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RESEARCH ARTICLE

Protective Effect of Probiotics in Combination with Vaccination on Antibody Response, Biochemical and Hematological Indices in Broilers

Noreen Sarwar [1]*, Atif Mehmood[2], Ali Sheraz[2] and Muhammad Noman[2]

[1]University of Veterinary and Animal Sciences, Lahore; [2]University of Lahore, Lahore, Pakistan
*Corresponding author: noreen.sarwar@uvas.edu.pk

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A B S T R A C T

In this study, the effect of multi strain probiotics was studied on antibody response, blood chemistry and hematological parameters in combination with vaccination. A total of sixty day-old broiler chicks were equally divided into four groups: Control, Probiotic, Vaccine and Probiotic + Vaccine. Probiotics were offered at 2g/500ml of water for 5 days/week up to 6 weeks. NDV vaccine was given by eye drops at 0.025ml/bird at day 7. At 21st day NDV Lasota vaccine was offered in drinking distil water at 0.50ml /bird. IBDV vaccine was administered by drinking water at 10th, 22nd and 30th day of experiment. Blood samples were collected at 15, 30 and 45 days to measure antibody response, blood chemistry and hematological parameters. Antibody titer against NDV vaccine and IBD vaccine showed decline in control and probiotic groups. In case of NDV vaccine, there was no significant difference in Vaccine group (GMT=$\log_2$ $^{7.33}$) and Probiotic + Vaccine group (GMT=$\log_2$ $^{7.66}$) at day 45th of experiment. For IBDV vaccine, there was significant difference at 45th day in ELISA titer of vaccine group (2233) and Probiotic+Vaccine group (4206). Cholesterol and ALT showed significant decrease in PV group at 45th day. Significant increase in WBC count was observed in PV group. Level of significance was $P \leq 0.05$. Probiotics when administered along with vaccine showed improvement in antibody titer but no harmful increase or decrease in blood chemistry and hematological parameters was observed.

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INTRODUCTION

Poultry is the fastest growing sector all over the world. Effective and balanced diet is very important factor for cost effective poultry production. Several hormones and antibiotics are used as growth promoter but due to development of resistance against antibiotics and presence of their residues in feed, probiotics are safe and better choice as feed additives (Bidarkar *et al*., 2014). Probiotics are emerging as alternatives prophylactics with additional benefits such as enhanced immune response, toxin binding and increased digestion (Pedroso *et al*. 2013, Jadhave *et al*., 2015).

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. These are nontoxic and nonpathogenic microorganisms which when administered through digestive route produce health benefits. Probiotic level also effects on blood parameters, growth performance, cecal microbiota, carcass traits and immune response of broiler (Pourakbari *et al*., 2016). Probiotic species belonging to *Streptococcus*, *Enterococcus*, *Candida*, *Lactobacillus, Bacillus*, *Aspergillus*, *Saccharomyces and Bifidobacterium h*ave a very good effect on the variety of intestinal micro flora, growth performance, nutrient digestibility, immunomodulation, intestinal histological changes, certain haematobiochemical constrains, carcase characteristics and to improve sensory characters of broiler meat (Kabir, 2009; Pournazari *et al*., 2017; Souza *et al.,* 2018).

Previously Kudair and Al- Hussary, 2010 observed vaccination against Newcastle disease (ND), Infectious bursal disease (IBD) and Infectious Bronchitis (IB) produces changes in the biochemistry of the serum. Moreover, it has resulted in the change of behavior of several enzymes including alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and aminotransferase (ALT) in adding to levels of

glucose, proteins (globulin, albumin), triglycerides, cholesterol and lipids. Oral administration of Lactobacillus, Bifidobacterium and Streptococcus resulted in increase in antibody response against ND vaccine (Talazadeh *et al*., 2016).

The present study was planned to investigate the combined effect of probiotics and vaccines on antibody response biochemical and hematological indices when offered along with NDV vaccine and IBDV vaccines.

