EFFECT OF MULTISTRAIN PROBIOTIC ON IMMUNE RESPONSE AND GROWTH OF BROILERS VACCINATED AGAINST NEWCASTLE DISEASE

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ABSTRACT: The objective of the study was to investigate the effect of a probiotic ongrowth and immune response of the broiler chicks vaccinated against Newcastle disease. Parameters of investigation were weight gain, feed conversion ratio (FCR), live to dressed body weight ratio; weight of various lymphoid organs i.e. Bursa of Fabricius, thymus and spleen; immune response of the broilers. The findings were compared with the cyclophosphamide (cyc.) treated Newcastle disease virus (NDV)-vaccinated; untreated and NDV-vaccinated; and the unvaccinated untreated control chicks. The probiotic treated chicks showed higher mean body weights, better FCR, higher NDV HI antibody titers, lesser overall mortality, no NDV post challenge mortality and no detrimental effects on their lymphoid organs, compared to the cyclophosphamide treated and untreated chicks. Probiotic had good effects on growth and immune response of the broilers.

Key words: probiotic, immunomodulation, cyclophosphamide, feed conversion ratio, NDV.

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INTRODUCTION

The poultry especially broilers serve as a viable and quick source to meet the animal protein shortage of human population because of their rapid growth potential. Pakistan is facing a problem of diet deficient in animal proteins (Economic Survey of Pakistan, 2006). Beside breeding and management strategies efforts should be directed to control infections which are the major contributing factors towards losses to farmers. Antimicrobial growth promoting agents have made a major contribution to profitability in intensive husbandry, but due to their role in escalating antibiotic resistance and their residual effects, the European Commission has decided to ban all commonly used feed antibiotics. Over the last three decades probiotics (direct feed microbes) which include *Lactobacillus* culture, have been used as an alternative of antibiotic therapy (Huyghebaert *et al.*, 2011).

Probiotics are viable single or mixed cultures of bacteria, beneficial to health of the host (Soomro *et al.* 2002). The microorganisms present in probiotic have rapid colonization ability in gut so that they can minimize pathogens by competitive exclusion and are stable at intestinal pH. In addition probiotic regulate intestinal microorganisms and improve feed conversion efficiency. They act as alternative tool to help in colonization of normal microflora in gut of newly-hatched chicks (Rajmane, 2000).

The probiotic used in this study contains *Streptococcus salivarius, Lactobacillus delbruckii sub specie bulgaricus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, Candida pintolepesii, Aspergillus oryzae* in isolated forms. The objective of the present project was to study immunomodulatory effect of probiotic on broiler chicks vaccinated against Newcastle disease, the effect on lymphoid organs and on feed conversion efficiency.

MATERIAL AND METHOD

A total of 280 commercial one day old broiler chicks were obtained from a commercial hatchery and offered water and feed ad libitum upto 52 days of their age. The probiotic was offered via feed to the broiler chicks from day 1 to day 52.

At day 1, chicks were randomly divided into seven experimental groups i.e. A, B, C, D, E, F and G (n=40 per group). Probiotic was administered @ 50 grams/ton of feed for broilers in group A and B. It was given @ 150 grams/ton of feed for broilers of groups C and D. No probiotic was offered to birds of group E, F and G. All the chicks in groups A, C and E were administered with cyclophosphamide as immunosuppressive agent @ 3mg/bird/day for first four consecutive days. Chicks in group G were not administered any treatment (no probiotic, no cyclo. and no vaccination).

All the chicks in groups A, B, C, D, E and F were primed with live NDV vaccine i.e. Mukteswar strain @ 0.05 ml/bird via intraocular route at day 1. The chicks were boosted with live NDV vaccine i.e. Mukteswar strain through intraocular route on day 7 (Rehmani, 1996). A second NDV vaccination booster was given on day 21 using Mukteswar live NDV vaccine through the

subcutaneous route of wing web. The birds of these groups were also vaccinated against Infectious Bursal Disease (on 10th and 19th day of age through eye dropping and drinking water respectively), Avian Influenza (on 11th and 20th day of age via Subcutaneous route) and Infectious Bronchitis (on 1st day through eye dropping).

Live body weights of chicks in all groups were determined on day 7, 14, 21, 28, 35, 42 and 52. 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 group. Feed conversion ratio (FCR) of each group of experimental chicks was calculated on day 52 (Ososanya *et al.*, 2013).

FCR = feed consumed (grams) / Weight gain (grams)

Blood samples from the chicks in various experimental groups were collected on day 1, 7, 14, 21, 28, 35, 42 and 52. Haemagglutination Inhibition test was performed to determine the serum anti-NDV HI antibody titer (Feberwee *et al.* 2009). A total of three birds from each treatment group were killed by dislocating atlanto-axial joints at day 14, 28, 35 and 42. The lymphoid organs i.e. Bursa of Fabricius, spleen and thymus were separated, cleared off fat and tissue debris and weighed for calculation of respective lymphoid organ body weight index according to following formula.

