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IMMUNOPHYSIOLOGICAL AND PRODUCTIVE RESPOSE OF BROILER CHICKS TO DIETARY SUPPLEMENTATION WITH MULTI-ENZYME AND / OR PROBIOTICS

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ABSTRACT: The main objectives of this study were to elucidate the effect of multi-enzyme and/or probiotics supplementation on performance, antioxidant status, some blood biochemical parameters and immune responses of broiler chickens. A total of 120, unsexed day-old, broiler chicks were randomly assigned into four groups of 30 chicks in five replicates, six birds each. The first group was used as control and fed the basal diet while, chicks in the 2nd,3rd and 4th groups were fed the basal diet supplemented with multi enzymes (Avizyme at 0.20 g/kg diet), probiotics (Biacton at 0.50 g/kg diet) either singly or in combination. Results showed that live body weight and body weight gain were significantly improved by dietary treatments compared to control group. All treatments had significantly increased dressing and total edible parts (%) and decreased abdominal fat, with the best results being achieved by the combined multi-enzyme and probiotic supplementation. Blood constituents (plasma proteins and lipid fractions, thyroid hormones, especially T_3 and glucose) were significantly affected by treatments. There are significant improvements in hematological traits including RBCs and WBCs counts, hemoglobin concentration, PCV (%), MCV, MCH and lymphocytes (%). Antioxidant status indices and enzymes including TAC, GSH, and SOD did not significantly affected by different treatments, however, glutathione peroxidase (GPX) activity was significantly higher in broilers fed the supplemented diets compared to the control one. Immune responses in terms of the main lymphoid organs weight (Bursa and Thymus), immunoglobulins (α–globulin, β–globulin, γ-globulin, IgA, and IgG), Phagocytic activity (PA), Phagocytic index (PI), lysozyme activity (LA), Bactericidal activity (BA) and Lymphocyte transformation test (LTT) were significantly improved by treatments compared with the control. It is concluded that a mixture of 0.2g of multi-enzyme plus 0.5g of probiotics /kg diet could be used to improve growth traits and enhance immunity of broiler chicks.

Keywords: Enzymes – probiotics - immune response -blood parameters - broilers.

INTRODUCTION

Nowadays, poultry industry facing numerous challenges, the most important one is the health risks and diseases that caused by pathogenic bacteria, fungi and viruses. These challenges elevate poultry mortality, economic cost and decrease of meat quality and quantity. Antibiotics were the most commonly used supplements to poultry diets due to their role as growth promoters through limiting the growth of pathogenic microorganisms and their toxins, enhancing the growth performance and carcass quality (Slizewska et al., 2006). Nevertheless, there are many problems of using antibiotics as growth promoters because its effect on human health, residues in meat and establishment of antibiotic-resistant strains of bacteria (Afsharmanesh and Sadaghi, 2014). Thus, the European Union (EU) banned the use of antibiotic growth promoters in poultry nutrition since 2006 (Dankowiakowska et al., 2013) in accordance with the directive low No.A5-0373/2002. This prohibition initiates poultry producers to search for alternative growth promoters to antibiotics. Probiotics and prebiotics were the best choice in this respect, where probiotics addition to poultry diets caused an improvement in growth and feed conversion ratio (Kalavathy et al., 2003 and Mountzouris et al., 2010). Moreover, probiotics are used to help maintain healthy microbial balance within the gastrointestinal tract and inhibit a wide range of gram – negative and gram-positive bacteria (Jin et al., 1997). This is accomplished through three main mechanisms: competitive exclusion, bacterial antagonism and stimulation of immune system (Hume, 2011 and Ohimain and Ofongo, 2012). Furthermore, several studies revealed that probiotics can enhance humeral immune response and immune organs function. In this respect, Shoeib et al. (1997) reported that the bursa of Fabricius of probiotic-fed chickens showed an increase in the number of follicles with high plasma cells reaction in bursa medulla. Additionally, Tollba and Mahmoud (2009) observed that erythrocytes and lymphocytes were significantly increased while heterophils count decreased due to probiotic feeding. However, Mountzouris et al. (2010) found that probiotic inclusion to poultry diets had no effect on systemic humeral immune response because the concentration of IgA, IgM, IgG and total immunoglobulins did not differ between probiotic fed and control group.

Another practical approach to enhance productive performance of broiler chicks was the use of exogenous enzymes in poultry diets. It is well know that enzymes, even in small quantity, can stimulate and/or accelerate the rate of chemical reactions that transform as dietary substance into production of biological importance for broilers growth and production (Gupta et al., 2014 and El- Sanhoury et al., 2017).

Thus, exogenous enzymes have been reporter to improve nutrients utilization and increasing the digestibility of fibrous materials (Anjum and Chaudhry, 2010). Indeed, the high inclusion rate of cereal grains in broiler diets may result in poor growth and feed conversion ratio; sticky droppings; increase of pathogenic bacteria in the intestine and carcass downgrades (Choct, 2006 and Attia et al., 2014). Such negative effects are usually associated with the presence of high levels of non–starch polysaccharides (NPS) in cereal gains such as corn, wheat and barley (Olukosi et al., 2007). Amerah et al. (2017) studied the effect of an

enzyme complex (containing protease; amylase; beta-glucanase; xylanase; pectinase; celluloseand phytase) addition to broiler diets and found that enzymes had a positive effect on live body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR).

