

Effects of Dietary Supplementation of a Mixture of Synbiotic and Some Digestive Enzymes on Performance, Behaviour and Immune Status of Broiler Chickens

¹Abeer H. Abdel Razeq and ²M.A. Tony

¹Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt

²Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: This study aimed at studying the effects of a mixture of synbiotic and some feed enzymes (Avi-bac[®]) on broiler chickens performance, behaviour and immune status. Two hundred and twenty five 1-d-old chicks (Hubbard breed) were randomly distributed into three treatment groups (3 replicates each) using 25 chicks/replicate on floor pens. Control (C) birds were offered non-supplemented basal diets. Treatments 1 and 2 (T1 and T2) were fed diets containing Avi-bac[®] 250 and 500 g/ton feed respectively. Feed and water were offered *ad-libitum* for 35 days experimental period. Feed consumption and body weight were recorded weekly to calculate body gain and feed conversion. Feeding, drinking and resting behaviour were observed. Blood samples were collected by time intervals to evaluate the immune status of the birds against some vaccines and to evaluate the stress conditions. At the end of the experimental period 9 birds were chosen randomly from each group to compare carcass yield. The results showed that body weight was significantly ($p<0.05$) improved in chicks fed on Avi-bac[®] containing diets compared with control ones. Supplementation of diets with Avi-bac[®] increases significantly feed intake ($p<0.05$) and improved feed conversion. The best feed conversions were recorded in supplemented groups. Dressing percentage and liver weights were non-significant differ between groups. However, abdominal fat content was reduced significantly ($p<0.05$) in both supplemented groups. Birds in supplemented groups recorded low level of cortisol and H/L ratio. Supplementation of the broiler diets was significant enhance immune responses measured against vaccines used. It can be concluded that, using mixtures of synbiotics and digestive enzymes act synergistically as feed additives and reflected positively on zootechnical performance of broiler chickens, reduce stresses and enhance immune status.

Keywords: Behaviour, broiler, immunity, performance, synbiotic

INTRODUCTION

Substitution of conventional antibiotic growth promoters with probiotics has received much attention in the recent past. Probiotics have been utilized to improve bird performance by maintaining the normal microflora of the gut. The main action of probiotics is a reinforcement of the intestinal mucosal barrier against deleterious agents (Fioramonti *et al.*, 2003). Meanwhile, prebiotics have been defined as “a non-digestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestine” (Gibson and Roberfroid, 1995; Young *et al.*, 1998). Synbiotics is defined as a mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and/or by selectively stimulating their growth improving the host’s welfare (Awad *et al.*, 2009). The efficacy of probiotics may be potentiated by several methods: The selection of more efficient strains;

gene manipulation; the combination of several strains; and the combination of probiotics and synergistically acting components such as prebiotics and digestive enzymes (Gibson and Roberfroid, 1995; Batavani, 2010). Recent researches and development of synbiotic products has been increasingly focused on functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity and improved immune status in broiler chicks. Mohnl *et al.* (2007) found that the synbiotic product had a comparable potential to improve broiler performance as avilamycin (an antibiotic growth promoter). Elijah and Ruth (2012) concluded that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. However, there is scarce information available to date on synbiotics and its possible mechanisms in broiler chickens (Patterson and Burkholder, 2003). Low information is available regarding the effect of adding synbiotic products in combination with feed enzymes to broiler diets and their effects on the zootechnical

performance and immune responses of broiler chickens. Based on this concept, the goal of the present study was to investigate the effects of synbiotic product with some feed exogenous enzymes on broiler performance, feeding behaviour and some immune parameters of broiler chickens.

MATERIALS AND METHODS

Avi-bac®: Avi-bac® is a commercial combination of *Lactobacillus*, *Bifido* bacteria, oligosaccharides derived from yeast cell wall with some digestive enzymes (amylase, cellulase, beta-glucanase and hemicellulase). Avi-bac® manufactured by ProByn International Inc., USA.

Experimental birds: Two hundred and twenty five 1-d-old chicks (Hubbard breed) of both sexes were obtained from a commercial local hatchery. Chicks were marked, weighed and randomly divided into 3 dietary treatments (75 chicks/group) and housed in floor pens (3 pens for each treatment) at the Poultry Rearing Centre, Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt.

Birds in all experimental groups were vaccinated against Newcastle and Gumboro diseases according to the vaccination program showed in Table 1.