Organ body weight index (OBWI) = lymphoid organ weight (gms) / body weight (gms)

A total of five birds from each experimental group were selected randomly and exposed to field isolate of Newcastle Disease Virus at dose rate of 1000 EID_{50} per ml at day 45. Each bird was injected 1 ml of inoculums intraperitoneally and kept under observation for10 days.

The pathogenicity of field virus was recorded as follows:

- Embryo Infective Dose 50 (EID_{50}) was determined according to Swayne and Beck (2004) using 9 – 11 day old embryonated chicken eggs and was found as 10^{-668} / 0.1 ml.
- Mean Death Time was 59 hours as calculated according to Kim *et al.* (2007).
- Intracerebral Pathogenecity Index was found to be 1.46 as calculated according to Panda *et al.* (2004).

The economics of probiotic usage in broilers feed was also calculated (Asghar *et al.*2002).

Analysis of Variance was used to compare data collected from different treatment groups regarding weigh gain and weight of immune organs. The statistical differences among various treatment groups were determined using Least Significance Difference (LSD) test at the 5% probability level as illustrated by Meier (2006). ^{a,b,c,d,e,f,g} Any two means carrying the same superscript were not significantly different from each other at 5% probability level, using LSD test.

RESULTS AND DISCUSSION

Probiotic treated groups exhibited higher mean body weights compared to cyclo.-treated and untreated groups from day 1 to day 52. At day 42, group B had over 328 grams higher mean weight than the cyclo – treated chicks of group E and over 203 grams higher weight than that of untreated control chicks of group G (Table-1). Group B and D (probiotic treated but no cyclo-treated) exhibited higher live-to-dressed body weight ratios as compared to probiotic untreated chicks in Group F and G. These findings were corroborated by the trials conducted by Gao *et al*. (2008), Zhou *et al.* (2010) and Liu *et al*. (2012) who found similar results in their findings. Alkhalfa *et al*. (2008) reported that the birds fed on probiotic levels 1 and 0.8 g/kg diet exhibited higher body weight among chicken groups at 6 weeks of age and Improved feed conversion was noticed in birds fed a diet supplemented with probiotic. Gao *et al*. (2008) concluded that the dietary Yeast Culture affected immune functions, digestibility of Ca and P, and intestinal mucosal morphology of broilers. Growth performance was also optimized at 2.5 g/kg of YC in the present study.

Feed Conversion Ratio values of chicks in various treatment groups showed that the probiotic administered chicks in treatment groups B and D (probiotic but no cyc. – treated) had better FCR compared to cyclo.-treated chicks in groups A, C, E and untreated control chicks in G as shown in Table -2. Improvement in growth performance and feed efficiency of broiler chickens fed probiotics was thought to be induced by the total effects of probiotic action including the maintenance of beneficial microbial population, improved feed intake and digestion, and altering bacterial metabolism as reported by Awad *et al.* (2009). Alkhalfa *et al.* (2010), Gao *et al.* (2008) and Liu *et al.* (2012) reported similar findings in their experimental trials.

At day 14 the highest Mean + S.E value of Bursal Body Weight Index (2.04 + 0.10) was observed for group F (untreated but vaccinated). On subsequent days, there was an overall increase in BBIX values of probiotic treated cyclo. treated and cyclo. untreated chicks compared to immunosuppressed but probiotic untreated chicks. The BBIX values of all groups were statistically non-significant at $P \geq 0.05$. Kalavathy (2003) concluded the same result in his experimental study.

At day 14 the Thymus Body Weight Index values of group C (immunosuppressed and probiotic treated) was significantly better than group A (immunosuppressed but probiotic treated), B (probiotic treated but not immunosuppressed) and group D (probiotic treated but not cyclo. treated). It possibly explained that probiotic helped in weight gain of thymii of immunocompromised chicks of group C. In later days the mean TBIX values showed non-significant difference among various treatment groups (Table-3).

The Spleen Body Weight Index values of various groups were not significantly ($P \geq 0.05$) different from each other throughout the trial (Table-3). There was a possibility that in our study effects induced by probiotics could not be observed by determining the SBIX and the microscopic changes (Not investigated in present work) might be present. Shamoon et al. (2012) reported that probiotic slightly increased thymus weight of immunocompromised chicks and mean spleen weights of the treatment groups were not significantly ($p> 0.05$) different. 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.

