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Immunomodulatory and Growth-Promoting Effect of a Probiotic Supplemented in the Feed of Broiler Chicks Vaccinated Against Infectious Bursal Disease

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ABSTRACT

This study was designed to investigate the effects of a probiotic plus an immunomodulatory product on the growth performance, immune response and net economic returns of broiler chicks vaccinated against Infectious bursal diseases virus (IBDV). A flock of 350 day-old chicks were equally and randomly distributed in seven groups, each comprising 50 birds. A mixture of microorganisms ([1]Probiotic) or Cyclophosphamide (immune suppressor) was offered through feed supplementation to different groups. The Probiotic, consisting of *Lactobacillus* species, *Bifidobacterium*, *Streptococcus salivarius* and *Enterococcus faecium*, in addition to *Aspergillus oryza* and *Candida pintolopessii*. Compared with control treatment group, the probiotic-supplemented chicks had better feed conversion ratio (FCR) (1.938 and 1.959), with significantly heavier live body weight 2141.0±34.2 and 2120.3±33.2, respectively. Similarly, their antibody titers against IBDV were significantly higher (941 and 832) on day 35. No morbidity and mortality were observed in these groups. This study suggests that a product composed of a probiotic and cyclophosphamide enhanced the growth rate as well as the immunity against infectious bursal disease virus of broilers chicks.

INTRODUCTION

Rapidly-growing human population and better living standards in various regions of the world demands increased food supply, especially of meat. The diet of Pakistanies is generally deficient in animal proteins (Finance Division, Pakistan, 2006). Broilers, because of their rapid growth, may be a viable and quick source to meet this animal protein shortage. In order to enhance productivity, in addition of breeding and management strategies, cheap and good-quality broilers feeds should be introduced.

Probiotics are viable single or mixed cultures of bacteria, beneficial to health of the host (Somroo *et al*. 2002). They contain naturally occurring microorganisms with a short generation time, rapid colonization ability in gut that can minimize pathogens by competitive exclusion and are stable at intestinal pH. Moreover, they regulate intestinal microorganisms and improve feed conversion efficiency. They have also been used as alternative tools for helping to colonize newly-hatched chicks with normal microflora (Rajmane, 2000). It has been suggested by many researchers that probiotics are convincing alternatives for antibiotics as therapeutic and growth-promoting agents (Cavazzoni *et al*.1998). Cyclophosphamide causes strong immunosupression not only in animals and mice but it is also considered to be equally responsible for the reduction in humoral immune response through oral administration in humans (Putnam *et al*, 1975).

[1] PROTEXIN Hilton Pharmaceuticals, Pakistan.

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The multi-strain probiotic contains seven strains of bacteria viz *Lactobacillus* spp, *Bifidobacterium* and *Streptococcus thermophillus*, and two fungi, *Aspergillus oryza* and *Candida pitolopessi*, which are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird, and human sources. It is reported to be safe, non-toxic and residual free. The product is compatible with all feeds and feed ingredients, like vitamins, minerals and some antibiotics. It can be used in a wide range of circumstances, either to improve the general health of animals, address specific problems or to maximize animal performance. Under general conditions, the probiotic has been promoted to improve health naturally, stimulate appetite, aid in the establishment of gut flora in immature animals such as day-old chicks, calves, lambs, kids and kittens, reestablish gut microflora after antibiotic treatment, optimize digestion of feed and reduce stress (Rajmane, 2000).

The continuous use of sub-therapeutic levels of antibiotics in animal feeds may lead to the development of drug-resistant microorganisms, which could then pass the resistance on to infectious microorganisms in humans. Recently, the European Union banned all human medicine-related antibiotics for sub-therapeutic use in livestock feeds to reduce the potential of resistance to antibiotics in humans. A possible alternative to antibiotics for growth promotion and improvement of feed efficiency in domestic avian species is feeding living microbial cultures (Jin *et al.,* 1997). Manystudies have been performed to measure the effect of probiotics on the growth performance of animals, including broiler chickens, in controlled environments, but there is little information available on this aspect when broiler is reared in our local open shed environment.

The current study was designed to investigate the effects of a probiotic on the growth performance, immune response to infectious bursal disease vaccine in terms of antibody titers and mortality or morbidity after challenge with infectious bursal disease virus.

