohammad *et al.*, (2017) revealed that multi-enzymes insignificant effect on weights of carcass, thigh, breast and drumsticks of broilers. Similarly, Sugiharto *et al.*, (2018) indicated that multi-strain probiotic did not have significant effects on percentages of thigh, breast, abdominal fat and drumsticks of broilers. In addition, Tang *et al.*, (2021) proved that probiotic had insignificant effects on dressed carcass and abdominal fat % of broilers. Also, Yaqoob *et al.*, (2022) showed that multi-enzymes supplementation did not significantly impact on relative weights of dressed carcass, breast, thigh, drumsticks and abdominal fat for broilers.

The enhancement in carcass characteristics (dressed carcass, breast, thigh and drumsticks %) by using probiotic and enzymes might be related to an improvement in the nutrients utilization and may be due to more edible muscle mass. Better fleshing and a more advantageous meat-to-bone ratio in the treated groups may be responsible for the higher dressed yield in the groups supplemented with enzymes and probiotics. Zhou *et al.*, (2015) reported that probiotics could promote intestinal digestion and nutrient absorption and further enhance muscle tissue development through improving the intestinal microflora and composition. Adding exogenous enzymes enhances the energy availability and use of nutrients, thus enhances feed conversion ratio (Shirmohammad and Mehr, 2011), then improve carcass quantity. As seen by Attia *et al.*, (2012), the increase of the intestinal villi, the increase in nutrient release caused by the addition of enzymes made more nutrients available for absorption and, consequently, for biochemical reactions that encourage anabolic reactions and muscle growth. Alam *et al.*, (2003) illustrated that higher carcass yield by addition of enzymes in diet may be due to higher fat deposition in carcass. The decreasing in abdominal fat may be attributed to the beneficial impact of probiotics and enzymes on the distribution of fats inside the body (Ali *et al.*, 2018). Since the primary fat deposition in broiler chickens is abdominal fat, which appears to be closely related to total carcass fat, indicating the fact that probiotics enhance efficient energy usage (Santoso *et al.*, 1995).

**Table 3. The effect of probiotics and enzymes on weights of non-edible parts as percentage of live body weight**

Items	Treatment			SEM	Sig
	C	T1	T2		
<b>Blood</b>	2.78	2.73	2.78	0.10	NS
<b>Feather</b>	5.03	5.08	5.05	0.22	NS
<b>feet and sank</b>	3.46	3.30	3.50	0.08	NS
<b>Head</b>	1.89	1.89	1.91	0.04	NS
<b>Proventriculus</b>	0.33	0.34	0.35	0.02	NS
<b>Pancreas</b>	0.27	0.26	0.28	0.03	NS

C= Control, T1= Probiotic, T2= Enzymes, SEM= Standard error of means, Sig= significance, NS= not significant.

### Nonedible parts

Data presented in Table (3) revealed that supplementation of 1g probiotics or 0.5g enzymes per kg diets for broiler chickens did not have any significant ( $P>0.05$ ) effect on relative weights of non-edible parts (blood, feather, (feet and sank), head, proventriculus and pancreas).

Our findings are in agreement with those obtained by Algedawy *et al.*, (2011) indicated that differences in relative weights of non-edible parts (blood, feathers and pancreas) were not significant due to addition of probiotic or enzymes in diets of broilers. Also, Shabani *et al.*, (2012) reported that weights of pancreas were not significantly ( $P>0.05$ ) affected by adding of probiotic in diets for broilers. Sugiharto *et al.*, (2018) indicated that multi-strain probiotics had insignificant effects on percentages of proventriculus and pancreas of broilers. Moreover, Bharathidhasan *et al.*, (2009) and Bromfield *et al.*, (2021) showed that adding enzyme in diets of broilers did not have statistical effects on pancreas and proventriculus percentages. Similar results obtained by Ali *et al.*, (2018), who revealed that using of probiotic or multi-enzymes in diets of broilers had insignificant effects on relative weights of proventriculus and pancreas. Additionally, relative weight of feet and shank of broilers was not significantly affected by enzymes (Hajati, 2010 and Houssein *et al.*, 2019) or probiotics supplementation (Tang *et al.*, (2021).

