*2011 International Conference on Life Science and Technology*
*IPCBEE vol.3 (2011) © (2011) IACSIT Press, Singapore*

# Humoral Immune Responses of Broiler Chickens Fed with Antibiotic and Neem Fruit Powder (*Azadirachta Indica*) as Feed Additive Supplemented Diet

Nasir Landy[1*], Gholamreza. Ghalamkari[2], Majid . Toghiani[2] and Faramarz Fekri Yazdi[1]
1Young researcher club of Islamic Azad university- Khorasgan Branch, Isfahan, Iran
2. Assistance professor Islamic Azad university -Khorasgan Branch, Isfahan, Iran
* Corresponding E-mail address: n_landy1984@yahoo.com

*Abstract*—**This experiment was conducted to examine the effect of different levels of neem (Melia Azadirachta) in comparison with an antibiotic (flavofosfolipol) on humoral immune responses of broiler chicks. In this trial 192 one-day-old broiler chicks (Ross 308) were weighted and randomly assigned to the 4 treatment groups, each with 4 replicates with 12 broilers. The 4 treatments were as follows: 1. Basal diet (control); 2. Basal diet + antibiotic (4.5 mg flavofosfolipol/kg diet) ; 3. Basal diet + neem fruit (7 g/kg diet) ;4. Basal diet + neem fruit (12 g/kg diet). The broiler tested for antibody titer against Sheep Red Blood Cell (SRBC), following SRBC injection and Newcastel Disease Virus (NDV), Avian Influenza (AI) following vaccination. At 28 (for AI, NDV) and 31(for SRBC) days blood sample were taken and analyzed for immune response. Results showed that, neem fruit (7 mg/kg) treated broiler had highest antibody titres against SRBC and AI.7 g/kg neem fruit to diet resulted higher antibody titres against SRBC than that 12 g/kg diet neem treatment($P < 0.05$).Antibody titres against AI of birds given 7 g/kg diet neem fruit supplemented diet was significantly higher than those bird fed with basal diet and antibiotic ($P < 0.05$). Used of 7 g/kg diet Neem led to the highest antibody titres against Newcastel virus compared to other groups but not significantly. In conclusion, these results are discussed in light of the possible immunopotentiating effects of neem fruit.**

***Keywords-Melia Azadirachta; Broiler; Humoral immune responses***

## I. INTRODUCTION

For many years, growth promoter feed additives have been included in poultry diets to promote growth, protect health and maximize the genetic potential of poultry. These, antibiotics have been used at sub therapeutic doses in animal feed, including poultry diets, for over five decades to prevent disease, promote growth and feed conversion efficiency [1]. Antibiotics exerted their effect by stabilizing the intestinal microbial flora thereby preventing proliferation of specific intestinal pathogens [2]. Today, the non-prescription use of antibiotics in poultry feeds has been eliminated or severely limited in many countries because of concerns related to development of antibiotic-resistant human pathogenic bacteria and legislative action to limit their use in probable in many others. A complete ban on antibiotics in poultry feeds was brought in to force on January 1st by the European Union; thus, all of the antibiotics used at sub-therapeutic levels for growth promotion (AGP's) were with drawn. The ban on AGP's has driven and prompted the search and development of alternatives like probiotics, yeast cultures, organic acids, prebiotics, enzymes, botanicals including extracts and essential oils of some herbs and spices [3]. Phytogenic feed additives used in animal feeding to improve performance and immune responses of poultry.The neem tree *Melia Azadirachta* from the family Meliaceae contains azadirachtin- a biologically active compound which is responsible for its varied medicinal uses [4]. Neem dry leaves fed to broilers have been reported to significantly enhance the antibody titres against new castle disease virus (NDV) antigen and also potentiated the inflammatory reactions to 1. Chloro-2,4-di- nitro benzene (DNCB) inskin contact test [5]. In other trial Mice injected with neem oil showed enhanced phagocytic activity and expression of MHC class-II antigens [6] .Despite these findings, there is a dearth of information on the possible immunopotentiating effects of neem dry fruit powder in broiler chicks. The present study was, planned to investigate the effect of herb (neem fruit) in comparison with flavofosfolipol on humoral immune responses of broiler chicks.

