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Effect of Dietary Supplementation of Probiotics (Addon Poultry Max) on Biochemical and Immune Parameters in Commercial Broiler Chicken

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A B S T R A C T

Two hundred and forty, day old chicks were distributed randomly into four treatments with three replicates in each treatment (20 birds in each replicate) and were fed standard feed. The trial was studied for 42 days which comprised of four dietary treatments wherein T1 served as basal diet. Basal diet+ 250g/ton (AGP), basal diet + Addon Poultry Max (250g/ton) and basal diet + Probiotics (Addon Poultry Max) (250g/ton) and Enrofloxacin (10ml/100kg BW) levels were designated as T2, T3 and T4 respectively. Effects of different treatment groups on serum biochemical (total protein, cholesterol) parameters and immune response (CMI to PHA-P, HI to ND vaccine) were evaluated. The humoral immune response to SRBC indicated insignificant ($P>0.05$) influence, while CMI response with P-HAP was significantly ($P<0.05$) higher in broilers raised on Probiotics (Addon Poultry Max) supplemented groups compared to the control group. Concentration of serum cholesterol was not affected due to treatments. However, numerically lower total serum cholesterol values were observed in Probiotics (Addon Poultry Max) supplemented when compared to control group. The highest serum total protein value was observed with Probiotic (Addon Poultry Max) (5.49 g/100ml) followed by T4 group (4.95 g/100ml) during sixth week. Hence, it can be concluded that, supplementation of Probiotic (Addon Poultry Max) as alternative to antibiotic can be used for improving performance of broiler chicken.

Introduction

Broiler production has become an important economic activity all over the world in the last few decades. India, large with an annual production (BAHS, 2015) of around 73.21 billion eggs and 3.725 million metric tons of poultry meat, ranks 3rd in egg production and 7th in broiler meat production, respectively, in the world. Antibiotics have been used as growth promoters for more than 5 decades in the feed industry to get advantage of increasing protection against some diseases, toxins, increasing nutrients absorption in

intestine. However, because of the possible development of resistance by pathogenic bacteria against antibiotics, their efficacy was reduced besides public health impact due to their residues in eggs and meat. The Probiotics are reported to regulate gut integrity, enhance useful microbial environment, reduce digestive disorders, improve nutrient absorption and utilization, increases production and check the mortality. The Probiotics may provide an alternative to the administration of sub therapeutic levels of antibiotics in preventing the colonization of the gastro intestinal tract by unfavorable microorganisms. Probitoics act in tandem with adhesion receptors on the gut epithelium, with nutrients, produce antibacterial substances, stimulate immunity and reduce mortality. The supplementation of Probiotics to the diet significantly improved the live weight and feed conversion ratio of the chicken (Roozbeh *et al.,* 2012).

Antibiotics and Probiotics are used separately as feed additives in poultry rations for the positive growth response but the information on combined feeding of those feed additives is limited further more information is available on alternative feeding of antibiotic and Probiotics and viceversa. Similarly more information is also available on the immune - modulating effect on Probiotics.

Hence, the present study was designed to explore the influence of antibiotic & Probitoics (Addon Poultry Max) and their combination feeding on broiler immune performance and biochemical parameters of blood.

Materials and Methods

Two Hundred and Forty birds were housed in each deep litter with an average floor space of 82 square inches or 205 sq. cm per bird. Feed and water were offered *ad lib*. and the birds were raised under identical management conditions. The routine vaccination schedule was followed. A growth rial was conducted in randomized block design, comprising of four dietary treatments, where in first treatment (T1) served as basal diet. Basal diet + 250g/ton (AGP), basal diet +250g/ton Probiotic (Addon Poultry Max), (basal diet + 250g/ton of Probiotic (Addon Poultry Max) and Enrofloxacin 1oml/100kg BW) levels were designated as T1, T2, T3, and T4, respectively. Three replicates were allocated to each of the treatments, employing twenty birds/replicate. Probiotic is proprietary commercial Add on Poultry Max product, prepared by Virbac Indian Private limited company, Mumbai. It is a mixture of *Bacillus coagulans*$1.5X10^9$ *Bacillus licheniformis* $0.8X10^9$, *Bacillus subtilis* $1.5X10^9$ *Lactobacillus acidophilus* 0.2X10.

Immuneparameters

The effect of feeding of Addon Poultry Max on immune response of broilers was studied by measuring the following parameters.

