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Effect of Organic Acids with Probiotic Supplementation on Immunity and Blood Biochemical Status of Broiler Chicken

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ABSTRACT

The present research was conducted to study the effect of organic acids with probiotic supplementation on immunity and blood biochemical status of broiler chicken. A total number of 300 birds were reared for a period of forty two days with dietary treatments; T_0 - control diet as per BIS (2007), T_1 - control + sodium diformate @ 0.2%, T_2 - control + sodium diformate @ 0.2 + probiotic @0.02%, T_3 - control + blends of organic acids @0.2%, T_4 - control + blends of organic acids @0.2% + probiotic @0.02%. Each treatment consist of sixty birds with four replicates containing fifteen birds per replicate. Two birds from each replicate of average body weight were selected and blood samples at the end of 42 days were collected to determine immune titre and blood biochemical parameters. Immune titre of birds were found to be non-significant with increased antibody titre in all treatment groups than control. Blood biochemical parameters viz., total protein, albumin, globulin and BUN were found to be significant. However there was decreased level of cholesterol in all treatment groups than control.

Introduction

Poultry is one of the quickest growing industry in India and furthermore world. Present day modern poultry industry has accomplished marvelous gains in the proficient and efficient creation of high quality and safe chicken meat, eggs and poultry byproducts. Meanwhile as making gains underway and effectiveness, the industry needs to increase the prosperity and thriving of the birds and limit the effect of the business on the earth. The use of feed additives has been a basic bit of gaining this ground. Feed additives are essentially included to enhance the productivity of the bird's development as well as laying capacity, prevent diseases and enhance feed utilization. Antibiotic feed additives as growth promoters have for quite some time been added to poultry feed to stabilize the intestinal microbial flora, improve the general performances and prevent some specific intestinal pathogens (Hassan *et al.,* 2010). The prohibition on use of antibiotics as growth promoters in European Union since 2006 grasped the finding of differentiating alternative to antibiotics in farm animal nutrition (Attia *et al.*, 2006, 2012; El-Deek *et al.,* 2011). Antibiotic-resistant bacteria (whose number is growing well ordered) are considered furthermore a social issue with a high reasonable impact due to the extending number of hospitalizations. The finding of natural molecule as an elective choice to anti-microbials could upgrade welfare both in birds and people. A couple of different choices to anti-microbial growth promoters have been proposed, for instance organic acids (Kral *et al.,* 2011), probiotics (Capcarova *et al.,* 2008), phytogenic feed additives (Gálik and Rolinec, 2011), products bees (Petruska *et al.*, 2012) and enzymes (Bentea *et al.,* 2010). Basically, organic acids incorporate carboxylic acids and unsaturated fats having a chemical formula of R-COOH, where R represents to chain length of the acids. Organic acids have growth-promoting properties (Fascina *et al.*, 2012), likewise its utilization could stimulate the natural immune response (Lohakare *et al.*, 2005; Abbas *et al.*, 2013). Probiotics are either single as well as blend of live microbial culture which elevate health benefits to the host (Fuller, 1992). Strategy for probiotics activity incorporates competition with receptor sites in the intestinal tract, production of specific metabolites (short organic fatty acids, hydrogen peroxide, other metabolites possessing antimicrobial activity) and immune stimulation effect (Madsen *et al.*, 2001; Sherman *et al.,* 2009). *Saccharomyces* known to offer a good quality protein and B-complex vitamins. Due to immunomodulatory properties, yeast extract, the non-anti-microbial product is recommended to be the potential non-anti-microbial option for diminishing pathogenic micro-organisms in turkey production (Huff *et al.,* 2010). Microencapsulation of probiotic can be utilized to upgrade the viability during processing and also for the targeted delivery in gastrointestinal tract. Considering the wide scope for the research of combination of single or blends of organic acids with probiotic to give optimum synergistic effect on immunity and blood biochemical performance of broiler chicken, the present study is planned.

