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Effect of Dietary Supplementation of Probiotic (Problend) on Immune Status, Biochemical Profile and *E. coli* Counts in Commercial Broiler Chicken

R. Shirisha*, Krishnadaida[1], M.V. L.N. Raju[2], S. Sai Reddy[3] and V. Ravinder Reddy[1]

[1]Department of Poultry Science, P.V.N.R Telangana Veterinary University, College of Veterinary Science, Rajendranagar, Hyderabad-30. Telangana, INDIA

[2]Directorate on Poultry Research, ICAR, Rajendranagar, Hyderabad-30, INDIA

[3]Department of Animal Genetics and Breeding, P.V.N.R Telangana Veterinary University College of Veterinary Science, Rajendranagar, Hyderabad-30. Telangana, INDIA

**Corresponding author: R Shirisha; Email: sirisharamayan55@gmail.com*

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ABSTRACT

An experiment was conducted to study the influence of dietary supplementation, of graded level of probiotics on the immune response, serum biochemical (total protein, cholesterol), and *E.coli* counts in commercial broiler chicken. A total of 240 unsexed broiler chicks were distributed randomly in to six dietary treatments and reared commercial broiler chicks under uniform management conditions from day old to six weeks of age to evaluate the immune response (Cell mediated immunity (CMI) to phyto heamagglutinin-P(PHA-P), Humoral immunity (HI) to new castle disease vaccine(NDV), serum biochemical (total protein, cholesterol), and *E.coli* counts. The humoral immune response to Sheep-Red blood cells (SRBC) as studied at 42 days old indicated insignificant (P>0.05) influence due to dietary treatments, while CMI response to PHA-P was significantly (P<0.05) affected, but no specific trend could be attributed. The relative weights of bursa were significantly (P<0.05) higher in probiotic group compared to control. The concentration of serum cholesterol and total protein were not affected due to treatments. At 42 days of age, significantly (P<0.05) reduced *E. coli* counts were observed in small intestine and excreta of broilers, supplemented with dietary probiotics. It was concluded that commercial broilers can be reared profitably to improve immunity and reducing harmful bacterial load in the intestine and excreta by supplementation of probiotics at graded levels.

Keywords: Probiotic, immunity, *E.coli* counts, commercial broiler, biochemical profile

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. This is mainly due to the advent of development in the field of genetics, nutrition and relatively shorter time for meat production. Lower cost and greater demand for broiler meat has been observed due to consumer preference. 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. Problend is proprietary commercial probiotic product, prepared by biofactor inputs private limited company, Hyderabad. It is defined as live microbial feed supplements which imparts beneficial effect to host by improving its intestinal microbial balance. Their mode of action is by "competitive exclusion of harmful pathogens". It is a mixture of *Bacillussps* (*B. megaterium*, *B. subtilis*, *B. clausii*, *B. pumilus, B. licheniformis*, *B. polymyxa*, *B. amyloliquifaciens) Lactobacillus sps.* (*L. sporogenes, L. casi, L. acidophilus, L. rhamnosus, L. bulgaricus) and yeast culture (S. cerevisiae, S. boulardi).* The probiotic is reported to regulate gut integrity, enhance useful microbial environment, reduce digestive disorders, improve nutrient absorption and utilization, improves immunity, increases production and check the mortality. The supplementation

of probiotic to the diet significantly improved the live weight and feed conversion ratio of the chicken (Roozbeh Shabani *et al*., 2012b).

MATERIALS AND METHODS

A total of 240 commercial broiler birds were randomly allotted to 48 battery brooder cell with an average floor space of 82 square inches per bird. The experiment was conducted from day old to six weeks of age. Probiotic (Problend) was supplemented to a maize-soyabean meals diet at 6 graded levels each (100, 200, 300 g/ton of probiotic, 200g/ton of commercial product and a probiotic (300 g/ton) + antibiotic (100 g/ton) against a basal diet. All the 6 diets (Table 1) were *Iso-nitrogenous* and *Iso-caloric*. The Nutrient composition of dietary treatments was given in Table 2. Each diet was fed *ad-libitum* to 8 replicates consisting of 5 birds per replicate.