Humoral immune response to NDV

Blood samples were collected from eight birds individually from each dietary group at 42^{nd} day of age and antibodies specific for Newcastle disease Vaccine were measured in serum of chicks by haemagglutination inhibition (HI) test and were expressed as SRBC titers log2 (Allan *et al.*,1978).

Evaluation of cell mediated immuneresponse

Cell mediated immune (CMI) response was evaluated by cutaneous basophilic hypersensitivity (CBH) test by injecting 100µg phytohaemagglutin – P (PHA-P) in 0.1ml of NSS into toe web of eight birds from each dietary group. Thickness was measured

at 24 hours after injection and CBH was calculated using the formula (Edelman *et al.*, 1986).

Serum parameters

On day 21st and 42nd, blood from one representative bird from each replicate was collected in a clean sterile glass tube and kept in a slanted position at room temperature to facilitate the separation of serum for estimation of cholesterol and Total protein by using spectrophotometer with commercially available kits (Arkray Health care private Limited).

Serum analysis

2 ml of blood was collected from the wing vein using 2-3 ml syringe into an eppondrof tube. Then the blood was incubated from 2-3 hrs at 37°C and then centrifuged at 10,000 rpm for 6 minutes. Then the serum was separated and collected into another eppendrof tube and stored at-20°C for serum analysis.

Cholesterol

Cholesterol was estimated in serum using kit number- 83LS100-40 (Arkray Healthcare private Limited). The reagent of 1000 µl was taken in a cuvette, to it, 10 µl of sample was added. This was incubated for 10 minutes and read at 505 nm (409- 510nm). Then the optical density values were noted down and calculated.

Total protein

Total protein was estimated in serum using kit number- 71LS200-40 (Arkray Healthcare private Limited). The reagent of 1000µl was taken in a clean cuvette (by rising with distilled water), and10µl of serum sample was added and mixed well and incubated at 20-26°C for 30 minutes and then read at 578 nm (550-580 nm). Then the optical density values were noted down and calculated.

Results and Discussion

Immune organs

The present experiment revealed that the data on immune competence in broiler chicken was influenced by different dietary treatments with Probiotic (Addon Poultry Max) fed diets are presented in Table 1. The study revealed no significant difference in the relative weights of spleen and thymus at 42nd day of age. The relative spleen weight was higher in control (0.133g), then followed by Probiotc (Addon Poultry Max @ 250g/ton (0.1145). The relative thymus weight was higher in Probiotc (Addon Poultry Max) (0.347g) and least in control group. However, the supplementation of Probiotc (Addon Poultry Max s significantly) ($P<0.05$) improve the relative weight of bursa at 42 dosage. The higher bursa weight was observed with Probiotc (Addon Poultry Max) (250g/ton) when compared to all other group.

Immune organs of broilers were not significantly influenced by all the dietary supplementations of Probiotc (Addon Poultry Max) and their combination with antibiotic. But bursa was highest in Probiotc (Addon Poultry Max) supplemented group. These findings are in agreement with the results of Rama Rao *et al.*, (2004) who observed higher lymphoid organ (bursa, spleen) weights in broilers fed Probiotics diet. Contrary to these findings, Panda *et al.*, (1999) observed lack of difference in the live weight of spleen and bursa in Probiotics supplemented groups.

Humoral immune response

The data on humoral immunity was evaluated in terms of antibody response to Sheep Red Blood Cell at 42nd of age in broiler chicken as

influenced by different dietary treatments with Probiotics (Addon Poultry Max) fed diets are presented in Table 2. The mean, log2 antibody response to SRBC were not significant at 42nd day of age and mean log2 titer value was higher in Probiotic (Addon Poultry Max @ 250g/ton (9.75) and low in antibiotic @ 250g/ton (9.25) group.

The humoral immunity was evaluated in terms of antibody response to SRBC at 42nd day of age using HA assay. The mean log2 titer values were higher in all test diets compared to control. Similar findings were also observed by Panda *et al.*, (1999) who reported that supplementation of Probiotics did not have any significant effect on antibody production against SRBC.

In contrary to this Kabir *et al.*, (2004); Khaksefidi and Ghoorchi (2006); Nayebpor *et al.*, (2007) observed that significantly (P<0.01) higher antibody production in experimental birds as compared to control group.

