

## Evaluation of The Immunological and Growth Enhancing Effect of Probiotic Used in Poultry Ration

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### ABSTRACT

A total of 60 one day old broiler chicks (Cobb 500) were used, the chicks were first weighted and randomly assigned into three experimental groups (20 chicks per each), the first group (G1) was negative control and received basal diet, the second one (G2) was a positive control and received basal diet and challenged orally with mixed culture of *Salmonella Pullorum* and *Salmonella Gallinarum* ( $1 \times 10^8$ /ml) at 25 days of age, the third one (G3) received the probiotic mix from 1 day of age this mix contained: *Bacillus subtilis* MORI 91 [ $2 \times 10^8$  colony forming unit (cfu)/g], *Clostridium butyricum* M7 ( $2.06 \times 10^8$  cfu/g), *Lactobacillus plantarum* K34 ( $2 \times 10^8$  cfu/g), and challenged orally with mixed culture of *S. Pullorum* and *S. Gallinarum* at 25 days of age. The results of the cytokines and inflammatory markers were detected as follows: the first group gave (105, 89.5, and 68.1), the second group gave (160, 135, and 115), and the third group gave (85, 65.3, and 56.3) for IFN- $\gamma$ , IL12, and IL4 respectively. The results of body weights and feed conversion ratios were: the first group gave (1550 and 1.91), the second group gave (1300 and 1.8), and the third group gave (1800 and 2.01) respectively. Furthermore, the results of mortalities, and PM lesions were: the first group showed 4/20 (20%) dead chicks with enteritis, while the second group showed 7/20 (35%) dead chicks with enteritis and enlargement with necrosis of the liver, spleen and kidneys, and the third group showed 2/20 (10%) dead chicks with enlargement of the liver, spleen and kidney. For the hematological parameters the first group gave ( $30.65 \pm 0.85$ ,  $2.82 \pm 0.31$ , and  $9.25 \pm 0.16$ ), the second group gave ( $28.26 \pm 0.76$ ,  $2.62 \pm 0.23$ , and  $8.75 \pm 0.26$ ), and the third group expressed ( $34.85 \pm 1$ ,  $3.66 \pm 0.25$ , and  $10.5.13 \pm 0.37$ ) for PCV, mean RBCs ( $10^6 \mu\text{l}^{-1}$ ), and hemoglobin, respectively. There was a significant difference in the results of cytokines, body weight, feed conversion ratios, mortality rate, PM lesions, and hematological parameters between the probiotic treated and the control groups with  $P < 0.05$ . It was concluded that the probiotic mix enhanced the growth and hematological parameters, also it lowered the inflammatory markers and the mortalities.

**Keywords:** Probiotic, Growth parameters, Cytokines, Hematological parameters.

### INTRODUCTION

The uncontrolled utilization of antimicrobials, lead to the propagation and shedding of substantial amounts of antimicrobial-resistant bacteria which can directly and indirectly infect humans, and as commensals, which may carry transferable resistance determinants across species borders and could reach humans through multiple routes of transfer. These pathways

include not only food, but also water, sludge, and manure used for fertilization of food crop soils. The continued use of antimicrobials in food animals will increase the pool of resistance genes, as well as their density (Marshall and Levy, 2011; Roushamet *al.*, 2018).

Whereas, the fear of the appearance of antibiotic resistant microbes in human

health, the interest in natural feed additives with immunomodulatory properties has increased significantly. This interest in nutritional immunomodulators is particularly meaningful in conditions of intensive poultry production, where many stressful factors, for instance the high stocking density has a negative influence on animal health. Nutritional immunomodulation may be defined as diet supplementation with specific nutrients or activities to affect some effective aspects of immune function in order to achieve an intended goal (Korver, 2012). Probiotics are microorganisms when being supplemented to poultry, may have a positive effect in the prevention and treatment of infections. Finally, the probiotics have been found to be effective in the treatment of some gastrointestinal diseases and could be considered to be therapeutic agents (Marteau *et al.*, 2001). Salmonellosis is one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced meat and egg production (Haideret *al.*, 2007).

