

Effect of a Herbal Supplement Livol on the Growth Performance and Antibody Response Against Infectious Bursal Disease Virus in Broiler Chicks

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Abstract.- Current study was conducted to evaluate the effect of two commercially available immunostimulants Livol (herbal supplement) and Immunotone (selenium and vit. E) on growth performance and humoral response against IBD virus vaccine. In the present study 150 broiler chicks were purchased and divided into five groups namely A, B, C, D and E (30 chicks in each group). The birds of groups A, B and E were vaccinated with infectious bursal diseases (IBD) virus vaccine (Busaplex) on 1st day of age. Birds of groups A and C were supplemented with Immunotone while group B and D were supplemented with Livol. The effect of immunostimulants on humoral immune response was evaluated by recording weekly serum antibody titers against IBD through indirect haemagglutination (IHA) test. No significant difference was found in the body weight of broiler chicks on 7, 14, 21 and 28 days of age among the different treatment groups but the body weight of groups B and D receiving Livol was significantly increased ($P < 0.05$) after 35 days. No significant difference was found in the feed conversion ratio (FCR) during treatment period but on day 35 Livol treated groups B and D showed significantly higher FCR 1.52 ± 0.02 and 1.48 ± 0.02 , respectively. The cumulative mean titers for IHA for different treatment groups A, B, C, D and E were 43.3, 51.4, 12.3, 14.1 and 40.1, respectively ($P < 0.05$). These results indicate that Livol supplementation may help to increase post vaccination humoral immune response against IBD in broiler chicks.

Keywords: Broiler chicks, IBD, immunostimulant, immunotone, Livol.

INTRODUCTION

Intensive farming, semi-vertical integration system and lack of adaptation of biosecurity measures, this country 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 and results in immunosuppression and failure to develop satisfactory immunity (Amin *et al.*, 1991). The target organ of infectious bursal disease virus (IBDV) is the bursa of Fabricius which is specific reservoir for B lymphocytes. IBDV causes destruction and depletion of B cells in bursa of Fabricius (Mazariegos *et al.*, 1990). Immunosuppression induced by IBDV cause apoptosis (Vasconcelos and Lam, 1994).

Immunostimulation of a bird may lead to increased phagocytosis by macrophages and increase in antibody production (Spallholz *et al.*, 1973). There are many immunostimulating substances that have been used in poultry with success. Some of these agents include levamisole, vitamin E and selenium (Koller, 1982; Bashir, 1994). 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). The objective of present study was to evaluate the effect of two therapeutic substances on growth performance and humoral immune response against IBD in broiler chicks.

MATERIALS AND METHODS

Experimental design

A total of 150 day old broiler chicks were divided in to five groups A, B, C, D and E each having 30 birds. Birds of groups A and C were

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supplemented with Immunotone (ingredients are selenium and vitamin E) @ 2ml/ 6 liter of water. Birds of group B and D were supplemented with Livol (herbal supplement ingredients are *Andrographis paniculata*, *Azadirachta indica*, *Betafin*, *Magnifera indica*, *Terminalia chebula*, *Terminalia arjuna*, *Eclipta elba* and *Solanum nigrum*) @ 1ml/ liter (Qamar *et al.*, 2011) of water from day one to 42 of age. The birds of groups A, B and E were vaccinated against IBD on day one using Bursaplex vaccine of IBD. All the groups were maintained under standard housing and management condition.

Body weight and feed consumption

Individual body weight of the chicks in each replicate in all treatment groups were recorded on day 7, 14, 21, 28 and 35. Feed consumption of all treatment groups was recorded on day 7, 14, 21, 28 and 35 and the mean total feed consumption per bird was calculated as described by Nidaullah *et al.* (2010).

Measurement of serum antibody titer

Blood samples were obtained at weekly intervals from randomly selected 5 birds of each group from day 1 to 42. IHA antibody titers against IBD were measured in serum samples as described by Rahman *et al.* (1994). Geometric mean titers (GMT) of each group were calculated at each week.

Statistical analysis

The collected data was analyzed by ANOVA using the SAS software (SAS Institute, 1996), while the mean differences among various treatments were separated by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Body weight