Increased titer values against SRBC might be due to the effect of Probiotics (Addon Poultry Max), on immune system or improved intestinal absorption of some nutrients such as Zn, Cu and Se. The higher lymphoid organ weight (bursa and spleen) also supports the increased immune response in test groups. The reduced gut pH and pathogenic bacteria in intestine, increased weight of lymphoid organs which might have acted indirectly to enhance the immune competence in broilers.

Table.1 Effect of Probiotic (*Addon Poultry Max)* on relative immune organ weights of broiler chicken at 42days of age (N=6)

Diets	Relative weights		
	Spleen	**Thymus**	**Bursa**
T1	0.0919	0.322	**0.074^{b}**
T2	0.1101	0.323	**0.084^{b}**
T3	0.1330	0.347	**0.100^{b}**
T4	0.1145	0.334	**0.098^{b}**
P Value	0.345	0.984	**0.001**
SEM	**0.005**	**0.015**	**0.011**

Table.2 Effect of dietary inclusion of *Addon Poultry Max* on Immune Response in broiler chicken at 42nd day of age (N=8)

Diets	*PHA-P response (thickness index)	SRBC titers (log2)
	6th week	**6th week**
T1	109.0 ^{b}	**9.5**
T2	154.0 ^{b}	**9.25**
T3	113.9 ^{b}	**9.75**
T4	126.3^{b}	**9.75**
P Value	0.001	**0.64**
SEM	**28.64**	**1.129**

*****PHA-P: Phytohaemaglutinin-phosphate Show level of significance @ P>0.01

Table.3 Effect of dietary inclusion of *Addon Poultry Max* on Serum biochemical profile in broiler chicken at 21st and 42nd day of age (N=8)

Diets	Cholesterol (mg/100ml)		Total Protein (g/100ml)	
	3rd week	6th week	3rd week	6th week
T1	199.1	184.0	3.53	4.66
T2	192.9	182.3	3.55	4.71
T3	178.5	180.3	3.79	5.49
T4	187.5	182.0	3.70	4.95

Mean bearing at least one common superscript in a column do not differ significantly (P>0.05)

Cell Mediated Immunity

The present experiment revealed that the data in Table 2. There is no significant difference was observed in PHA-P (thickness index) response among all dietary treatments but numerically higher PHA-P (thickness index) response was noticed in all test diets compared to control group at the age of 42nd day.

Cell mediated immunity was evaluated in terms of CBH response at 42nd day of age by injecting PHAP into inter digital space. There was significant difference in CBH response among the treatments, but higher CBH response was noticed in all test diets compared to control. These results concur with the reports of Verduczo *et al.*, (2009) who observed that supplementation of yeast cell significantly increased cell mediated immune response in terms of cutaneous basophilic hypersensitivity test at 21st day of age compared to control.

Biochemical parameters

Cholesterol and total protein

The total serum cholesterol in broiler chicken as influenced by various dietary treatments was presented in Table 3. The study revealed that there was no significant difference among different dietary treatments. However, numerically lower total serum cholesterol values observed in Probiotics (Addon Poultry Max) supplemented group at both third (178.5 mg/100ml) and sixth week (180.3 mg/100ml) ofage when compared to control group. Highest cholesterol was recorded in control group in both 3rd and 5th week respectively.

The data on total protein (g/100ml) as influenced by various dietary treatments is presented in the Table 3. The study revealed that there was no significant difference among different dietary treatments. The highest serum total protein value was observed with Probiotics (Addon Poultry Max) (3.79 g/100ml) group in third week and during sixth week (5.49 g/100ml) followed by combined antibiotic and Probiotic (Addon Poultry Max) group during third week (3.70 g/100ml) and during sixth week (4.95 g/100ml). Least total protein was recorded in control group in both 3rd week and 5th week when compared to all other groups.

Supplementation of Probiotics and combination with Probiotics and antibiotic in broiler diets did not influence the cholesterol and total protein at 42 day of age which may be due to improved metabolic activities by various supplements tested in the study. Similar findings were reported by Panda *et al.*, (2006); Similarly Ashayerizadeh *et al.*, (2009); Shareef and Al-Dabbagh (2009); Shanmuga and Saravana (2013) observed that the total cholesterol was decreased, while total protein was increased, at the inclusion of 1.5% of probiotics (*Saccharomyces cerevisiae*).

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