MATERIALS AND METHODS

Experimental design: Sixty, day old broiler chicks were distributed into four groups; Control (C), Probiotic (P), Vaccination (V) and Vaccination + Probiotics (VP). The groups were housed separately, with adequate temperature, lighting, ventilation and feed.

Probiotics including *Bifidobacterium, Lactobacillus paracasei, Lactobacillus casei, Streptococcus thermophiles, Lactobacillus acidophilus* were offered at the ratio of 2g/500ml of water for 5 days/week up to 6 weeks. Vaccination against Newcastle disease (ND) and infectious bursal disease (IBDV) was carried out in vaccine group 3. At day 7, a dose of Newcastle disease vaccine (NDV) was given to the broilers by eye drops at the rate of 0.025ml/bird. At day 21 NDV (Lasota) was provided in drinking, distil water at the rate of 0.50ml/bird. Group V was vaccinated with infectious bursal disease (IBDV) (Lohmann Animal Health Heinz-Lohmann-Strasse 4 D-27472 Cuxhaven Cuxhaven ME D-27472 Germany) by drinking water at 10th, 22nd and 30th day. Probiotics were offer to V+P group as offered to P group. VP group was vaccinated at the same rate as V group was. No treatment was given to control group.

Sample collection: Blood samples were collected randomly from brachial wing veins of birds at day 15, 30 and 45. To determine specific antibody titers against NDV, serum samples were subjected to hemagglutination inhibition test (Choi *et al*., 2013; Khurshid and Rhaman, 2018). IgG in sera against IBDV were detected by enzyme linked immunosorbent assay (Idexx® Bursa Ag capture test kit, Lyon, France) (Yurong *et al*., 2005; Talebi *et al*., 2008; Daodu *et al*., 2018). Serum samples were subjected to biochemical analysis for Albumin, Uric acid, Cholesterol, ALT and AST. Blood samples were processed in hematology analyzer (Sysmex XP-100) for hematological parameters (HGB, HCT, WBC, RBC, MCH, MCHC) 15, 30 and 45 days.

Statistical analysis: All the data were subjected to analysis of variance using SPSS software and least significant design (LSD) test was used to determine the means of different treatments. The level of significance was P≤0.05 (Talebi *et al.,* 2008).

RESULTS

Antibody titer against NDV vaccine: Mean HI antibody titer of broiler chicks against NDV in control group showed a decline in maternal antibody titer. GMT value at day 15 was $\log_2$ $^{2.66}$ while at day 45 it was $\log_2$ $^{1.33}$. Probiotic group also showed decrease in GMT value from 2.66 at day15 to 1.66 at day 45. The decrease in GMT values in probiotic group was less steep than control group. In vaccinated group, highest GMT value was observed as $\log_2$ $^{7.33}$ at day 45. Probiotic + vaccine group showed relatively increase GMT value i.e. $\log_2$ $^{7.66}$ but statistically there was no significant difference (P≤0.05) (Fig. 1).

Antibody titer against IBDV vaccine: Maternal antibodies showed decline in control and probiotic groups. In control group ELISA titer was decreased from 553 to 215 at day 15th to day 45th. Probiotic group also showed decrease in GMT values from 580 to 285 at 15th to 45th day. Vaccine group showed the highest ELISA titer value as 2233 at 45th day. Probiotic + Vaccine group showed increase in ELISA titer that was 4207 day 45th. Significant increase in antibody titer was observed as compared to vaccine group (P≤0.05) (Fig 2).

Effect on Blood chemistry: At 15th and 30th day no significant difference was observed in uric acid, Albumin, AST, ALT and Cholesterol values in C, P, V and PV groups (P≤0.05). At 45th, uric acid values were significantly different in C, P and V groups while no significant difference was observed in P and PV group. No significant difference was observed in albumin values. ALT and cholesterol showed significant decrease in PV group. AST values were significantly different in V and PV groups only (Table 1).