Table 1: Chemical composition of experimental ration

Diets	Probiotic in diet
Control	Basal diet without probiotic
Probiotic	Basal diet + probiotic100g/ton
Probiotic	Basal diet+ probiotic 200g/ton
Probiotic	Basal diet+ probiotic 300g/ton
Commercial Probiotic	Basal diet+ commercial probiotic 200g/ton
Probiotic and Antibiotic	Basal diet+ probiotic 300g/ton plus antibiotic 100g/ton

Table 2: Ingredient Composition of Basal Diets (in kgs) fed to the commercial broilers from 0-42 days

Ingredient	Prestarter (0-14d)	Starter (15-28d)	Finisher (29-42d)
Maize	53.7	56	59.1
Oil	1.6	4	4
Soyabean meal	40	35	32.2
Shell grit	1.65	1.83	1.75
Dicalcium phosphate	1.85	1.95	1.89
Salt	0.4	0.4	0.4
DL-Methionine	0.21	0.19	0.15
L-Lysine HCl	0.11	0.14	0.15
Trace Mineral Mixture	0.1	0.1	0.1
Vitamin AB2D3K	0.02	0.02	0.02
Vitamin B-Complex	0.025	0.025	0.025
Coccidiostat	0.05	0.05	0.05
Antibiotic	0.05	0.05	0.05
Choline chloride(50%)	0.1	0.1	0.1
Toxin binder	0.1	0.1	0.1
Tylosine	0.05	0.05	0.05
Total	100	100	100
Nutrient composition			
ME(kcal/kg)	2911	3070	3106
Crude protein (%)	22.56	21.74	19.51
Lysine (%)	1.3	1.21	1.02
Methionine (%)	0.55	0.50	0.45
Calcium (%)	1.0	1.06	1.01
Available phosphorous(%)	0.45	0.46	0.45

*Vitamin premix provided per kg diet: Vitamin A 200000IU, Vitamin D3 3000IU, Vitamin E 10mg, Vitamin K 2mg, Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg, and Niacin 15mg.

*Trace mineral provided per kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg, Iodine 1mg and Selenium 0.1mg.

Collection of immune organs. The immune organs collected are bursa, thymus and spleen at the end of the experiment (42 days) on five birds from each replicate.

Immune parameters

The effect of feeding of probiotic 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 42nd 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 $\log_2$ (Allan *et al.,*1978).

Evaluation of Cell mediated Immune response

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).

$$CMI = \frac{\text{Post injection skin thickness of toe web}}{\text{Pre injection skin thickness of toe web}} \times 100$$

Biochemical 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).

***Escherichia coli* Count**

Two birds from each dietary treatment were slaughtered on 42day and 1.0gm of intestinal and fecal contents were collected aseptically from each bird and suspended in 9ml of nutrient broth. Serial dilution of each sample were made in nutrient and *E. coli* counts were recorded on EMB agar by surface spread method. The number of colonies was expressed as 1og 10 value.

RESULTS AND DISCUSSION

The results of weight of immune organs in broiler chicken was influenced by different dietary treatments fed with probiotic diets are presented in Table 3. There is no significant difference in the relative weights of spleen and thymus at 42 d of age. However, the supplementation of probiotics significantly (P<0.05) improve the relative weight of bursa at 42 d of age. The higher bursa weight was observed with probiotic (100g/ton) when compared with control 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 probiotic diet. Contrary to these findings, Panda *et al*. (1999) observed lack of difference in the live weight of spleen and bursa in probiotic supplemented groups.

Table 3: Effect of *probiotic* on relative immune organ weights of broiler chicken at 42 days of age (N=6)

Diets	Levels (g/ton)	Relative weights		
		Spleen	Thymus	Bursa
Control	0	0.1330	0.322	0.100[b]
Probiotic	100	0.1059	0.307	0.153[a]
Probiotic	200	0.1145	0.334	0.098[b]
Probiotic	300	0.1054	0.342	0.092[b]
Commercial Probiotic	200	0.1101	0.347	0.074[b]
Probiotic and Antibiotic	(300 + 100)	0.0919	0.323	0.084[b]
P Value		0.345	0.984	0.001
SEM		0.005	0.015	0.011

The data on humoral and cell mediated immunity were evaluated in broiler chicken supplementation with probiotic presented in Table 4.