This study was planned to evaluate the efficacy of a multistrain probiotic for improving the growth parameters and some hematological parameters in broiler chickens. Added to that, this work was also aimed to study the effect of the multistrain probiotic in lowering the inflammatory markers, mortalities, and the subsequent lesions.

## MATERIALS AND METHODS

### *Birds and experimental design*

A total of 60 one day-old commercial broiler chicks (Cobb 500) obtained from a local hatchery (National Poultry Company), the chicks were weighted and randomly assigned into three experimental groups (20 chicks per each) as shown in Table 1, and the composition of the basal diet shown in (Table 2).

### *Feed additive probiotic*

using the *Bacillus subtilis* MORI 91, *Clostridium butyricum* M7, *Lactobacillus plantarum* K34.

### *Collection of the blood samples:*

the collected blood samples were divided into two parts (1<sup>st</sup> part for testing the hematological parameters; PCV, hemoglobin, and RBCs count) and (the 2<sup>nd</sup> part for separation of the serum by centrifugation for cytokine ELISA tests) for detecting the cytokines and inflammatory markers following the instructions of the manufacturer of (Cusabio ELISA kits for poultry cytokines).

### *The body weights and feed conversion ratios:*

On a weekly basis, 5 birds from each group were randomly selected and weighed and the feed intake per each group was recorded at the same time. Feed intake was determined as the difference between the amount of feed supplied and the remaining feed at the end of each week for each repetition. Body weight (the weight of the birds at the end of each week) and body weight gain (the difference between the two successive weights) were calculated. The feed conversion ratio (FCR) was calculated as the ratio between feed intake and body weight gain at the end of each week.

### *The mortality percentage and PM lesions:*

the mortalities were recorded daily, and the post-mortem lesions observed in intestine, liver, spleen and kidney were reported for each group.

### *Statistical analysis:*

the results were represented as geometric mean, the analysis was done by applying a one-way analysis of variance together with Duncan's multiple range test for testing the significant differences among the different treatment groups, the results were considered significant at  $P < 0.05$ .

## RESULTS

It was clear that the second group gave high levels of all tested cytokines with a significant difference between all groups with  $P < 0.05$  (Table 3). Concerning the hematological parameters, the result of PCV, Mean RBCs, Hemoglobin concentration proved a significant difference between all groups with  $P < 0.05$ . The group received the probiotic mix

(G3) gave the highest percentage in hematological parameters (Table 4).

The body weights and feed conversion ratios for the group received the probiotic mix (G3) gave higher results and there was a significant difference compared to another groups  $P <$

0.05 (Table 5). Regarding mortalities and PM lesions observed, the group received the probiotic mix (G3) gave the lowest mortality rate with minimal changes in the vital organs (Table 6), and a significant difference between all groups for mortalities and PM lesions with  $P < 0.05$ .

**Table (1).** The tested broiler chicken groups

Groups	Number of chicks	Treatment
Negative control group (G1)	20	Received basal diet
Positive control group (G2)	20	Received basal diet and challenged orally with mixed culture of <i>S. Pullorum</i> and <i>S. Gallinarum</i> ( $10^8$ cfu/each) at 25 days of age
The group received the probiotic mix from 1 day of age <i>Bacillus subtilis</i> MORI 91 ( $2 \times 10^8$ colony forming unit (cfu)/g), <i>Clostridium butyricum</i> M7 ( $2.06 \times 10^8$ cfu/g), <i>Lactobacillus plantarum</i> K34 ( $2 \times 10^8$ cfu/g) used at adose of 0.5 g/kg feed daily, (G3).	20	Received basal diet and challenged orally with mixed culture of <i>S. Pullorum</i> and <i>S. Gallinarum</i> ( $10^8$ cfu/each) at 25 days of age

**Table (2).** The composition of the basal diet:

Ingredients	Starter ration	Grower finisher ration
Corn %	52.8	58.94
Soybean meal %	35.2	30
Corn gluten %	6	5
Soya oil %	2.6	2.57
Common salt %	5.304	0.3
Lysine %	5.1	0.1
Methionine %	5.20	0.15
Di calcium phosphate %	1.6	1.7
Limestone %	1.2	1.118
Broiler premix %	5.304	0.30