Average body weight (g) in each week of different treatment groups A, B, C, D and E of broiler chicken was 884±15.85, 899±19.80, 895±18.84, 923±18.04 and 858±24.88, respectively (Table I). On day 7 no significant difference was observed in body weight of broiler chicken in different treatment groups (Table I). On 14 day group A (immunotone treated) showed significantly higher body weight compared to other treated

groups ($P<0.05$). On day 21, 28 and 35 group D (only Livol treated) showed significantly ($P<0.05$) higher body weight compared to unvaccinated group C. On day 21, 28 and 35, group B (Livol treated) showed significantly ($P<0.05$) higher body weight compared to other vaccinated groups A (immunotone treated) and E (only vaccinated). These findings are in agreement with Narahari (1992), El-Deek *et al.* (2003) and Wheeler (2006) that herbal liver supplementation in broiler serve as growth promoter. On day 35 unvaccinated group D (1892.2±17.6) and vaccinated group B (1862±28.4) had no significant difference in body weight of broiler chicken as shown in Table I. The findings of present study are supported by the observation of Qamar *et al.* (2011), who reported that herbal liver tonics supplemented (1ml/l) in broiler diet may serve as growth promoters. In this study vitamin E along with selenium (immunotone) had little effect on body weight compared to livol which is in line with the study outcomes of Salman *et al.* (2007) who showed that vitamin E along with selenium (immunotone) had little effect on weight of birds.

Feed conversion ratio

Mean feed conversion ratio (FCR) of treatment groups A, B, C, D and E of broiler chicken was 1.17±0.31, 1.16±0.28, 1.18±0.24, 1.15±0.22 and 1.20±0.31 respectively (Table II). On day 7 and 14 no significant difference was observed in FCR of broiler chicken in different treatment groups. On day 21, 28 and 35, group D (only Livol treated) showed significantly ($P<0.05$) high FCR compared to unvaccinated group C. Results of present study is justified by the result of Chand *et al.* (2005), who fed 0, 0.5, 1.0, 1.5, 2.0 and 2.5% *Berberis lyceum* to broilers and observed best feed efficiency. On day 21, 28 and 35, the group B (Livol treated) showed significantly high ($P<0.05$) FCR compared to other vaccinated groups A (Immunotone treated) and E (only vaccinated). On day 35 unvaccinated group D (1.48±.02) and vaccinated group B (1.52±.026) had no significant difference in FCR of broiler chicken. Present study is also in agreement with the result of Ravikumar *et al.* (2002) who treated the birds with Liv.52 and observed higher average live weight in treated birds compared to control.

Table I.- Body weight in grams (Mean ±S.E) of broiler chicken in different treatment groups from day 7 to 35 of age.

| Groups | Body weight in grams | | | | | Average body weights |
|--------|-----------------------|-------------------------------------|-------------------------|------------------------|--------------------------|----------------------|
| | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | |
| A | 162±4.77 ^a | 478 ^a ±10.8 ^a | 705±26.3 ^b | 1269±14.2 ^b | 1806±23.2 ^c | 884±15.85 |
| B | 160±17.4 ^a | 453±17.2 ^{ab} | 734±13.5 ^{ab} | 1288±22.5 ^b | 1862±28.4 ^a | 899±19.80 |
| C | 147±15.5 ^a | 410±16.5 ^b | 772±14.8 ^a | 1329±19.1 ^a | 1818±28.3 ^b | 895±18.84 |
| D | 149±17.0 ^a | 413.4±13.6 ^b | 778.4±24.6 ^a | 1384±17.4 ^a | 1892.2±17.6 ^a | 923±18.04 |
| E | 155±24.4 ^a | 465.4±19.7 ^a | 700±17.9 ^b | 1196±36.4 ^c | 1772±26.0 ^c | 858±24.88 |

Values with different superscripts within a column differ significantly (P<0.05)

Treatment Groups: A, Vaccinated and Fed with Immunotone; B, Vaccinated and Fed with Livol; C, Unvaccinated and fed with Immunotone; D, Unvaccinated and fed with Livol; E, Vaccinated without Immunostimulant

Table II.- Feed conversion ratio (Mean±S.E) of broiler in different treatment groups from day 7 to 35 of age.

| Groups | Feed Conversion Ratio | | | | | Mean FCR |
|--------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|-----------|
| | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | |
| A | 0.80±0.07 ^a | 0.90±0.08 ^a | 1.28±0.014 ^b | 1.32±0.01 ^b | 1.55±0.03 ^{ab} | 1.17±0.31 |
| B | 0.82±0.05 ^a | 0.94±0.07 ^a | 1.23±0.02 ^{ab} | 1.30±0.02 ^{ab} | 1.52±0.02 ^{ab} | 1.16±0.28 |
| C | 0.88±0.08 ^a | 1.05±0.11 ^a | 1.17±0.03 ^a | 1.26±0.02 ^{bc} | 1.54±0.02 ^{ab} | 1.18±0.24 |
| D | 0.87±0.09 ^a | 1.04±0.10 ^a | 1.16±0.04 ^a | 1.21±0.04 ^c | 1.48±0.02 ^b | 1.15±0.22 |
| E | 0.84±0.12 ^a | 0.90±0.08 ^a | 1.29±0.02 ^b | 1.4±0.039 ^a | 1.58±0.03 ^a | 1.20±0.31 |

Values with different superscripts within a column differ significantly (P<0.05)

For treatment groups see Table I.