## II. MATERIALS AND METHODS

192 one-day-old broiler chicks (Ross-308) were weighted and randomly assigned to the 4-treatment group, each with 4 replicates with 12 broiler chickens. A commercial antibiotic growth promoter and feed additives were supplemented to no additive added basal diet. The 7 treatments were as follows:

1. Basal diet (control)
2. Basal diet + antibiotic (10 mg flavofosfolipol/kg diet)
3. Basal diet + neem dry fruit powder (7 g/kg diet)
4. Basal diet + neem dry fruit powder (12 g/kg diet)

The birds were fed a starter diet form days 1-14, a grower diet from days 14- 28 and finisher from days 28-42 (Table 1). The diets were formulated to meet the requirements of broilers as recommended by the Catalog Ross (2007).

TABLE1..THE INGREDIENT AND CHEMICAL COMPOSITION OF BASAL STARTER, GROWER AND FINISHER DIETS

| Ingredients (g/kg) | Starter | Grower | Finisher |
|---|---|---|---|
| Corn | 537.3 | 533 | 561.5 |
| Soybean meal | 400 | 396 | 370 |
| Oil | 20 | 35 | 35 |
| DCP | 19.3 | 17.1 | 15.6 |
| $Caco_3$ | 10.5 | 8.7 | 8.5 |
| NaCl | 3.5 | 3 | 3 |
| Mineral-Premix[1] | 2.5 | 2.5 | 2.5 |
| Vitamin-Premix[2] | 2.5 | 2.5 | 2.5 |
| DL-Methionine | 3.1 | 2 | 1.4 |
| L-Lysine | 1.3 | - | - |
| Calculated composition (mg/kg) | | | |
| M.energy (kcal/kg) | 2870 | 2980 | 3000 |
| Crude protein | 22.16 | 22 | 21 |
| Calcium | 0.86 | 0.751 | 0.7 |
| Av.phosphorus | 0.495 | 0.446 | 0.414 |
| Meth.+cysteine | 1.012 | 0.89 | 0.8 |
| Lysine | 1.339 | 1.198 | 1.13 |

1-To provide the following per kg of diet: Vit A 10,000 IU, vitamin D3 2000 IU, vitamin E 5 IU, vitamin K 2mg, riboflavin 4. 20mg; vitamin B12 0.01mg; pantothenic acid 5mg; nicotinic acid 20mg; folic acid, 0.5mg.
2- To provide the following per kg of diet: ; choline 3mg; Mg 56mg; Fe 20mg; Cu, 10mg; Zn 50mg; Co 125mg; Iodine 0.8mg.

All of the dietary feed additives were added at the expense of saw dust. Birds were allowed to free access to feed and water during the 42-d growout period. The lighting cycle was 23 h/d maintained. The ambient temperature in experimental house was maintained at 32°C during the first week and gradually decreased by 3°C in the second and third week, and fixed at 22°C thereafter.

Chicks were vaccinated against Infectious Bursal Disease at day 14 and 21, New Castle Disease (HB1) and Avian Influenza (oil emulsion H9N2 vaccine) at day 9 and New Castle Disease (La sota) at day 21.

At 28d of age two broiler from each replicate of treatments were randomly selected blood samples for antibody analysis, for Newcastel Disease Virus (NDV) and AI were taken by puncture of the brachial vein .Serum antibody titers against NDV, AI were measured by the hemagglutination inhibition test (HI), and HI antibodies were then converted into log.At25 d of the experiment, 2 birds per each group were injected in the right wing vein with 1 mL 1% Sheep Red Blood Cell (SRBC). One week after injection blood samples were taken and plasma was collected.antibody titer against SRBC was done by HA method.

## III. STATISTICAL ANALYSIS

Data were subjected to one –way analysis of variance using SAS statistical package (version 6.08 1989).significant effect of dietary treatments were compared with Duncan Values with different superscript differ significantly ($p < 0.05$) between treatments

## IV. RESULT AND DISCUSSION

Humoral immune responses of broiler chickens including, antibody titers against SRBC, AI and NDV presented in Table 2. There was no significant major difference in antibody titres against NDV between treatments, on other hand AI vaccination increased antibodies in groups treated with 7 g/kg diet Neem fruit than other treatments. As it was higher than birds fed control diet and antibiotic group ($p<0.05$) . The highest antibody titers against SRBC obtain in broilers fed diet contained 7 g/kg Neem fruit. Neem (7 g/kg diet) treatment significantly enhanced the antibody titres against SRBC than that treated with 12 g/kg Neem fruit ($P < 0.05$).In addition, use of 7 g/kg Neem fruit in broiler diets promoted better immune responses than other treatments.