Therefore, the present study was carried out to further investigate the physiological and immunological responses of broiler chicks to dietary supplementation with probiotics, or multi – enzyme preparation, either singly or in combination. Blood parameters and oxidative status indicators were also studied.

MATERIALS AND METHODS

The present study was conducted at the Poultry Research Station, Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Egypt, from Mach to April 2017.

One hundred and twenty unsexed one-day-old Cobb broiler chicks obtained from commercial hatchery, were randomly distributed into four groups of 30 chicks, in five replicates of 6 birds each. Chicks were submitted to the following dietary treatments: the 1st group was used as control and fed the basal diet without any supplementation while chicks in the 2nd,3rd and 4th groups were fed the basal diet supplemented with multienzyme (Avizyme at 0.20 g/kg diet, MEnz), probiotics (Biacton at 0.50 g/kg diet, Prob) and multienzyme plus probiotics (0.20 g Avizyme /kg diet + 0.50g/kg Biacton, MEnz+Prob), respectively. The enzyme complex preparation Avizyme® is a product of Danisco Animal Nutrition, Marlborough, and Wiltshire, UK. It contains 1500 U/g endo-1, 4-β-xylanase, 2000 U/g α-amylase and 20000 U/g protease and its recommended dose for broiler and turkey diets is 0.20 g per kg diet.

Biacton is a product of Chemvet DK A/S, Denmark. It consists of Lactobacillus farciminis CNCM MA67/4R at a concentration of 1 x 109 CFU g-1, together with the carrier materials from waxy maize corn starch (19.8%), yeast extract (7.5%), oil-free soybean lecithin (2.4%), and corn starch (70.1%). Its recommended dose for use in broiler diets is 0.5 kg per ton feed. The experimental diets were formulated according to NRC (1994) Table 1. The chicks were housed in wire cages (60 × 50 × 40 cm) provided with galvanized feeders and automatic nipple drinkers in semi-opened room equipped with two exhaust fans to keep normal ventilation. They reared on similar managerial conditions and fed the experimental diets (ad libitum) and given free access to water. A light schedule similar to commercial conditions was applied until the 7th day being 23 h light followed by 20 h light from the 8th day until 3 days before slaughter (8-32 days of age). The average outdoor minimum and maximum temperature and relative humidity during the experimental period was 22C^{o} and 24 C^{o} and 55.7 % and 58.7%, respectively. The brooding temperature (indoor) was 32, 30, 27 and 24-21 C^{o} during 1-7, 8-14, 15-20 and 21-35 days of age (declined gradually). Chicks were vaccinated against the most common diseases such as Newcastle Disease; Infectious Bursal Disease (IBDV) and Infectious Bronchitis (IB).

Data collection:

Growth performance traits including live body weight (LBW) at 1, 21 and 35 days of age were recoded for each group. At 35 d of age, 5 chicks were taken randomly from each treatment, slaughtered; plucked and eviscerated then

the dressed weight was obtained. The dressed carcasses were then divided into front (breast) and hind (Femur) parts and their weights were recorded. Liver, gizzard, heart and spleen were separated and individually weighed. The carcass parts weights were expressed as relative to live body weight. A sample of breast and thigh meat (50:50 basis) were analyzed for dry matter (DM), protein, fat and ash according to AOAC (2004). Meat tenderness and water holding capacity (WHC) were measured according to the method of Volvoinskaia and Kelman (1962). Color intensity of meat and drip were determined according to the method of Husani et al. (1950), whereas pH value was measured by a pH meter as described by Aitken et al. (1962).

Blood Parameters:

Blood samples were collected in heparinized tubes from five birds / treatment group at 35 d of age. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at -18 °C until analysis. Biochemical constituents of plasma were measured by using available Commercial Kits. Glucose concentration (mg/dl) according to Trinder (1969), total protein (g/dl) according to Henry et al. (1974), albumin (g/dl) according to Doumas (1971), and different types of globulin (α-globulin, β-globulin and γ-globulin) according to Bossuyt et al. (2003), serum globulin concentration was calculated. Moreover, serum levels of creatinine and urea were also determined using method of Bartles et al. (1972), triglycerides according to Fossati and Prencipe (1982), total cholesterol according to Stein (1986), HDL-cholesterol according to Lopez-Virella et al., (1977), and LDL-cholesterol according to Friedewald et al. (1972). Alkaline phosphatase (ALP) concentration was determined according to the colorimetric method of Bauer (1982).

Besides, the activity of serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT), were estimated according to Reitman and Frankel (1957) using commercial kits. Serum samples were assigned also for determination of total antioxidant capacity (TAC) according to Koracevic et al. (2001), superoxide dismutase (SOD) activity according to Misra and glutathione peroxidase (GPX) activity according to Paglia and Valentine (1967) and blood reduced glutathione (GSH) concentration according to Ellman, (1959). Phagocytic activity and index were determined according to Kawahara et al. (1991). Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells, while Phagocytic index (PI) = Number of yeast cell phagocytized/ Number of phagocytic cells. Plasma Immunoglobulins (IgY, IgM and IgA) were also determined using commercial ELISA kits (Biomedical Company, USA) according to Bianchi et al. (1995).

