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RESEARCH ARTICLE

Effect of Immunostimulants on Humoral Response against Infectious Bursal Disease in Broilers

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A B S T R A C T

In poultry industry, infectious bursal disease (IBD) is one of important viral disease causing high mortality and immunosuppression in chicken. Current experiment was conducted to see the effect of two commercially available immunostimulants Livol (herbal supplement) and immunotone (selenium and vit E) in conjunct with IBD virus (IBDV) vaccine in broilers. A total of 360 one-day old broiler chicks were divided into six equal groups, i.e., A, B, C, D, E and F. On day 0, birds in groups A, B and E were vaccinated against IBD. Chicks in groups A and C were treated with Immunotone (a commercial product) through drinking water and birds of groups B and D were supplemented with Livol. The effects of immunostimulants against IBD were evaluated through ELISA by recording weekly serum antibody titer till 42 days. On days 17, 23 and 29, five birds from each replicate pen were killed for histopathological studies and bursa/body weight ratio. Significantly ($P<0.05$) higher ELISA antibody titer were observed in group B (treated with Livol) as compared to group F (control). Histopathology revealed varying degree of lympho-proliferative changes in bursa of Fabricius and spleen with increase number of lymphocytes in groups B and D. Bursa/body weight ratio was highest in birds of group D whereas lowest was observed in those of group E. It was concluded that Livol supplementation may have potent immunomodulatory effect in chickens.

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INTRODUCTION

Intensive farming, semi-vertical integration system and lack of adaptation of biosecurity measures, Pakistan has become an ideal place for infectious diseases (Subtain *et al*., 2011). Infectious bursal disease (IBD) is commonly encountered lymphocytolytic disease that adversely affects the defensive mechanism of birds resulting in immunosuppression consequently failure to develop satisfactory immunity (Amin *et al*., 1991; Ou *et al*., 2013; Rashid *et al*., 2013). Infectious bursal disease virus (IBDV) damage the bursa of Fabricius in broiler chicken, specific reservoir for B lymphocytes (Mazariegos *et al*., 1990). Immunosuppression induced by IBDV cause apoptosis and made the broiler chicks unable to respond towards different vaccines, therefore, birds become more susceptible to various viral and bacterial infections (Vasconcelos and Lam, 1994). Mucosa associated lymphoid tissue of duodenum, jejunum and caeca are the first site of IBDV replication. Virus multiplies rapidly in immature B cells of bursa of Fabricius and other lymphoid organs like spleen and thymus, where degree of replication is lesser (Tanimura *et al*., 1994; Rautenschlein *et al*., 2001). Proper vaccination and monitoring of immune response of birds after vaccination are key steps to control the infectious diseases and immunosuppression (Arshad *et al*., 2005). A number of immunostimulants are used to improve the humoral immune response to minimize the immunosuppressive effects of IBD vaccine. In commercial poultry farming, a number of immunomodulatory products have been used like Livol (Beenish *et al*., 2013) herbal medicine (Khushdil *et al*., 2012; Li *et al*., 2013), Lisovit ® (Qayyum *et al*., 2012), organic selenium (El-Sheikh *et al*., 2010) and probiotic (Naseem *et al*., 2012) with success. The ultimate success of any therapeutic substance used in commercial poultry flocks, is based not only on the direct effect on a pathogen but also substantiation by an effective immune response (Qureshi, 1999). Main objective of present experiment was to see the effect of two immunostimulatory products

on humoral immunity against IBD vaccinated broiler chickens and to investigate the IBDV distribution in lymphocytes of lymphoid organs.

