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Immunomodulatory Effects of Multistrain Probiotics (Protexin™) on Broiler Chicken Vaccinated Against Avian Influenza Virus (H9)

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Abstract: This study was undertaken to evaluate the effects of probiotics (Protexin) on the immune response of broiler chicks. The parameters of investigation were hemagglutination inhibition HI titer of antibodies against avian influenza virus and post field AIV challenge. The findings were compared with the cyclophosphamide treated AIV-vaccinated; untreated and AIV-vaccinated and unvaccinated and control birds this investigation revealed that protexin treated chicks have higher AIV-HI antibody and no AIV post challenge mortality compared to the cyclophosphamide treated and untreated chicks the overall finding of this study clearly demonstrate that the use of this multistrain probiotics has good effect on immune response of broiler chicks.

Key words: Immunity, avian influenza virus, protexin

Introduction

Irrational use of antibiotics as growth promoter and as therapeutic agent in livestock and poultry is one of the hot issues. International institutions and organizations related to public health are showing deep concern to reduce the use of antibiotics in the feed of animals and poultry. This absurd use of antibiotics is not only developing an increase in microbial resistance to antibiotics but also the presence of antibiotic residues in animal products is a matter of public health importance. An International Study Group on Antimicrobial Strategies (ISGNAS) has also mentioned the increased microbial resistance to antibiotics as a serious problem. To develop rational alternative of antibiotic to control microbial diseases is under consideration (Araneo *et al*., 1996). It has been suggested by many workers that probiotics are convincing alternative for antibiotics as therapeutic and growth promoting agent (Cavazzoni *et al*., 1998 , Eren *et al*., 1999 and Martins *et al*., 2005).

Use of probiotics in the feed of food producing animals and birds is gaining momentum. Since 1973, probiotics, which include Lactobacillus cultures, have been used as an alternative for antibiotic therapy. Tortureo (1973) led the way in the use of lactobacillus acidophilus cultures as growth promoter in chicks.

Probiotics are single or mixed cultures of microbes have beneficial effect on the health of the host (Soomro *et al*., 2002). These microorganisms, when fed, improve the properties of indigenous microflora (Havenaar and Huisin't Veld, 1992) and the feed conversion ratio of the host (Dhingra, 1993; Rajmane, 2000).

Lactic acid bacteria including *Lactobacilli, Streptococci,* and *Bifidobacteria* are commonly used organisms in probiotics preparations. Some non lactic acid bacillus species are also used as probiotics. *Saccharomyces cerevisiae* and *Saccharomyces boulardii* are the examples of yeast also used in preparation. All these microbial species are obtained from the soured and fermented milk, yogurt, cheese and intestinal contents of poultry gut (Fuller, 1997; Medina *et al*., 2001)

Probiotics used as feed additive has a good impact on the performance of poultry (Stavric and Kornegay, 1995) these live organism after residing intestinal tract and their metabolites can act as immunomodulatory agent by activating specific and non-specific host immune responses in chicks, which help in prevention and control of various infectious diseases. (Kostiuk *et al*., 1992; Koenen *et al*., 2004).

Protexin™ is one of the commercial preparations of probiotics available in Pakistan marketed by M/S Protexin International limited, UK. It contains *Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, Candida pintolepesii, Aspergillus oryzae* in isolated forms. The present project was undertaken to study immunomodulatory effect of protexin on broiler chicks vaccinated against avian influenza virus (H9)

Materials and Methods

A total of 280 day-old broiler chicks Hubbard were procured from market and divided equally into seven groups i.e. A, B, C, D, E, F, and G, each group containing forty chicks. These chicks were reared in Experimental Rooms of Department of Microbiology , University of Veterinary and Animal Sciences, Lahore, Pakistan.

Birds of group A and B were given multistrain probiotic (Protexin.™) throughout rearing period @ 50 gm/ton feed and of C, D @ 150 gm/ton feed and E, F and G groups were not offered with Protexin. Birds of groups A, C, and E were given cyclophosphamide (immunosuppreser) @ 3 mg/bird/day for four consecutive days.

Table 1: Avian influenza virus (H9) geometric mean haemagglutination inhibition antibody (gm-hi) titers of chicken in various experimental groups at weekly intervals

Age in days	GM HI titer in treatment groups						
	A	B	C	D	E	F	G
1	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
21	12	38	12	25	7	14	0
28	14	64	13	54	10	43	0
35	18	92	15	81	10	65	0
42	20	102	15	95	12	8	0
49	34	259	18	189	15	68	54

Table 2: Post-AIV-challenge morbidity and mortality in various experimental groups

Post challenge period in (days)	Morbidity / Mortality in Experimental Groups						
	A	B	C	D	E	F	G
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	1/0
3	-	-	-	-	-	-	3/0
4	-	-	-	-	2/0	-	0/1
5	-	-	-	-	1/0	1/0	-
6	1/0	-	1/0	-	1/1	-	-
7	-	-	-	-	-	-	-
8	-	-	2/0	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
Total	1/0	-	3/0	-	4/1	1/0	4/1

Vaccination: Chickens of groups A, B, C, D, E and F were vaccinated against avian influenza virus. The chickens were primed with the alum precipitated AIV killed vaccine at the age of 13 days whereas boosting was done with oil based killed AIV vaccine at the age of 21 days.