Diets and feeding program: Corn-soybean meal based diets were formulated to cover the nutrient requirements for Hubbard broilers (Hubbard manual). Three stages diets (starter, grower and finisher) in the form of mash and water were provided *ad-libitum* during the 35 days experimental period (Table 2).

Control (C) birds were offered non-supplemented basal diets. Treatments T1 and T2 were fed basal diets containing Avi-bac® 250 and 500 g/ton feed respectively.

The individual body weight for all birds in the replicates as well as the rest of feed was recorded weekly. The consumed feed for each replicate was divided by the number of birds per replicates to calculate consumption/bird/week. Body weight gain and feed conversion were calculated.

Behavioural observations: Scan sampling was used for 5 weeks according to Sandilands *et al.* (2005). Birds were observed 3 days/week. Behavioural observation was 10 min/replicate/day. Observations were in the morning (9.00 am) and at afternoon (2.00 pm). The number of birds performing each of behaviour was recorded. Results were expressed as the percentage of birds performing the behaviour/total observed birds (Reiter and Bessei, 2000).

Table 1: Vaccination programme

| Age (days) | Vaccine* | Application |
|------------|---------------------------------------|----------------|
| 7 | Bivalent hitchner B ₁ + IB | Eye dropping |
| 14 | Hitchner B1 | Eye dropping |
| 16 | Gumboro D78 | Drinking water |
| 21 | Lasota | Drinking water |
| 28 | Gumboro D78 | Drinking water |

*: Vaccines were obtained from Intervet, Inc., Egypt

Table 2: Composition percentage and calculated nutrients profile of the basal diets

| Ingredients % | Starter (1-20 day) | Grower (21-30 day) | Finisher (31-35 day) |
|-----------------------------|--------------------|--------------------|----------------------|
| Yellow corn | 51.70 | 56.15 | 61.15 |
| Corn gluten meal | 5.00 | 5.00 | 5.00 |
| Soybean meal (44% CP) | 37.30 | 31.50 | 25.90 |
| Soy oil | 2.20 | 3.50 | 4 |
| Dicalcium phosphate | 1.60 | 1.60 | 1.70 |
| Limestone | 1.40 | 1.45 | 1.44 |
| Common salt | 0.40 | 0.40 | 0.40 |
| DL-methionine | 0.05 | 0.05 | 0.06 |
| L-lysine | 0.05 | 0.05 | 0.05 |
| Vitamin and mineral premix* | 0.30 | 0.30 | 0.30 |
| Calculated analysis: | | | |
| ME (Kcal/kg) | 2951.80 | 3049.55 | 3124.07 |
| Crude protein % | 23.20 | 21.29 | 19.00 |
| Crude fat % | 6 | 6.92 | 8 |
| Crude fiber % | 4.50 | 4.80 | 5.20 |
| Calcium % | 1 | 1 | 1 |
| Non-phytate phosphorus % | 0.45 | 0.45 | 0.45 |

*: Per kg premix: 1 200 000 IU vit. A, 350 000 IU vit. D₃, 4 000 mg vit. E, 250 mg vit. B₁, 800 mg vit. B₂, 600 mg vit. B₆, 3.2 mg vit. B₁₂, 450 mg vit. K₃, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, 40 mg Co

Blood samples: Blood samples for serum separation were collected from all groups (5 samples/replicate) weekly. Antibody titres against Newcastle and Gumboro vaccines were measured. Cortisol level in serum as a stress indicator was done as well.

Heparinised blood samples were collected from all groups (5 samples/replicate) at 5th and 12th days post the first and second doses of Newcastle vaccines. These samples were used for separation of mononuclear cells responsible for phagocytic activity according to Gross and Siegel (1983).

Carcass yield: Nine birds from each group (3 birds/replicate) were chosen randomly to compare live body weights, dressing percentage, liver weights and abdominal fat.

Statistical analysis: All data were statistically analyzed using IBM SPSS® version 19 software for personal computer (2010). Means were compared by one way ANOVA (p<0.05) using Post Hoc test according to Snedecor and Cochran (1980).

RESULTS

The results of zootechnical performance for Control (C) group and both treated groups T1 and T2 are shown in Table 3.

Results of behavioural pattern measured are listed in Table 4.