Materials and Methods

Experimental birds

A total of 350 commercial day-old broiler chicks was obtained from a grandparent hatchery of Big Bird chicks and raised in the experimental open-sided poultry house of the Microbiology Department, University of Veterinary and Animal Sciences, Lahore, Pakistan Water was provided *ad libitum*, but the feed offered to the experimental birds was measured. The commercial feed was provided by [2]National feed and the mixture of (microorganisms) by Hilton Pharma.

Table 1 - Composition of Protexin™, Hilton Pharmaceuticals, Private, Limited-Pakistan.

Sr No	Composition (100 Gm)	Concentration
1	Lactobacillus plantarum	1.89 x 10 ^{10} cfu/ kg
2	Lactobacillus delbrueckii subsp. Bulgaricus	3.09 x 10^{10} cfu/kg
3	Lactobacillus acidophilus	3.09 x 10 10 10 cfu/kg
4	Lactobacillus rhamnosus	3.09 x 10^{10} cfu/kg
5	Bifidobacterium bifidum	3.00 x 10 cfu/kg
6	Streptococcus salivarius subsp. Thermophilus	6.15 x 10^{10} cfu/kg
7	Enterococcus faecium	8.85 x 10^{10} cfu/kg
8	Aspergillus oryza	7.98 x 10^{10} cfu/kg
9	Candida pintolopesii	7.98 x 10^{9}cfu/kg

The effect of the probiotic was evaluated by offering the product via feed to the broiler chicks from day 1 to day 49, as suggested by the manufacturer. The parameters used to evaluate the probiotic effects on broiler chicks were body weight gain (BWG); weight of lymphoid organs, such as bursa of fabricius, thymus and spleen; immune response to IBDV vaccinations; overall mortality and mortality and morbidity after challenge with virulent IBDV. The findings were compared with immune-suppressed (cyclophosphamide-treated) birds, non-treated birds, non-vaccinated and non-treated birds (negative control) and vaccinated (35 days of age) and non-treated (positive control) birds, totaling seven treatment groups with eight chicks each.

Experimental groups:

On the first day, chicks were randomly divided into seven groups as described above and probiotic (50 or 150 g/ton) or cyclophosphamide (3 mg/bird) were mixed with the feed and offered to the different groups according treatment. The negative control group was non-treated and non-vaccinated. All the groups, except for the positive control group, were vaccinated against Infectious bursal disease (IBD), infectious bronchitis (IB) and avian influenza (AI) viruses. The vaccination against IBDV was performed on days 7 and 12. The IB

[2] National Feed Industry Lahore, Pakistan

vaccination was given on day one. Vaccination against AI was performed on days 11 and 20.

Body weight and feed intake

Eight chicks were randomly selected according to weight on the first day of their age and then subsequently each week up to 49 days. Feed was daily weighed and offered to the birds in each treatment group throughout the experiment and feed residues were weighed to determine the daily feed intake per bird. Feed conversion ratio was weekly calculated as the amount (in grams) of feed consumed to produce 01 gram of live weight, as described by Morgan & Lewis (1962).

Feed Conversion = Feed intake / weight gain.

Determination of antibody titers and lymphoid organs weight

On day 1, five experimental chicks from each group were randomly selected for bleeding (jugular vein) and thereafter, on days 7, 14, 21, 28, 35 and 42, to check for anti-IBD antibodies. The serum and lymphoid organs were separated accordingly. Serum samples were stored at –20 °C till further use. Antibody titer against IBDV was determined by indirect hemagglutination inhibition (IHA) assay, as described by Gold & Fudenberg (1976).

The IHA antibody titer of each serum sample was expressed as the reciprocal of its end-point dilution. Similarly, the lymphoid organs (bursa of fabricious, spleen and thymus) were separated and weighed to calculate respective lymphoid organ body weight ratio, as discussed by Giambrone & Closser (1990).

Experimental challenge

A total of ten birds from each experimental group were randomly separated on day 35, and challenged with a field isolate of [3]IBDV at dose of 100 EID_{50} ($10^{-4.3}$ EID_{50} per ml). Each bird was given 01 ml of inoculum intra-peritoneally and kept under observation for 10 days.

Statistical analysis

Data obtained from the treatment groups was compared by analysis of variance. The statistically significant differences among treatment means were determined using the least significant difference test at 5% probability level, as defined by Steel & Torrie (1982).

