

Immunomodulatory effect of *Eleutherococcus senticosus* on Newcastle disease vaccine in broiler

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Abstract

The study was conducted to evaluate the effects of *Eleutherococcus senticosus* (ES) extract on the serum antibody response to Newcastle Disease Virus (NDV) vaccination, phagocytic index (PI) and stress index (SI) in broiler chicks. Two hundred one-day old broiler chicks (Ross-308), were randomly divided into two main groups GA and GB (Each one 100 birds), each of GA and GB chicks were divided to 4 subgroups, 25 birds each, The 4 subgroups were symbolically named G1, G2, G3 and G4 for birds of GA, according to the three vaccination methods and control, respectively. As well G5, G6, G7 and G8 were referred to the birds of GB. Subgroups subjected to different methods of vaccination against ND (live attenuated Lasota strain) at 10 days-old, orally (G1&G5), oculonasal (G2&G6) and S/C (G3&G7) with killed Lasota strain, and non-vaccinated control (G4&G8). Daily oral administration of 0.05 ml of 100% ES extract concentration/ bird, to all birds of G1, G2, G3 and G4 to the end of study. The results of present study showed that the ES significantly increased specific antibody titer against (NDV) vaccination, increase phagocytic activity of heterophils and decrease stress index at ($P < 0.05$).

Key words: *Eleutherococcus senticosus*, Newcastle disease, Elisa test, Phagocytic index, Stress Index.

تأثير التعزيز المناعي لنبات الجنسنغ السيبيري على لقاح مرض النيوكاسل في فروج اللحم

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الخلاصة

تم اجراء هذه الدراسة لتقييم تأثير مستخلص نبات الجنسنغ السيبيري على معيار الاجسام المضادة ضد فايروس مرض النيوكاسل ومعامل البلعمة ومعامل الكرب في فروج اللحم. تم استخدام 200 طير من فروج اللحم بعمر يوم واحد نوع روز(308)، حيث قسمت عشوائيا الى مجموعتين رئيسيتين (كل مجموعة احتوت على 100 طير)، وكل مجموعة رئيسية قسمت بدورها الى 4 مجاميع ثانوية (25 طير لكل مجموعة ثانوية). المجاميع الثلاثة الاولى لكل مجموعة رئيسية لقحت بلقاح مرض النيوكاسل (عتره لاسوتا) بعمر 10 ايام عن طريق الفم (G1 و G5) والتقطير بالعين والمنخر (G2 و G6) والحقن تحت الجلد (G3 و G7) على التوالي، والمجموعتين الثانويتين المتبقية (G4 و G8) لم تلقح بلقاح النيوكاسل واعتبرت كمجاميع سيطرة. وكذلك تمت معاملة المجاميع الاربعه الثانوية التابعة للمجموعة الرئيسية الاولى بمستخلص الجنسنغ السيبيري بجرعة 0.1 مل عن طريق الفم ويوميا الى نهاية الدراسة. اظهرت نتائج هذه الدراسة إن استخدام مستخلص الجنسنغ السيبيري أدى الى زيادة معنوية لمعيار الاجسام المضادة ضد لقاح مرض النيوكاسل وزيادة فعالية البلعمة لخلايا الهيتروفيل وكذلك يعمل على انخفاض معامل الكرب عند مستوى معنوية ($P < 0.05$).
الكلمات المفتاحية: الجنسنغ السيبيري ، مرض النيوكاسل ، اختبار الاليزا ، معامل البلعمة ، معامل الكرب.

Introduction

Newcastle disease (ND) is one of the most dominant avian viral diseases in birds that causes economic losses in the poultry sectors (1). The severity of disease depends on virus

strain, species of bird, age, immune status and the conditions may also greatly affect the signs of disease (2). Modulation of immunity is useful in antimicrobial therapy and vaccine

development (3). Immunomodulators are substances that are able to regulate or modulate immune responses. The use immunomodulators has increased particularly in poultry production around the world (4), and improve immune functions and resistance against infections in chickens and other domestic animals (5). Immunomodulator stimulates leucocytes, particularly cells of the macrophage system, and stimulate and potentiates the immune system of the bird (6). Interest in using immunomodulators to improve cellular and humoral immune functions and resistance against infections in chickens and other domestic animals has increased in the last decade (7). Many nutrients are capable of modulating the immune system (8). *Eleutherococcus senticosus* (ES) is one of the promising medicinal plants. It is significant for its secondary metabolites, called glycosides – eleuterosides. Secondary metabolites in the plant possess a wide range of beneficial effects on human organism and animals (9). ES has antioxidant, antiglycemic, anti-stress, and antibacterial, modulate and stimulate immunity, it has also the ability to reduce insulin levels, it has an inhibitory effect against free radicals (10,11,12). ES also called Siberian Ginseng, *Acanthopanax senticosus*, and *Ciwujia* in Chinese (13). Also it has been reported that the root bark and stem bark of ES have immuno-stimulating action (14, 15).