MATERIALS AND METHODS

Experimental design: A total of 360 day old chicks were reared in to six groups A, B, C, D, E and F each group replicated three times with 60 birds per pen. Broiler chicks in groups A and C were treated with Immunotone (containing selenium and vitamin E) as per manufacturer recommendations i.e. @ 2ml/6 liters of water. Birds of group B and D were given Livol (herbal supplement ingredients are *Andrographis paniculata*, *Azadirachta indica*, *Betafin*, *Magnifera indica, Terminalia chebula, Terminalia arjuna, Eclipta elba* and *Solanum nigram*, again according to manufacturer recommendations @ 1ml/7liter of water from day one to 42 of age. On day 0, chicks in groups A, B and E were vaccinated against IBD, using Bursaplex vaccine of IBD. Group F served as negative control i.e. without any supplement and/ or vaccine. The study was conducted using a randomized complete block design. Trial was conducted in an environmentally controlled poultry house where temperature, ventilation and other requirements were managed as per standard husbandry practices. Fresh wood shaving to the depth of 10cm was provided as bedding.

Measurement of antibody titer: Five birds were randomly selected for collection of blood from each group at 0, 7, 14, 21, 28, 35 and 42 days of age. To detect the antibody directed against IBDV a commercial ELISA kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA) was used. The procedure was conducted as described by manufacturer's recommendations.

Bursa to body weight ratio and histopathology: At days 17, 23, 29, post IBDV vaccination, five broiler chickens were randomly selected from each replicate group and were slaughtered humanely. Bursa to body weight ratio were estimated as described by Debnath *et al*. (2005). For histopathology, five broiler chickens were selected randomly from each treatment groups and slaughtered at 17, 23 and 29 days of age. After postmortem examination, bursa of Fabricius and spleen were collected, gross lesions observed and tissues samples were fixed in 10% formalin to be used for histopathology. Tissues samples were embedded in paraffin and tissues sections were stained through hematoxylin and eosin. Bursal lesions, lymphocytic depletion and other histological changes in bursa of Fabricius were scored by the pattern described by (Raue *et al*., 2004). Lymphocytic depletion and bursal damage were scored according to following criteria: 0=Apparently normal lymphoid follicles; 1=Mild lymphoid depletion indicated by just thinning of lymphocyte population without any sign of focal necrosis or remarkable edema; 2=Moderate lymphoid depletion along with focal necrosis and interfollicular edema; 3=Severe lymphoid depletion virtually leaving no lymphocyte but only reticular cell and 4=Atrophy of follicles usually with cystic spaces, in folding of epithelium and marked fibroplasias. The prepared tissue sections were later examined using a binocular microscope stereo-microscope (Olympus BX 41, U-LH100HG, Olympus optical, Co. Ltd) connected by camera (Spot Idea^{TM} 28.2-5MP) to computer idea, Version 4.7) using different magnifications. The collected data were compared using a randomized block analysis of variance (ANOVA) using the SAS software (SAS Institute, 1996) with significance level $P<0.05$.

RESULTS

The present study was conducted to determine the effect of two immunostimulatory products on humoral immunity against IBD vaccinated broiler chickens. ELISA was used to measure the serum antibody titer against IBD on 0, 7, 14, 21, 28, 35 and 42 days of age. On all experimental days, group B had significantly ($P<0.05$) higher geometric mean titer (GMT) against IBD virus (Table 1). In all vaccinated groups, antibody titer declined from day 14th, followed by an increase at day 21. Thereafter titer gradually increased from 35th day of age and continued till 42 day of experiment (Table 1).

Bursa to body weight (B/BW) ratio was significantly ($P<0.05$) high in unvaccinated groups D (Livol treated) on all experimental days as compared to unvaccinated group C (Immunotone treated) and F (without supplement and vaccination). Significantly high bursal weight observed in vaccinated group B (Livol treated) as compared to other vaccinated groups A and E (Table 2).