Statistical Analysis:

Data were analyzed by the GLM procedure (Statistical Analysis System (SAS), 2002) using one-way ANOVA with the following model:

$Y_{ik} = \mu + T_i + e_{ik}$

Where Y is the dependent variable; μ is the general mean; T_i is the effect of experimental treatments; e_{ik} is the experimental random error.

Before analysis, all percentages were subjected to arcsine transformation ($\log_{10} x + 1$) to normalize data distribution. The significance of the differences among means was determined using Duncan's new multiple range test (Duncan, 1955) (at $P < 0.05$).

RESULTS AND DISCUSSION

Results showed that the initial live body weight (LBW) of chicks did not differ between all treatments indicative of normal distribution of chicks within treatments (Table 2). However, at 21 days of age birds fed the basal diet supplemented with MEnz and Prob either singly or in combination (MEnz+Prob) had significantly heavier LBW compared with the control group. The heavier LBW was recorded for chicks of the MEnz+Prob treatment group, being higher by 9.41,3.21 and 3.72% than those fed the control, MEzn and Prob supplemented diets, respectively. Similarly, at 35 days of age, LBW of MEnz+Prob-fed chicks was significantly higher than the other treatment groups and the control one. The percentage of this increase was 14.59; 8.68 and 7.76 % compared with chicks of the control, MEnz and Prob –fed groups, respectively. A similar trend was also observed for body weight gain (BWG) where chicks fed the experimental diets achieved the best gain compared with the control group. Thus, during the period from 0-21d, the control chicks had the lowest ($P< 0.05$) BWG. Moreover, during the next period of growth (21-35 d), chicks fed the MEzn + Prob diet had significantly higher BWG than the other treatment groups (MEzn and Prob). This increase in BWG was also recorded for the entire period of the experiment (0-35d) where the MEzn + Prob had significantly the best BWG followed by those fed Prob and then MEnz diets. The control chicks had the lowest BWG during the whole period. It appears from the previous results that the improvement in LBW and BWG of chicks is due to the beneficial effects of both the MEnz and or Prob either singly or in combination. The role of lactobacillus bacteria (the main component of the Prob used in our study) in promoting broiler growth may be related to its effect on digestive tract microflora either by competitive exclusion or antagonism. It is possible also that the enhanced growth performance of chicks may be due to reduction of bacterial utilization of essential nutrients, by altering intestinal PH and stimulating digestive enzymes synthesis and release. This concept was reported by many authors who postulated several mechanisms for probiotics effect on performance of broiler chicks (Denli et al.,2003; Stanley et al.,2004 and Madkour et al.,2008).

On the other hand, enzymes supplementation to broiler diets was known as a practical approach to enhance digestion of fibrous compounds present in cereal grains and their cell wall. Since, yellow corn is the main ingredient in poultry nutrition, enzymes has better influence in facilitating nutrients digestion, mainly because broilers lack enzymes that digest non-starch polysaccharides (NSP) presented in cereal grains. Thus the improvement of growth (LBW) by MEnz supplementation is due to its content of xylanase, alpha amylase and protease which facilitates digestion of cereals (corn) and enhanced nutrients availability and digestibility of diets. These results are in harmony with those reported by Olukosi et al. (2007) and Bedford and Partridge (2010) who used enzyme mixtures containing Xylanase, Protease and α-amylase in poultry diets.

The improvement in the body weight in this study may be due to the increased efficiency of digestion and nutrient absorption processes due to presence of both enzyme and probiotic bacteria. Edens (2003) reported that the inclusion

of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. As a consequence, there is an increase in the efficiency of digestion and nutrient absorption processes.

Carcass Characteristics:

Table 3 shows the effect of different treatments on carcass traits and some organs weight of broiler chickens. Results revealed a significant increase in the dressing percentage of chicks from different treatments compared by the control ones. This was also observed for the total edible parts, although the value of the control chicks did not differ significantly from that of the Prob- fed chicks. The relative weight of goblets (heart, liver and gizzard) and pancreas were not significantly influenced by treatments. Moreover, abdominal fat weight was significantly low in all probiotic and multi enzymes-supplemented groups when compared with control group. This may reflect the positive effect of both probiotic and enzymes on repartitioning of fats in the body. Since abdominal fat represents the main fat deposition in broiler chickens and it seems to be directly related to total carcass fat indicating the fact that probiotics enhance efficient energy usage which in close agreement with the results by Santoso et al.(1995) .Our results are also in accordance with several studies that reported lowering of abdominal fat by probiotic supplementation (Anjum et al., 2005 and Mehr et al., 2007). This effect was previously explained by Santoso et al. (1995) who demonstrated that the reduction of abdominal fat could be related to a decrease in the activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis, after probiotic supplementation.