Table III.- Geometric mean IHA antibody titer (Mean±S.E) against infectious bursal disease virus from day 7 to 42 of age.

| Groups | Geometric mean IHA antibody titer | | | | | | CMT |
|--------|-----------------------------------|-----------------------|----------------------|----------------------|-----------------------|----------------------|-----------|
| | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 | |
| A | 31±1.73 ^{ab} | 20±1.41 ^{aa} | 2±1.41 ^{aa} | 30±1.41 ^b | 68±1.41 ^{ab} | 89±1.41 ^b | 43.3±26.1 |
| B | 33±1.0 ^a | 22±1.41 ^a | 24±1.41 ^a | 36±1.41 ^a | 72±1.41 ^a | 20±2.82 ^a | 51.4±27.7 |
| C | 28±1.41 ^b | 17±2.0 ^a | 10±1.41 ^b | 7±1.18 ^c | 6±1.41 ^c | 6±1.41 ^d | 12.3±8.70 |
| D | 30±1.41 ^{ab} | 18±2.6 ^{aa} | 12±1.41 ^b | 9±2.0 ^c | 8±1.41 ^c | 8±1.41 ^d | 14.1±8.60 |
| E | 30±1.41 ^{ab} | 20±1.41 ^{aa} | 21±1.0 ^{aa} | 28±1.41 ^b | 65±2.0 ^b | 77±2.23 ^c | 40.1±24.4 |

Values with different superscripts within a column differ significantly (P<0.05). CMT: Cumulative Mean Titer

For treatment groups see Table I.

Geometric mean titer

In the present study, the geometric mean titer (GMT) against IBD measured through IHA test (Table III). The highest cumulative mean titers were recorded in group B (51.4±27.7), followed by groups A (43.3±26.1), E (40.1±24.4), D (14.1±8.6) and C (12.3±8.7). The vaccinated groups A, B and E revealed significant increase (P<0.05) in geometric mean antibody titer compared to unvaccinated groups C and D on 7, 14, 21, 28, 35 and 42 days of age. The results of present study are supported by the observation of Sadekar *et al.* (1998) who

reported medicinal herbs observed significant effect on the immune performance against IBD. In the present study the group A supplemented with Immunotone showed moderate protective serum antibody titer (P<0.05) compared to control groups C, D and E. Current trial support Madron and Vrzgulova (1988) statement, who reported that selenium supplementation enhanced the natural resistant of animals by increasing response of the organism to antigenic stimuli. The GMT was significantly (P<0.05) recorded in group B supplemented with Livol throughout the

experimental period as compared to group A. The present results are supported by the observation of Sarang and Durrani (2005) who reported that neem leaves infusion is better to enhance immunomodulatory response against IBD. *Withania somnifera* is known to positively moderate the immune system of man and animals (Kuttan *et al.*, 1996). Other ingredients of Livol (*Magnifera indica*, *Azadirachta indica*, *Eclipta Elba* and *Terminalia chebula*) are also reported to have immunomodulatory properties (Biswas *et al.*, 2002; Ravikumar *et al.*, 2002). Each of these herbs has been scientifically proven to boost immunity in poultry (Makare *et al.*, 2001; Rajak *et al.*, 2004; Sembulingam *et al.*, 2005).

CONCLUSIONS

The findings of present investigation indicate that Livol has potentiating effect on humoral immune response in chickens. It could be concluded that administration of these herbal supplements has a potent immunomodulatory effect and evoke the immune response in chickens. Livol supplementation (@1ml/litter through drinking water) can be beneficial and may help to increase post vaccination antibodies production against IBD in broiler chicks resulting in better disease control at farm level.

