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IMMUNOMODULATORY EFFECTS OF LISOVIT® IN RESPONSE TO NEWCASTLE DISEASE AND INFECTIOUS BURSAL DISEASE VACCINES IN BROILERS

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ABSTRACT

The present study was designed to investigate effects of Lisovit® (Biomin, Austria) on the immune system of broiler chickens. For this purpose, 270 (day-old) broiler chicks were randomly divided into three groups A, B and C of 90 birds each. The birds were vaccinated against Newcastle Disease (ND) on day 3 (intraocular) and day 18 (drinking water) of age and against Infectious Bursal Disease (IBD) on day 8 (intraocular) and day 21 (drinking water) of age. Group A served as control, while birds of groups B and C were offered Lisovit® (Biomin, Austria) at the dose rate of 100 and 200 mg/kg body weight (b.wt.) respectively on days 22, 24 and 26 of age. The serum samples were collected at day 21, 31 and 41 of age and subjected to haemagglutination inhibition (HI) and indirect haemagglutination (IHA) antibody titres for ND and IBD vaccines, respectively. On day 13 post vaccination against ND, maximum values of the geometric mean haemagglutination inhibition (GMHI) antibody titres of groups A, B and C were 33.51,185 and 244, respectively. Whereas on day 10 post vaccination against IBD, maximum values of geometric mean indirect haemagglutination (GMIHA) antibody titres of groups A, B and C were 35.09, 255.19 and 387.01, respectively. The group C achieved significantly ($p<0.05$) higher antibody titres than group B and A. Feed conversion ratio (FCR) of group C was found better than group B and A. Mortality rate upto 42 days of age was significantly ($p<0.05$) lower in group C (2.2%), followed by B (13.3%) and highest in A (29.0%). These results indicate that Lisovit® at the dose rate of 200mg/kg b.wt. can be used to enhance antibody titres against ND and IBD vaccines, to reduce mortality rate and to improve FCR in broiler chickens.

Key words: Lisovit®, antibody titre, FCR, mortality rate, broilers.

INTRODUCTION

Poultry keeping is the dominant form of poultry production in the developing world. It is one of the organized and vibrant sectors of agriculture industry in Pakistan. According to the Economic Survey of Pakistan, 2009, this sector generates employment (direct / indirect) and income for about 1.5 million people and the poultry meat contributes 23.8 percent of total meat production in Pakistan. Infectious diseases such as Newcastle disease (ND), Infectious Bronchitis (IB), Infectious Bursal disease (IBD), Egg Drop Syndrome, Hydropericardium Syndrome (HPS) and Avian Influenza (AI) are few of the major constraints hindering its further progress. ND and IBD cause huge economic losses in terms of high mortality, morbidity, loss of growth and decreased egg production. Outbreaks mostly occur in unvaccinated broilers and layers causing 100% morbidity and 25% mortality (Shabbir *et al.*, 2008). However, these diseases also have been found to be common even in the vaccinated flocks due to improper immune response and vaccine failures (Numan *et al*., 2005).

Minimizing the immunosupression through immunization of the birds at proper time against ND and IBD, and monitoring the post vaccination immune response are the key steps to the control of infectious diseases (Arshad *et al.*, 2005), However, strategies to control immunosupression are largely based upon vaccination programs only and not upon attainment of appropriate humoral responses. Utilization of immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious diseases. Immunostimulation comprises a prophylactic and therapeutic concept aimed at stimulation of the non-specific and specific immune response (Hyde and Patnode, 2001). Many immunostimulating substances have been used in poultry with success such as levamisole, vitamin E and selenium (Siegel, 2001; Hussain *et al*., 2004; Ševcíková, 2006), ascorbic acid and vitamin D (Lohakare *et al.*, 2005; Ajakaiye *et al.*, 2010), and herbal extracts such as polydispersed beta (1-4) linked acetylated mannan (acemannan) and oil extracted propolis which significantly alter antibody titres to NDV as well as to IBD and AI vaccines (Taheri *et al.*, 2005; Awaad *et al*., 2010).

Lisovit® (Biomin, Austria) contains non antibody protective (NAP) proteins such as muramidase (lysozyme), peroxidase, immune stimulating herbal

extracts and vitamins A, C and E. The objective of the present study was to evaluate the effects of Lisovit® supplementation on humoral immune response against ND and IBD vaccines, feed conversion ratio (FCR) and mortality rate in broiler chicks.

MATERIALS AND METHODS

Experimental birds: A total of 270 (day-old) broiler chicks were divided randomly into three groups A, B and C, each having 90 birds. The birds were vaccinated against ND on day 3 (intraocular) and day 18 (drinking water) of age by using Lasota strain and against IBD on day 8 (intraocular) and day 21 (drinking water) of age by using hot strain. Group A served as control, while birds of groups B and C were offered Lisovit® (Biomin, Austria) at the dose rate of 100 and 200 mg/kg body weight (b.wt.), respectively on days 22, 24 and 26 of age. All the groups were maintained for 6 weeks under standard housing and management conditions in the Dept. of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

Measurement of serum antibody titers: Blood samples of five birds from each replicate were collected from the wing vein at the day 21, 31 and 41 of age. The samples were immediately transferred into sterile test tubes and serum was harvested. Antibody titres against NDV were measured in experimental birds through haemagglutination inhibition (HI) test (Hussain *et al.*, 2004) and against IBD vaccine through indirect haemagglutination (IHA) test (Hussain *et al.*, 2003).

