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

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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

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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, cellualse, 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 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-lysin	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. D_3, 4 000 mg vit. E, 250 mg vit. B_1, 800 mg vit. B_2, 600 mg vit. B_6, 3.2 mg vit. B_{12}, 450 mg vit. K_3, 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 5^{th} and 12^{th} 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±3.600[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

	HI serum newcastle antibodies titre			ELISA gumboro antibodies titre		
Age (week)	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
5th days post 1st 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]
12th days post 1st 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]
5th days post 2nd 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]
12th days post 2nd 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±3.77%) while they was (53.51±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 significally enhance IgG response, also to Haghighi *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 5^{th} day post 1^{st} and 2^{nd} vaccination. Also at 12^{th} day post 2^{nd} 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.

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