**Table 4. The effect of probiotics or enzymes on weights of immune organs as percentage of live body weight**

Items	Treatment			SEM	Sig
	C	T1	T2		
Spleen	0.19	0.20	0.20	0.01	NS
Bursa	0.13	0.13	0.13	0.01	NS

C= Control, T1= Probiotic, T2= Enzymes, SEM= Standard error of means, Sig= significance, NS= not significant.

### Immune organs

Relative weights of spleen and bursa as affected by probiotics or enzymes are shown in Table (4). The present study indicated that the relative weights of spleen of broiler chicks at 35 days of age tended to be higher in the probiotic (T1) and enzymes (T2) treatments than in their control (C), however, data revealed that the differences in values of relative weights of bursa and spleen were not significant ( $P>0.05$ ) among all experimental groups.

The present results are also in accordance with those of several studies which reported that the relative weights of bursa and spleen for broilers were not significantly affected by multi-enzymes (Vahid *et al.*, 2012; Metwally *et al.*, 2020 and Yaqoob *et al.*, 2022) or probiotics supplemented (Sugiharto *et al.*, 2018; Hidayat *et al.*, 2020).

On the other hand, Algedawy *et al.*, (2011) showed that average weights of spleen and bursa were significantly increased ( $P<0.05$ ) with probiotic supplementation as compared to exogenous enzyme mixture for broilers. Sadeghi

*et al.*, (2015) showed that dietary inclusion of probiotics increased the relative weight of spleen, but had no effect on the relative weight of bursa. Also, Ali *et al.*, (2018) revealed that bursa % was significantly ( $P < 0.05$ ) increased by using of multi-enzymes in the diets of broilers.

### Carcass quality

The impact of probiotics or enzymes on carcass quality (chemical and physical characteristics) are shown in Table (5). Data revealed that the probiotics or enzymes did not have any significant ( $P > 0.05$ ) effect on studied physical characteristics (drip loss, cocking loss and WHC). Also, our results revealed that the differences in moisture and ash percentages were not significant ( $P > 0.05$ ) among all groups. While the crude protein percentage was significantly ( $P \leq 0.05$ ) higher in T1 and T2 treatments as compared to the control group, with no significant ( $P > 0.05$ ) differences between T1 and T2 treatments. Ether extract was significantly ( $P \leq 0.05$ ) decreased at using probiotics or enzymes, however, the differences were not statistically between T1 and T2 treatments, or between T2 and the control groups.

**Table 5. The effect of probiotics or enzymes on carcass quality (%)**

Items	Treatment			SEM	Sig.
	C	T1	T2		
<b>Chemical composition</b>					
Moisture,	71.34	71.09	70.96	4.81	NS
Crude Protein	21.77 <sup>b</sup>	23.08 <sup>a</sup>	22.89 <sup>a</sup>	2.26	*
Ether extract	4.10 <sup>a</sup>	3.05 <sup>b</sup>	3.43 <sup>ab</sup>	0.89	*
Ash	1.16	1.19	1.18	0.34	NS
<b>Physical characteristics</b>					
Drip Loss	2.43	2.30	2.34	0.86	NS
Cooking Loss	18.09	17.91	18.00	1.09	NS
WHC	73.60	74.00	73.76	3.27	NS

C= Control, T1= Probiotic, T2= Enzymes, SEM= Standard error of means, WHC= Water holding capacity, Sig= significance, NS= not significant, \*= significant

The results of this study are in harmony with those of Pelícia *et al.*, (2004) who found that multi-enzyme addition to broilers diet resulted in significantly higher meat protein than control. In addition, Zhou *et al.*, (2015) reported that probiotic had insignificant effect on WHC % for broilers. Also, Habib (2016) showed that the WHC % of breast meat for broilers was not significantly affected by enzymes supplementation. Moreover, Mohammad *et al.*, (2017) revealed that multi-enzymes did not prove any significant effects on cocking loss, WHC, ash, moisture and ether extract % in meat of broilers. Moreover, Eltrefi *et al.*, (2017) showed that using of probiotic in diets of broilers had insignificant effects on moisture and ash in breast meat. Additionally, Sugiharto *et al.*, (2018) indicated that multi-strain probiotics did not show significant effects on drip loss % for broilers. Also, Houssein *et al.*, (2019) reported that using of enzymes in diets of broiler chicks had insignificant effects on cocking loss %, WHC %. As well as,



Tang *et al.*, (2021) noted that probiotic had in insignificant effects on moisture and ash % in thigh or breast muscles of broilers. Also, Bromfield *et al.*, (2021) showed that enzyme supplementation to diets of broilers did not have significant effects on moisture, ether extract and ash %.