Effect on hematological indices: At 15th and 30th day no significant difference was observed in WBC, RBC, HB, HCT, MCV, MCH and MCHC in all groups. At 45th day only WBC values showed significant difference while (P≤0.05). Highest value was observed in PV group followed by V, P and control groups. HB value in control group was significantly different from P, V and PV groups. Highest HB value (7.1) was observed in PV group. HCT values in P, V and PV groups were significantly different from C group but there was no significant difference in experimental groups. MCV, MCH and MCHC values showed no significant difference (P≤0.05) (Table 2).

DISCUSSION

Probiotics are biotherapeutic agents that enhance growth and immunity (Dhama *et al*., 2011, Rajput *et al*. 2013). The use of probiotics for poultry is based on the knowledge that the gut flora is involved in resistance to enteric infections including *E. coli*, *Salmonella* and *Campylobacter* (Stern *et al.,* 2001). In present study *Bifidobacterium, Lactobacillus paracasei, Lactobacillus casei, Streptococcus thermophiles, Lactobacillus acidophilus* were used as probiotics. *Enterococcus*, *Bacillus*, *E. coli*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Lactococcus*, *Pediococcus* species, *Saccharomyces boulardii* have been used as a probiotics (Mountzouris *et al.,* 2007, Rajput *et al*., 2013; Souza *et al*., 2018). *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, along with *Streptococcus faecalis* showed considerable increase in growth and immunity in the chicken (Hamid *et al.,* 2006).

Table 1: Effect of probiotics and vaccines on blood chemistry of chicks in different groups at different days

Groups	Uric Acid (mg/dl)	Albumin (g/dl)	ALT (IU/L)	AST (I/L)	Cholesterol (mg/dl)
At 15th day					
C	3.46±0.1[a]	8.1±0.5[a]	1.23±0.1[a]	193±3.1[a]	136±7.2[a]
P	3.73±0.7[a]	8.26±1.8[a]	1.56±0.4[a]	188±5.1[a]	127±11.1[a]
V	3.8±0.9[a]	7.06±0.1[a]	0.13±0.05[a]	183±16.09[a]	104±7.54[a]
PV	4.2±0.5[a]	7.96±0.2[a]	1.13±0.5[b]	186±15.0[a]	138±25.2[a]
At 30th day					
C	5.5±0.6[a]	9.1±1.0[a]	0.96±0.0[a]	187±5.6[a]	221±2.5[a]
P	4.7±0.7a[b]	9.06±1.8[a]	1.76±0.5[a]	185±6.8[a]	192±17.5[a]
V	3.8±0.3[b]	8±0.8[a]	1.43±0.4[a]	182±20.2[a]	182±37.01[a]
PV	5.03±0.4[a]	8.63±0.6[a]	0.2±0.1[a]	155±31.7[a]	174±12.0[a]
At 45th day					
C	6.7±0.2[b]	7.4±0.5[a]	6.6±0.3[b]	295±04.0[a]	228±05.5[b]
P	5.25±0.1[a]	9.85±1.7[a]	6.4±0.5[a]	293±2.0[a]	231±8.0[a]
V	7.03±0.1[c]	9.7±0.4[a]	9.1±0.2[c]	295±2.0[b]	294±03.78[c]
PV	3.9±0.6[a]	9.2±0.1[a]	2.76±0.1[b]	137±5.0[a]	199±1.52[b]

Values (mean+SD) in each column having different superscript differ significantly (P≤0.05); C = Control; P= Probiotics; V= Vaccines; PV = Probiotic+ Vaccines.