Histopathological lesions in bursa of Fabricius and spleen scored at 17, 23 and 29 days of age, revealed increased number of lymphocytes in Livol and Immunotone treated groups. Histopathologically, varying degree of lymphoproliferative changes were observed in both bursa and spleen (Table 3). Birds of Group-D (unvaccinated control) showed the active follicle consist of lymphoid cells with interfollicular tissue and shown no obvious histopathological lesion (Fig. 1a). In Group A (Immunotone treated), bursal samples showed mild lymphocytic damage at medullary region of bursal follicle. Bursal samples from Group B (Livol treated) showed lymphocytic hyperplasia and lymphoblast activation. Moderate lymphocytic depletion was observed in lymphoid follicles in Group E (Fig. 1b). The unvaccinated control group D did not show any histopathological lesion in spleen (Fig. 1c). Birds of groups A (Immunotone supplemented), and B (Livol supplemented) showed lymphoblastic activation. Various degrees of congestion observed in spleen of Group A. The vaccinated group E (only IBDV vaccinated) showed focal hemorrhages (Fig. 1d) whereas spleen of group F (without supplement and vaccination) was normal as no lesions were observed.

DISCUSSION

IBD causes the considerable economic losses through high mortality and immunosuppression in poultry. In Pakistan commercially available vaccines are abruptly used to control different viral diseases but unfortunately failure of these products occur from time to time. Therefore, present study was conducted to determine the

Table 1: Geometric mean ELISA antibody titers against infectious bursal disease virus (IBDV)

Groups	Days					
	7	14	21	28	35	42
A (IBDV vaccinated and Immunotone)	2857±119.5^{b}	1719±62.5^{a}	1815±53.8^{b}	2342±54.0^{b}	3373±26.8^{b}	3479±27.2^{b}
B (IBDV vaccinated and Livol)	3254±41.8^{a}	1815±53.8^{a}	1964±28.5^{a}	2614±90.2^{a}	3752±80.1^{a}	3890±36.4^{a}
C (Immunotone)	2629±31.4^{c}	1104±20.2^{d}	659±23.6^{e}	194±25.0^{e}	77±4.2^{e}	72±4.7^{e}
D (Livol)	2996±14.4^{b}	1246±23.2^{c}	883±33.6^{d}	365±27.5^{d}	204±10.5^{d}	185±7.8^{d}
E (IBDV vaccinated)	3008±16.5^{b}	1541±29.2^{b}	1645±26.6^{c}	2084±18.2^{c}	2965±24.2^{c}	3021±19.6^{c}
F (without supplement and vaccination)	2600±31.4^{c}	1100±20.2^{d}	650±23.6^{e}	100±25.0^{e}	77±4.2^{e}	72±4.7^{e}

Values (mean±SE) bearing different superscripts in a column differ significantly (P<0.05).

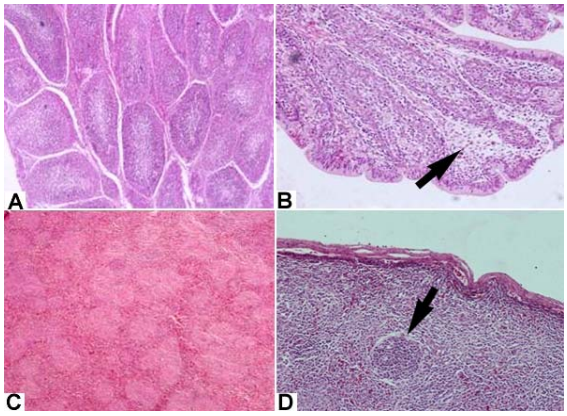

Fig. 1: Histopathology of Bursa of Fabricius showing A) active follicle containing lymphocytes in group D and B) follicular necrosis in group E (arrow). Spleen of chicken showing C) no pathological changes (group unvaccinated-Control) and D) lymphoblast activation (arrow) and slight congestion (group A vaccinated). H & E; 400X.

Table 2: Bursa/body weight ratio of different treatment groups on different days of age in broiler chickens

Groups	17th day	23rdday	29th day
A	0.97±0.10^{d}	1.01±0.05^{d}	1.13±0.15^{d}
B	1.08±0.05^{c}	1.22±0.04^{c}	1.53±0.19^{c}
C	1.02±0.07^{b}	1.53±0.14^{b}	1.90±0.07^{b}
D	1.12±0.14^{a}	1.65±0.02^{a}	1.95±0.12^{a}
E	0.52±0.01^{e}	0.60±0.02^{e}	0.67±0.01^{c}
F	1.02±0.07^{b}	1.50±0.14^{b}	1.80±0.07^{b}

Values (mean±SE) bearing different superscripts in a column differ significantly (P<0.05). Group A: IBDV vaccinated and Immunotone treated; group B: IBDV vaccinated and Livol treated; group C: immunotone treated only; group D: Livol treated only; group E: IBDV vaccinated only and group F without supplement and vaccination.