On the other hand, Pelícia *et al.*, (2004) observed lower meat moisture for broilers received multi-enzyme in their diet compared with control. Additionally, Zhou *et al.*, (2015) and Tang *et al.*, (2021) reported that probiotic significantly decreased drip loss % and cocking loss% in breast and thigh muscles of broilers. Also, Mohammad *et al.*, (2017) revealed that multi-enzymes had insignificant effects on crude protein in meat of broilers. Moreover, Eltrefi *et al.*, (2017) showed that using of probiotic in diets of broilers had insignificant effects on crude protein, ether extract% in breast meat. Yaqoob *et al.*, (2022) showed that multi-enzymes supplementation significantly ( $P < 0.05$ ) decreased cocking loss % of breast meat of broilers.

Increase of protein content of meat may be due to probiotic and enzymes enhancement of the digestion of nutrients, increase digestive enzyme activity, which enhance digestibility of protein and starch and enhance the absorption of nutrient. Falaki *et al.*, (2010) reported that probiotics increase protein availability, improve nutrient intake, and increase nitrogen stability, all of which can have a significant impact on carcass quality. Toghyani *et al.*, (2011) explained that improvement the carcass traits may be associated to the prevention of intestinal pathogen colonization and better nutrient utilization (protein and energy) of the diet when prebiotics were added to the broiler diet. According to Popova (2017), probiotic feeding regimens have a natural potential to improve poultry meat quality in vivo due to the improvement of the intestinal microbiota and the decrease in the intestinal load of pathogenic bacteria which in turn improve the health and performance of the birds as well as the quality of their meat.

**Table 6. The effect of probiotics and enzymes on intestine microflora**

Treatment \ Items	Total count (log <sup>10</sup> CFU/g)	<i>Lactobacillus</i> (log <sup>10</sup> CFU/g)	<i>Coli form</i> (log <sup>10</sup> CFU/g)	Lacto/Coli (log <sup>10</sup> CFU/g)
C	8.25 <sup>a</sup>	6.16 <sup>c</sup>	3.95 <sup>a</sup>	1.58 <sup>c</sup>
T1	6.54 <sup>c</sup>	7.69 <sup>a</sup>	3.29 <sup>b</sup>	2.35 <sup>a</sup>
T2	7.60 <sup>b</sup>	6.97 <sup>b</sup>	3.37 <sup>b</sup>	2.08 <sup>b</sup>
SEM	0.25	0.29	0.16	0.21
Sig.	***	**	**	**

a, b and c: Means with different superscripts in the same column are significantly different ( $P < 0.05$ ). C= Control, T1= Probiotic, T2= Enzymes, SEM= Standard error of means, Sig.= significance, \*\*= highly significant, \*\*\* =very highly significant

### Intestinal microflora

The results shown in Table (6) indicate that the highest ( $P < 0.05$ ) total count of bacteria was recorded for the control group, followed by those of T2 group, and the lowest ( $P < 0.05$ ) total count of bacteria was recorded for T1 group. *Lactobacillus* bacteria count was significantly ( $P < 0.05$ ) higher in T1 group,

followed by T2 group, while the lowest ( $P < 0.05$ ) *Lactobacillus* bacteria count was evaluated in the control group. The highest ( $P < 0.05$ ) *coliform* bacterial count was recorded in the control group as compared to T1 and T2 groups, however, no statistical ( $P > 0.05$ ) differences were proved between T1 and T2 groups *incoliform* bacterial count. The present study illustrated that the Lacto: Coli ratio was significantly ( $P < 0.05$ ) higher in T1, followed by T2 treatment, and the lowest ( $P < 0.05$ ) value of Lacto: Coli ratio was evaluated for the control group.