Table 3: Performance parameters measured (means±S.E.)

| Age (week) | Parameter | C | T1 | T2 |
|------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 1 | Body weight (g/bird) | 149.96±0.48 ^a | 154.70±0.24 ^b | 155.98±0.19 ^c |
| | Feed intake (g/bird) | 129.39±0.39 ^a | 130.79±0.17 ^b | 129.14±0.18 ^a |
| | Body weight gain (g/bird) | 99.74±0.43 ^a | 104.42±0.36 ^b | 105.68±0.32 ^c |
| | FCR | 1.30 | 1.25 | 1.22 |
| 2 | Body weight (g/bird) | 480.68±0.27 ^a | 490.52±0.24 ^b | 491.02±0.32 ^b |
| | Feed intake (g/bird) | 497.03±0.50 ^a | 500.79±0.22 ^b | 500.26±0.61 ^b |
| | Body weight gain (g/bird) | 330.72±0.66 ^a | 335.82±0.44 ^b | 335.04±0.42 ^b |
| | FCR | 1.50 | 1.49 | 1.49 |
| 3 | Body weight (g/bird) | 820.46±0.19 ^a | 839.12±0.11 ^b | 838.86±0.19 ^b |
| | Feed intake (g/bird) | 544.05±0.28 ^a | 550.93±0.14 ^b | 553.52±0.27 ^c |
| | Body weight gain (g/bird) | 339.78±0.20 ^a | 348.60±0.32 ^b | 347.84±0.38 ^b |
| | FCR | 1.60 | 1.58 | 1.59 |
| 4 | Body weight (g/bird) | 1290.04±0.05 ^a | 1298.14±0.10 ^b | 1297.92±0.05 ^b |
| | Feed intake (g/bird) | 734.88±0.23 ^a | 739.34±0.33 ^b | 739.58±0.29 ^b |
| | Body weight gain (g/bird) | 469.58±0.17 ^a | 459.02±0.14 ^b | 459.06±0.17 ^b |
| | FCR | 1.56 | 1.61 | 1.61 |
| 5 | Body weight (g/bird) | 1950.32±0.08 ^a | 1999.94±0.10 ^b | 2000.16±0.10 ^b |
| | Feed intake (g/bird) | 1110.30±0.60 ^a | 1158.46±0.27 ^b | 1166.17±0.29 ^c |
| | Body weight gain (g/bird) | 660.28±0.12 ^a | 701.80±0.12 ^b | 702.24±0.10 ^c |
| | FCR | 1.68 | 1.65 | 1.66 |
| Total | Body weight (g/bird) | 1950.32±0.08 ^a | 1999.94±0.10 ^b | 2000.16±0.10 ^b |
| | Feed intake (g/bird) | 3015.65±0.52 ^a | 3080.31±0.38 ^b | 3088.67±0.34 ^c |
| | Body weight gain (g/bird) | 1900.10±0.21 ^a | 1949.66±0.31 ^b | 1949.86±0.27 ^c |
| | FCR | 1.59 | 1.58 | 1.58 |

Figures in the same row with different superscript letters are statistically significantly different (p<0.05)

Table 4: Feeding, drinking and resting behaviours (mean±S.E.)

| Parameter (%) | Age (week) | C | T1 | T2 |
|--------------------|------------|-------------------------|--------------------------|-------------------------|
| Feeding behaviour | 1 | 10.21±1.03 | 10.57±1.76 | 11.56±1.76 |
| | 2 | 9.35±2.01 | 10.16±1.44 | 10.35±0.96 |
| | 3 | 10.16±1.06 | 11.16±1.06 | 11.95±0.79 |
| | 4 | 8.29±1.85 | 8.53±1.52 | 8.61±1.58 |
| | 5 | 6.94±0.97 | 7.85±1.33 | 7.86±0.70 |
| | Overall | 9.10±1.38 | 9.65±1.42 | 10.06±1.05 |
| Drinking behaviour | 1 | 2.02±0.05 | 2.67±0.67 | 2.83±1.02 |
| | 2 | 4.18±0.07 | 4.35±1.30 | 5.29±1.05 |
| | 3 | 6.08±0.87 | 6.38±0.61 | 6.66±2.99 |
| | 4 | 4.30±0.13 | 4.44±0.59 | 5.44±0.59 |
| | 5 | 4.40±0.34 | 4.49±1.11 | 4.84±0.50 |
| | Overall | 4.18±0.48 | 4.47±0.86 | 5.01±1.12 |
| Resting behaviour | 1 | 39.08±2.49 | 39.33±4.83 | 41.22±1.40 |
| | 2 | 45.00±2.63 ^a | 55.65±3.48 ^b | 60.59±4.61 ^b |
| | 3 | 49.27±3.48 ^a | 59.95±3.60 ^b | 67.14±3.01 ^b |
| | 4 | 62.79±5.46 ^a | 70.27±1.85 ^{ab} | 78.04±2.55 ^b |
| | 5 | 71.40±3.69 ^a | 80.41±2.05 ^{ab} | 88.28±7.27 ^b |
| | Overall | 53.51±3.55 ^a | 61.12±3.16 ^a | 67.06±3.77 ^b |