Results revealed also that the primary immune organs (thymus and bursa) weights (%) were significantly increased in response to different dietary supplements, especially Prob either alone or with the enzyme compared by the other treatments. However, spleen (%) as a secondary lymphoid organ was not influenced. The increase in the immune-related organs weight may reflect better immunity status of broilers in the treatment groups compared with the control group. The significant increase in weight of thymus and bursa may be attributed to the effect of probiotic bacteria on the functional activities of the immune system responses which led to increase in the number of lymphocytes in the primary lymphoid organs. Since both of thymus and bursa are involved in the development and differentiation of T and B lymphocytes which responsible for cell – mediated and humoral immunity.

Meat quality:

The chemical and physical characteristics of broilers meat as affected by dietary enzymes and probiotic supplementation are shown in Table 4. It is clear from the results that meat moisture was significantly lower for broilers fed the MEnz; Prob and both of them compared by the control group. Similarly, meat protein (%) of treated chicks was significantly higher compared to the control group. On the other hand, data revealed non-significant effect of treatments on meat lipids and ash (%) as well as on physical characteristics of meat (WHC, color, tenderness and pH). These results agree with Pelícia et al.(2004) who found that multi-enzyme addition to broilers diet resulted in significantly lower meat moisture than the un-

supplemented control but, multi-enzyme significantly induced higher meat protein than un – supplemented diet.

Although there has been an increasing interest in dietary probiotics in the poultry industry, there are little or no published reports on the impact of dietary probiotics feeding on meat quality attributes (Kim et al., 2016). Though, some studies noted that there was no effect of probiotics on chicken meat quality (Pelícia et al., 2004 and Zhang et al., 2012).

Blood Parameters:

The blood parameter responses to dietary treatments are illustrated in Tables 5, 6 and 7. It is clear from the results that plasma total protein, globulin and A/G ratio were significantly improved by treatments compared with the control values (Table 5). This effect was not observed for plasma albumin level, urea, creatinine, and their ratio. This may indicate that blood protein metabolites did not influenced by treatments, while the higher plasma protein level increase parallel the enhanced growth of broiler chicks. On the other hand, all dietary supplements increased plasma glucose concentration, especially in the ME+Pro-supplemented group, while plasma total lipids, triglycerides, cholesterol and LDL were significantly decreased compared by the control chicks (Table 6). Furthermore, chicks fed the basal diet supplemented with MEnz and Pro either singly or in combination had significantly higher T3 (but not T4) level than the control group. This holds true as T3 is the more active thyroid hormone in birds.

In general, the significant increase in blood glucose concentration of the treated groups as compared with the control are agreement with Hussein (2014) who found higher blood glucose concentration in broilers fed on diets supplemented with probiotics. This increase might be related to an improvement in the nutrients utilization and the role of the added enzymes in enhancing glucogenolysis and the consequent increase in glucose absorption from the intestinal tract and utilization, which was supported by the previous results by Das et al. (2005).

The effect of probiotics on decreasing plasma total lipids, cholesterol and LDL was also reported by many researchers (Mohan et al., 1995 and Panda et al., 2006). Since, the significant reduction in plasma lipid fractions of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation which in accordance with the previous findings by Mohan et al. (1995, 1996). Other researchers have reported a simultaneous decrease of blood triglyceride content (Kalavathy et al. 2003 and Mansoub 2010).

The observed increase in plasma thyroid hormones (T4 and T3) in the treated groups as compared with the control group was similar to the results obtained by Chotinsky and Mihaylov (2013) who showed a significant increase in serum level of T3 with the supplementation of probiotics in the diet of broiler chickens. The present study reflects the beneficial effects of probiotics on thyroid gland activity, and/or the influence of biological supplementations on the level of thyroid hormones in the blood and provides new interesting data about a possible causal relationship between the growth promoting effect of probiotics and thyroid hormones. Depending on the previous results, it can be concluded that the observed significant increase in the T4 and T3 in the treated groups as comparison with control group in this

study is logic since it is necessary for most body functions because they directly affect a number of physiological and metabolic processes (McNabb, 2000). In this respect, Dawson et al. (1994) showed a significant positive correlation between thyroxine and body weight.

On the other hand, the effect of enzyme supplements to diets on increasing plasma proteins and decreasing lipids levels was evident in our study. This favor effect of enzyme addition might suggest that dietary enzymes can play a significant role in both lipid and protein metabolism. Thus, enzyme supplementation to diets is employed to increase the availability of non-starch polysaccharides, protein and other macronutrients that are entrapped by intact cell wall structure or viscous polymers that resistant to digestion by the endogenous host enzymes (Frigard et al., 2007 and El-Sanhoury et al.,2017). Moreover, Probiotic may change enzymes, which are associated in regulating cholesterol synthesis, oxidation or elimination for lowering cholesterol in laying hens (Denli et al., 2003).

Results of the present study showed also that the normal liver function-indicator enzymes activity (ALT, AST and ALP) were not significantly influenced by different treatments. Similarly, antioxidant status indices and enzymes including TAC, GSH, and SOD did not significantly affected by different treatments, except for glutathione peroxidase (GPX) activity which was significantly higher in blood plasma of broilers that fed the supplemented diets compared to the control one (Table 7).