Similar findings were observed by Djouvinov *et al.*, (2005), who stated that total bacterial count and coliform bacteria in intestinal were significantly ( $P < 0.05$ ) decreased but *Lactobacillus* population was significantly ( $P < 0.05$ ) increased by adding probiotic at 300g/ton feed for ducklings as compared to control diet. Also, Teo and Tan (2007) explained that coliform bacteria count for male broilers (Ross) chicks received probiotic contain *B. subtilis* at levels  $10^8$  and  $10^9$ CFU/kg diets remarkably ( $P < 0.05$ ) decreased compared with control at 42 days of age. Additionally, Mountzouris *et al.*, (2010) illustrated that populations of coliforms spp. in caecum of broilers (Cobb) were significantly ( $P < 0.05$ ) decreased by adding probiotic) at concentration of  $10^{10}$ CFU/kg diet. Moreover, they also showed that broilers received probiotic (had the highest ( $P < 0.05$ ) *Lactobacillus* spp. concentration than control group at 42 days of age. Also, Kazemi *et al.*, (2019) stated that using of probiotic at level 150g/ton feed significantly ( $P < 0.05$ ) increased *Lactobacillus* population in ileum of broiler chickens (Ross 308) as compared to control at 42 days of age. In contrast, Cengiz *et al.*, (2015) revealed that probiotic at levels of 1 and 0.5g/kg starter and finisher diets, respectively for broilers (Ross 308) did not have significant ( $P > 0.05$ ) effects on total aerobic and *Lactobacilli* bacterial counts during the period from 1 to 42 days of age.

As for enzyme effects on bacterial count, enzymes supplementation at level 200g/ton feed of broiler chickens significantly ( $P < 0.05$ ) increased *Lactobacillus* and decreased coliform bacteria count in ileum during the period of 1-49 days of age (Ohimain and Ofongo 2013). Shakouri *et al.*, (2009) indicated that supplementation of enzymes for broilers (Cobb) did not have statistical ( $P > 0.05$ ) effects on *Lactobacillus*, coliform and total bacteria count in ileum during the period of 1-28 days of age.

It's possible that the considerable rise in *Lactobacilli* colony count in the probiotic group is related to the fact that it helps to balance the intestinal microecosystem by regulating harmful bacteria through a competitive reaction that boosts the number of helpful bacteria. Probiotics have a number of important mechanisms of action, including an antagonistic effect on pathogen bacteria by altering gut pH, a direct antimicrobial effect by secreting products that inhibit their development, such as bacteriocins, organic acids, and hydrogen peroxide, production of short chain fatty acids in the intestine, regulation of the host's immune system, normalisation of gut microbiota, and various metabolic effects (Emanuel and Adrian, 2010 and Ferreira *et al.*, 2011). According to Spring *et al.*, (2000), the significant reduction in *coliform* bacteria count can be due to that probiotics contain various beneficial bacteria that coat the intestinal villi and

prevent *coliform* bacteria from sticking to the intestinal wall of broilers. The reduction in pH of the GUT is considered as an effective means of preventing potentially pathogenic bacteria such as *coliform* and *salmonella* from entering the lower part of the GIT (Bjerrum *et al.*, 2005).

## Conclusion

Based on the results of this study, it can be concluded that adding of 1g probiotics or of 0.5g multienzymes /kg diet are recommended to obtain the best carcass quantity and quality, also they enhance *Lactobacillus* bacterial count in intestine of broiler chickens (Ross-308) at 0-5 weeks of age.