Figures in the same row with different superscript letters are statistically significantly different (p<0.05)

Table 5: Physiological stress indicators measured (means±S.E.)

| Parameter | C | T1 | T2 |
|------------------|------------------------|------------------------|------------------------|
| H/L ratio | 0.65±0.06 ^a | 0.40±0.05 ^b | 0.38±0.05 ^b |
| Cortisol (ng/mL) | 6.42±0.18 ^a | 3.51±0.15 ^b | 3.50±0.17 ^b |

Figures in the same row with different superscript letters are statistically significantly different (p<0.05)

Table 6: Serum antibodies titers against newcastle and gumboro vaccines

| Age (week) | HI serum newcastle antibodies titre | | | ELISA gumboro antibodies titre | | |
|------------|-------------------------------------|-----|-----|--------------------------------|-------|-------|
| | C | T1 | T2 | C | T1 | T2 |
| 0 | 7.2 | 7.2 | 7.2 | 0.482 | 0.482 | 0.482 |
| 1 | 4.2 | 4.2 | 4.8 | 0.372 | 0.411 | 0.417 |
| 2 | 4.8 | 5.0 | 4.6 | 0.261 | 0.256 | 0.269 |
| 3 | 7.0 | 6.8 | 7.8 | 0.342 | 0.321 | 0.419 |
| 4 | 7.2 | 7.7 | 8.0 | 0.435 | 0.446 | 0.486 |
| 5 | 6.6 | 6.6 | 7.8 | 0.443 | 0.445 | 0.531 |

The results of physiological stress indicators in the blood are tabulated in Table 5.

Immunological parameters measured are listed in Table 6, 7 and 8.

Figures in the same row with different superscript letters are statistically significantly different (p<0.05).

Table 8 illustrates the results of carcasses yield and some organs weights at the end of the experimental period.

DISCUSSION

Recent researches and development of synbiotic products have been increasingly focused on functional benefits including enhancement of performance, resistance to gastrointestinal bacterial infection and improved immune status in broiler chicks. The

Table 7: Phagocytic activity post newcastle vaccines

| | Phagocytic % | | | Phagocytic index | | |
|--|----------------------|----------------------|----------------------|------------------------|------------------------|------------------------|
| | C | T1 | T2 | C | T1 | T2 |
| 5 th days post 1 st vaccination | 54±1.67 ^a | 59±0.33 ^b | 65±0.88 ^c | 0.40±0.03 ^a | 0.56±0.02 ^b | 0.62±0.02 ^c |
| 12 th days post 1 st vaccination | 53±1.22 ^a | 54±3.18 ^b | 55±2.40 ^c | 0.37±0.04 ^a | 0.36±0.05 ^b | 0.41±0.05 ^c |
| 5 th days post 2 nd vaccination | 55±1.80 ^a | 61±0.33 ^b | 66±0.67 ^c | 0.41±0.03 ^a | 0.60±0.01 ^b | 0.62±0.02 ^c |
| 12 th days post 2 nd vaccination | 54±1.67 ^a | 56±0.98 ^b | 62±0.36 ^c | 0.43±0.04 ^a | 0.41±0.02 ^b | 0.58±0.02 ^c |

Table 8: Carcass yield at 35 days of age

| Parameter | C | T1 | T2 |
|---------------------|---------------------------|---------------------------|---------------------------|
| Live BW (g) | 1950.21±0.10 ^a | 2000.32±0.11 ^b | 2000.65±0.16 ^c |
| Dressing weight (g) | 1478.26±0.30 ^a | 1518.24±0.18 ^a | 1520.49±0.12 ^a |
| Dressing weight (%) | 75.80 | 75.90 | 76.00 |
| Liver weight (g) | 46.20±0.24 ^a | 46.50±0.15 ^a | 46.70±0.11 ^a |
| Liver weight (%) | 2.37 | 2.32 | 2.33 |
| Abdominal fat (g) | 15.72±0.62 ^a | 10.45±0.21 ^b | 10.12±0.13 ^b |