Table 4: Effect of dietary inclusion of *probiotic* at graded levels on Immune Response in broiler chicken at 42nd day of age (N=8)

Diets	Levels (g/ton)	*PHA-P response (thickness index)	SRBC titers (log_2)
		6th week	6th week
Control	0	109.0[b]	9.5
Probiotic	100	120.1[b]	10.25
Probiotic	200	154.0[a]	9.25
Probiotic	300	165.4[a]	9.75
Commercial Probiotic	200	113.9[b]	9.75
Probiotic and Antibiotic	(300 + 100)	126.3[b]	9.75
P Value		0.001	0.64
SEM		28.64	1.129

*PHA-P: Phytohaemaglutinin-phosphate

level of significance at the rate of P>0.01

The mean, log_2 antibody response to SRBC were not significant at 42 d of age and mean log_2 titer value was higher in probiotic at the rate of 100g/ton (10.25) and low in probiotic at the rate of 200g/ton (9.25) when compared to control group. Similar findings were also observed by Panda *et al*. (1999) who reported that supplementation of probiotic did not have any significant effect on antibody

production against SRBC. Increased titer values against SRBC might be due to the effect of probiotics, on immune system or improved intestinal absorption of some nutrients such as Zn, Cu and Se. 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. 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 21 d of age compared to control.

The serum cholesterol and total protein in broiler chicken as influenced by various dietary treatments was presented in Table 5. The serum cholesterol and total protein no significant difference among different dietary treatments, at third and sixth week of age. Similar findings were reported by Panda *et al. (*2006), Ashayerizadeh *et al.* (2009), and Shareef and Al-Dabbagh (2009). Shanmuga Priya and Saravana Babu (2013) observed that the total cholesterol was decreased, while total protein was increased, at the inclusion of 1.5% of probiotic (*Saccharomyces cerevisiae*).

Table 5: Effect of dietary inclusion of *probiotic* at graded level on Serum biochemical profile in broiler chicken at 21st and 42nd day of age (N=8)

Diets	Levels (g/ton)	Cholesterol (mg/100ml)		Total Protein (g/100ml)	
		3rd week	6th week	3rd week	6th week
Control	0	187.5	182.3	4.83	4.71
Probiotic	100	199.1	182.9	3.55	4.66
Probiotic	200	205.2	183.3	2.95	4.58
Probiotic	300	178.5	180.3	3.70	4.95
Commercial Probiotic	200	191.9	185.5	3.92	4.35
Probiotic and Antibiotic	(300 +100)	192.9	184.0	3.79	5.49
P Value		0.333	0.999	0.604	0.326
SEM		3.49	3.19	0.29	0.15

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

The result of the *Escherichia coli* counts in the intestine and excreta in broilers presented in Table 6. The *Escherichia coli* counts (mean log10 of cfu/ml) in the intestine and excreta were significantly (P<0.05) lower in the groups supplemented with probiotics compared to the control group without probiotic supplementation. The results of this study are in agreement with the findings of Panda *et al.* (2001), Rama Rao *et al.* (2004), Tollba and Mahmoud (2009) and Jeong and Kim (2014).

The reduced bacterial count in broilers might be due to anti bacterial activity of probiotic through reducing the pH of gastro intestinal tract and microbial cytoplasm there by inhibiting the growth of pathogenic intestinal bacteria.

Table 6: Effect of *probiotic* on *Escherichia coli* (log10 of cfu/ml count) in the intestine and excreta of broiler chicken at 42 days of age (N=6)

Diets	Levels (g/ton)	*E. coli* (log 10 of cfu/ml count)	
		Intestine	Excreta
Control	0	6.04^{d}	6.26^{c}
Probiotic	100	5.36^{bc}	5.81^{bc}
Probiotic	200	5.32^{b}	5.77^{a}
Probiotic	300	5.15^{a}	5.65^{b}
Commercial probiotic	200	5.40^{bc}	5.35^{a}
Probiotic and Antibiotic	(300 + 100)	5.64^{bc}	5.72^{ab}
P Value		0.05	0.048
SEM		0.052	0.102

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

CONCLUSION

Supplementation of probiotics was more effective among all the dietary treatments in terms of better immune response, and reduced *E. coli* counts in intestinal contents and excreta at 42 d of age. It can be concluded that, supplementation of probiotics as alternative to antibiotic can be used for improving performance of broiler chicken

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