Additionally, there were no significant differences in the activities of ALT, AST and ALP among treatment groups, indicative of normal liver function in broiler chicks that fed multi-enzyme or Probiotic-supplemented diets compared to control chicks. This may be, a valuable tool for determination of a safe inclusion rate for these additives. Based on our findings, Probiotic and multi-enzymes administration at the levels applied in this study may not exert adverse effects on broiler chickens. Similar results were reported by Lee et al. (2010) who found insignificant differences in the activities of AST and ALT among treatment groups with multi enzymes. Also, El-Baky (2013) and Panda et al. (2006) observed no effects on blood alkaline phosphatase activities in probiotic supplemented treatments compared with the controls.

The hematological parameters presented in Table 8 had an interesting view as they summarize the previously observed enhancement of all productive, physiological and immunological traits, since blood indices are considered as the best mirror of normal body- functions. This holds true as our results showed significant increases in RBCs count, hemoglobin, PCV, MCV, MCH, WBCs total count, lymphocytes and monocytes (%) in boilers fed the supplemented diets as compared to control group.

The previous results would be explained as the supplementation of basal diets with multi-enzymes or probiotics might resulted in better iron salt absorption from the small intestine and better produce of vitamins B that affecting positively blood-cell forming processes. This observation was supported by the findings of Kander (2004) who reported similar results.

The hematological results of the present study are in close agreement with Chuka (2014) and Paryad and Mahmoudi (2008) who found that WBC's count was higher in broiler chicks fed different levels of probiotics than those fed diets without

probiotics. Also, Abdollahi et al. (2003) reported that supplementation of broiler diets with probiotics increased leukocytes number. Also, Cetin et al. (2005) observed in turkey that the probiotic supplementation caused statistically significant increases in the erythrocytes count, hemoglobin concentration and hematocrit values.

In addition, Strompfová et al. (2006) reported a significant increase in the concentrations of hemoglobin, hematocrit value and red blood cell count after application of probiotic. The previous study would be explained as the supplementation of multi probiotic to the basal diet resulted in better iron salt absorption from the small intestine and better produce of vitamins B that affecting positively blood-cell forming processes (Kander, 2004).

Moreover, increased blood WBC's count might be related to the production of more immune cells (Gaggìa et al., 2010) that play an important role in defending the biological system against different diseases (LaFleur and LaFleur, 2008).These results are in agreement with the earlier findings of Jin et al. (1997) who reported that probiotic increased the hematological profile of poultry either due to its direct effects on hemopioetic organs or the indirect effects on the intestinal micro flora. However, hematological parameters are always influenced by environmental changes and nutrition. Mehri et al. (2010) reported that the addition of enzyme products to commercial soybean diets significantly increased lymphocytes, and decreased heterophils and H: L ratio. They explained that enzyme is an anti-nutritional factor existing in many legumes and reduction in innate immune stimulation associated with a reduction in the enzyme content of substrate entering the intestinal tract in chickens. Similar results were reported by Olukosi et al. (2007) who found that treatments with multi enzymes and probiotic did not significantly influence on eosinophils and monocytes.

Concerning the effect of different treatments on immune- related blood parameters, our data revealed that feeding diet with different supplements increased significantly plasma globulins, in terms of α–globulin, β–globulin, γ-globulin, IgA, and IgG (but not IgM)as compared to the control group (Table 9). Moreover, the response(s) of all immunological indexes in terms of LA, BA, LTT, PA, PI and LTT to different dietary supplements was significantly better than chicks - fed the basal control diet. It is well known that the specific use of probiotics aims to modulate the host immune response to potentially harmful antigens via increasing plasma immunoglobulins level. Since, globulins are water-insoluble proteins (Singh et al., 2001), and among the different types of globulin are α–globulin, β–globulin and γ-globulin which considered the main globulins in the blood (Hodek and Stiborova, 2003). Therefore, an elevated amount of globulin may represent an increase in the ability to produce additional IgG and IgM. The increase in oxidative stress causes decreased immunoglobulin levels and antioxidant enzymes (Ercal et al., 2000) and consumption of fat that contributes the alteration of immunoglobulin levels (IgG and IgM).Dietary probiotic significantly increased serum IgA and IgG concentrations of broilers. This concept was supported by the findings of Stanley et al. (2004) who found that enzymes and probiotics supplementation greatly increased disease resistance. They

focused on investigating gastrointestinal flora and mucosal cellular interaction when using probiotics as a substitute for antibiotics. Since, Probiotics may exert its beneficial effects and modulate the immune system of the host against potentially harmful antigen via activation of lymphocytes and antibody production.

The present result agrees with Tollba and Mahmoud (2009) who reported that a significant increase in counts of lymphocytes due to feeding dietary probiotic compared with control diet. The mode of action of probiotics was suggested by Sissons (1988) and Makled (1991) via producing antibiotic substances, inhibiting harmful bacteria, altering microbial metabolism, decrease intestine pH and simulating the immune system. Similarly, Olabisi and Peter (2008) reported the production of high level of serum IgG after oral inoculation of probiotic in layer chickens and they believed that Probiotics can enhance the immune response in broilers. So that probiotics resulted in an enhancement of broiler humoral immune response (Huanget al., 2004). Furthermore, Gao et al. (2007) and El-Sanhoury and Ahmed (2017) suggested that dietary supplementation of enzyme preparations containing xylanase enhanced immune responses of broiler chicks.