Table 3: Histopathological bursal and spleen lesion scoring of different treatment groups on different experimental days

Groups	Bursa			Spleen		
	17 day	23 day	29 day	17 day	23 day	29 day
A	1	2	2	1	2	2
B	1	1	1	1	1	1
C	0	1	1	0	1	0
D	0	0	0	0	0	0
E	2	2	3	2	2	1
F	0	0	1	0	1	0

Histopathological assessment of the experimental parameters was graded as: 0 = showing no changes; 1= mild changes, 2 = moderate changes and 3 = severe changes; Group A: IBDV vaccinated and immunotone treated; group B: IBDV vaccinated and Livol treated; group C: immunotone treated only; group D: Livol treated only; group E: IBDV vaccinated only and group F without supplement and vaccination.

immunostimulatory effect of two commercially available products against IBD vaccine. Results of present study indicated the highest antibody titer in group B supplemented with Livol. It has been well described previously that medicinal herbs have significant effect on humoral immunity against IBD vaccines and weight gain of broiler birds (Sadekar *et al*., 1998; Qayyum *et al*., 2012).

In present study group A supplemented with Immunotone showed moderate protective serum antibody titer (P<0.05) compared to groups C, D, E and F. Natural immunity of broiler birds increased by selenium supplementation thus enhancing the response against viral antigens (Shekaro *et al*., 2012). The highest antibody titer against IBD observed in group B throughout the experiment compared to group A. Livol contains *Azadirachta indica* that stimulates the immune system through enhancing the phagocytic activity and antigen presenting ability of macrophages and cytokines (Thatte and Dhanukar, 1997). Production of certain cytokines like interleukins-1 (IL-1), IFNγ and TNF-α gets stimulated indicating activation of T helper cells (Th1) type of responses. These cytokines also stimulate certain white blood cells (WBCs) to become more effective killer. In addition to activation interferons, interfere with the production (replication) of viruses (Mahima *et al*., 2013). Bursa and spleen are responsible for antibody production in chicken and their appropriate size are indicative of effective coverage against diseases, which can be confirm by enhance antibody titer (Nidaullah *et al*., 2010). Broiler chicken fed Livol (Herbal supplement) showed significant increases in bursa to body weight ratio. Eucalyptus and peppermint oils increase bursa to body weight ratio as compared with untreated control (Awaad *et al*., 2010). *Withania somnifera*, major constituent of Livol is known to positively moderate the immune system of man and animals (Kuttan *et al*., 1996). Different active ingredients present in Livol like *Magnifera indica, Azadirachta indica, Eclipta Elba* and *Terminalia chebu* has been scientifically proven to boost immunity in poultry (Zhai *et al*., 2011; Khushdil *et al*., 2012; Awaad *et al*., 2013). Livol supplemented groups had higher relative organ to body weight ratio indicating stimulating effect of Livol against immunosuppressive effect of IBD vaccines in birds. After inoculation of virulent strain of infectious bursal disease virus causes T lymphocytes suppression in spleen (Rautenschlein *et al*., 2001).

In the present study, bursa of Fabricius of all the birds of group E showed lymphoid necrosis at cortical and medullar regions. Infectious bursal disease virus appear first in other hematogenic organs like kidney, spleen and liver via the blood after infection followed by bursa where mass replication occur (Zhang *et al*., 2001). Within bursa of Fabricius immature B-cells is believed to be first site of replication for IBD virus where virus causes damage to B lymphocytes in lymphoid follicles (Chen *et al*., 2009). Serious necrosis and atrophy of lymphoid follicles in IBD virus infected chicken has been observed in bursa (Elankumaran *et al*., 2001; Hoque *et al*., 2001; Rautenschlein *et al*., 2001). Lymphocyte proliferation and modulation of number of lymphocytes in bursa and spleen

were detected in Livol treated groups. It has been reported that immunostimulants protected bursa and other lymphoid organs from damage in infections such as IBD (Mushtaq *et al*., 2003).