Table 2: Effect of probiotics and vaccines on hematological parameters of chicks in different groups at different days

Groups	WBC	RBC	HB	HCT	MCV	MCH	MCHC
At 15th day							
C	223±2.0[d]	1.98±0.61[a]	6.0±0.0[b]	20.4±0.51[a]	128.1±1.04[a]	38.5±0.51[b]	28.6±1.52[a]
P	256±3.0[b]	2.17±0.1[a]	9.93±0.1[a]	29.3±1.1[b]	129.9±0.05[a]	41.46±1.2[ab]	31.9±1.6[a]
V	249±1.52[c]	2.46±0.29[a]	9.0±1.0[a]	29.3±1.1[b]	128.1±2.2[a]	41.8±0.72[a]	30.8±1.75[a]
PV	229±1.5[a]	2.2±0.1[a]	9.06±0.9[a]	26.16±0.7[a]	129.5±3.5[a]	41.9±1.7[a]	29.7±2.6[a]
At 30th day							
C	126±0.5[a]	2.63±0.1[a]	10.2±0.7[a]	31.05±2.4[ab]	116±1.0[a]	37±0.5[a]	32.6±0.4[a]
P	126.5±0.1[a]	2.77±0.1[a]	10.3±0.1[a]	31.8±0.6[a]	113±3.0[a]	37±0.1[a]	32.5±0.4[a]
V	127±0.94[a]	2.63±0.2[a]	9.3±0.1[a]	27.5±0.5[b]	110.5±0.5[a]	38.2±0.3[a]	33.7±0.7[a]
PV	126.5±0.73[a]	2.55±0.05[a]	11.2±0.3[a]	33.4±1.7[a]	117±2.0[a]	39.5±1.5[a]	33±0.7[a]
At 45th day							
C	122±1.4[a]	3.36±0.4[c]	6.3±0.7[c]	12.11±0.9[b]	10.6±0.7[b]	53.8±0.4[a]	52.8±1.5[a]
P	141.3±2.3[b]	4.8±0.3[b]	9.83±0.3[ab]	26.9±1.8[a]	12.4±0.4[ab]	50.7±4.0[ab]	52.9±1.7[a]
V	156.3±2.5[c]	5.4±1.0[b]	8.8±0.1[b]	24.4±0.4[a]	13.3±0.4[ab]	45.0±4.0[ab]	52.1±0.8[a]
PV	171±3.0[d]	7.1±0.3[a]	10.8±0.7[a]	23.86±1.5[a]	15.7±2.9[a]	41.67±0.9[b]	68.1±7.4[a]

Values (mean+SD) in each column having different superscript differ significantly (P≤0.05); C = Control; P= Probiotics; V= Vaccines; PV = Probiotic+ Vaccines.

In present study antibody response against NDV vaccine in control group showed a decrease in maternal antibodies. In P group decrease was less steep might be because probiotic reduced the degradation of antibodies. Bioactive peptides formed during lactic acid fermentation could contribute to the immunomodulatory effects of probiotic bacteria (Leblanc *et al.,* 2004) and T cell or B cell mediated immune response can be produced by the interaction between pathogens and host cells (Haghighi *et al.,* 2006). PV group showed increase in titer as compared to V group but difference was not significant. Talebi *et al.* (2008) reported similar results that probiotics and vaccination when given simultaneously produced better antibody response. The increase in antibody titer in PV group was might be because of immunomodulation of different subsets of immune system cells. The results are against the findings of Talazadeh *et al.* (2016) that aqua blend probiotics showed significant increase in antibody titer against NDV vaccine.

Antibody titer against IBDV vaccine showed significant increase in Probitic + Vaccine group as compared to vaccine group. Immunostimulation by probiotics depends upon number and type of probiotics. The findings were in accordance with that of Talebi *et al.* (2008). Talazadeh *et al.* (2016) observed significant increase in antibody titer against NDV vaccine but no significant increase in antibody titer against Avian influenza virus vaccine. It might be due to orally administered probiotics which can enhance the systemic antibodies in case of some antigens (Haghighi *et al.,* 2005).

Hematological indices like WBC, RBC and HB values showed increase at day 45th of experiment in probiotic+ vaccine group. While HCT, MCHC and MCH did not show any notable change. HCT did not show notable changes while others showed significant increase in parameters matched with control. Owosibo *et al.* (2013) observed decrease in lymphocytes, HGB and RBC valuese after 6 weeks of probiotic treatment.