Findings revealed that dietary supplementation with both multi-enzyme or probiotic preparations either singly or in combination could improve the most important productive and immune-physiological aspects of broiler chicks reared under the prevailing conditions of our study. Generally, under the prevailing conditions of the present study the best results could be achieved by the combined ME+Pro inclusion to diets.

Table (1): Ingredients, determined and calculated analysis (%) of the basal diets

Ingredients	Diet (% as fed)	
	Starter (1-21 d of age)	**Grower (22-35 d of age)**
Corn, Grain	53.60	56.30
Soybean meal (44% CP)	31.00	20.00
Wheat Bran	0.00	5.00
Gluten Meal (60% CP)	5.00	5.00
Full fat soybean seed	4.00	7.00
Vegetable oil	2.75	3.00
Dicalcium phosphate	1.80	1.60
Vit+min premix*	0.30	0.30
Limestone	1.00	1.00
NaCl	0.30	0.45
DL-Methionine	0.15	0.20
L-Lysine HCl	0.10	0.15
Total	100.00	100.00
Determined[1] and calculated[2] composition (% as fed)		
Nutrient	Supplied	Supplied
Dry matter[1]	86.59	86.66
ME (kcal/kg)[2]	3061	3107
Crude protein[1]	23.16	20.56
Ether extract[1]	5.88	6.83
Crude fiber[1]	3.68	3.64
Calcium[2]	0.91	0.85
Ash[1]	6.09	5.97
Available phosphorus[2]	0.49	0.47
Lysine[2]	1.22	1.08
Methionine[2]	0.55	0.56

* Vitamins and minerals mixture provide per kg of diet: Vit. A (as all-trans-retinyl acetate); 12000 IU; Vit. E (all rac-α-tocopheryl acetate); 10 IU; k_3 3mg; Vit.D_3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; Choline chloride, 500 mg; Vit. B_{12}, 10µg; Vit. B_6, 1.5 mg; Thiamine (as thiamine mononitrate); 2.2 mg; Folic acid, 1 mg; D-biotin, 50µg. Trace mineral (mg / kg of diet) Mn, 55; Zn, 50; Fe, 30;Cu, 10; Se, 0.1 and Ethoxyquin 3mg.

Table (2): Effect of dietary supplementation with multi enzyme and/or probiotics on live body weight and weight gain of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
Body Weight (g)						
1 d	48.47	50.31	49.67	49.04	1.45	0.222
21 d	765^{b}	811^{a}	807^{a}	837^{a}	24.43	0.001
35 d	1672^{c}	1763^{b}	1779^{b}	1916^{a}	45.02	0.005
Body Weight Gain (g)						
1-21 d	716^{b}	761^{a}	758^{a}	788^{a}	26.33	0.05
21-35 d	907^{c}	952^{b}	971^{b}	1079^{a}	38.79	0.05
1-35 d	1624^{c}	1713^{b}	1729^{b}	1867^{a}	48.50	0.05

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM=Standard error of mean; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics.

Table (3): Carcass characteristics and relative weight of immune organs of broilers fed different dietary supplements.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
Carcass characteristics :						
Dressing, %	72.44^{b}	79.29^{a}	76.01^{a}	78.79^{a}	1.483	0.018
Total edible parts %	75.82^{b}	82.90^{a}	79.39^{ab}	82.34^{a}	1.523	0.017
Heart, %	0.455	0.476	0.432	0.437	0.022	0.484
Gizzard, %	1.070	1.106	1.103	1.193	0.045	0.290
Proventriculus, %	0.283	0.314	0.338	0.364	0.020	0.061
Liver, %	1.853	2.029	1.843	1.920	0.081	0.377
Abdominal fat, %	1.44^{a}	0.76^{b}	0.78^{b}	0.73^{b}	0.130	0.003
Pancreas, %	0.127	0.147	0.139	0.137	2.246	0.754
Immune organs :						
Spleen, %	0.079	0.072	0.079	0.068	0.013	0.920
Bursa, %	0.036^{b}	0.052^{a}	0.037^{b}	0.050^{a}	0.003	0.004
Thymus, %	0.377^{b}	0.397^{ab}	0.443^{a}	0.464^{a}	0.023	0.051

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM=Standard error of mean; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics.

Table (4): Chemical and physical characteristics of meat as affected by different treatments.