TABLE 2. THE EFFECT OF DIETARY INCLUSION OF FEED ADDITIVES ONSERUM ANTIBODY TITERS (IGG) AGAINST NEWCASTLE DISEASE VIRUS (NDV) , AVIAN INFLUENZA (AI) AND SHEEP RED BLOOD CELL (SRBC) IN BROILERS SUPPLEMENTED .

| TRearments | Antibody titer | | |
|---|---|---|---|
| | NDV[1]($log_2$) | AI[1]($log_2$) | SRBC[2]($log_2$) |
| Control | 7.5a | 3.42c | 7abc |
| flavofosfolipol | 6.8a | 4.37bc | 7.5ab |
| 7 g/kg diet | 7.87a | 6.25a | 8a |
| 12 g/kg diet | 7.85a | 5.37ab | 5.7c |
| SEM | 0.48 | 0.34 | 0.76 |

[a-c]Mean values followed by the same letters in the column do not differ according to Duncan test
[1]Antibody titers by the hemagglutination inhibition test($log_2$)
[2]Antibody titers by the hemagglutination test($log_2$)

in other study, concluded that neem leaf preparation might be a potential immune adjuvant for inducing active immunity tumor antigens [7].Talwar et al showed that , use of neem oil causes an elevation of both immunoreactive and bioactive TNF-alpha and gamma-interferon in serum , and immunomodulator of plant origin are potentially usable for termination of pregnancy [8].Adjustment with our result in other trial In tests for humoral immune responses, neem treated mice had higher IgM and IgG levels[9].Neem is such a powerful booster that some researcher have attributed its contraceptive properties for both male and female to an enhanced immune system. while scientists have not yet identified specifically how neem work, neem boosting both the lymphocytic and cell mediate immune systems , at the same time it kills or slows the growth of many disease – causing organism such as bacteria, virus and fungus. The results from this trial showed that the substitution of the antibiotic by the neem diets resulted in significantly higher humoral immune responses in broiler chicks.

## REFERENCES

.


[1] R. M. Engberg, M. S. Hedemann, T. D. Leser and B. B. Jensen, "Effect of Zinc bacitracin and Salinomycin on Intestinal Microflora and Performance of Broilers, "Poultry Science, vol79, Sep. 2000, pp.1311-1319.

[2] W. J. Visek, "The Mode of Growth Promotion by antibiotics, "Journal of Animal Science, vol.46,1978, pp. 1447-1469.

[3] D. M. Hooge, "Dietary Alternatives for Imoroving Live Performance of Antibiotic- Free Poultry, " Zootecnica International, March 2006, pp 42-51.

[4] A. William, U. K. Sandable, M. U. Gambo and B. Paul-Bokko, “ Studies on Haematological Parameters of the Domestic , ” Journal of Experimental and Applied Biology, vol. 1. 2000, pp. 107-109.

[5] R. D. Sadekar, A. Y. Kolte, B. S. Barmase and V. F. Desai, “ Immunopotentiating Effect of Azadirachta Indica (Neem) Dry Leaves powder in Broilers , ” Indian Journal .ofExperimental Biology, vol. 36. Nov. 1998, pp.1151-1153.

[6] S. N. Upadhyay, S. Dhawan, S. Garg and G.P. Talwar, “ Immunomodulatory Effects of Neem (Azadirachta Indica) oil, ” International Journal pf Immunopharmacol, vol. 14, Oct. 1992, pp. 1187-1193.

[7] R. Baral, I. Mandal and U. Chattopadhyay, “ Immunostimulatory Neem Leaf Preparation Acts as an Adjuvant to Enhance the Efficacy of Poorly Immunogenic B16 Melanoma Surface Antigen Vaccine , ” International Journal of Immunopharmacol, vol. 5, Jul.2005, pp. 1343-1352.

[8] G. P. Talwar, S. Shah, S. Mukherjee and R. Chabra , “ Induced Termination of Pregnancy by Purified Extracts of Azadirachta Indica (Neem), ” Am J Reprod Immunol, vol. 37, Jun. 1997, pp.485-491.

[9] A. Ray, B. D. Banerjee and P. Sen, “Modulation of Humoral and Cell-Mediated Immune Responses by Azadirachta Indica (Neem) in Mice, ”Indian Journal pf Experimental Biology, vol. 34, Jul. 1996, pp. 698-701.