FCR and mortality rate: FCR was calculated at the 42nd day as total feed consumed divided by the weight of live and dead birds whereas the mortality rates were calculated group wise.

Statistical analysis: HI antibody titres against ND and IHA antibody titres against IBD vaccines were statistically converted into geometric mean titres (GMT's) for each group (Banda *et al*., 2008). The data of all groups was compared by analysis of variance. Statistically significant differences among various treatment means were determined by using Least Significant Difference test (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

The HI titers against NDV were determined on day 03, 13 and 23 (Table I) post vaccination. The group C achieved significantly ($P<0.05$) higher titers than group B and A. The results of study are in accordance with the findings of Boa-Amponsem *et al.* (2000) who reported high levels of vitamin E to be immunostimulatory. Similarly, Swain *et al.* (2000) reported that vitamin E added to inactivated and emulsified vaccines e.g. ND and IBD vaccines enhanced the immune response to viral antigens in chickens. The results obtained during present investigation are also in agreement with the findings of Leshchinsky and Klasing, (2001) who reported that high doses of vitamin E improved antibody production to viral and bacterial antigens. Lin *et al.* (2002) also reported increased antibody titers against NDV with dietary vitamin A along with significantly improved egg laying performance in laying hens. Results are in line with the finding of Wu *et al.,* (2000), who reported a positive correlation between antibody titers and ascorbic acid supplementation of 1000ppm in 1-day old chicks. The perceptible reason for enhanced antibody production is increased number of lymphocytes and hence enhances immune response with increased Lisovit® supplementation.

The IHA titers against IBD vaccine were determined at day 0, 10 and 20 (Table II) post vaccination. The group C achieved significantly ($P<0.05$) higher titres than group B and A. The findings of the present study are supported by Sava *et al.* (1995) who studied that muramidase, following oral administration, acted as an activator of a number of immunity enhancing host responses. Similarly, Lin *et al.* (2002) has reported that both humoral and cellular immune responses were modulated by vitamin A. The results of the study indicated that immunomodulatory property is antigen dependant as described by Taheri *et al.* (2005) and Awaad *et al.,* (2010). The increased antibody titre may be because of boosted humoral response attained by the immunostimulatory effect of Lisovit® whereas the non-satisfactory antibody levels in group A may be attributed to low quality vaccines in developing countries, immune compromised health status of animals, or enhanced immunosupression because of heat stress and water deprivation.

The FCR was non-significantly ($P>0.05$) higher in group C whereas the mortality rate was significantly ($P<0.05$) lower in group C both against NDV and IBD vaccines. The results of the study are similar to the findings of Lohakare *et al.* (2005) who reported that ascorbic acid improved the feed conversion ratio when added to the poultry feed. Ather (2000); Villar *et al.* (2002); Lohakare *et al.* (2005); reported that vitamins C, D and E and poly herbal premix had regulatory effects on performance in broilers in terms of weight gain when added in feed under commercial growing conditions. The findings of the present study are coincided with the results of Friedman and Sklan, (1997) who reported that dietary level of vitamin A (500 mg/kg b.wt.) had maximized the growth and feed efficiency of broiler chickens. The findings of the present study are similar to the findings of the Mahmoud *et al.* (2004) who reported significant differences in mortality rates among groups supplemented with or without vitamin C and a 500mg/kg vitamin as dietary supplement showed less severe stress

response after exposure to high temperatures. Similarly, a high mortality rate and challenge by the viral diseases has been reported by Dalloul *et al*., (2003) in vitamin A deficient animals.

Conclusion and Recommendations: Humoral immunity is a key concept in the proper protection against ND and IBD vaccines. From the above discussion, it can be inferred that the Lisovit® supplementation at higher doses not only enhances antibody titers against ND and IBD, but also improves FCR and decreases mortality rates in broiler chickens. It is recommended that in order to maintain sufficiently high post-vaccination antibody levels in flocks of birds, commercially available immunostimulatory chemical agents/substances and herbal extracts may be used as an adjunct therapy to the vaccination.

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Table I: Feed conversion ratio (FCR), mortality rate and geometric mean haemagglutination inhibition (GMHI) titres against Newcastle disease vaccine in birds of three groups

			GMHI titers days after vaccination_		
Groups	**FCR on day 42**	**Mortality Rate**	**3**	**13**	**23**
A	1.90	29.97^{c}	7.29^{a}	33.51^{a}	12.69^{a}
B	1.85	13.3^{b}	7.99^{a}	185^{b}	84^{b}
C	1.82	2.2^{a}	8.00^{a}	244^{c}	140^{c}

Mean values having different superscripts within a column differ significantly (P<0.05).

Table II: Feed conversion ratio (FCR), mortality rate and geometric mean indirect haemagglutination (GMIHA) titres against infectious bursal disease vaccine in birds of three groups

			GMIHA titres days after vaccination		
Groups	**FCR on day 42**	**Mortality Rate**	**0**	**10**	**20**
A	1.90	29.97^{c}	5.27^{a}	35.09^{a}	29.11^{a}
B	1.85	13.3^{b}	5.03^{a}	255.19^{b}	101.56^{b}
C	1.82	2.2^{a}	5.54^{a}	387.01^{c}	121.77^{c}

Mean values having different superscripts within a column differ significantly (P<0.05).

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