Materials and Methods

An experiment was conducted to study the effect of organic acids with probiotic supplementation on immunity and blood biochemical status of broiler chicken. A total 300 broilers were reared for six weeks with dietary treatments, T_0 (control diet as per BIS, 2007), T_1 (control plus sodium-diformate @ 0.2%), T_2 (control plus sodium diformate @ 0.2% plus probiotic @ 0.02%), T_3 (control plus blends of organic acid @ 0.2%), T_4 (control plus blends organic acid @ 0.2% plus probiotic @ 0.02%) as shown in table 1. Each treatment consisted of sixty birds with four replicates containing fifteen birds in each replication. The Probiotic contained encapsulated *Saccharomyces cerevisiae* @ 1 x 10^{10} CFU/g. Blends of organic acid consisted of buffered organic acids like Calcium Propionate, Sodium Formate, Fumaric acid, Sorbic acid and Citric acid in equal quantity. The birds were reared on deep litter system and standard managemental practices were followed during the entire experimental period. Individual body weight of each bird was recorded at weekly interval and bodyweight gain was calculated as mean of each replicate. The birds from the experimental trial were assessed for the antibody titer against the New Castle Disease (ND) at 42 days of age. Blood samples from eight birds of each treatment (two from each replicate with average body weight) were collected toward the finish of test. The blood samples were collected via wing vein from each bird and serum was isolated by centrifugation at 3000 RPM for 10 minutes and kept at - 20^0C till further investigation. These serum samples were used for Haemagglutination Inhibition (HI) test as per procedure of O.I.E (1992) to detect the antibody titer against New Castle Disease. Two fold serial dilutions of antigen and serum was used as antigen for HI test. The HI titer was expressed as $\log_2$ value of the highest dilution of serum causing complete inhibition of 4HA unit of antigen and given in table 2. Biochemical parameters included estimation of Total protein, Albumin, BUN, Serum cholesterol utilizing biochemical kits produced by AGD Biomedicals PVT.LTD with the assistance of AGD Biochemistry Auto-analyzer whereas serum Globulin was determined by subtracting Albumin from total protein and depicted in table 2. The collected data during the study was analyzed statistically as per Snedecor and Cochran (1994) by utilizing Statistical Package for the Social Sciences (SPSS) Version 17.0.

Results and Discussion

Average mean values of HI titres indicated non-significant differences in different groups and values ($\log_2$) recorded as 4±0.42, 4.13±0.52, 4.25±0.49, 4.38±0.6 and 5±0.68 in T_0, T_1, T_2, T_3 and T_4 with pooled mean value 4.35±0.24 treatment respectively The values of serum total protein (mg/dl) were T_0 - 2.95±0.25, T_1 -3.14±0.16, T_2-3.24±0.26, T_3-3.69±0.14 and T_4 - 4.06±0.1 with mean 3.41±0.1. Among all the Treatment group T_4 fed with mixture of organic acids and probiotic differed significantly from T_0, T_1 and T_2 with numerically highest value among all the treatments however lowest serum total protein value was observed in control group. The values of serum albumin (mg/dl) for treatment group T_0 to T_4 with mean were 1.36±0.1, 1.43±0.1, 1.54±0.12, 1.82±0.12, 1.86±0.1 and 1.6±0.06 respectively. The data pertaining values of serum albumin subjected to analysis of variance found to be significant. The highest value for serum albumin was observed in T_4 group. Whereas lowest value was observed in treatment group T_0 fed normal diet as per BIS, 2007. The data pertaining values of serum globulin (mg/dl) subjected to analysis of variance found to be significant. The values for treatment group T_0 to T_4 with mean (mg/dl) were 1.59±0.21, 1.71±0.18, 1.7±0.16, 1.87±0.07, 2.2±0.04 and 1.81±07. It was observed that group T_4 fed with blends of acidifier with probiotic showed numerically highest value among all the treatment group. However control group showed numerically lowest value. The values for treatment groups of serum cholesterol (mg/dl) were T_0-229.88±7.43, T_1-220.38±13.96, T_2-212.08±26.67, T_3-201.44±12.73 and T_4-192.32±15.19 with mean 211.22±7.29. Non-significant differences were found among the treatments. Highest numerical value of serum cholesterol was observed in control group (229.88 mg/dl) whereas lowest value of serum cholesterol was in group T_4 (192.32mg/dl). It was observed that all treatment groups recorded lower value for serum cholesterol as compared to control. The data pertaining values of BUN (mg/dl), subjected to analysis of variance found to be significant. The values of BUN for different treatment groups were T_0 -1.02±0.04, T_1-0.87±0.03, T_2 -0.85±0.02 T_3 -0.77±0.02 and T_4 -0.7±0.02 with mean 0.84 ±0.02.Group T_4 significantly lower BUN as compared to control. It was observed that all treatment groups recorded lower value for BUN as compared to control.

The results of the immune parameter corroborates with Ozek *et al*., (2011) who found organic acid and essential oil mixture + organic acid supplementation in diets in summer season on immune response of laying hens.

Table.1 Composition of broiler ration

Ingredient	Pre-Starter					Starter					Finisher				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
Maize	46.2	46.2	46.2	46.2	46.2	49	49	49	49	49	54	54	54	54	54
Soya (DOC)	43.5	43.5	43.5	43.5	43.5	40.6	40.6	40.6	40.6	40.6	35.1	35.1	35.1	35.1	35.1
Soya oil	5.57	5.57	5.57	5.57	5.57	6.3	6.3	6.3	6.3	6.3	6.92	6.92	6.92	6.92	6.92
L-Lysine	0.01	0.01	0.01	0.01	0.01	-	-	-	-	-	-	-	-	-	-
DL-Methionine	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
LSP	1.13	1.13	1.13	1.13	1.13	1.15	1.15	1.15	1.15	1.15	1.1	1.1	1.1	1.1	1.1
DCP	2.01	2.01	2.01	2.01	2.01	1.86	1.86	1.86	1.86	1.86	1.79	1.79	1.79	1.79	1.79
Trace-min mix	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit mix	0.30	0.30	0.30	0.30	0.30	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat*	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder*	0.10	0.10	0.10	0.10	0.10	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium diformate*	-	0.2	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.2	-	-
Probiotic*	-	-	0.02	-	0.2	-	-	0.02	-	0.02	-	-	0.02	-	0.02
Acid Mixtures*	-	-	-	0.2	0.02	-	-	-	0.2	0.2	-	-	-	0.2	0.2
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CP (%)	23	23	23	23	23	22	22	22	22	22	20	20.1	20.1	20.1	20.1
ME (Kcal/kg)	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200