## References

- Alam, M.J., Howlider, M.A.R., Pramanik, M.A.H. and Haque, M.A. (2003). Effect of exogenous enzyme in diet on broiler performance. *International Journal of Poultry Science*, 2(2): 168-173.
- Algedawy, S.A., Soliman, M.K., Elashmawy, I.M. and El-Sayed, Y.S. (2011). Effect of commercial probiotic and exogenous enzymes on the growth performance, immune response, and hemato-biochemical parameters of broiler chickens. In *Proceedings of the 4<sup>th</sup> Scientific Conference of Animal Wealth Research in the Middle East and North Africa*, Foreign Agricultural Relations (FAR), Egypt, 3-5 October 2011 (pp. 430-445). Massive Conferences and Trade Fairs.
- Ali, R.A.M., El-Shafey, A.S. and El-Kelawy, M.I. (2018). Immuno physiological and productive response of broiler chicks to dietary supplementation with multi-enzyme and/or probiotics. *Egyptian Poultry Science Journal*, 38(4): 1047-1067.
- Anjum, M. S., and Chaudhry, A.S. (2010). Using enzymes and organic acids in broiler diets. *The Journal of Poultry Science*, 47: 79-105.
- AOAC, (2004). *Official methods of analysis*. 18th ed., Association of Official Analytical Chemists, Washington, DC, USA.
- Attia, Y.A., El-Tahawy, W.S., Abd Al-Hamid, A., Hassan, S., Nizza ,A. and El-Kelawy, M.I. (2012). Effect of phytate with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. *Italian Journal Animal Science*, 11: 303-308.
- Bharathidhasan, A., Chandrasekaran, D., Natarajan, A., Ravi, R. and Ezhilvalavan, S. (2009). Effect of enzyme supplementation on carcass quality, intestinal viscosity and ileal digestibilities of broilers to nutrient reduced diet. *Tamilnadu Journal of Veterinary and Animal Sciences*, 5(6): 239-245.
- Bjerrum, L., Pedersen, K., and Engberg, R.M. (2005). The influence of whole wheat feeding on *Salmonella* infection and gut flora composition in broilers. *Avian Diseases*, 49(1): 9-15.
- Bromfield, J.I., Hoffman, L.C., Horyanto, D. and Soumeh, E.A. (2021). Enhancing growth performance, organ development, meat quality, and bone mineralisation of broiler chickens through multi-enzyme super-dosing in reduced energy diets. *Animals*, 11(10): 2791.

- Cai, K., Shao, W., Chen, X., Campbell, Y., Nair, M., Suman, S.P., ... and Schilling, M.W. (2018). Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. *Poultry Science*, 97(1): 337-346.
- Cengiz, Ö., Köksal, B.H., Tatlı, O., Sevim, Ö., Ahsan, U., Üner, A.G. and Önel, A.G. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. *Poultry Science*, 94(10):2395-2403.
- Deniz, G., Orman, A.B.D., Cetinkaya, F., Gencoglu, H., Meral, Y. and Turkmen, I.I. (2011). Effects of probiotic (*Bacillus subtilis* DSM 17299) supplementation on the caecal microflora and performance in broiler chickens. *Revue de Medecine Veterinaire*, 162(11): 538-545.
- Dhawan, S., and Kaur, J. (2007). Microbial mannanases: an overview of production and applications. *Critical Reviews in Biotechnology*, 27(4): 197-216.
- Difco, (1998). *Difco Manual*. 11<sup>th</sup> Ed., Difco Laboratories. Division of Becton. Dickinson and Company, Sparks, Maryland, USA.
- Djouvinov, D., Boicheva, S., Simeonova, T. and Vlaikova, T. (2005). Effect of feeding lactina probiotic on performance, some blood parameters and caecal microflora of mule ducklings. *Trakia Journal of Sciences*, 3(2): 22-28.
- Duncan, D.B. (1955). Multiple range and multiple tests. *Biometrics*, 11: 1-42.
- Eltrefi, A.M. E., Mohamed, A.A., Habib, A.B., Abu shulukh, E.S., Abubaker, A.A., Mohammed, M.F., Ibrahim, G.A. and Abdelwahid, H.H. (2017). Effect of different levels of probiotic, biogen, on performance and carcass characteristics of broiler chickens, *International Journal of Recent Trends in Engineering and Research*, 3(7):82-90.
- Emanuel, V., and Adrian, V. (2010). The influence of prebiotics on bacteriocin synthesis using the strain *Lactobacillus paracasei* CMGB16. *African Journal of Microbiology Research*, 4(7): 534-537.
- Falaki, M., Shargh, M., Dastar, B. and Zrehdaran, S. (2010). Effects of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. *Journal of Animal and Veterinary Advances*, 9(18): 2390-2395.
- Ferreira C.L., Salminen, S., Grzeskowiak, L., Brizuela, M., Sanchez, L., Carneiro and H. and Bonnet, M. (2011). Terminology concepts of probiotic and prebiotic and their role in human and animal health. *Revista de Salud Animal*, 33(3): 137–146.
- Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology*, 66(5): 365-378.
- Furrie, E., Senok, A.C., Frank, D.N., and Sullivan, K.E. (2006). Pondering probiotics. *Clinical Immunology – Journals*, 121:19–22.
- Ghareeb, K., Awad, W.A., Sid-Ahmed, O.E. and Böhm, J. (2014). Insights on the host stress, fear and growth responses to the deoxynivalenol feed contaminant in broiler chickens. *PloS one Journal*, 9(1): e87727.