At 45th day the significant decrease in values of uric acid, ALT, AST and cholesterol was observed in Probiotic+Vaccine group. No significant change was observed in albumin values. The results agreed with Hashemzadeh *et al.* (2013) findings that cholesterol decreased by probiotics and vaccine action in the treatments having no significant effect on albumin in serum and significant decrease effect was observed in uric acid. The highest level of uric acid was observed in control group while low values of uric acid were observed in experimental batch (Hashemzadeh *et al.* 2010). Similarly, Pourakbari *et al.* (2016) observed decrease in cholesterol and triglyceride level. Owosibo *et al.* (2013) found that cholesterol level reduced significantly and no significant difference was observed in albumin. Owosibo *et al.* (2013) did not observe significant difference in ALT, AST, Uric acid, Albumin.

Our results for cholesterol disagreed with Islam *et al.* (2004) who reported that cholesterol level was increased. The decrease in cholesterol level might be because of hydrolysis of bile and decrease in cholesterol producing enzyme.

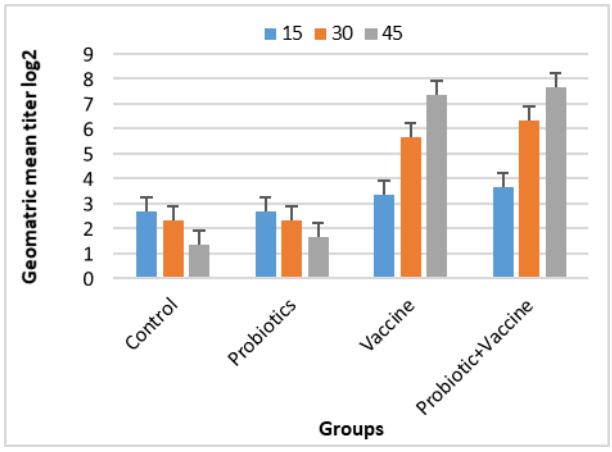

Fig. 1: Mean HI antibody titer against NDV vaccine in response to probiotics and/or vaccine in broiler chicks.

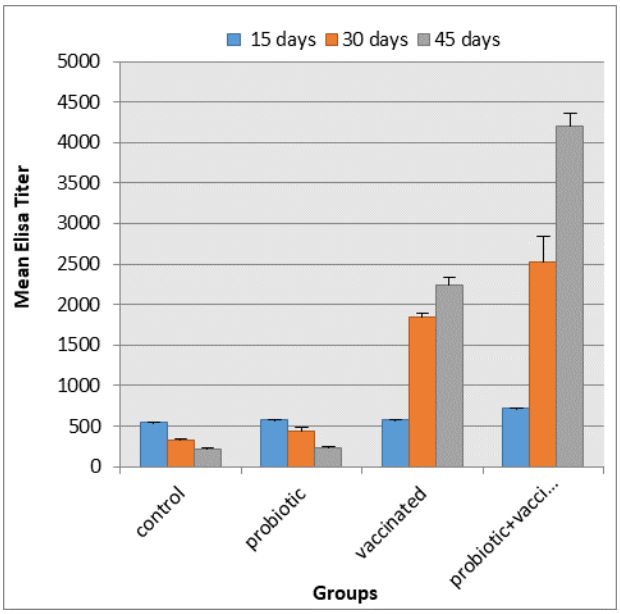

Fig. 2: Mean ELISA antibody titer against IBDV vaccine in response to probiotics and/or vaccine in broiler chicks.

Conclusions: It is concluded that as Probiotic+ Vaccinated group showed increase in GMT value and showed better immune response and no harmful effect was observed on hematological and biochemical indices so probiotics may be used in feed of broiler. Better combination of probiotic strains might be needed to enhance the immune response and performance of broiler chicks.

Authors contribution: NS conceived the idea, designed the study and was involved in data analysis and write up. AM, AS and MN executed the experiment.

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