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The Effect of Levamisole on Growth Performance, Humoral Immunity and Blood Biochemical Profile in Broiler Chickens

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ABSTRACT

Levamisole is an anthelmintic drug commonly used for the treatment of parasitic diseases. It triggers immunity in livestock. The research was conducted to evaluate the levamisole (LMS) effect's on growth performances, humoral immunity and selected hemato-biochemical parameters in broilers chickens. A total of 40, thirteen days old "Lohman" broiler chicks were reared up to 33 days with routinely vaccinated. The chicks were randomly divided into four equal groups: A, B, C and D (n=10). The group A was considered as control (neither vaccinated, not treated). The other three groups were vaccinated with Newcastle disease and infectious bursal disease live vaccine on 15 days of age. Group B was only vaccinated. Group C and D were provided with levamisole hydrocloride (Hcl) at a dose rate of 3 mg / kg and 10 mg / kg body weight, respectively through drinking water from 16th day at twenty four hours interval for five successive days. Results showed that LMS treated broilers had significantly ($p<0.01$) higher antibody titer compared to non-treated group. Body weight and body weight gain were significantly ($p<0.05$) higher in LMS treated (low and high doses) groups. Broilers treated with LMS had significantly ($p<0.05$) improved FCR. Hematological parameters (TEC, Hb and PCV) didn't differ statistically among the groups. Creatinine values were in normal range among the treated groups. Broilers treated with high doses (10 mg/kg) of LMS showed increase level of serum alanine transaminase (ALT) and aspartate transaminase (AST) levels. Serum total cholesterol, LDL cholesterol and triglycerides were also significantly increased in levamisole treated group (10 mg / kg) whereas HDL cholesterol was not significantly differed ($p<0.05$). Overall, this work explores the use of levamisole @ 3 mg/kg body weight in stimulating immune response, promoting growth performance but higher doses of LMS may be detrimental to poultry health.

Introduction

Levamisole is an imidazole-thiazole group derivate. It is an effective and safe broad- spectrum anthelmintic commonly used in both veterinary and human medicine. Earlier studies have shown that levamisole can enhance both cellular and humoral immune responses in normal chickens (Oladele *et al*., 2012; Habibi *et al*., 2012). LMS has also been shown with several species to be a potent immune stimulant in the modulation of leukocyte cytotoxic activity, phagocytosis, respiratory burst, macrophage activating factor and antibody response (Mulero *et al.,* 1998, Cuesta *et al.,* 2002). Effects of LMS as an adjuvant in vaccination efficacy have been debated (Morrison *et al.,* 2001). Cell-mediated immunity can be ameliorated by LMS's inclusion in DNA based vaccines and chemically killed viral vaccines (Jin *et al.,* 2004 and Kang *et al.,* 2005). Through dietary administration, LMS has been reported to enhance antibody response to hepatitis B vaccination in humans. Another study has reported that LMS injected with a DNA vaccine against the foot-and-mouth disease virus (FMDV) stimulated both humoral and cellular immune responses in conjunction with strong production of interferon (IFN) (Jin *et al.,* 2004).

In the last few years, commercial poultry farming has been developing very rapidly and poultry diseases are the major constraints for the development of the poultry industry (Karim, 2003). This profitable sub-sector is seriously interrupted by a number of infectious and contagious diseases such as Newcastle disease (ND), infectious bursal disease (IBD), salmonellosis, fowl cholera, infectious coryza, chronic respiratory disease, aspergillosis, coccidiosis, helminthiasis, etc. Though

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vaccination program has been taken as a preventive measure against most pathogenic viral diseases, use of immunomodulators with vaccines including Newcastle disease (ND) vaccine may increases the protective level of vaccines. Maternal antibodies protect birds during the first few weeks of age and can interfere with the development of humoral immunity; however, they cannot avoid the rapid establishment of the vaccine protection. Since ND can cause up to 100% mortality, a regularly implemented vaccination program is necessary to prevent outbreaks in chickens. Vaccination success depends on particular factors such as special antigens, immunogenicity of antigens and immune stimulants. Since the emergence of antibiotic-resistant microorganisms and antibiotic residues in meat are the most limiting matter to their expanded usage, vaccination with immune stimulants for the prevention of diseases in poultry is considered as an most effective measure. LMS has been documented to have immunostimulant properties but its role in growth performance and effects on hemato-biochemical parameters in broiler need to be readdressed. Therefore, the research was designed to assess the effect of LMS on humoral immunity, body growth and blood biochemical profile (lipid profile, creatinine, AST and ALT) in broilers treated with LMS.