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REFERENCES

- AMIN, M., RANA, A.N. AND HUSSAIN, T., 1991. *Prevalence of Gumboro disease in broilers and around Multan*. Annual Progress Report of Poultry Development Center, Rawalpindi, pp.69-72.
- BASHIR, F.K., 1994. *Effect of vitamin E deficiency and excess on immune system of broiler chicken*. M.Sc. (Hons.) thesis, Department of Veterinary Pathology, College of Veterinary Sciences Lahore, Pakistan.
- BISWAS K., CHATTOPADHYAY, I., BANERJEE, R.K. AND BANDYOPADHYAY, U., 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). *J. Curr. Sci.*, **82**: 1336-1345.
- CHAND, N., DURRANI, F.R., MIAN, M.A. AND DURRANI, Z., 2005. Effect of different levels of added *Berberis lycium* on the performance of broiler chicks. *Int. J. Biotechnol.*, **2**: 971-974.
- EL-DEEK, A.A., ATTIA, Y.A. AND HANNFY, M.M., 2003. Effect of aniseed (*Pimpinella anisum*), ginger (*Zingiber officinale roscoe*) and fennel (*Foeniculum vulgare*) and their mixture on performance of broilers. *Arch. Geflügelk.*, **67**: 92-96.
- KOLLER, L., 1982. Chemically induced immunomodulation. *J. Am. Vet. Med. Assoc.*, **181**: 1102-1106.
- KUTTAN, G., 1996. Use of *Withania somnifera* as an adjuvant during radiation therapy. *Ind. J. exp. Biol.*, **34**: 854-856.
- MADRON, P. AND VRZGULOVA, N., 1988. Vitamin E and selenium increase the natural resistance of farm animals. *Veterinarstvi*, **38**: 369-371.
- MAKARE, N., BODHANKAR, S. AND RAIGARI, V., 2001. Immunomodulatory activity of alcohol extract of *Mangifera indica* in mice. *J. Ethnopharmacol.*, **78**: 133-137.
- MAZARIEGOS, L.A., LUKERT, P.D. AND BROWN, J., 1990. Pathogenicity and immunosuppressive properties of infectious bursal disease "intermediate" strain. *Avian Dis.*, **34**: 203-209.
- NARAHARI, D., 1992. Performance promoting ability of 'Activity Forte' in broilers. *Poult. Adv.*, **25**: 37-38.
- NIDAULLAH, H., DURRANI, F.R., AHMAD, S.I., JAN, U. AND GUL, S., 2010. Aqueous extract from different medicinal plants as anticoccidial, growth promotive and immunostimulant in broilers. *J. Agric. Biol. Sci.*, **5**: 53-58.
- QAMAR, M.F., ZAFAR, M., ANJUM, A.A., NAJEEB, M.I. AND MAQBOOL, A., 2011. Role of herbal polysaccharides as growth promoters in broilers (Lahore). *Sci. Int.*, **23**: 157-159.
- QURESHI, A.A., 1999. Gumboro disease in Pakistan. *Poult. Intl.*, **38**: 42-43.
- RAHMAN, S.U., ASHFAQUE, M. AND SAEED, J., 1994. Infectious bursal disease antibody titration using indirect haemagglutination test. *Pak. Vet. J.*, **14**: 101-103.
- RAJAK, S., BANERJEE, S.K., SOOD, S., DINA, A.K., GUPTA, Y.K., GUPTA, S.K. AND MANLIK, S.K., 2004. *Emblica officinalis* causes myocardial adaptogen and protects against oxidative stress in ischaemic reperfusion injury in rats. *Phytother. Res.*, **18**: 54-60.
- RAVIKUMAR, B.K. AND BHAGAWAT, V.G., 2002. Efficacy of Liv.52 Protec liquid, a herbal growth promoter in commercial broiler production. *Poult. Adv.*, **5**: 7-8.
- SADEKAR, R.D., KOLTE, A.Y., BARMASE, B.S. AND DESI, V.F., 1998. Immunopotentiating effects of

- Azadirachta indica* (Neem) dry leaves powder in broiler, naturally infected with IBD virus. *Indian J. exp. Biol.*, **36**: 1151-1153.
- SALMAN, M., SELCUK, Z. AND MUGLALI, O.H., 2007. Effect of vitamin E and selenium on performance, plasma and tissue GSH-Px activity in broilers. *Istanbul Vet. Sci.*, **3**: 25-34.
- SARANG, M.J. AND DURRANI, F.R., 2005. *Immunomodulatory and growth promoting effect of Neem (Azadirachta indica) leaves infusion in broiler chicks*. MSc (Hons) Thesis. NWFP Agricultural University, Peshawar.
- SAS INSTITUTE. 1996. *SAS User's Guide: Statistics*. Version 7.0. SAS Institute Inc., Cary, NC.
- SEMBULINGAM, K., SEMBULINGAM, P. AND NAMASIVAYAM, A., 2005. Effect of *Ocimum sanctum* Linn. on the changes in central cholinergic system induced by acute noise stress. *J. Ethnopharmacol.*, **96**: 477-482.
- SPALLHOLZ, H.E., MARTIN, J.L. AND GERLACH, M.C., 1973. Enhanced immunoglobulin M and immunoglobulin G antibody titer in mice fed selenium. *Infect. Immun.*, **8**: 841.
- SUBTAIN, S.M., CHAUDHRY, Z.I., ANJUM, A.A., MAQBOOL, A. AND SADIQUE, U., 2011. Study on pathogenesis of low pathogenic avian influenza virus H9 in broiler chickens. *Pakistan J. Zool.*, **43**: 999-1008.
- VASCONCELOS, A.C. AND LAM, K.M., 1994. Apoptosis induced by IBDV. *J. Gen. Virol.*, **75**: 1803-1806.
- WHEELER, G.E., 2006. Use of a herbal supplement to reduce the effects of stress in intensively reared chickens. *Ind. J. Indig. Med.*, **11**: 51-60.

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