- Habib, A. B., Mohamed, A.A., Eltrifi, A.M., Abu-Shullukh, E.S. and Abubaker, A.A. (2016). Effect of feed supplemented with xylam enzyme on performance, carcass characteristics and meat quality of broiler chicks. *Journal of Applied Veterinary Sciences*, 1(1): 15-20.
- Hajati, H. (2010). Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. *American Journal of Animal and Veterinary Sciences*, 5(3): 221-227.
- Hamm R. (1960). Biochemistry of meat hydration. *Advances in Food Research*; 10(2):335-443.
- Hassan, M. R., and Ryu, K.S. (2012). Naturally derived probiotic supplementation effects on physiological properties and manure gas emission of broiler chickens. *Journal of Agriculture and Life Science*, 46(4): 119-127.
- Hidayat, M.N., Malaka, R., Agustina, L. and Pakiding, W. (2020). Effect of probiotic *Lactobacillus paracasei* on hematology and relative weight of lymphoid organs of broiler. In *IOP Conference Series: Earth and Environmental Science*. 492(1): 012127.
- Hussein, E.O., Suliman, G.M., Abudabos, A.M., Alowaimer, A.N., Ahmed, S.H., El-Hack, A., ... and Laudadio, V. (2019). Effect of a low-energy and enzyme-supplemented diet on broiler chicken growth, carcass traits and meat quality. *Archives Animal Breeding*, 62(1): 297-304.
- Kaushal, S., Sharma, R.K., Singh, D.V., Shukla, S.K., Kumar, S., Palod, J. and Singh, M.K. (2019). Performance, carcass characteristics and economics of broiler chickens fed dietary enzymes and probiotic. *Iranian Journal of Veterinary Research*, 20(4): 293.
- Kazemi, S.A., Ahmadi, H. and Karimi-Torshizi, M.A. (2019). Evaluating two multistrain probiotics on growth performance, intestinal morphology, lipid oxidation and ileal microflora in chickens. *Journal of Animal Physiology and Animal Nutrition*, 103(5):1399-1407.
- Lee, K., Lillehoj, H.S. and Siragusa, G.R. (2010). Direct-fed microbials and their impact on the intestinal microflora and immune system of chickens. *The Journal of Poultry Science*, 47:106-114.
- Mahajan, P., Sahoo, J. and Panda, P.C. (2000). Effect of probiotic (Lacto-Sacc) feeding and seasons on the different quality characteristics of poultry meat. *Indian Journal of Poultry Science*, 35(3): 297-301.
- Meng, X., and Slominski, B.A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poultry Science*, 84(8): 1242-1251.
- Metwally, M.A., Farghly, M.F.A., Ismail, Z.S.H., Ghonime, M.E. and Mohamed, I.A. (2020). Effect of different levels of optizyme and phytase enzymes and their interactions on the performance of broiler chickens fed corn/soybean meal: 3. European production efficiency factor, european broiler index and some immune organs. *Egyptian Journal of Nutrition and Feeds*, 23(3): 507-514.