Materials and Methods

Experimental birds

The research was conducted at the experimental shed of the Department of Physiology, Faculty of Veterinary Science of Bangladesh Agricultural University, Mymensingh during the period from 12 March to 10 April 2019. A total of forty (40) apparently healthy "LOHMAN" broiler chicks of thirteen days old were purchased from Rashid Hatchery Ltd, Mymensingh.

Experimental design

The experiment was conducted in a completely randomized design. On the 14th day of age, chicks were randomly divided into four groups (group A, B, C and D). Each group contained 10 birds. The birds of groups A, B, C and D were kept in separate cages. Group A was kept as control, i.e. neither vaccinated nor treated; it was served as control group. Group B, C and D was administered with Newcastle disease vaccine at recommended dose as one drop on right eye of each bird. Group B was only vaccinated and not treated with levamisole. Group C and group D were vaccinated and treated with levamisole in a dose of 3 mg /kg and 10 mg /kg body weight, respectively orally with drinking water at 24 hours interval between doses for five successive days. At 16th day of age, treatment was started and ended at the age of 21 days. Birds received their freshly prepared daily medication in the morning hour of each day. The concentration of levamisole in the water to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication.

Levamisole

Avinex® vet powder was purchased as a source of levamisole from Renata Animal Health Ltd. Each gram powder contains 300 mg levamisole Hcl.

Management of experimental birds

Managements and rearing of birds was strictly followed according to standard broiler farming system. Birds were vaccinated against ND, IBD and IB as per manufacturers' instructions. Strict biosecurity measures were taken during the experimental periods. Birds were supplied with grower diet for ten days (13-23 days) and finisher feed for last ten days (23-33 days). Feed and drinking water were supplied *ad libitum*.

Measurements of body weight

The body weight of each bird was measured weekly at 14th, 21st, 28th and 33rd days of age using digital balance and total body weight gain was calculated as follows: body weight gain= final body weight – initial body weight. FCR was determined by the formula: total feed consumed by birds divided total body weight gain. Feed consumption is the amount of feed consumed by the birds in a period of time. Feed intake was calculated as the difference between the amount of feed supplied to the birds and the amount of feed that remained at the end of each feeding period (Yi *et al.,* 2018; Khalil *et al.,* 2020).

Blood collection

Blood samples were collected from each bird at slaughter after completion of experiment. About ten to 12 mL blood was collected from each bird. Five to 6 mL of the blood was kept in a sterile test tube containing anticoagulant (3.8% trisodium citrate solution) at a ratio of 1:10 and remaining of the blood was taken in a another sterile test tube without any anticoagulant for separation of serum.

Preparation of serum

Test tubes containing blood without anticoagulant were kept in a slanting position at room temperature. These samples were refrigerated overnight at 4°C. The separation of serum from the clotted blood was achieved following centrifugation at 1000 rpm for 15 min as described by Salahuddin *et al.* (2012). These cell free serum samples were preserved at –20°C for further biochemical analysis.

Hemato-biochemical analysis

The hematological parameters (Hb, TEC and PCV) were performed within two hours of collection according to standard procedures described earlier (Das *et al.,* 2014 and Haque *et al.,* 2017). The biochemical tests were performed in Professor Muhammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh. The serum total cholesterol, triglycerides, high density lipoproteins (HDL), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed with a UV spectrophotometer T80 (PG instruments, Great Britain). Specific reagents from High Technology Incorporation (HTI), USA were used for each test.

Measurement of humoral immunity

Humoral immunity was measured by detecting antibody titer against ND vaccine by performing hemagglutination inhibition test as per procedure described earlier (Ambali *et al.,* 2017). Shortly, a preparation of ND virus with known HA titer was obtained. Then test serum was diluted two fold. e.g. from 1:4 to 1:1024. A fixed amount of virus was added to every well of a 96-well plate, equivalent to 4 HA units (varies according to the virus), except for the serum control wells. The plate was then allowed to stand at room temperature for 60 minutes (time varies according to specific requirements). Red blood cells (RBC) was added and incubated at 4°C for 30 minutes. The wells were observed.

Statistical analysis

Data were stored in Microsoft Excel-2013 and imported to the software Graph Pad Prism 8 for analysis. Results are expressed as the mean +SEM. One way analysis of variance (ANOVA) was used for data analysis (Steel and Torrie, 1980). Significant differences between the groups were detected in the ANOVA using Tukey's multiple comparsion test. $P<0.05$ was considered as statistically significant