**Table (3).** Cytokines and inflammatory markers

Groups	Number	IFN- $\gamma$	IL-12	IL-4
Negative control group (G1)	16	105 $\pm$ 0.02	89.5 $\pm$ 0.01	68.1 $\pm$ 0.2
Positive control group (G2)	13	160 $\pm$ 0.01	135 $\pm$ 0.05	115 $\pm$ 0.2
Received the probiotic mix (G3)	18	85.2 $\pm$ 0.1	65.3 $\pm$ 0.1	56.3 $\pm$ 0.1

**Table (4).** Results of some hematological parameters

Groups	Hematological parameters		
	PCV	Mean RBCs ( $10^6 \mu\text{l}^{-1}$ )	Hemoglobin
Negative control group (G1)	30.65 $\pm$ 0.85	2.82 $\pm$ 0.31	9.25 $\pm$ 0.16
Positive control group (G2)	28.26 $\pm$ 0.76	2.62 $\pm$ 0.23	8.75 $\pm$ 0.26
Received the probiotic mix (G3)	34.85 $\pm$ 1	3.66 $\pm$ 0.25	10.5 $\pm$ 0.37

**Table (5).** Results of body weights and feed conversion ratios

Groups	Tested number	Body weight	Feed conversion ratio
Negative control group (G1)	16	1550±2.5	1.91 ± 0.1
Positive control group (G2)	13	1300±3.1	1.83 ± 0.03
The group received the probiotic mix (G3)	18	1800±2.75	2.01 ± 0.1

**Table (6).** Results of mortalities and PM lesions

Groups	Mortality	PM lesions			
		Intestine	Liver	Spleen	Kidney
Negative control group (G1)	4/20 (20%)	Enteritis	Enlarged	Enlarged	Enlarged
Positive control group (G2)	7/20 (35%)	Enteritis	Enlarged with necrotic foci	Enlarged with necrotic foci	Enlarged
Received the probiotic mix (G3)	2/20 (10%)	Enteritis	Enlarged	Enlarged	Enlarged

## DISCUSSION

The application of probiotics in the poultry industry enhanced the growth, immunological, and hematological parameters (Khan *et al.*, 2011). Cytokines are immunoregulatory peptides with relatively small molecular weights that participate in the innate and adaptive immune responses. The interleukin-2 (IL-2) is a Th-1-associated cytokine that plays a central role in the adaptive immunity. The interleukin-12 (IL-12) is an important cytokine required for the initiation and regulation of cellular immunity through the differentiation of naïve T cells into Th-1 cells, which is crucial for host resistance to many microbial pathogens (Park *et al.*, 2008). The interferon- $\gamma$  (IFN- $\gamma$ ) regulates acquired immunity by activating lymphocytes and enhancing the expression of MHC class II antigens. In addition to that, the IFN- $\gamma$  is a common marker of cellular immunity and high levels have been correlated with protective immune responses to coccidial and other enteric infections (Lee *et al.*, 2008). Members of the tumor necrosis factor superfamily play crucial roles in both innate and adaptive immunity, including inflammation, apoptosis, and cell proliferation (Kaiser and Stäheli, 2008).

The data of cytokines presented in the (Table 3) agreed with Haghghi *et al.*, (2008) who found that the depression of IL-12 and IFN- $\gamma$  expression is associated with probiotic-mediated reduction in the intestinal colonization with *Salmonella* serovar Typhimurium. Also, the immunomodulatory

effects of the used probiotic agreed with Lee *et al.*, (2010) who found that the *Bacillus* based diet can augment protective immunity against enteric pathogens in chickens. The obtained data agreed with Khan *et al.*, (2011) who confirmed that the supplementation of probiotic in layer diets did not appear to cause any adverse effects on immunity compared with negative control. The gained data disagree with Rajput *et al.*, (2017) who found that *S.boulardii* and *B.subtilis* B10 may have a role to induce mucosal immunity by activation of the TLRS and cytokines expressions in broilers. Also the data of cytokines disagreed with Gadde *et al.*, (2017) who concluded that *B.subtilis* supplementation altered intestinal immune activity and influenced gut barrier integrity through increased tight junction gene expression, and hence increased IL2 and IFN- $\gamma$  expression, while in IL4 was found to be up regulated with *B.subtilis* strain treated group. The gained data about the increased levels of PCV, RBC, and hemoglobin in the (Table 4) were similar to Onifade, (1997), and Okeyet *et al.*, (2015) who found that there is a positive correlation between dietary level of *Saccharomyces cerevisiae* probiotic with hematological indices like PCV, RBC, and hemoglobin in broiler chickens.