Figures in the same row with different superscript letters are statistically significantly different (p<0.05)

consumption of a probiotic in combination with a suitable prebiotic (synbiotic) and some digestive enzymes can result in synergistic effects which improves the functions and shelf life of probiotic (Zanoni *et al.*, 2008). The impact of probiotic supplementation in combination with prebiotic and some digestive enzymes (Avi-bac[®]) on broiler chicken performance in the current study revealed that the parameters studied were significantly (p<0.05) affected by the treatments. The means of live body weight and weight gain over the course of the experiment were significantly improved (p<0.05) for broilers fed treated diets compared with non supplemented control group. The treated groups (T1 and T2) recorded significant improvement (p<0.05) in body weight gain (1949.66±0.31 and 1949.86±0.27 g/bird, respectively) compared with the control group (1900.10±0.21 g/bird). From the obtained results it is clear that the improvement in chicken performance were also influenced by the dose of the additive used. Treated groups consumed diets contained synbiotic were recorded the highest body gain and improved feed conversion than that of control group. Synbiotic products contain viable bacterial cultures that establish early in the gut while the prebiotic present in them serve as a source of nutrient for the probiotics in addition to dietary sources (Mohnl *et al.*, 2007; Zhang *et al.*, 2006). The probiotics and/or synbiotics could have positive effects on bacterial population such as *Lactobacillus* sp., in the gastrointestinal tract (Smirnov *et al.*, 2005) and the addition of probiotic to diets has been found to improve growth performance (Jin *et al.*, 1997; Wenk, 2000). Gut microflora changes actively by adding prebiotics and significantly reduces gut pH which improve chicks performance through influencing gut microbial population (Rahmani and Speer, 2005). Prebiotics increase useful microorganism (Spring *et al.*, 2000) and improves bird immunity (Shashidhara and Devegowda, 2003). Consequently, improves body weight gain in the total rearing period (Parks *et al.*, 2001). Hooge (2004) reported that positive effects of

mannan oligosaccharides on chick performance could be more visible during stressful, high temperature as that situation in Egypt, high density and weak management conditions. Prebiotics are potential alimentary supplements which reduce harmful effects of putrefactive factors and increases nutrition output (Fooks and Gibson, 2002). When the bird gut is infected by pathogen bacteria, lymphocytes aggregate in that position and mucosa layer thickness increases, thus absorbance of nutrients reduces (Gunal *et al.*, 2006). So prebiotics consumption is effective on feed intake and improvement of production through reducing pathogen population. Also it has been reported that using prebiotics increases nutrient absorbance area via increasing gut length and thus improves bird performance (Santin *et al.*, 2001).

Important effects of supplementing digestive enzymes include: improved digestibility of nutrients, reduced small intestine fermentation and increased caecal fermentation (Choct *et al.*, 1999a, b). The increased microbial activity in the caeca is likely a result of poorly absorbed products of enzymatic degradation entering the caeca where they stimulate bacterial fermentation (Bedford, 2000). This aspect of enzyme activity may resemble the mode of action of prebiotics. The possibility of producing enzymes targeted at specific results has been reported (Choct, 2006).

In the present study the effects of the synbiotic and enzyme mixture on the feeding, drinking and resting behaviours of the broilers were also studied (Table 4). The analysis of data clarified that the probability of birds feeding, drinking was increased after the dietary supplementation, with the higher probability of resting. From the first day of the supplementation till the last, the supplemented birds increased their visits to the feeders and drinkers and their activity. It also observed that the overall means of feeding and drinking behavioural patterns were not significantly (p<0.05) affected by the treatment, where the birds of T2 group recorded more proportions of overall means of feeding, drinking (10.06±1.05 and 5.01±1.12%, respectively) versus (9.65±1.42 and 4.47±0.86% for T1 group and 9.10±1.38 and 4.19±0.48% for control group, respectively).

Dietary synbiotic supplementation improves the feeding behaviour of the broilers. The chicks from the supplemented groups (T1 and T2) visited more often the feeders during the whole experiment. Dietary

synbiotic supplementation increased also the visiting rate to the drinkers and this phenomenon was enhanced at the higher inclusion dose of the additive. It was rather expected though, since there is a direct relationship between feeding and drinking in birds and the two parameters, i.e., probability of a chicken feeding and drinking were highly and significantly correlated.