A low Geometric Mean Haemagglutination Inhibition antibody titer in sera of one day-old chicks ranging from 77-79 was observed. Highest antibody titers were observed at day 35 in group B and group D, which showed significantly higher titers compared to group F, A, C, E and G. Therefore probiotic seemed to have immunomodulatory effect on immune response of both immunocompetent and immunosuppressed chicks (Table-4). These findings are in agreement with those reported by Gao *et al.* (2008) and Naseem *et al.* (2012). Naseem *et al.* (2012) who conducted trials and reported highest indirect hemagglutination inhibition (IHA) antibody titer ie 941 against infectious bursal disease virus (IBDV) observed on day 3 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 P50 (Protexin at the recommended dose and no cyclophosphamide treatment). Li *et al.* (2009) also stated that treatment groups getting feed supplemented with Astragalus polysaccharides (APS) , 4×10^{10} cfu (colony forming units) probiotics and both, showed a siginificant increase (P 0.05) in Newcastle disease antibody titer , ANAE+ T-lymphocyte percentage, immune organ relative weights, histological changes as well as *Lactobacilli* and *Bacillus cereus* numbers. Similarly, Mohiti *et al.* (2007) and Noverr and Huffnagle (2004) also reported that resident microbiota played a pivotal role in shaping the immune system repertoire.

Five birds from each group were exposed to field isolate of Newcastle Disease Virus on day 45. After 10 days of observations it was found that no mortality and/or morbidity was observed in probiotic treated groups B and D. Group A and C showed very little mortality as compared to cyclo.-treated group E and control group. The probiotic treated chicks had better protection as compared to those of control groups helping the vaccinated chicks to completely resist the field NDV challenge. However the cyclo-treated NDV vaccinated and untreated NDV vaccinated chicks had post NDV challenge mortality. The use of probiotic was found economically feasible as the probiotic treated groups showed better growth and production performance i.e. body weight gain, FCR, dressed meat to body weight ratio, HI antibody and overall low mortality.

Table-1. Mean body weights (grams) of chicks in various experimental groups on day 42.

Groups	Body Weight (gms)	Day 42
A	Mean + SE	$1889.33+67.40^{c}$
	Range (Min-Max)	1380-2225
B	Mean + SE	$2141.00+34.25^{a}$
	Range (Min-Max)	1840-2430
C	Mean + SE	$1957.50+39.52^{b}$
	Range (Min-Max)	1780-2080
D	Mean + SE	$2120.33+33.26^{a}$
	Range (Min-Max)	1985-2355
E	Mean + SE	$1812.77+42.58^{c}$
	Range (Min-Max)	1410-2170
F	Mean + SE	$1962.17+47.29^{b}$
	Range (Min-Max)	1730-2410
G	Mean + SE	$1937.84+17.62^{b}$
	Range (Min-Max)	1801-2070

Table-2. FCR values of various experimental groups on day 52

Groups	Ration Consumed (grams)	Mean Weight Gains (grams)	FCR
A	4575	2310	1.9805
B	4870	2512	1.9386
C	4490	2258	1.9880
D	4830	2465	1.9594
E	4695	2225	2.1101
F	5110	2444	2.0908
G	5020	2344	2.1416

Table-3. Mean bursal body weight index (bbix), spleen body weight index (sbix) and thymus body weight index (tbix) of various experimental groups on day 14.

	Mean + Standard Error		
GROUPS	BBIX	SBIX	TBIX
A	$0.58 + 0.10^{ab}$	$0.53 + 0.13^{a}$	$2.17 + 0.16^{a}$
B	$1.94 + 0.13^{b}$	$0.52 + 0.14^{a}$	$3.02 + 0.32^{a}$
C	$1.08 + 0.37^{ca}$	$0.52 + 0.09^{a}$	$3.93 + 0.71^{b}$
D	$1.84 + 0.12^{db}$	$0.55 + 0.12^{a}$	$3.10 + 0.26^{a}$
E	$0.39 + 0.05^{e}$	$0.52 + 0.13^{a}$	$1.31 + 0.18^{a}$
F	$2.04 + 0.10^{fb}$	$0.55 + 0.11^{a}$	$4.05 + 0.34^{b}$
G	$1.93 + 0.06^{gb}$	$0.55 + 0.12^{a}$	$4.44 + 0.38^{b}$

Table-4. Newcastle disease virus geometric mean haemagglutination inhibition antibody titers of chickens in various experimental groups.

Age in days	GM HI Titers of Treatment Groups						
	A	**B**	**C**	**D**	**E**	**F**	**G**
1	78^{a}	79^{a}	77^{a}	78^{a}	79^{a}	79^{a}	79^{a}
7	60^{a}	59^{a}	42^{a}	52^{a}	45^{a}	37^{a}	52^{a}
14	137^{a}	111^{ab}	194^{bc}	147^{bc}	128^{a}	239^{c}	37^{d}
21	84^{a}	181^{b}	97^{a}	147^{b}	45^{ac}	119^{b}	20^{c}
28	64^{a}	145^{b}	23^{c}	128^{b}	97^{a}	123^{b}	21^{c}
35	162^{a}	832^{b}	194^{a}	941^{b}	111^{c}	675^{d}	27^{c}
42	$56a^{b}$	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}

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