Conclusion: The findings of present investigation indicated that Livol has potentiating effect on humoral immunity in broiler chickens. It may be concluded that supplementation of Livol (herbal product) may have a potent immunostimulatory effect, therefore can be helpful in ameliorating the negative and/or harmful effects of IBDV vaccination.

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REFERENCES

Amin M, AN Rana and T Hussain, 1991. Prevalence of Gumboro disease in broilers and around Multan. Annual Progress Report, Poultry Development Center, Rawalpindi, Pakistan, pp: 69-72.

Arshad M, M Siddique, M Ashraf and HA Khan, 2005. Effect of selenium supplementation on antibody titers against infectious bursal disease vaccine in broiler chicks. Pak Vet J, 25: 203-204.

Awaad MHM, AM Atta, MA Elmenawey, GB Gharib, A Wafaa, Abd El-Ghany and AA Nada, 2013. The effect of a combination of D-Glucan and *Propionibacterium granulosum* on productive performance and immune modulation of immuno-compromised and non-immuno-compromised broiler chickens. Vet World, 10:31-38.

Awaad MHH, GA Abdel-Alim, KSS Sayed, S Kawkab, A Ahmed, AA Nada, AS Metwalli and AN Alkhalaf, 2010. Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens. Pak Vet J, 30: 61-66.

Beenish Z, RK Muti, A Asim, ZD Aneela, A Sohaib, HS Muhammad and M Khalid, 2013. Effect of a herbal supplement Livol on the growth performance and antibody response against infectious bursal disease virus in broiler chicks. Pak J Zool, 45: 1387-1391.

Chen L, MJ Ran, XX Shan, M Cao, P Cao, XM Yang and SQ Zhang, 2009. BAFF enhances B-cell-mediated immune response and vaccine-protection against a very virulent IBDV in chickens. Vaccine, 27: 1393-1399.

Debnath S, EH Chowdhury, D Begum, K Alam, S Mojumder and MR Islam, 2005. Management of Infectious bursal disease using a modified quaternary ammonium derivative in broiler chickens. Bangladesh Vet J, 39: 67-76.

El-Sheikh AMH, EA Abdalla and MH Maysa, 2010. The effect of organic selenium supplementation on productive and physiological performance in a local strain of chicken. Egyptian Poult Sci J, 30: 517-533.

Elankumaran S, RA Heckert and L Mours, 2001. Persistence and tissue distribution of a variant strain of infectious bursal disease virus in commercial broiler chickens. Proc Int Symp on Infectious Bursal Disease and Chicken Infectious Anaemia. Rouischholzhausen, Germany, pp: 353-565.

Hoque MM, AR Omar, LK Chong, M Hair-Bejo and I Aini, 2001. Pathogenicity of Sspl-positive infectious bursal disease virus and molecular characterization of the VP2 hypervariable region. Avian Pathol, 30: 369-380.

Khushdil M, C Naila, K Sarzamin and SQ Muhammad and JT Abdul, 2012. Comparative effect of different schedules of administration of medicinal plants *(Allium sativum, berberislycium, Eclipta alba* and *Mangiferaindica)* infusion on the immunity and overall performance of broiler chicks. *Sarhad* J Agric: 28: 319-326.

Kuttan G, 1996. Use of Withania somnifera as an adjuvant during radiation therapy. Indian J Exp Biol, 34: 854-856.

Li XT, B Wang, JL Li, R Yang, SC Li, M Zhang, W Huang and L Coa, 2013. Effect of Dangibuxxuie Tang, a Chinese herbal medicine on growth performance and immune response in broiler chicken. Biol Res, 46:183-188.

Mahima, A.K. Verma, R. Tiwari, K. Karthik, S. Chakraborty, R. Deb and K. Dhama, 2013. Nutraceuticals from fruits and vegetables at a glance: A review. J Biol Sci, 13: 38-47.