These observations may be attributed to high palatability and digestibility of dietary nutrients than in control diets. So treated birds visited more often feeder and consumed a smaller amount of diet and this will reflected on improved feed utilization and conversion.

These results were coinciding with George *et al.* (2010), who found that the chicks from the oregano supplemented groups visited less often the feeders during the whole experimented by the birds, because if oregano essential oil dietary supplementation was desirable, the average visits should have risen.

The overall proportion of birds of T2 group showed significant ($p<0.05$) higher proportions of resting behaviour than those of control group, where the overall values for resting was ($67.05\pm 3.77\%$) while they was ($53.51\pm 3.55\%$) for control group.

From the observed results, it is clear that the resting behaviour is related to feeding behaviour and consequently to body weights as during resting birds take this pattern to reduce their activities in a manner that would converse which may be affected performance of birds later. These findings are in agreement with those of Hocking *et al.* (1996) and Webster (2000) who found that there was relation between resting behaviour and body weight of the birds.

Physiological stress indicators in blood (Table 5) significantly increased ($p<0.05$) H/L ratio in control group 0.65 ± 0.06 versus 0.40 ± 0.05 and 0.38 ± 0.05 in T1 and T2 groups respectively. The cortisol level was also high in control group compared with the treated ones. It is obviously that increasing the dose of synbiotic used decreasing the stress indicators in the blood for the tested parameters. This may be due to the anti-stress effects of the probiotic as explained by Gross and Siegel (1983) and Fayed and Tony (2008).

In this study, we examined the effect of the Synbiotic on humeral immune response and peripheral blood mononuclear cells, the phagocytic percent and index of broiler chickens (Table 6 and 7). Concerning humeral immune response, high dose of Synbiotic (T2 group) improve the HI antibody titers for Newcastle Disease Virus (NDV) and ELISA antibody for Gumboro disease (IBV) comparing with that of control group. The result of group (T1) broiler chickens fed on diet containing 0.250 kg/Ton feed are fluctuated above and below that of the control group. These results are in agreement with that of Maassen *et al.* (2000) who recorded that, oral administration of lactobacillus strain

is significantly enhance IgG response, also to Haghghi *et al.* (2006) who found that probiotics enhance the systemic antibody response to some antigens in chickens and Talebi *et al.* (2008) who found that administration of a multi-strain probiotic improve the antibody responses to NDV.

Phagocytic percent and index of broiler chickens significant ($p<0.05$) increase in treated groups compared with control one at 5th day post 1st and 2nd vaccination. Also at 12th day post 2nd vaccination significantly increased as well. These results are in agreement with previous findings by Diaz-Rosales *et al.* (2006) who recorded that probiotic including increases the activities of phagocytes, also with Shimada *et al.* (2009) who reported that probiotics act on macrophages activity in a dose dependent manner. The activities of phagocyte may be explained as, the bacterial cell or bioactive peptide released during fermentation by lactic acid bacteria activate immune response through a dynamic interaction with specific Toll-like receptors on the surface of macrophage (it was known that the phagocytosis by macrophages is Toll-like receptors dependent) this interaction between host cells and pathogens or their structural components may play a critical role in the early innate immune response. The activation of the Toll-like receptors start signalling cascades that involve the activation of proteins and transcription factors inducing the secretion of pro-inflammatory and effectors cytokines which farther activate macrophage cells (Blander and Medzhitov, 2004).

In respect to carcass yield (Table 8) the current study demonstrates that there was no significant difference in dressing weights and liver weights between treated groups and control one. However there was a significant ($p<0.05$) reduction of abdominal fat in treated groups (T1 and T2) than that in control group. Abdominal fat decreased significantly by increase the dose of synbiotic used and these finding maybe reflect the utilization of feed energy to encourage the zootechnical performance of the broiler chickens. Our results are in line with that of Mirza (2009) and Lokman *et al.* (2012) who found that the dietary supplementation of broilers with probiotic and synbiotic decreased significantly the fat content of the carcass and had no significant effect on the heart, liver and gizzard percentage when compared with control at 42 days of age.

In conclusion the current study shows that the mixture of synbiotic and digestive enzymes could improve significantly broiler performance and feed conversion. The mixture used in this study had positive effects on broiler behaviour and can act as an anti-stress factor which could be reflected on the general health conditions, growth performance and carcass yield of broiler chickens. This study also provides evidence that the oral administration of Synbiotic (low and high

doses) to broiler chickens enhances immunity and improves humeral immune response represented by increase antibody response to NDV and IBV vaccine.

