African Journal of Pharmacy and Pharmacology Vol. 6(25), pp. 1860-1864, 8 July, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.294
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Effects of levamisole on the immune response of broilers against Newcastle disease vaccines

Mostafa Habibi[1]*, Mohammad Amin Zandieh[1], Hasan Ghahri[2], Reza Salman Zadeh[2] and Farid Yeganeh[2]

[1]Young Researchers Club, Urmia Branch, Islamic Azad University, Urmia, Iran.
[2]Department of Animal Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran.

Accepted 12 April, 2012

The efficacy of levamisole (LMS) hydrochloride, a standard immune modulator, was conducted on 240 broilers using different commercial Newcastle disease (ND) vaccines. La sota, Clon 30 and Avinew vaccines were used in this study. Treated groups received 30 mg/kg of LMS in drinking water for 2 days before and 2 days after each vaccination. Vaccination program were based on routine vaccination in Urmia area. Blood samples were obtained from wing vein on 1st, 14th, 28th and 42nd days. Antibody responses were measured using haemagglutination inhibition (HI) technique. The results of this study show that LMS increased the antibody levels against ND and significant results were seen in Avinew (28th day) and La sota (28th and 42nd days) vaccinated groups. Although, other treated groups showed higher antibody rate compare with control groups; no significant results were documented in those groups. In conclusion, LMS can stimulate immune system which causes better response to vaccination. Further studies are needed to evaluate other effective factors for each of the best results of LMS inclusion in broiler diet.

Key words: Broiler, levamisole, antibody titer, Newcastle, haemagglutination inhibition (HI) technique.

INTRODUCTION

Traditionally, synthetic chemicals and antibiotics have been used to prevent or treat poultry diseases and have achieved at least partial success. However, the emergence of antibiotic-resistant microorganisms and antibiotic residues in meat are the most limiting matter to their expanded usage. Therefore, vaccination against specific pathogens has been developed with variable degrees of successes. Such successes depend on the particular factors such as special antigens, immunogenicity of antigens and immune stimulants. Use of immune stimulants for the prevention of diseases in poultry is considered an effective and improving area. Immuno stimulants are natural or synthetic substances able to enhance the non-specific and/or the specific immune responses (Anderson, 1992).

Levamisole (LMS) is a synthetic antihelmintic drug for animals against stomach, intestinal and lungworms (JECFA, 1991). The immuno-stimulatory effect of LMS was first reported by Renoux and Renoux (1971) and was found to enhance the protection of a *Brucella* vaccine in mice. This phenomenon was confirmed with rainbow trout in the late 1980s (Siwicki, 1989). LMS has also been shown with several species to bea potent immune stimulants in modulation of leukocytecytotoxic activity (Cuesta et al., 2002), phagocytosis (Mulero et al., 1998; Findlay and Munday, 2000), respiratory burst (Siwicki, 1989; Mulero et al., 1998), antibody response (Jeney and Anderson, 1993; Cuesta et al., 2004) and macrophage activating factor (Mulero et al., 1998). Effects of LMS as an adjuvanton vaccination efficacy have been debated (Morrison et al., 2000, 2001). Previous studies have demonstrated that cell-mediated immunity can be ameliorated by the inclusion of LMS in DNA based vaccines and chemically killed viral vaccines (Jin et al., 2004; Kang et al., 2005).

Administration of LMS through dietary supplementation has been reported to enhance antibody response to

*Corresponding author. E-mail: mostafa_nic@yahoo.com. Tel: +98 914 140 1593. Fax: +984413460980.

Table 1. Vaccination agenda.

Group	1 day	10 days	15 days	21 days
A	H120/Spray[1]	Avinew	GambroGM/97	Avinew
B	H120/Spray	La sota	GambroGM/97	La sota
C	H120/Spray	Clone30	GambroGM/97	Clone30

1-Razi-IranCo.

hepatitis B vaccination in humans (Kayatas, 2003B). Another study has reported that LMS injected with a DNA vaccine against the foot-and-mouth disease virus (FMDV) stimulated both humeral and cellular immune responses in conjunction with strong production of interferon (IFN) (Jin et al., 2004).