- Midilli, M., and Tuncer, Ş.D. (2001). The effects of enzyme and probiotic supplementation to diets on broiler performance. *Turkish Journal of Veterinary and Animal Sciences*, 25(6): 895-903.
- Mohammad, A.A., Habib, A.B., Eltrefi, A.M., Abu Shulukh, E.S. and Abubaker, A.A. (2017). Research article effect of different levels of multi-enzymes (Natuzyne Plus®) on growth performance, carcass traits and meat quality of broiler chicken. *Asian Journal of Animal and Veterinary Advances*, 13: 61-66.
- Montanhini Neto, R., Ceccantini, M.L. and Fernandes, J.I.M. (2013). Immune response of broilers fed conventional and alternative diets containing multi-enzyme complex. *Brazilian Journal of Poultry Science*, 15: 223-231.
- Mountzouris, K.C., Tsitsirikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G. and Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulin, and cecal microflora composition. *Poultry Science*, 89(1): 58-67.
- N.R.C (1994). *Nutrient Requirements of Poultry*. 9<sup>th</sup> Review Edition, National Academy Press, Washington, D.C.
- Ohimain, E.I., and Ofongo, R.T. (2013). Effect of enzyme supplemented diet on gut microflora, digesta pH and performance of broiler chickens. *The Journal of Microbiology, Biotechnology and Food Sciences*, 3(2): 127.
- Pelícia, K., Mendes, A., Saldanha, E., Pizzolante, C., Takahashi, S., Moreira, J., Garcia, R., Quinteiro, R., Paz, I. and Komiyama, C. (2004). Use of prebiotics and probiotics of bacterial and yeast origin for free-range broiler chickens. *Revista Brasileira de Ciência Avícola*, 6: 163-169.
- Popova, T. (2017). Effect of probiotics in poultry for improving meat quality. *Current Opinion in Food Science*, 14: 72-77.
- Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M., Alagawany, M., Abd, El-Hack M., and Swelum, A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. *Poultry Science*, 99(12): 6946-6953.
- Rinkinen, M., Jalava, K., Westermarck, E., Salminen, S. and Ouwehand, A.C. (2003). Interaction between probiotic lactic acid bacteria and canine enteric pathogens: a risk factor for intestinal *Enterococcus faecium* colonization. *Veterinary Microbiology*, 92(1-2): 111-119.
- Sadeghi, A.A., Shawrang, P. and Shakorzadeh, S. (2015). Immune response of Salmonella challenged broiler chickens fed diets containing Gallipro®, a Bacillus subtilis probiotic. *Probiotics and Antimicrobial Proteins*, 7(1): 24-30.
- Santoso, U., Tanaka, K. and Ohtani, S. (1995). Effect of dried Bacillus subtilis culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. *British Journal of Nutrition*, 74: 523-529.
- Sarangi, N.R., Babu, L., Kumar, A., Pradhan, C.R., Pati, P.K. and Mishra, J.P. (2016). Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth

- performance and carcass characteristics of broiler chickens. *Veterinary World*, 9(3): 313.
- SAS (1998). *SAS Users Guide: Statistics*. SAS Institute Inc., Cary, NC.
- Shabani, R., Nosrati, F.J. and Kioumars, H. (2012) The effect of probiotics on carcass and internal organs of broilers. *Annals of Biological Research*, 3: 5475-5477.
- Shakouri, M.D., Iji, P.A., Mikkelsen, L.L. and Cowieson, A.J. (2009). Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *Journal of Animal Physiology and Animal Nutrition*, 93(5): 647-658.
- Shirmohammad, F. and Mehri, M. (2011). Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks. *African Journal of Biotechnology*, 10(38):7541-7547.
- Spring, P., Wenk, C., Dawson, K.A. and Newman, K.E. (2000). The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella challenged broiler chicks. *Poultry Science*, 79: 205-211.
- Sugiharto, S., Isroli, I., Yudiarti, T. and Widiastuti, E. (2018). The effect of supplementation of multistrain probiotic preparation in combination with vitamins and minerals to the basal diet on the growth performance, carcass traits, and physiological response of broilers. *Veterinary World*, 11(2): 240.
- Tang, X., Liu, X. and Liu, H. (2021). Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. *Frontiers in Veterinary Science*, 8.
- Teo, A.Y. and Tan, H.M. (2007). Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). *Journal of Applied Poultry Research*, 16(3): 296-303.
- Toghyani, M., Toghyani, M. and Tabeidian, S.A. (2011). Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. In *International Conference on Food Engineering and Biotechnology*. 9:82-86.
- Vahid, K., Abolghasem, G., and Hassan, K. (2012). Immune response and ileal microflora in broilers fed wheat-based diet with or without enzyme Endofeed W and supplementation of thyme essential oil or probiotic PrimaLac®. *African Journal of Biotechnology*, 11(81): 14716-14723.
- Viveros, A., Brenes, A., Arija, I., and Centano, C. (2002). Effect of microbial phytase supplementation on mineral utilization and serum enzyme activities. In broiler chicks fed different levels of phosphorus. *Poultry Science*, 81:1172-1183.
- Yaqoob, M.U., Yousaf, M., Iftikhar, M., Hassan, S., Wang, G., Imran, S., and Wang, M. (2022). Effect of multi-enzymes supplementation on growth performance, meat quality, ileal digestibility, digestive enzyme activity and caecal microbiota in broilers fed low-metabolizable energy diet. *Animal Bioscience*, 35(7): 1059.