Group G acted as a control group neither vaccinated nor given protexin and cyclophosphamide.

Blood sampling for haemagglutination inhibition test: Blood samples from 10 chicks of each group were collected at weekly intervals from day 1 to day 47. The serum was separated. Collected sera were used for determining serum antibody titers against AIV by hemagglutination inhibition test. (Alexander and Chettle, 1977)

Experimental challenge: A total of 5 birds from each treatment group were challenged with field AI virus at the dose of 1000 EID_{50} per ml at 45th day of age. Each bird was injected 1 ml of inoculum intraperitoneally and kept under observation for next 7 days. These challenged birds were bled and there serum was separated and stored at -20°C. The data obtained were analyzed statistically (Steel and Torrie, 1982).

Results

Avian influenza virus (H9) geometric mean haemagglutination inhibition antibody (GM-HI) titers of chicken in various experimental group, at weekly intervals, are summarized in Table 1.

The maternal geometric mean haemagglutinating inhabitation antibody titer against avian influenza (H9) virus in chicken at the day one, seven and 14 were zero in groups A, B, C, D, E, F and G.

Geometric mean avian influenza (H9) virus haemagglutinating inhabitation antibody titer of chicks in experimental groups A, B, C, D, E, F and G at day 21 were 12, 38, 12, 25, 7, 14 and 0 respectively indicating development of titer post vaccination in sera of all treatment groups except in G group (unvaccinated group).

At day 28, the geometric mean avian influenza (H9) virus haemagglutinating inhabitation antibody titer of chicks in experimental groups A, B, C, D, E, F and G were 14, 64, 13, 54, 10, 43 and 0 respectively. The cyclophosphamide treated groups (A, C, and E) had lower geometric men titer as compared to protexin^{TM} treated groups.

At day 35, the geometric mean avian influenza (H9) virus haemagglutinating inhabitation antibody titer of chicks in experimental groups A, B, C, D, E, F and G were 18, 92, 15, 81, 10, 65 and 0 respectively. The cyclophosphamide treated groups (A, C, and E) had lower geometric men titer as compared to protexin^{TM} treated groups.

At day 42, the geometric mean avian influenza (H9) virus haemagglutinating inhabitation antibody titer of chicks in experimental groups A, B, C, D, E, F and G were 20, 102, 15, 95, 12, 8 and 0 respectively. The cyclophosphamide treated groups (A, C, and E) had lower geometric men titer as compared to protexin^{TM} treated groups.

At day 49, the geometric mean avian influenza (H9) virus haemagglutinating inhabitation antibody titer of chicks in experimental groups A, B, C, D, E, F and G were 34, 259, 18, 189, 15, 68, and 54 respectively. This sudden increase in titer is a consequence of challenge with live avian influenza virus (H9)

Discussion

The immune response after vaccination is an elegant tool for studying the effect of probiotics both in human and animal subjects. In our study, for evaluating the effect of probiotics on immune system experimental, chicks were vaccinated with alum precipitated killed Avian influenza (H9) vaccine on day 13 and boosting was done with oil based killed Avian influenza (H9) vaccine on day 21

Avian influenza virus (H9) geometric mean haemagglutination inhibition antibody (AIV-HI) titers were zero. Absence of maternal antibodies indicates that parent stock was not vaccinated with avian influenza (H9) vaccine. AIV-HI titers were also zero on day 14 because first vaccination was done on day 13. Highest

GM-HI titres were observed in sera of birds in-group B (Protexin at recommended dose and no cyclophosphamide treatment) group D (Protexin at higher dose and no cyclophosphamide treatment) group F (no Protexin and no cyclophosphamide treatment). It was observed that titers in sera of birds of group B and D were significantly higher than that of group F whose birds received only vaccine and no other treatment. Similarly GM-HI titres of group A (Protexin at recommended dose and cyclophosphamide treated) and C (Protexin at higher dose and cyclophosphamide treated) are higher than that of group E (No Protexin and but cyclophosphamide treated). Low titers in these groups indicate that cyclophosphamide caused immunosupression. Perdigon *et al.* (1991) reported *Lactobacillus casei* significantly increased the amount of Ig A in response to *Salmonella typhimurium* and protected the mice against enteric infection. Zulkifli *et al.* (2000) reported the lactobacillus culture treated birds mounted a higher serum antibody response than the oxytetracycline treated and control birds. Overall this study indicate that the protexin had an immunomodulatory effect on immune response of both immunocompetent and immunosupressed birds. These findings are in agreement with those reported by Perdigon *et al.* (1991), Panda and Chawak (1996), Zulkifli *et al.* (2000) and Dalloul *et al.* (2003).