Results and Discussion

Effects of levamisole on live body weight of broilers

Effects on body weight gain of different groups of broiler chickens are presented at Table 1. On the 14th day of age, prior to treatment, it was observed that the body weight of different group of birds were more or less similar and not statistically significant ($P>0.05$). After 7 days of treatment, body weight in all the treated groups B, C and D was found higher than that of non-vaccinated control group. It is reported that levamisole treated group shows better weekly average body weight than the control group (Oladele et al., 2012). On the 28th day of age, it was observed that the body weight of LMS treated group were higher than the non-treated group and the data were statistically significant ($p<0.05$). On the 33rd day of age the body weight were increased in all groups. The highest body weight was recorded in group D (Vac+LMS-10) and the lowest body weight was in group A (control) (Table 1). However, no significant difference observed between two treatment groups. The increased rate of body weight gain in the treated group might be due to an increased feed utilization, digestion, absorption and metabolism of supplied feed nutrient since levamisole can stimulate active transport of oxygen molecules across the cell membrane (Alishahi *et al.,* 2012). Levamisole also enhances resistance to stressors such as oxygen deficiency, temperature, salinity as well as improvement of both humoral and cell mediated immunity promoting feed conversion ratio (FCR), body weight and other growth parameters of broiler chickens (Kim and Rajapakse, 2005).

Use of levamisole after vaccination exhibited an increase in the body weight gain and profit margin of the broiler chickens (Table 2). LMS used in the drinking water at the dose of 3 mg/kg or 10 mg/kg was found to be more beneficial in gaining weight than untreated group A and B. The findings of current study are similar to that of Porchezhian & Punniamurthy, 2006 who investigated that LMS given orally in different dose levels to broilers had positive effect on performance parameters ($P<0.05$) including reduced cumulative feed intake and enhanced cumulative feed efficiency as well as cumulative body weight gain or a better flock performance of broiler chickens. However, LMS, in the present study, showed less effective in improving FCR of broiler chickens. These obtained results are not consistent with the study of Funde, 2005**,** who reported significantly better FCR in LMS treated group compared to control group.

Effects of levamisole on hematological parameters

Hematological parameters (Hb, TEC and PCV) values were more or less similar in all group of birds indicating that treating with levamisole in post vaccination birds has no effect on increasing or decreasing cellular volume (Table 3). These results of Hb, TEC and PCV disagree with the report of Hoque *et al.,* 2006 and Begum *et al.,* 2010, which stated that, Hb, PCV and TEC values were increased after administration of levamisole.

Effects of levamisole on biochemical parameters of broiler chickens

Two important liver enzymes (ALT and AST) were increased significantly in the levamisole treated group D (Vac + LMS 10) but not in group C than control group of broiler chickens which indicated that liver supposed to be damaged upon supplementation of high doses of levamisole (10 mg/kg) **(**Table 4). The same result also stated by Hrvoje *et al*., 2016.

Table 1. Effects of levamisole on live body weight (g) (Mean ± SEM) in broiler chickens

Groups	Body weight (g) (Mean ± SEM)			
	14th day (g)	21st day (g)	28th day (g)	33nd day (g)
Group A (Control + No Vac)	521.00 ± 2.92 [a]	945 ± 28.45[b]	1290 ± 32.52[a]	1463 ± 83.52[a]
Group B (Control + Vac)	507.00 ± 4.40 [a]	1050 ± 31.25[a]	1425 ± 40.25[b]	1673 ± 90.78[b]
Group C (Vac + LMS-3)	491.00 ± 12.29 [a]	1040 ± 29.56[a]	1460 ± 45.55[c]	1738 ± 66.00[c]
Group D (Vac + LMS-10)	499.00 ± 6.96 [a]	1035 ± 30.44[a]	1472 ± 47.05[c]	1772 ± 124.52[c]

* Values with dissimilar letters in a column differs significantly (p<0.05)

Table 2. Effects of levamisole on body weight gain (g) (Mean ±SEM) and FCR in broiler chickens of the experimental groups

Groups	Body weight gain (g)	% Body weight gain	FCR
Group A (Control + No Vac)	854.00± 111.14[a]	181.51± 21.76[a]	1.69±0.01[a]
Group B (Control + Vac)	1183.00± 106.02[b]	233.68± 20.89[b]	1.49± 0.04[b]
Group C (Vac + LMS-3)	1212.00± 87.82[b]	242.66± 17.46[b]	1.47± 0.05[b]
Group D (Vac + LMS-10)	1554.00±667.43[c]	310.52±78.67[c]	1.51± 0.02[b]

* Values with dissimilar letters in a column differs significantly (p<0.05)

Table 3. Effects of levamisole on hematological parameters (Hb, TEC, PCV) (Mean ± SEM) in broiler chickens

Groups	Hb (g %)	TEC (million / µL)	PCV (%)
Group A (Control + No Vac)	6.88 ± 0.15	2.20 ± 0.11	28.40 ± 1.03
Group B (Control + Vac)	7.64 ± 0.26	2.86 ± 0.14	29.40 ± 2.40
Group C (Vac + LMS-3)	7.42 ± 0.35	2.54 ± 0.16	29.20 ± 1.80
Group D (Vac + LMS-10)	7.52 ± 0.46	2.72 ± 0.10	30.60 ± 2.50