Items	Control	MEnz	Prob	ME+Pro	SEM	SIG
Chemical composition of meat :						
Moisture, %	71.02[a]	66.01[b]	65.71[b]	65.09[b]	0.875	0.001
Protein , %	19.24[b]	20.82[ab]	20.80[ab]	22.32[a]	0.525	0.007
Fat, %	8.240	8.380	8.620	8.140	0.209	0.423
Ash, %	2.660	2.200	3.020	2.380	0.227	0.097
Physical characteristics of meat						
pH	6.290	6.340	6.372	6.344	0.060	0.812
Color	0.196	0.191	0.198	0.197	0.005	0.734
Tenderness	2.550	2.570	2.644	2.542	2.246	0.487
WHC	4.392	4.414	4.452	4.432	0.052	0.866

[a,b,c,d] Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM=Standard error of mean ;MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics; WHC= water holding capacity

Table (5): Effect of different treatments on plasma protein fractions of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
Total protein (g/dl)	5.793[b]	6.367[a]	6.208[a]	6.250[a]	0.110	0.011
Albumin (g/dl)	3.253	3.405	3.150	3.250	0.070	0.120
Globulin (g/dl)	2.540[b]	2.962[a]	3.058[a]	3.000[a]	0.101	0.009
A/G ratio	1.286[a]	1.166[ab]	1.030[b]	1.087[b]	0.051	0.016
Urea (mg/dl)	26.67	26.78	27.08	26.17	0.667	0.806
Creatinine (mg/dl)	0.833	0.817	0.875	0.792	0.042	0.576
Urea/ Creatinine	32.79	32.86	30.99	33.49	1.842	0.796

[a,b,c,d] Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM= Standard error of mean; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics.

Table (6): Effect of different treatments on plasma glucose level, plasma lipids and thyroid gland hormones concentration of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
Glucose (mg/dl)	196^{c}	206^{ab}	203^{bc}	211^{a}	2.471	0.004
T. Lipid (mg/dl)	482^{a}	427^{b}	424^{b}	438^{b}	9.29	0.001
Triglycerides (mg/dl)	161	158	161	162	2.819	0.769
Cholesterol (mg/dl)	181^{a}	169^{b}	172^{b}	167^{b}	2.694	0.013
HDL(mg/dl)	47.59	47.08	46.67	49.92	1.075	0.183
LDL(mg/dl)	100.67^{a}	90.77^{b}	93.13^{ab}	84.48^{b}	3.115	0.017
T3 (ng / ml)	1.54^{b}	1.66^{a}	1.87^{a}	1.84^{a}	0.037	0.025
T4 (ng / ml)	7.46	7.81	7.79	8.07	0.155	0.091

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM=Standard error of mean; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics.

Table (7): Effect of different treatments on the liver function or antioxidant- related enzyme activities of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
ALT(U/L)	25.87	23.92	25.42	25.75	1.003	0.508
AST(U/L)	63.00	59.42	59.83	60.25	1.193	0.180
ALT/AST	0.412	0.402	0.425	0.426	0.013	0.528
ALP (U/100ml)	10.52	10.80	9.45	10.80	0.439	0.135
TAC (mg/dl)	411	429	421	420	8.119	0.499
GPX (U/dl)	0.397^{c}	0.474^{a}	0.438^{b}	0.451^{ab}	0.008	0.003
GSH (mg/dl)	983	990	989	988	18.429	0.993
SOD (U/dl)	244	250	247	250	4.521	0.709

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM=Standard error of mean; ; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics; AST=aspartate amino transferase; ALT=alanine amino transferase; TAC=total antioxidant capacity; GPX =glutathione peroxidase; GSH= glutathione; SOD=superoxide dismutase.

Table (8): Effect of different treatments on hematological traits of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
RBC's(10^6/mm^3)	2.217^c	2.633^a	2.467^b	2.608^a	0.043	0.001
Hemoglobin (g/100ml)	11.00^b	12.42^a	11.50^b	12.05^{ab}	0.275	0.011
PCV %	32.17^b	36.83^a	33.75^b	36.17^a	0.626	0.002
MCV(micron^3)	145.11^a	139.88^b	136.81^b	138.69^b	4.037	0.001
MCH(ug)	49.62^a	47.17^b	46.62^b	46.20b	1.033	0.011
MCHC (mg%)	34.20	33.71	34.07	33.36	0.629	0.780
WBC's (10^3/mm^3)	22.33^b	26.42^a	24.73^a	24.92^a	0.657	0.004
Lymphocytes (%)	37.33^b	40.50^a	40.75^a	40.17^a	0.789	0.025
Monocytes (%)	13.83	13.54	13.50	14.08	0.484	0.812
Basophils, (%)	0.800	0.900	0.800	0.550	0.139	0.360
Eosinophils, (%)	12.25	12.35	12.50	12.25	0.508	0.983
Heterophiles, (%)	35.78	32.72	32.45	32.95	1.318	0.284
H/L ratio	0.965	0.811	0.797	0.824	0.050	0.100

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM= Standard error of mean; ; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics; RBC's=red blood cell; PCV=packed cell volume; MCH=mean corpuscular hemoglobin; WBC's=white blood cell, MCV=Mean cell volume, MCH = Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration.

Table (9): Effectof different treatments on immune-related measurements of broiler chicks.