Post avian influenza (H9) virus challenge mortality and morbidity in various treatment groups: Five birds from each group were challenged on day 45. After 10 days of observations it was found that no mortality and morbidity was observed in protexin treated birds i.e. birds of groups B and D. While chicks of groups A and C, who received protexin and cyclophosphamide treatment, had very little mortality as compared to cyclophosphamide treated (group E) and control groups. The Protexin treated chicks had better protection as compared to those of control groups helping the vaccinated chicks to completely resist the field AIV challenge. However the cyclophosphamide-treated AIV vaccinated and untreated AIV vaccinated chicks did have post AIV challenge mortality and morbidity

References

Alexander, D.A. and N.J. Chettle, 1977. Procedures for the haemagglutination and the haemagglutination inhibition test for Avian Infectious Bronchitis Virus. Avian. Pathol., 6: 9-17.

Araneo, B.A, J.J. Cebra and J. Beuth, 1996. Problems and priorities for controlling opportunistic pathogens with new antimicrobial strategies: an overview of current literature. Microbiol. Virol. Parasitol. Inf. Dis., 431-465.

Cavazzoni, V., A. Adami and C. Castrovilli, 1998. Performance of broilerchickens supplemented with *Bacillus coagulants* as probiotic. Br. Poult. Sci., 39: 526-539.

Dhingra, 1993. Probiotics in poultry diet. Poult. Adv., 26: 43-45.

Dalloul, R.A., H.S. Lillehoj, T.A. Shellem and J.A. Doerr, 2003. Intestinal immunomodulation by vitamin A deficiency and lactobacillus-based probiotic in Eimeria acervulina-infected broiler chickens. Avian Dis., 47: 1313-20.

Eren, M., G. Deniz, H. Biricik, S.S. Gezen, I.L. Turkmen and H.M. Yavuz, 1999. Effects of supplementation of zinc bacitracin, mannanoligosaccharide and probiotic in broiler feeds on fattening performance. Veteriner Fakiiltesi Dergisi, Uludag Universitesi, 18: 73-84.

Fuller, R., 1997. Probiotics 2: applications and practical aspects. Published by Chapman and Hall, Lon., UK, pp: 1-209.

Havenaar, R. and J.H.J. Huisin't Veld, 1992. Probiotics; a general view in the Lactic Acid Bacteria, Vo1.1. The Lactic Acid Bacteria in Health and Disease; ed. B.J.B. Wood, Elsevier App. Sc., Barking, pp: 151-170.

Koenen, M.E., J. Kramer, R. van der Hulst, L. Heres, S.H. Jeurissen and W.J. Boersma, 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. Br. Poult. Sci., 45: 355-66.

Kostiuk, O.P., C.Z. Cherryshora and A.P. Volokha, 1992. The current concepts of the influence of *Lactobacilli* on the immune system of the human body. Fiziol. Zh., 43: 106-115.

Martins, F.S., R.M. Nardi, R.M. Arantes, C.A. Rosa, M.J. Neves and J.R. Nicoli, 2005. Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. J Gen Appl. Microbiol., 51: 83-92.

Medina, R., M. Kat, S. Gonzalz and G. Oliver, 2001. Characterization of the Lactic acid bacteria in ewe's milk and cheese from North West Argentina. Food Prot., 64: 559-563.

Panda, B.K. and M.M. Chawak, 1996. Immune modulating effect of someindigenous growth promoters in commercial broilers. Ind. J. Poult. Sci., 31: 213-215.

Perdigon, G., S. Alvarez and A.H. Pesce DeRuis, 1991. Immunoadjuvant activity of oral *Lactobacillus casei* : influence dose on the secretory immune response and protective capacity in intestinal infections. J. Dairy Res., 58: 485-496.

Rajmane, B.V., 2000. Efficacy of protexin on performance of broilers Parel Mumbai, Bombay Vet. College, Born. Vet., 14: 542.

Soomro, A.H., T. Masud and H.A. Rathore, 2002. Application of probiotics culture. J Am. Vet. Adv., 1: 40-42.

Stavric, S. and E.T. Kornegay, 1995. Microbial probiotics for pigs and poultry biotechnology in animal feeds and animal feeding. R.J. Wallace and A. Chesson, eds. V.C.H., Weinheim, Germany., pp: 205-231.

Steel, R.G.D. and J.H. Torrie, 1982. Principles and Procedures of Statistics. A Biochemical Approach. McGraw Hill International Book Company, U.S.A., pp: 362-364.

Tortureo, F., 1973. Influence of implantation of *Lactobacillus* in chicks on the growth, feed conversion, malabsorption of fat-syndrome and intestinal flora. Poult. Sci., 52: 197-203.

Zulkifli, I., N. Abdullah, N.M. Azrin and Y.W. Ho, 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. Br. Poult. Sci., 41: 593-597.