Materials and methods

Experimental design:

Two hundred one day-old broiler chicks (Ross-308), were randomly divided into two main groups GA and GB (Each one 100 birds). Each of GA and GB chicks were divided to 4 subgroups, 25 birds each, the sub groups were symbolically named G1, G2, G3 and G4 for birds of GA, according to the three vaccination methods and control,

Results

The effects of *Eleutherococcus senticosus* on titer of ND antibodies, phagocytic and stress indexes in broiler chickens during the different phases of experiment are shown in (Table 1), (Table 2), and (Table 3). Titer of

respectively. As well G5, G6, G7 and G8 were referred to the birds of GB. Subgroups subjected to different methods of vaccination against ND at 10 days-old, orally (G1 & G5), ocularnasal (G2&G6) using live attenuated Lasota strain, killed Lasota strain via S/C (G3& G7), and non-vaccinated control (G4&G8). Daily oral administration of 0.05 ml of 100% ES extract concentration/ bird, to all birds of G1, G2, G3 and G4 to the end of study. Feed and drinking water were given ad-libitum with feed through space held constant for all birds. Extra care was taken to secure biosecurity during the course of the experiment. Blood sampling, serum for Enzyme Linked Immunosobent assay (ELISA): The blood were collected randomly from wing vein. At days 10, 21, 28, 35, and 42 of experiment. Blood samples were taken randomly (N=6 per group). The sera from these samples were separated by centrifugation, collected in Eppendorf tube, and stored at -20 C° until further use. For humoral immune response: use Indirect ELISA for NDV antibodies titer measurement: The serum samples were tested with NDV specific ELISA (Synbiotics Co., San Diego, CA, USA) according to the manufacturer's protocol using an automated micro-plate reader (ELx800, BIO-TEK Instruments Inc., Winooski, Vermont, USA). Blood samples (2ml) from a wing vein of six birds of each treatment were collected at 14 days and 28 days of age after starting the experimental diet and vaccination program on day 10 of age. The blood samples were placed onto labeled slides and smears were fixed to determine the Stress index, from the data H/L ratio according to (16) and the Phagocytic index according to (17). Two way analysis of variance (ANOVA) was applied for statistical analysis of data using Sigma Stat (Jandel scientific software V3.1), and $P<0.05$ was considered as statistically significant.

ND antibodies test results showed at the age of 10 days and there is a significant difference ($P<0.05$) between the G1 and G5 only when comparing the groups GA and GB. Results did not show a significant

difference when compared between GA subgroups. But when comparing among the GB subgroups has significant difference was found ($P<0.05$) between the G5 when compared with the G6 and G7, as well as the comparison between the G7 and G8. At 21 days, comparison between the GA & GB subgroups appeared significant difference ($P<0.05$) between the G1 and G5, as well as between the G2 and G6. There is also a significant difference ($P<0.05$) among the four subgroups of GA, as well as in the GB groups. At 28 days found significant difference ($P<0.05$) between groups G1 and G5 only when comparing the subgroups of GA and GB. There is also a significant difference ($P<0.05$) among the four subgroups of the GA group, as well as in the GB group. Statistical analysis of the results were similar at the age of 35 and 42 days, and found a significant difference ($P<0.05$) when compared among GA & GB groups, except the comparison between the G4 and G8. The results also showed that there were significant differences ($P<0.05$) when compared among the four subgroups under each of the GA group and the GB except the comparison between the G6 and G7 group and at the age of 35 days (Table 1). For PI test, the results showed a significant

difference ($P<0.05$) at 14 days of age in G1, G2, G3 subgroups when compared with the subgroups G5, G6, G7, respectively. At age 28 days results revealed significant effect ($P<0.05$) between G1, G2, G3, G4 when compared with G5, G6, G7, G8, respectively. When comparing groups within the GA was found significant difference ($P<0.05$) among all subgroups except the comparison between (G1, G2) and (G3, G4) at the age of 14 and 28 days, and subgroups within GB at 14 days old revealed was not significant difference between the groups G5, G6, G7, while these subgroups morally differenced ($P<0.05$) when compared with the G8, at 28 days observed significant difference between the four subgroups in GB ($P<0.05$) except for the comparison between the G6 and G7 (table 2). Measuring stress Index at the age 14 days and 28 days appeared significant difference ($P<0.05$) between the G1, G2, G3, G4 when compared with the G5, G6, G7, G8 respectively. The comparison within subgroups of GA and GB have shown significant difference only between G3 and G4 ($P<0.05$) at 14 days. But at 28 days old, the results showed a significant difference at the level of ($P<0.05$) between the vaccinated subgroups in GA and GB compared to the control subgroups (Table 3).