REFERENCES

- Awad, W.A., K. Ghareeb, S. Abdel-Raheem and J. Böhm, 2009. Effects of dietary inclusion of probiotics and synbiotics on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poultry Sci.*, 88: 49-56.
- Batavani, A., 2010. The Composition of Probiotics and Prebiotics of Immune System Stimulants. Publisher of Research Group of Itook Farda.
- Bedford, M.R., 2000. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimize subsequent problems. *World Poultry Sci. J.*, 56: 347-365.
- Blander, J.M. and R. Medzhitov, 2004. Regulation of phagosome maturation by signals from toll-like receptors. *Science*, 304: 1014-8.
- Choct, M., 2006. Enzymes for the feed industry: Past, present and future. *World Poultry Sci. J.*, 62: 5-15.
- Choct, M., R.J. Hughes and M.R. Bedford, 1999a. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine and ileal and caecal volatile fatty acid production in chickens fed wheat. *Br. Poult. Sci.*, 40: 419-422.
- Choct, M., R.J. Hughes and M.R. Bedford, 1999b. Effects of xylanase on individual bird variation, starch digestion throughout the intestine and ileal and caecal volatile fatty acid production in chickens fed wheat. *Br. Poult. Sci.*, 40: 419-422.
- Diaz-Rosales, P., I. Salinas A. Rodriguez, A. Cuesta, M. Chabrillon and M.C. Balebona, 2006. Gilthead seabream (*Sparus aurata* L.) innate immune response after dietary administration of heat-inactivated potential probiotics. *Fish Shellfish Immunol.*, 20: 482-92.
- Elijah, I.O. and T.S.O. Ruth, 2012. The effect of probiotic and prebiotic feed supplementation on chicken health and gut microflora: A review. *Int. J. Anim. Vet. Adv.*, 4(2): 135-143.
- Fayed, R.H. and M.A. Tony, 2008. Effect of probiotic supplementation as an anti-stress factor on growth performance, behaviour and carcass traits of broiler chickens. *Proceeding of the 1st Mediterranean Summit of WPSA*, pp: 518-524.
- Fioramonti, J., V. Theodorou and L. Bueno, 2003. Probiotics: What are they? What are their effects on gut physiology? *Best Pract. Res. Clin. Gastroenterol.*, 17: 711-724.
- Fooks, L.J. and G.R. Gibson, 2002. Probiotics as modulators of the gut flora. *British J. Nutr.*, 88(1): 39-49.
- George, K.S., Z. Costantinos, D. Nikolaos, A. Iosif and G.D. Band Stelios, 2010. Effects of oregano essential oil dietary supplementation on the feeding and drinking behaviour as well as the activity of broilers. *Int. J. Poultry Sci.*, 9(4): 401-405.
- Gibson, G.R. and M.B. Roberfroid, 1995. Dietary manipulation of the human colonic microbiota, introducing the concept of prebiotics. *J. Nutr.*, 125: 1401-1412.
- Gross B. and H.S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.*, 27(4): 788-795.
- Gunal, M., G. Yayli, O. Kaya, N. Karahan and O. Sulak, 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broiler. *Int. J. Poultry Sci.*, 5: 149-155.
- Haghighi, H.R., J. Gong, C.L. Gyles, M.A. Hayes, H. Zohu, B. Sanei, J.R. Chambers and S. Sharif, 2006. Probiotics stimulate production of natural antibodies in chickens. *Clin. Vaccine Immunol.*, 13(9): 975-980.
- Hocking, P.M., C.E. Channing, D. Waddington and R.B. Jones, 1996. Age related changes in fear, sociality and pecking behaviours in two strains of laying hen. *British Poult. Sci.*, 42: 414-423.
- Hooge, D., 2004. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993-2003. *Int. J. Poult. Sci.*, 3(3): 163-174.
- Jin, L.Z., Y.W. Ho, N. Abdullah and S. Jalaludin, 1997. Probiotic in poultry: Modes of action. *World's Poult. Sci. J.*, 53: 351-368.
- Lokman, T.A.B., G.S. Muneer and T.T. Ihsan, 2012. Effect of probiotic supplementation on broiler performance. *College of Veterinary Medicine, University of Duhok, Al-Barwary*, ISSN: 2222-1263.
- Maassen, C.B.C., V. Holten-Neelen, F. Balk, M.J. Den Bak-Glashouwer, R.J. Leer, J.D. Laman, W.J. Boersma and E. Claassen, 2000. Strain dependent induction of cytokine profiles in the gut by oral administered Lactobacillus strains vaccine. *Lett. Appl. Microbiol.*, 48: 797-800.
- Mirza, R.A., 2009. Using prepared synbiotic in early feeding of broiler chicken and its effect on some productive, physiological and histological performance and carcass characteristics. *M.Sc. Thesis, College of Agriculture University of Salahaddin- Erbil*.
- Mohnl, M., Y. Acosta Aragon, A. Acosta Ojeda, B. Rodriguez Sanchez and S. Pasteiner, 2007. Effect of synbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. *Poult. Sci.*, 86 (suppl. 1): 217.