***Over and above

Table.2 Immune and blood biochemical parameters

Treatments	HI Titre (Log_2)	Total Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Cholesterol (mg/dl)	BUN (mg/dl)
T0	4^{a}±0.42	2.95^{a} ± 0.25	1.36^{a} ± 0.1	1.59^{a} ± 0.21	229.88^{a} ± 7.43	1.02^{d} ± 0.04
T1	4.13^{a}±0.52	3.14^{ab} ± 0.16	1.43^{a} ± 0.1	1.71^{a} ± 0.18	220.38^{a} ± 13.96	0.87^{c} ± 0.03
T2	4.25^{a}±0.49	3.24^{ab} ± 0.26	1.54^{ab} ± 0.12	1.7^{a}± 0.16	212.08^{a} ± 26.67	0.85^{bc} ± 0.02
T3	4.38^{a}±0.6	3.69^{bc} ± 0.14	1.82^{b} ± 0.12	1.87^{ab} ± 0.07	201.44^{a} ± 12.73	0.77^{ab} ± 0.02
T4	5^{a}±0.68	4.06^{c} ± 0.1	1.86^{b} ± 0.1	2.2^{b} ± 0.04	192.32^{a} ± 15.19	0.7^{a} ± 0.02
Pooled Mean	4.35±0.24	3.41± 0.1	1.6± 0.06	1.81± 0.07	211.22± 7.29	0.84± 0.02

Treatment in column bearing common superscripts doesn't differ significantly (P<0.05)

No significant differences were observed in antibody titer level of Newcastle disease virus (NDV), however, supplementing essential oil mixture and organic acid in layer diet tend to improve antibody responses against NDV.

Similar results were found to Manafi *et al.,* (2015, 2018), Yakhkeshi *et al.,* (2011), Hassanein and Soliman (2010) .In contrast to above results Flamand *et al.,* (2014) found no effect on immune response by administering the organic acid blends.The results obtained in present study for serum total protein are in accordance with Yesilbag and Colpan (2006) who studied the effects of organic acid supplemented diets (0.5, 1.0, and 1.5 %) on serum parameters in 20 week old 180 Lohmann LSL white layer hens showing significant increase in total protein. Linear increase in serum total protein was observed by Soltan (2008). This effect of organic acid supplementation on protein metabolism may be related to improvement of intestinal amino-acids absorption in acidic conditions that consequently enhances protein synthesis. Whereas Hassanein and Soliman (2010) reported that total protein levels of birds fed 0.4% (3.82), 0.8% (3.65) and 1.2% (3.97) yeast was lower than the control (4.16). Similarly Brzoska *et al.,* (2013) reported no significant differences between the control and experimental chickens for serum total protein. Naveenkumar *et al.,* (2018) also reported non-significant differences for serum total protein. The results of serum albumin were found to be similar with Yesilbag and Colpan (2006) who found significant increase in serum albumin. Soltan (2008) also reported linear increase of serum concentration of albumin. However Hassanein and Soliman (2010) reported decreased value of serum albumin of treatment group than control. Results of the present study for serum globulin are in agreement with Abdel-Fttah *et al.,* (2008) who supported that dietary organic acid supplementation increases serum globulin level significantly compared to control diet which improve the immune response. Globulin level has been used as indicator of immune responses and source of antibody production. Griminger (1986) stated that high globulin level signify better disease resistance and immune response. The outcome of present study for serum cholesterol level found to be similar with Abdel-Fttah *et al.,* (2008) where serum cholesterol level decreases significantly in treatment diets supplemented with organic acid. Hassanein and Soliman (2010) observed cholesterol levels of layers fed yeast-supplemented diets were lower than the control. Kazempour and Jahanian (2017), Ramigani *et al.*, (2015) also reported decrease in serum cholesterol levels in organic acid supplemented groups. However Yesilbag and Colpan (2006), Adil *et al.,* (2010) and Brzoska *et al*., (2013) reported no difference in value of total cholesterol on different dietary treatment supplemented with organic acids. The values of results for BUN were found to be similar with biochemical profile of chicken given by Abdi-Hachesoo (2011).

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