Newcastle disease (ND) is a contagious and the most economically important disease in poultry industry throughout the world which is caused by the virus Paramyxovirus type 1 (Ali et al., 2004). It is a virus which has three pathotypes: lentogenic (avirulent), mesogenic and velogenic (most virulent) (King, 2005). Maternal antibodies protect birds during the first weeks of age and can interfere with the development of humeral immunity; however, they cannot avoid the rapid establishment of the vaccine protection (Kouwenhoven, 1993). Since ND can have upto 100% mortality, a regularly implemented vaccination program is necessary to prevent outbreaks in chickens (Yousuf, 2005). The purpose of this study was to evaluate the effect of the LMS as immune stimulants on antibody titers using different kind of Newcastle vaccines used in field.

MATERIALS AND METHODS

Birds and housing

In this study, 240 Ross broiler chicks on their 1st day in three groups (A, B and C) were used. Each group divided into two subgroups (experimental and control with 4 replication each) in which control groups received no LMS treatment but experimental groups received 30 mg/kg b.w. of LMS in drinking water for 2 consecutive days before and 2 days after each vaccination. Vaccination program was routine in our area which is shown in Table 1. Dietary and environmental conditions were identical in all groups. Dietary supplement was adequate based on that proposed by NRC (1994).

Sera collection

The birds bled from wing vein on 1st, 14th, 28th and 42nd days. Sera were obtained from the clotted blood after slanting the syringes containing the blood and allowing it to stay overnight. The samples were then clarified by centrifugation at 3000 rpm for 5 min. Sera samples were stored at -20°C.

Haemagglutination inhibition (HI) technique titration

HI was used for the detection of antibody level against ND as described by Anene and Onuoha (1999).

Statistical analysis

All values are reported as mean ± standard deviation (SD). The statistical differences among groups were assessed using Duncan multiple range test and analysis of variance (ANOVA). A value of $P<0.05$ was considered significant. Statistical analysis was performed using SAS 9.1 for Windows.

RESULTS

The ND-HI titer before vaccination (Day 1) showed that the chicks possessed a uniformly level of maternal derived antibody (MDA) titer in all groups. Antibody titer against Newcastle vaccination after oral administration of LMS (30 mg/kg b.w) is shown in Table 2 and Figure 1. The results demonstrated that LMS was effective in this study. The changes in antibody titers against Newcastle vaccinal viruses were measured by HI method which is a usual approach to trace antibody levels in serum. Almost all experimental groups showed higher level of antibody titer than control groups. Avinew vaccinated group showed significant results only in 28th day of study. Although no level of significance was seen in clone 30 vaccinated groups; treated groups had better results compare to control groups. Based on results obtained from Table 2 in vaccinated birds with La sota, the best results were documented. 28th and 42nd days of study showed significant antibody titers results compare with control or other treated groups.

DISCUSSION

ND is one of the viral contagious diseases that infect all species of pet and wild birds. In order to control ND, the use of vaccines is necessary and effective vaccination depends on some critical factors which immune stimulators are one of them.

LMS is a synthetic compound that has been classically used as antihelmintic agent in animals. Moreover, LMS has proven immune stimulants properties (Cuesta et al., 2002). Recently, LMS as a chemical adjuvant has been investigated. A study has demonstrated that LMS stimulates T cell activation and increases the production of antibody using either a DNA vaccine or on inactive vaccine co-administered with LMS (Jin et al., 2004; Kang et al., 2005) which is correlated with our study especially in amelioration of antibody titer. In mammals, LMS has

Table 2. Antibody titers against Newcastle disease within a period of 42 days.

Vaccine group	Subgroups	1 day	14 days	28 days	42 days
Avinew	Control	5.38 ± 0.30	3.40^{c} ± 0.28	4.61^{c} ± 0.09	5.25^{c} ± 0.51
	Experiment	5.40 ± 0.38	3.48^{bc} ± 0.25	4.77^{b} ± 0.11	5.27^{c} ± 0.48
La sota	Control	5.34 ± 0.46	3.60^{ab} ± 0.42	4.81^{b} ± 0.13	5.56^{b} ± 0.14
	Experiment	5.31 ± 0.54	3.72^{a} ± 0.36	4.98^{a} ± 0.16	5.73^{a} ± 0.31
Clone30	Control	5.29 ± 0.39	3.33^{c} ± 0.27	4.51^{c} ± 0.22	5.13^{c} ± 0.35
	Experiment	5.36 ± 0.29	3.37^{c} ± 0.40	4.55^{c} ± 0.31	5.21^{c} ± 0.28
Significance		NS	*	**	*

*, All expressed as mean and standard deviation (SD); Mean in columns with different letters were significantly different (NS, Not significant; *p < 0.05; **p < 0.01).