RESULTS AND DISCUSSION

The groups supplemented with probiotic showed no mortality and morbidity, but the groups treated with cyclophosphamide or cyclophosphamide plus probiotic presented some sick chicks. It was observed that the probiotic-treated birds had overall lower mortality than the cyclophosphamide-treated and the non-treated and non-vaccinated chicks. The probiotic-treated groups had better protection as compared to those of control groups, helping the vaccinated chicks to completely resist the field IBDV challenge. However, the group treated with cyclophosphamide and vaccinated against infectious bursal disease virus (IBDV) and the group non-treated and vaccinated against IBDV presented mortality after the IBDV challenge (Table-2). These findings are consistent with those of Cavazzoni *et al*. (1998) and Rajmane (2000), who also reported relatively lower mortality in chickens offered a feed treated with *Bacillus coagulants*, rendering the bird more resistant and decreasing the microbial burden.

It is evident from this study that product added to feed as probiotic slightly increased thymus weight of immunocompromised chicks. Mean spleen weight of the treatment groups were not significantly ($p > 0.05$) different. Rhee *et al.* (2004) and Haghighi *et al*. (2005) reported that probiotic-treated chicks had significantly higher serum IgM against sheep red blood cells (SRBC) than untreated chicks. Similarly, Inooka & Kimura (1983) studied the effects of *Bacillus natto* in feed on the SRBC antibody response of broilers, and observed an increase in antibody production in the chicks receiving in-feed *Bacillus natto*, concluding that the effect of enhancement of antibody production might be associated with spleen and thymus development.

In the present study, in order to evaluate the effect of probiotics on the immune system, the experimental birds were vaccinated with a IBDV vaccine on days 7 and 12. A low geometric mean heamagglutination antibody titer in the sera of day-old chicks, ranging from 77-79, was observed. The highest indirect hemagglutination inhibition (IHA) antibody titer (941) against infectious bursal diseases virus (IBDV) was observed on day 3 day in the serum of birds in group P150 (Protexin at a higher dose and no cyclophosphamide treatment), followed by an antibody titer of 832 in group

[3] University Diagnostic Laboratory, University of Veterinary & Animal Sciences, Lahore-Pakistan.

P50 (Protexin at the recommended dose and no cyclophosphamide treatment). Similarly, the positive--control group (only vaccinated) also showed better IHA antibody titer (675) against IBDV as compared to the cyclophosphamide-treated group (111). However, on day 49, the highest IHA antibody titer (181) against IBDV was observed in the group probiotic 50 followed by the group probiotic 150 (111). The IHA antibody titer against IBDV in the group treated with recommended dose of probiotic is significantly different ($p<0.05$) from all other treated groups on day 49 (Table 3). These findings agree with those of Paola & Perdigon (2003), who reported that *Lactobacillus casei* significantly increased the amount of IgA in response to *Salmonella typhimurium* and protected mice against enteric infection. Zulkifli *et al*. (2000) also found that birds treated with a lactobacillus culture mounted a higher serum antibody response than the oxytetracycline-treated and the control birds. Similarly, Mohiti *et al.* (2007) and Noverr & Huffnagle (2004) also reported that resident microbiota play a pivotal role in shaping the immune system repertoire.

Table 2 - Infectious bursal disease virus (IBDV) post-challenge morbidity (M) and mortality (m) in various treatment groups.

Post-challenge period (days)	P50-C3 (M/m)	P50 (M/m)	P150-C3 (M/m)	P150 (M/m)	C3 (M/m)	Cont-V (M/m)	Cont-NV (M/m)
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	1/0
3	-	-	-	-	-	1/0	2/1
4	-	-	-	-	1/1	-	2/0
5	-	-	-	-	1/0	1/0	0/2
6	1/0	-	1/0	-	2/1	-	-
7	-	-	-	-	-	-	-
8			1/1		2/0	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
Total	**1/0 bird/s**	**-**	**1/1 bird/s**	**-**	**3/2 bird/s**	**1/0 bird**	**3/2 birds**

P 50 C3 = Group fed 50 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P50 = Group fed 50 g probiotics/ton of feed
P 150 C3 = Group received 150 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P150 = Group received 150 g probiotics/ton of feed
C3 = Group treated with cyclophosphamide at 3 mg/bird
Cont V = non-treated group
Cont NV = Non-treated and non-vaccinated group

Table 3 - Geometric mean Titer (GMT) using indirect heamagglutination inhibition (IHA) antibody titer against IBDV in different treatments.