Table (1): Newcastle disease antibody titers of vaccinated broiler chicks treated with *Eleutherococcus senticosus* extract at different ages.

| | Treatment | Age (day) | | | | |
|------------------|-----------|-------------------|------------------|------------------|------------------|------------------|
| | | 10 day | 21 day | 28 day | 35 day | 42 day |
| Interaction (GA) | G1 | 3733±102 Aa | 7061.2±270 Aa | 7956.6±86 Aa | 6891±210 Aa | 6021.8±236 Aa |
| | G2 | 3936.6±138 Aa | 5764.2±136 Ab | 6741.6±99 Ab | 5929.8±80 Ab | 5198.8±156 Ab |
| | G3 | 4013±100 Aa | 3376±151 Ac | 4619.8±167 Ac | 5522.4±198 Ac | 7030±59 Ac |
| | G4 | 3689±85 Aa | 2252.2±76 Ad | 1142.4±62 Ad | 921±44 Ad | 318.6±46 Ad |
| Interaction (GB) | G5 | 3131.2±58 Bb | 5978±92 Ba | 7121.6±116 Ba | 6048±89 Ba | 5097.4±193 Ba |
| | G6 | 3655.2±271 Aac | 4566±204 Bb | 6549.6±211 Ab | 4862.4±98 Bb | 3910.2±128 Bb |
| | G7 | 4041±76 Aa | 3113.4±117 Ac | 4310.8±199 Ac | 5116.4±125 Bb | 6514±117 Bc |
| | G8 | 3519.4±256 Abc | 1992.6±45 Ad | 1148.2±88 Ad | 700.8±37 Ac | 185.4±19 Ad |

A,B Values with a different superscript within GA and GB were significantly different ($P<0.05$).

a,b,c,d Values with a different superscript within subgroups of GA and GB were significantly different ($P<0.05$).

Table (2): Phagocytic index of vaccinated broiler chicks treated with *Eleutherococcus senticosus* extract at different ages.

| | Treatment | Age (day) | |
|------------------|-----------|-------------------|-------------------|
| | | 14 day | 28 day |
| Interaction (GA) | G1 | 37.286±1.15 Aa | 50.320±0.58 Aa |
| | G2 | 37.452±1.13 Aa | 49.572±0.33 Aa |
| | G3 | 40.326±0.53 Ab | 47.486±0.58 Ab |
| | G4 | 40.188±0.56 Ab | 45.964±0.88 Ab |
| Interaction (GB) | G5 | 45.210±0.27 Ba | 42.128±0.32 Ba |
| | G6 | 45.148±0.43 Ba | 39.514±0.35 Bb |
| | G7 | 46.364±0.35 Ba | 40.262±0.66 Bb |
| | G8 | 39.094±0.36 Ab | 33.540±0.23 Bc |

Table (3): Stress index of vaccinated broiler chicks treated with *Eleutherococcus senticosus* extract at different ages.

| | Treatment | Age (day) | |
|------------------|-----------|--------------------|--------------------|
| | | 14 day | 28 day |
| Interaction (GA) | G1 | 0.222±0.0035 Aa | 0.198±0.0050 Aa |
| | G2 | 0.221±0.0029 Aa | 0.193±0.0075 Aa |
| | G3 | 0.208±0.0026 Ab | 0.196±0.0066 Aa |
| | G4 | 0.183±0.0059 Ac | 0.166±0.0034 Ab |
| Interaction (GB) | G5 | 0.252±0.0021 Ba | 0.225±0.0049 Ba |
| | G6 | 0.252±0.0027 Ba | 0.220±0.0071 Ba |
| | G7 | 0.253±0.0022 Ba | 0.221±0.0086 Ba |
| | G8 | 0.246±0.0030 Ba | 0.246±0.0089 Bb |

A,B Values with a different superscript within GA and GB were significantly different ($P<0.05$).

a,b,c Values with a different superscript within subgroups of GA and GB were significantly different ($P<0.05$).