Mazariegos LA, PD Lukert and J Brown, 1990. Pathogenicity and immunosuppressive properties of infectious bursal disease "intermediate" strain. Avian Dis, 34: 203-209.

Mushtaq F, SA Khan, A Aslam, K Saeed, G Saleem and H Mushtaq, 2003. Effects of immunostimulants on broilers suffering from Infectious bursal disease. Pak Vet J, 23:36-40.

Naseem S, SU Rahman, M Shafee, AA Sheikh and A Khan, 2012. Immunomodulatory and growth-promoting effect of a probiotic supplemented in the feed of broiler chicks vaccinated against infectious bursal disease. Rev Bras Cienc Avic, 14: 109-113.

Nidaullah H, FR Durrani, SI Ahmed, U Jan and S Gul, 2010. Aqueous extract from different medicinal plants as anticoccidial, growth promotive and immunostimulant in broilers. J Agric Biol Sci, 5: 53-58.

Ou C, N Shi, Q Pan, D Tian, W Zeng and C He, 2013. Therapeutic efficacy of the combined extract of herbal medicine against infectious bursal disease in chickens. Pak Vet J, 33: 304-308.

Qayyum A, A Yousaf , T Ahmad, ZU Rehman and U Farooq, 2012. Immunomodulatory effects of Lisovit® in response to Newcastle disease and infectious bursal disease vaccines in broilers. J Anim Plant Sci, 22: 11-14.

Qureshi AA, 1999. Gumboro disease in Pakistan. Poult Int, 38: 42-43.

Rashid MH, C Xue, MT Islam, MR Islam, Z She and Y Cao, 2013. Comparative epidemiological study of infectious bursal disease of commercial broiler birds in Bangladesh and China. Pak Vet J, 33: 160-164.

Raue R, MR Islam, MN Islam, KM Islam, SC Badhy, PM Das and H Müller, 2004. Reversion of molecular engineered very virulent infectious bursal disease virus during infection of commercial chickens. Avian Pathol, 33: 181-189.

Rautenschlein S, HY Yeh and JM Sharma, 2001. A comparison of the immunopathogenesis of different IBDV strains. Proceeding of the II International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia. Rouischholzhausen, Germany, pp: 311-323.

Sadekar RD, AY Kolte, BS Barmase and VF Desi, 1998. Immunopotentiating effects of Azadirechta indica (Neem) dry leaves powder in broiler, naturally infected with IBD virus. Indian J Exp Biol, 36: 1151-1153.

Shekaro A, SB Oladele, PA Abdu and NDG Ibrahim, 2012. Effect of selenium on the susceptibility of vaccinated cockerels against infectious bursal disease. J Vet Adv, 2: 573-579.

Subtain SM, ZI Chaudhry, AA Anjum, A Maqbool and U Sadique, 2011. Study on pathogenesis of low pathogenic avian influenza virus H9 in broiler chickens. Pak J Zool, 43: 999-1008.

Tanimura N, K Tsukamoto, M Nakamura and S Yuasa, 1994. Pathological changes in specific–Pathogen free chickens experimentally inoculated with European and Japanese highly virulent strains of infectious bursal disease virus. International Symposium on Infectious Bursal Diseases and Chicken Infectious Anaemia, Rouishholzhausen, Germany, 143-154.

Thatte UM and SA Dhanukar, 1997. The Rasayana Concept: Clues from Immunomodulatory Iherapy. In: Immunomodulation, Upadhayaya, SN (ed). Narosa Publishing House, New Dehli, India, pp: 141-148.

Vasconcelos AC and KM Lam, 1994. Apoptosis induced by infectious bursal disease virus. J Gen Virol, 75: 1803-1806.

Zhang MF, GM Huang and S Qiao, 2002. Early stages of Infectious bursal disease virus Infection in chickens detected by in situ reverse transcriptase-polymerase chain reaction. Avian Pathol, 31: 593-597.