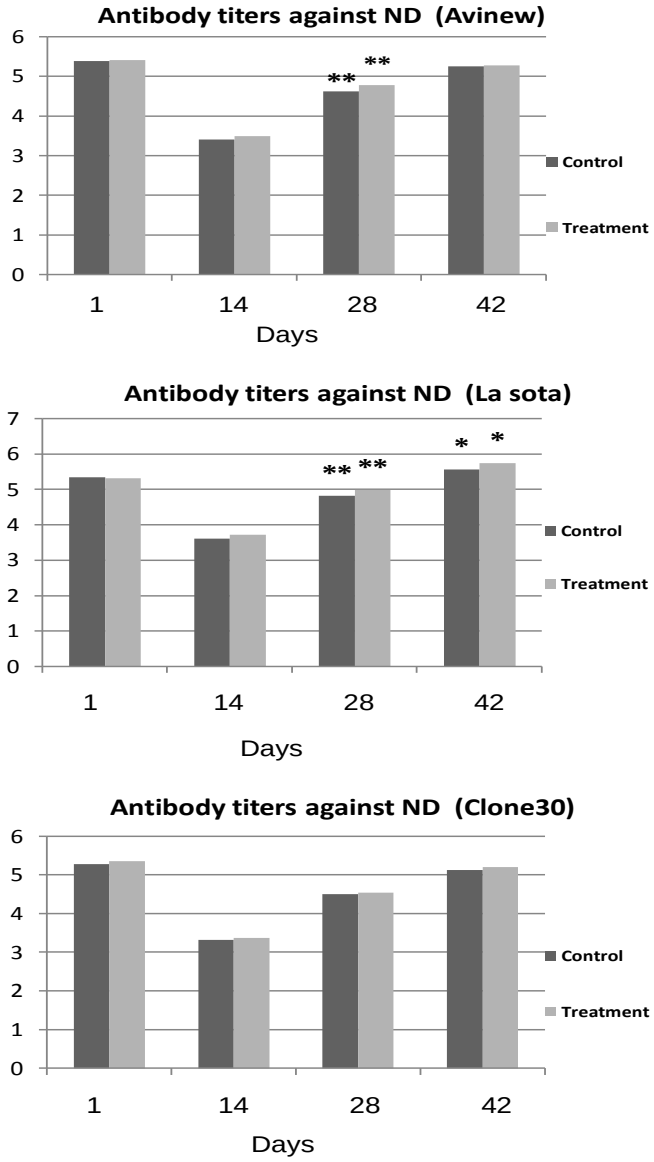

Figure 1. Effects of LMS on the antibody titers against Newcastle disease (ND). *p<0.05, **p<0.01.

been shown to increase serum lysozyme activity, serum antibody titers after immunization, the number of leucocytes, phagocyte activities, the expression of cytokines by macrophages, lymphocyte proliferation and anti-tumor responses (Tempero et al., 1995; Holcombe et al., 2001). In humeral immunity, we almost reached the same results in broilers based on results shown in Table 2 specially using La sota vaccine in which significant increased antibody titers on 28th and 42nd days were seen. Beside the activation of innate immunity, removal of any negative regulator(s) is also beneficial and has effect for the immune activation. LMS has been demonstrated by Song et al. (2006) previously to down-regulate the expression of suppressor of cytokine signaling 1 and 3 (SOCS 1 and 3), a key negative regulator of the JAK/STAT pathway. Also, down-regulating SOCS 1 by siRNA during the vaccination leads to a significantly increase of the antigen-specific immune responses (Song et al., 2006). LMS enhances the innate immune response as it does with the acquired response (acting as an adjuvant). It is documented that cells treated with LMS enhanced their cyclic guanosine monophosphate (cGMP) levels, which also increased microtubular assembly and cell mobility (Anderson et al., 1976); all these activities could be possible reasons of significant results in our study.

LMS as an immune stimulants can promote recovery from immune suppression states (Mulero et al., 1998; Sakai, 1999) and also can enhance both the innate and specific humeral and cellular immune responses (Li et al. 2006). Therefore, in condition of infection with immune suppressive disease such as infectious bursal disease (IBD) and chicken infectious anaemia (CIA), it could be much better to use LMS as vaccine adjuvant.