Items	Control	MEnz	Prob	ME+Pro	SEM	Sig
α–globulin (mg/dl)	1.078^b	1.199^a	1.167^a	1.165^a	0.041	0.025
β– globulin (mg/dl)	0.927^a	0.935^a	0.843^b	0.903^{ab}	0.024	0.051
γ –Globulin (mg/dl)	0.535^c	0.827^b	1.048^b	0.932^{ab}	0.070	0.001
LA (IU %)	0.507^b	0.570^{ab}	0.610^a	0.612^a	0.024	0.022
BA (%)	37.50^b	42.42^a	42.00^a	41.67^a	0.812	0.002
LTT(%)	25.83^b	27.83^a	27.45^a	27.80^a	0.469	0.025
PI (%)	1.383^b	1.792^a	1.692^a	1.717^a	0.050	0.001
PA (%)	17.17^b	20.67^a	20.75a	20.58^a	0.410	0.002
IgA (mg/100 ml)	77.83^b	83.33^a	80.17^{ab}	80.25^{ab}	1.325	0.052
IgG (mg/100 ml)	215^b	235^a	234^a	233^a	3.157	0.001
IgM (mg/100 ml)	928	978	977	975	17.82	0.181

^{a,b,c,d} Means in the same row followed by different letters are significantly different at ($P \leq 0.05$); SEM= Standard error of mean; ; MEnz = Multi-enzyme; Prob = Probiotics; MEnz+Prob = Multi-enzyme plus probiotics; PA= Phagocytic activity; PI= Phagocytic index; LA= lysozyme activity; BA= Bactericidal activity; LTT= Lymphocyte transformation test; IgA= Immunoglobulin A; IgG= Immunoglobulin G; IgM= Immunoglobulin M.

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الملخص العربي

الإستجابة الفسيولوجية والمناعية لإضافة مخلوط الإنزيمات أو محفزات النمو فى علائق بداري التسمين

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الإستجابة الفسيولوجية والمناعية لإضافة مخلوط الإنزيمات أو محفزات النمو فى علائق بداري التسمين
استهدفت الدراسة بيان التأثيرات الفسيولوجية والمناعية الناتجة عن اضافة مخلوط انزيمى او منشط نمو بصورة منفردة او خليط منهما الى علائق بدارى التسمين . تم استخدام عدد مائة وعشرون كتكوت غير مجنس عمر يوم حيث قسمت عشوائيا الى اربعة معاملات تجريبية، الاولى للمقارنة اما باقى المعاملات فقد تم إضافة مخلوط إنزيمى بواقع 2،جم/كجم عليقة أو محفز نمو بواقع 5،جم /كجم عليقة ثم خليط منهما بنفس النسبة وذلك للمجموعات الثانية والثالثة والرابعة على التوالى ، هذا وقد استمرت التجربة لمدة خمسة اسابيع تم خلالها تجميع البيانات وتحليلها معمليا واحصائيا

أوضحت النتائج حدوث تحسن معنوى فى وزن الجسم ومعدل الزيادة الوزنية نتيجة استخدام كل الاضافات السابقة مع تميز نسبى للمجموعة التى غذيت على خليط الانزيمات مع محفز النمو ، وجدت ايضا زيادة معنوية فى نسبة التصافى والأجزاء المأكولة من الذبيحة مع انخفاض نسبة دهن البطن وذلك فى كل المعاملات عن مجموعة المقارنة. بالنسبة لمكونات الدم حدثت زيادة معنوية فى بروتينات البلازما ونقص واضح فى الليبيدات مع ارتفاع معنوى لهرمون الغدة الدرقية ثلاثى اليود والجلوكوز فى الدم . وبالنسبة لصورة الدم حدثت زيادة فى عدد كرات الدم البيضاء والحمراء وهيموجلوبين الدم وحجم المادة الخلوية ودلائل الدم المختلفة وكذلك زيادة نسبة الخلايا الليمفاوية وذلك فى كل المعاملات مع تميز واضح لكتاكيت المجموعة الرابعة (مخلوط انزيمى + محفز نمو) ، بالنسبة للحالة التأكسدية العامة توضح الدلائل عدم وجود تغير معنوى فى معدل التأكسد الكلى وتركيز الجلوتاثيون وكذلك انزيم سوبرأكسيد ديسميوتيز بينما لوحظت زيادة معنوية فى نشاط انزيم جلوتاثيون بيروكسيدز .توضح النتائج أيضا حدوث تحسن معنوى فى الإستجابة المناعية للطيور المغذاة على العلائق التجريبية وذلك من ناحية زيادة الوزن النسبى للغدد الليمفاوية (البيرسا والثيموسية)، الجلوبيولينات المناعية (ألفا،بيتا،جاما)،النشاط الإلتهامى البلعمى، نشاط الليزوزومات وباقى دلائل المناعة الخلوية والدموية .

وقد خلصت نتائج البحث إلى أن استخدام مخلوط انزيمى + محفز نمو بالنسب الموجودة فى الدراسة له نتائج ايجابية على الأداء الإنتاجى والمناعى للطيور حتى عمر التسويق وبذلك توصى الدراسة بإضافة 2،جم من مخلوط الانزيمات مع 5،جم من محفز النمو لكل كيلو جرام من العليقة للحصول على أداء إنتاجى ومناعى متميز لبدارى التسمين .