- Zhou, X., Jin, E., Li, S., Wang, C., Qiao, E. and Wu, G. (2015). Effects of dietary supplementation of probiotics (*Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus natto*) on broiler muscle development and meat quality. *Turkish Journal of Veterinary and Animal Sciences*, 39(2): 203-210.
- Zhou, X., Wang, Y., Gu, Q. and Li, W. (2010). Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*, 89(3): 588-593.



## تأثير اضافة البروبيوتك أو الانزيمات في علائق دجاج التسمين على صفات الذبيحة والبكتريا المعوية

محمد أحمد الصغير<sup>1</sup>، يسن محروس محمد مهدي<sup>1</sup>، نادي محمد عيسى<sup>1</sup>، محمد الصغير محمد<sup>2</sup>

<sup>1</sup> قسم الإنتاج الحيواني- كلية الزراعة- جامعة الأزهر – أسيوط- مصر

<sup>2</sup> قسم إنتاج الدواجن - كلية الزراعة - جامعة أسيوط – أسيوط - مصر

### الملخص

الأهداف الرئيسية للدراسة الحالية هي تحديد كيفية تأثير البروبيوتك أو الإنزيمات على صفات الذبيحة والميكروفلورا المعوية لدجاج اللحم (Ross-308). تم استخدام عدد 135 كتكوت تسمين، عمر يوم، غير مجنس، تم توزيعها عشوائياً على ثلاث مجموعات تجريبية، وتم تقسيم كل مجموعة إلى ثلاث مكررات، كل منها 15 كتكوت. المجموعة الأولى تناولت العلائق الغذائية الأساسية (بادئ لمدة اسبوعين، نامي لمدة 3 اسابيع) فقط كمجموعة تحكم (C)، المجموعة الثانية (T1) تم تغذيتها على العليقة الأساسية مع اضافة بروبيوتيك (Guardizen-M) بمستوى 1 جم / كجم علف، بينما المجموعة الثالثة (T2) تم تغذيتها على العليقة الأساسية مع اضافة إنزيمات (Fra<sup>®</sup>Multizyme) بمستوى 0.5 جم / كجم علف. استمرت الدراسة لمدة 35 يوماً من العمر. أشارت النتائج إلى أن النسب المئوية للذبيحة والصدر والفخذ والدبوس كانت أعلى معنوياً ( $P \leq 0.05$ ) في المجموعتين T1 و T2 مقارنة مع مجموعة التحكم، كما انخفضت نسبة الدهون في منطقة البطن معنوياً ( $P \leq 0.05$ ) باستخدام البروبيوتيك أو الإنزيمات، أيضاً لم يتم الحصول على فروق معنوية في النسب المئوية للخصائص الفيزيائية للذبيحة (الفقد بالتنقيط، الفقد بالطبخ، معدل الاحتفاظ بالماء) بين جميع المجموعات. لم تتأثر نسبة الحوائج معنوياً باستخدام البروبيوتيك أو الإنزيمات. زادت بكتريا Lactobacillus المعوية معنوياً ( $P \leq 0.05$ ) وانخفضت بكتريا القولون معنوياً ( $P \leq 0.05$ ) في المجموعتين T1 و T2 مقارنة بمجموعة السيطرة. وفقاً لهذه النتائج، يمكن استنتاج أن استخدام 1 جرام من البروبيوتيك (Guardizen-M) أو 0.5 جرام إنزيمات (Fra<sup>®</sup>Multizyme) / كجم علف لها تأثيرات إيجابية على صفات الذبيحة وميكروفلورا الأمعاء لدجاج اللحم (Ross-308). وبالتالي، يوصى باستخدام هذه المواد تحت هذه المستويات في علائق دجاج التسمين سلالة (Ross-308).