Day	IHA Antibody Titers against IBDV per Treatment Group						
	P50-C3	P50	P150-C3	P150	C3	Cont V	Cont NV
7	78^{a}	79^{a}	77^{a}	78^{a}	79^{a}	79^{a}	79^{a}
14	60^{a}	59^{a}	42^{a}	52^{a}	45^{a}	37^{a}	52^{a}
21	137^{a}	111^{ab}	194^{bc}	147^{bc}	128^{a}	239^{c}	37^{d}
28	84^{a}	181^{b}	97^{a}	147^{b}	45^{ac}	119^{b}	20^{c}
35	162^{a}	832^{b}	194^{c}	941^{b}	111^{a}	675^{d}	27^{c}
42	56^{ab}	160^{c}	34^{bd}	147^{bc}	69^{a}	84^{a}	18^{d}
49	128^{a}	181^{b}	73^{c}	111^{a}	82^{c}	97^{c}	12^{d}

P 50 C3 = Group fed 50 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P50 = Group fed 50 g probiotics/ton of feed
P 150 C3 = Group received 150 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P150 = Group received 150 g probiotics/ton of feed
C3 = Group treated with cyclophosphamide at 3 mg/bird
Cont V = non-treated group
Cont NV = Non-treated and non-vaccinated group
Each value indicates the IHA GMT of the five samples repeated three times.
a,b,c,d Any two means carrying the same superscript are not significantly different from each other.

Groups treated with probiotic showed satisfactory feed conversion ratio (FCR) as compared to groups receiving both probiotic and cyclophosphamide. Probiotic-treated groups without cyclophosphamide presented better and satisfactory FCR, of 1.938 and 1.959, respectively, as compared to the cyclophosphamide-treated (1.980, 1.988 and 2.110, respectively) and control groups (2.090). Our findings are consistent with those of Nahashon *et al*. (1992). However, there are also conflicting research results concerning the effect of dietary probiotic supplementation. Leeson & Major (1990) suggested that only under stressful conditions

Table 4 - Live body weight of broiler chicks of the different groups at different ages.

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
P50-C3	48.78±1.3^{a}	137.7±3.6^{a}	335.7±9.2^{a}	533.5±15.0^{acf}	865.9±21.2^{ac}	1470.1±49.9^{ag}	1889.3±67.4^{ae}
P50	48.7±1.2^{a}	156.57±3.1^{b}	355.5±7.1^{ba}	628.6±12.4^{b}	948.6±18.3^{b}	1624.8±3.^{b}	2141.0±34.2^{b}
P150-C3	49.4±1.4^{a}	127.0±4.0^{c}	355.5±7.1^{ca}	503.3±19.7^{ca}	879.5±24.4^{cab}	1476.3±66.2^{cabg}	1957.5±39.5^{ca}
P150	48.8±1.3^{a}	158.6±3.1^{db}	355.5±7.1^{da}	603.2±13.4^{de}	944.0±19.7^{db}	1297.2±60.5^{d}	2120.3±33.2^{db}
C3	48.8±1.3^{a}	118.6±2.9^{ec}	283.4±8.2^{e}	518.6±14.9^{ea}	835.7±23.3^{ea}	1297.2±60.0$^{e\ d}$	1812.7±43.5^{e}
Cont	48.3±1.2^{a}	140.5±2.8^{fa}	344.5±7.1^{fa}	565.4±15.3^{fd}	866.7±11.1^{fad}	1554.7±44.1^{fab}	1962.1±47.2^{fac}
Cont NV	48.5±1.2^{a}	136.1±2.3^{gac}	350.9.5^{ga}	574.8±13.1^{gd}	715.9±27.8^{g}	1389.2±36.^{gfd}	1937.8±17.6^{gac}

P 50 C3 = Group fed 50 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P50 = Group fed 50 g probiotics/ton of feed
P 150 C3 = Group received 150 g probiotics/ton of feed & 3 mg cyclophosphamide/bird
P150 = Group received 150 g probiotics/ton of feed
C3 = Group treated with cyclophosphamide at 3 mg/bird
Cont V = non-treated group
Cont NV = Non-treated and non-vaccinated group
Each value indicates the IHA GMT of the five samples repeated three times.
a,b,c,d Any two means carrying the same superscript are not significantly different from each other.

coliform microorganisms increase in numbers and probiotics have measurable benefit.

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