* Values with dissimilar letters in a column differs significantly (p<0.05)

Table 4. Effects of levamisole on ALT, AST and creatinine (Mean ±SEM) in broiler chickens of the experimental groups

Groups	ALT (U / L)	AST (U / L)	Creatinine (mg / dL)
Group A (Control + No Vac)	3.17 ± 0.24a	10.15 ± 0.95a	0.72 ± 0.07a
Group B (Control + Vac)	5.53 ± 0.49b	11.55 ± 0.93a	1.00 ± 0.07b
Group C (Vac + LMS-3)	4.86 ± 0.56b	13.91 ± 1.39b	0.81± 0.01a
Group D (Vac + LMS-10)	6.66 ± 0.66c	16.58 ± 0.49c	1.07 ± 0.10b

* Values with dissimilar letters in a column differs significantly (p<0.05)

Table 5. Effects of levamisole on lipid profile (Mean ± SEM) in broiler chickens of the experimental groups

Groups	Total Cholesterol (mg / dl)	Triglyceride (mg / dl)	HDL (mg / dl)	LDL (mg / dl)
Group A (Control + No Vac)	115.27 ± 1.04a	94.63 ± 3.75a	40.67 ± 0.48	56.88 ± 1.02a
Group B (Control + Vac)	92.73± 3.39b	90.77± 1.11a	39.11± 0.68	43.25± 3.53b
Group C (Vac + LMS-3)	141.46± 5.55c	101.02± 2.18a	40.77± 0.98	73.49 ± 6.46c
Group D (Vac + LMS-10)	162.20± 11.30d	107.69± 4.38a	39.43 ± 0.99	97.24± 9.95d

* Values with dissimilar letters in a column differs significantly (p<0.05).

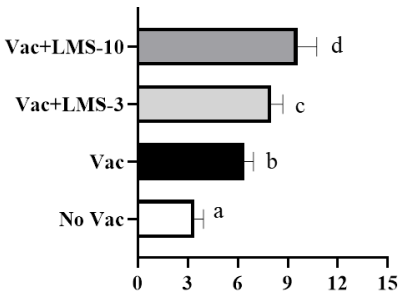

Figure 1. Effect of levamisole on antibody titer against ND vaccination in broiler chcikens. Values with dissimilar letters beside bar differs significantly (p<0.01).

The creatinine level of group D (vac + LMS 10) also found higher but not exceed than the normal level of creatinine (0.7-1.2 mg/dL). Supplementation of levamisole at high doses may affect the kidney (Table 4). In case of serum lipid profile, data revealed that high dose of LMS also increases total cholesterol and LDL cholesterol but didn't affect the triglycerides and HDL cholesterol levels (Table 5). The data indicated that use of LMS in high dose may affect the lipid profile. The recommended dose of 3 mg/kg or less may be useful to get better effect on lipid profile in broilers. The result of this study coincides with the findings of previous studies (Anderson *et al*., 1971).

Effects of levamisole on humoral immune response

The HI titer values were calculated in different groups (Figure 1). The obtained results from group A (Control +No Vac) was 3.40±0.55, B (Control + Vac) was 6.40±0.55, group C (Vac+LMS-3) was 8.00±0.71 and group D (Vac+LMS-10) was 9.60±1.14 HI units respectively. It was clearly found that the group D (Vac+LMS-10) showed highest antibody titer followed by group C. The data were statistically significant at $p<0.01$ level. These results revealed that, the administration of levamisole to Newcastle disease vaccinated birds orally (10 mg/kg body weights) or (3 mg/kg body weight) on 16-day old (the second day post vaccination) daily for five successive days resulted in potentiation of chicken immune response to Newcastle disease vaccination. This was evidenced by a significant increase in HI-geometeric means in levamisole treated groups versus non-treated groups (vaccinated only and control groups) (Figure 1). This result is in accordance with those obtained by Mulero *et al.*,1998; Li *et al.,* 2006 and Habibi et al., 2012 who reported LMS, as an immune stimulants, can promote recovery from immune suppression states and can enhance both the innate and specific immunity and humoral and cellular immune responses respectively. Therefore, in condition of infection with immune suppressive disease such as ND and IBD, it could be much better to use LMS as vaccine adjuvant.

Conclusion

The research study investigated the levamisole supplementation on growth performance, hemato-biochemical profile and humoral immunity in broilers and concluded that levamisole may have beneficial effect on performance and immunity in broilers and it can be used routinely to enhance immune response and boost productivity in broilers especially in the face of constant challenges to the immune system as those present in tropical environments. However, more precise investigation is necessary using large number of birds to draw a final conclusion.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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