LMS has been reported to increase humeral antibody response against La sota antigens in chickens (Cuesta et al., 1993). Chickens immunized with ND virus (NDV) developed a significantly higher level of HI antibodies when treated with LMS than untreated ones (Kulkarni et al., 1973). We confirmed the same results especially in La sota vaccinated group whose significant results were seen on 28th day of study until end of rearing period (42nd day). These correlations between our study and Kulkarni et al. (1973) studies completely showed accuracy of our study but in this study, we tried to find out the response of other vaccines beside La sota such as Avinew and Clone 30. A study revealed that LMS treatment of IBD virus (IBDV) infected chicks was able to restore their immune responses to sheep red blood cells (SRBC) to a level comparable to that of uninfected controls (Singh and Dhawedkar, 1993). It is interesting to note that the immune modulatory effect of LMS was observed only in birds which had undergone immune suppression due to prior IBDV infection. These findings confirmed the earlier observation that this drug did not increase the immune response above the normal level in immunologically competent hosts (Symoens and Rosenthal, 1977); therefore, usage of LMS while the flock is in immune suppression condition could be much beneficial than healthy situation. In a separate study, a higher functional antibody level (HI) in chickens has been seen (Yin et al., 2006). Based on this study, LMS can enhance lymphocyte proliferation both in mice and chickens (Yin et al., 2006) indicating its ability to induce cellular immunity, which was confirmed by its ability to induce a high level of IFN-gamma (IFN-γ). Furthermore, LMS do not only improve the humeral response but also induces a cell-mediated response to killed NDV vaccine, which in turn produces sustainable immune responses (Yin et al., 2006; Chawak et al. (1993). LMS may act as a multifunctional modulator after immunization to mediate the cell-mediated response of T cells, and at the same time promote activated B cells to produce antibody; this is another possible method of LMS to stimulate immune system. Higher functional antibody level (HI) in chickens which has also been seen in our study, completely proves mentioned notions.

Cytokines have critical roles in the development and maintenance of immune responses. IFN-γ, a signature Th1 cytokine, mediates the killing of organisms and is responsible for protection against a variety of intra-cellular infections. Interleukin-4 (IL-4), a Th2 cytokine, can promote B cell differentiation and enhance the production of antibodies by sensitized B cells (Belardelli, 1995) which can be another pathway of higher antibody levels in LMS treated groups because LMS stimulate the T cells to induce cytokines (Yin et al., 2006).

In summary, the results presented in our study demonstrate that LMS as an adjuvant can activate immune system via different pathways. Vaccination and immune stimulators are widespread areas in poultry industry and more investigation is needed based on other factors that affect immune responses.

REFERENCES

Ali A, Abdalla M, Mohammed M (2004). Interaction between Newcastle disease and infectious bursal disease vaccines commonly used in Sudan.Int. J. Poult. Sci., 3: 300-304.

Anderson DP (1992). Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture. Ann. Rev. Fish Dis., 2: 281-307

Anderson R, Glover A, Koornhof HJ, Rabson AR (1976). In vitro stimulation of neutrophil motility by levamisole: maintenance of cGMP levels in chemotactically stimulated levamisole-treated neutrophils. J. Immunol., 117(2): 428.

Anene L, Onuoha E (1999). Seroprevalence of Newcastle disease in local chickens from Southeast derived savannah zone of Nigeria. PathologieInfectiese, 52(3-4): 185-188.

Belardelli F (1995). Role of interferons and other cytokines in the regulation of the immune response. Apmis, 103(1-6): 161-179

Chawak M, Rajmane B, Rande A (1993). Effect of levamisole on performance and immunomodulation against Ranikhet disease in broilers under stress. Indian J. Anim. Sci., 63: 1060-1061.

Cuesta A, Esteban M, Meseguer J (2002). Levamisole is a potent enhancer of gilthead seabream natural cytotoxic activity. Vet. Immunol. Immunopathol., 89(3-4): 169-174.