It was clear that broiler chickens fed a basal diet containing probiotics had significantly higher body weights and feed conversion ratios compared with the control untreated group (Table 5). These results agree with the findings of Yeo and Kim (1997), Cavazzoni *et al.*, (1998), Mountzouris *et al.*, (2010), and

Shim *et al.*, (2010) who demonstrated that the growth promoting effects of probiotics on broiler performance come through the alteration in the intestinal microflora which led to suppression of growth of intestinal pathogens and enhancement of digestion and nutrient utilization. In addition to that, the obtained data were consistent with the findings of Jin *et al.*, (1998), Santin *et al.*, (2001), and Saied *et al.*, (2011) who reported that the feed intake and body weights of probiotic treated groups were significantly higher compared with the control group. The improved body weights and body weight gains of broilers fed on diets supplemented with probiotics might be due to lowering of the intestinal PH (Sandres, 1993), with subsequent improvement in nutrient digestion and utilization (Fuller, 1992) and inhibition of colonization of pathogenic bacteria such as *E.coli* and increased the population of nonpathogenic bacteria as lactic acid bacteria (Kabiret *et al.*, 2004). Also the improvement of growth performance might be due to limited effect on nutrient and energy cost for both growth and proliferation of live microbes in the gut, and the development of an immune response by the host, or to a more profound effect of the supplied microbes on gut health and function (Mountzouris *et al.* , 2010).

On the other hand, *Lactobacillus* spp is the most dominant bacterial species in the crop (Fuller, 1977; Mead, 1997; He *et al.*, 2000). It can inhibit the growth of pathogens (Fu *et al.*, 1999) by maintaining the normal microbial balance in the crop (Fuller,1973), which is important for broilers because the crop is the gateway for exotic bacteria to enter the intestines of birds. Also, *Lactobacillus* spp is the only organism normally present in the duodenum and small intestine (Barnes *et al.*, 1972).

The obtained results in (Table 5) showed that, the supplementation of broiler chickens diets with a probiotic mixture improved the cumulative feed conversion ratio compared with the control untreated groups. The improvement in the feed conversion ratio might be due to the better utilization of diet through modification of dietary proteins by intestinal microflora (Goldin and Gorbach, 1984). The obtained results were in agreement with that of Jin *et al.*, (1998), Mikulec *et al.*, (1999), Zulkifli *et al.*, (2000), and Balevli *et al.*, (2001) who demonstrated an

improvement in feed conversion ratio and relative growth rate for birds fed on diets supplemented with probiotics compared with the control groups. Also, the obtained results agreed with the previous findings of Talebi *et al.*, (2008), Awad *et al.*, (2009), Mountzouris (2010), and Pourakbari *et al.*, (2015) who stated that, probiotics improved feed conversion ratios of broiler chickens. Conversely, the data obtained in this study disagreed with that of Huang *et al.*, (2004) who reported that the addition of probiotics to the broiler diets had no significant effect on the feed conversion ratio. In addition to that, the obtained result disagreed with the findings of Saied *et al.*, (2011) who studied the effect of yeast culture on growth performance and revealed an increased feed intake and body weight without any significant effect on feed conversion ratio.

Concerning the results presented in (Table 6) which elucidates the mortalities from all the 3 groups: The lowered mortality results of the group treated with probiotics after challenge come in agreement with Khan *et al.* , (2011) who concluded that the feeding of probiotic did positively affect the immune system and reduced mortalities. Further studies are required for investigating the mechanisms mediated by the dietary supplementation of probiotics to enhance the protective innate immunity in a disease model which causes considerable economic impact in the poultry industry worldwide.

## CONCLUSION

The probiotic mix (*Bacillus subtilis* MORI 91, *Clostridium butyricum* M7, and *Lactobacillus plantarum*) enhanced the growth parameters and hematological parameters, also it lowered the inflammatory markers and the mortalities.

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