- Parks, C.W., J.L. Grimes, P.R. Ferket and A.S. Fairchild, 2001. The effect of mannan oligosaccharides, bambarmycins and virginiamycin on performance of large white male market turkeys. *Poult. Sci.*, 80: 718-723.
- Patterson, J.A. and K. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. *Poult. Sci.*, 82: 627-631.
- Rahmani, H.R. and W. Speer, 2005. Natural additives influence the performance and humoral immunity of broilers. *Int. J. Poult. Sci.*, 4(9): 713-717.
- Reiter, K. and W. Bessei, 2000. Effect of stocking density of broilers on temperature in litter and at bird level. *Archv. Fuer. Geflugelkunde*, 64: 204-206.
- Sandilands, V., B.J. Tolkamp and I. Kyriazakis, 2005. Behaviour of food restricted broilers during rearing and lay: Effects of an alternative feeding method. *Physiol. Behav.*, 85(2): 115-123.
- Santin, E., A. Maiorka, M. Macari, M. Grecco, J.C. Sanchez, T.M. Okada and A.M. Myasaka, 2001. Performance and intestinal mucosa development of broiler chickens fed diet containing *Scharomyces cerevisiae* cell wall. *J. Appl. Poult. Res.*, 10: 236-244.
- Shashidhara, R.G. and G. Devegowda, 2003. Effect of dietary mannan oligosaccharide on broiler rearing production traits and immunolity. *J. Poult. Sci.*, 82: 1319-1325.
- Shimada, T., C.L.C. Yuning, M. Chie, F. Kazutake, K. Yoshihisa and W. Jieshu, 2009. Immunomodulation effects of heat-treated *Enterococcus faecalis* FK-23(FK-23) in mice. *J. Nanjing Med. Univ.*, 23(3): 173-176.
- Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan and Z. Uni, 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. *J. Nutr.*, 135: 187-192.
- Snedecor, F.W. and W.G. Cochran, 1980. *Statistical Methods*. 7th Edn., Iowa State University Press, Ames. I.A.
- Spring, P., C. Wenk, K.A. Dawson and K.E. Newman, 2000. Effect of mannan oligosaccharide on different cecal parameters and on cecal concentration on enteric bacteria in challenged broiler chicks. *Poult. Sci.*, 79: 205-211.
- Talebi, A., B. Amirzadeh, B. Mokhtari and H. Gahri, 2008. Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. *Avian Pathol.*, 37(5): 509-512.
- Webster, A.B., 2000. Behavior of white leghorn laying hens after withdrawal of feed. *Poult. Sci.*, 79: 192-200.
- Wenk, C., 2000. Recent advances in animal feed additives such as metabolic modifier, antimicrobial agents, probiotics, enzymes and available minerals. (Review) *Asian-Aust. J. Anim. Sci.*, 13: 86-95.
- Young, R.J., D.B. Whitney, T.L. Hanner, D.L. Antonson, J.V. Lupo and J.A. Vanderhoof, 1998. Preventing of antibiotic-associated diarrhea utilizing *Lactobacillus GG*. *Gastroenterol. Int.*, 11(Suppl. 1): 86.
- Zanoni, S., A. Pompei, L. Cordisco, A. Amaretti, M. Rossi and D. Matteuzzi, 2008. Growth kinetics on oligo- and polysaccharides and promising features of three antioxidative potential probiotic strains. *J. Appl. Microbiol.*, 105: 1266-12.
- Zhang, G., L. Ma and M.P. Doyle, 2006. Effect of Probiotics, Prebiotics and Synbiotics on Weight Increase of Chickens (*Gallus domesticus*). Retrieved from: [http://www.ugacfs.org/research/pdf/poultry 2006.pdf](http://www.ugacfs.org/research/pdf/poultry%202006.pdf).