Cuesta A, Meseguer J, Esteban M (2004). Total serum immunoglobulin M levels are affected by immunomodulators in seabream

(Sparusaurata L.). Vet. Immunol. Immunopathol., 101(3-4): 203-210.
Findlay V, Munday B (2000). The immunomodulatory effects of levamisole on the nonspecific immune system of Atlantic salmon, *Salmosalar* L. J. Fish Dis., 23(6): 369-378.
Holcombe RF, Milovanovic T, Stewart RM, Brodhag TM (2001). Investigating the role of immunomodulation for colon cancer prevention: results of an *in vivo* dose escalation trial of levamisole with immunologic endpoints. Cancer Detect. Prevent., 25(2): 183.
JECFA, Joint FAO/WHO Expert Committee on Food Additives (1991). Levamisole. WHO Food Additive Series, 27: 75–101
Jeney G, Anderson DP (1993). Enhanced immune response and protection in rainbow trout to *Aeromonas salmonicida* bacterin following prior immersion in immunostimulants. Fish shellfish immunol., 3(1): 51-58.
Jin H, Li Y, Ma Z, Zhang F, Xie Q, Gu D, Wang B (2004). Effect of chemical adjuvants on DNA vaccination. Vaccine, 22(21-22): 2925-2935
Kang Y, Jin H, Zheng G, Xie Q, Yin J, Yu Y, Xiao C, Zhang X, Chen A, Wang B (2005). The adjuvant effect of levamisole on killed viral vaccines. Vaccine, 23(48-49):5543-5550.
King DJ (2005). Newcastle disease. In: Kahn, C.M. (ed), The Merck Veterinary Manual. 9th ed. Merck & Co., Inc, New Jersey, pp. 2255-2257.
Kouwenhoven B (1993). Newcastle disease. In: FERRAN, J.B.; Mc NULTY, M.S. Vírus infections of birds. Elsevier Science, Amsterdam, pp. 341-361
Kulkarni V, Mulbagal A, Paranjape V, Khot J, Manda A (1973). Immunostimulating effect of tetramisole on antibody formation against Newcastle disease virus in chicks. Indian Vet. J., 50:225-227
Li G, Guo Y, Zhao D, Qian P, Sun J, Xiao C, Liang L, Wang H (2006). Effects of levamisole on the immune response and disease resistance of Clariasfuscus. Aquaculture, 253(1-4): 212-217.
Morrison R, Nowak B, Carson J (2000). The effect of levamisole as an adjuvant on the humoral immune response of Atlantic salmon (*Salmosalar* L.). Bull. Eur. Ass. Fish Pathol., 20(3): 101-105.
Morrison R, Nowak B, Carson J (2001). The histopathological effects of a levamisole-adjuvanted Vibrio anguillarum vaccine on Atlantic salmon (*Salmosalar* L.). Aquaculture, 195(1-2): 23-33.
Mulero V, Esteban M, Munoz J, Meseguer J (1998). Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gilthead seabream (*Sparus aurata* L.). Fish shellfish immunol., 8(1): 49-62.
NRC (1994). Nutrient Requirements of poultry. 9th ed. Natl. Acad. Press, Washington, DC
Renoux G, Renoux M (1971). Immunostimulating effect of an imidotiiazole in the immunization of mice against *Brucella abortus* infection, pp. 349-350.
Sakai M (1999). Current research status of fish immunostimulants. Aquaculture, 172(1-2): 63-92.
Singh KCP, Dhawedkar R (1993). Immunomodulating effects of levamisole in chicks immunocompromised by infectious bursal disease virus. Trop. Anim. Health Prod., (1): 11-14.
Siwicki AK (1989). Immunostimulating influence of levamisole on nonspecific immunity carp (*Cyprinus carpio*). Dev. Comp. Immunol., 13(1): 87-91.
Song XT, Evel-Kabler K, Rollins L, Aldrich M, Gao F, Huang XF, Huang XF, Chen SY (2006). An alternative and effective HIV vaccination approach based on inhibition of antigen presentation attenuators in dendritic cells. PLoS Med., 3(1): 11.
Symoens J, Rosenthal M (1977). Levamisole in the modulation of the immune response: the current experimental and clinical state. J. Reticuloendothel. Soc., 21(3): 175-221.
Tempero MA, Haga Y, Sivinski C, Birt D, Klassen L, Thiele G (1995). Immunologic effects of levamisole in mice and humans: evidence for augmented antibody response without modulation of cellular cytotoxicity. J. Immunother., 17(1): 47.
Yin J, Jin H, Kang Y, Xiao C, Zhao L, Li X, Ding Z, Yang F, Zhu Q, Wang B (2006). Efficacy of modified levamisole adjuvant on inactivated virus vaccine.Viral Immunol., 19(3): 525-535.
Yousuf KM (2005). Control of Newcastle Disease in Village Chickens as a process. In: M.F. Wellington, (ed) Rural Poultry e-Newsletter 6th edition, International Rural Poultry Centre, KYEEMA Foundation, pp. 9-11.