Discussion

First time in Iraq used *Eleutherococcus senticosus* in poultry sector, until now studies didn't show the effect of ES on immunity of chickens. In the present study, the effect ES on the serum antibody response to NDV vaccination, phagocytic index and stress index were investigated. Generally vaccinated G1, G2 and G3 of GA treated with ES showed high significant values in comparison with untreated vaccinated G5, G6, G7 respectively, but highest significant values showed at 35 and 42 days due to immunomodulation effect of ES. Statistical analysis between subgroups of GA revealed significant differences at 21, 28, 35 and 42 days, and the highest value represented to G1 due to immunomodulatory effect of ES, these results agreement with (12), that referred to the *Acanthopanax senticosus* (also called *Eleutherococcus senticosus* or Siberian Ginseng) extract as dietary additive enhances the cellular and humoral immune responses of weaned piglets by modulating the production of immunocytes, cytokines and antibodies. Phagocytic index results show

significant differences all subgroups of GA and GB at 14 and 28 days except G4 & G8 at 14 days, results also revealed highest value in G1, G2, G3 & G4 at 28 days, that indicate to ES improve the activity of Phagocytic index. Diet supplementing with ES causes lower phagocytic index on day 14 than the basal diet group of birds and the reverse was true as bird aged to 28 day due to enhancing phagocytic function of heterophil immune cells. (18) Concluded that is largely due to a qualitative impairment of the avian innate host defenses characterized by a functional inefficiency of heterophils and macrophages for the first 7 to 14 days of life in chickens. Results of Stress index showed high significant differences between GA & GB at 14 & 28 days, results also revealed lowest value in G1, G2, G3 and G4 at 28 days, that indicate reduce the stress index due to ES has antioxidant, antiglycemic, anti-stress, antibacterial, immunostimulating effects, it has also the ability to reduce insulin levels, it has an inhibitory effect against free radicals (10,2).

References

1- Rasoli M, Yeap SK, Tan SW, Moeini H, Ideris A, Bejo MH, Alitheend NB, Kaiser P, Omar AR (2014)

Alteration in lymphocyte responses, cytokine and chemokine profiles in chickens infected with

- genotype VII and VIII velogenic Newcastle disease virus. *Comparative Immunology, Microbiology and Infectious Diseases*. 37: 11- 22.
- 2-Alexander DJ (2011) Newcastle disease in the European Union 2000 to 2009. *Avian Pathology*. 40: 547-558.
- 3-Bomminenia YR, Phama GH, Sunkaraa LT, Achantaa M, Zhang G (2014) Immune regulatory activities of fowlicidin-1, a cathelicidin host defense peptide. *Molecular Immunology*. 59: 55-63.
- 4-Porchezian T, Punniamurthy N(2006) Effect of oral levamisole hydrochloride on humoral immune response and serum proteins of broilers. *J. of Animal and Veterinary Advances*. 5: 873-874.
- 5-Hassan MG, Abdulla TA (2011) Effect of propolies feed supplemented on hygiene and performance of broiler chickens. *Iraq. J. of Veterinary Sci*. 25: 77-82.
- 6-Ganguly S (2013) Promising pharmaceutical effect of various biological and inorganic agents as feed supplements for livestock and poultry with discussion on research proven facts and establishment of concept. *Research in Pharmacy and Life Sci*. 1:115-120.
- 7-Ziaran HR, Rahmani HR, Palic D (2005) Effect of dietary oil extract of propolis on immune response and broiler performance. *Pakistan J. of Biological Sci*. 8: 1485-1490.
- 8-Korver D (2012) Implications of changing immune function through nutrition in poultry. *Animal Feed Sci and Technology*. 173: 54-64.
- 9-Eschbach LF, MJ Webster, JC Boyd, PD McArthur, TK Evetovich, (2000) The effect of siberian ginseng (*Eleutherococcus senticosus*) on substrate utilization and performance. *Int. J. Sport Nutr. Exerc. Metab.*, 10: 444-451.
- 10-Davydov M, AD Krikorian (2000) *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (*Araliaceae*) as an adaptogen. *J. Ethnopharmacol.*, 72(3): 345-93.
- 11-Miyanomae T, Frindel E (1988) Radioprotection of hemopoiesis conferred by *Acanthopanax senticosus* Harms (Shigoka) administered before or after irradiation. *Exp. Hematol*. 16:801-806
- 12-Xiangfeng Kong, Yulong Yin, Guoyao Wu Hejun Liu, Fugui Yin, Tiejun Li, Ruilin Huang, Zheng Ruan, Hua Xiong, Zeyuan Deng, Mingyong Xie, Yiping Liao, Sungwoo Kim (2007) Dietary supplementation with *Acanthopanax senticosus* extract modulates cellular and humoral immunity in weaned piglets. *Asian-Aust. J. Anim. Sci*. Vol. 20, No. 9:1453-1461.
- 13-Huang L¹, Zhao H, Huang B, Zheng C, Peng W, Qin L (2011) *Acanthopanax senticosus*: review of botany, chemistry and pharmacology. *Pharmazie*. 2011 Feb;66(2):83-97.
- 14-Wagner HM, Proksch A, Riess-Maurer I, Vollmar A, Odenthal S, Stuppne H, Juricic K, Le Turdu M, Fang JN (1985) *Arzneimittelforschung*. Immunostimulating action of polysaccharides (heteroglycans) from higher plants 35, 1069–1075.
- 15-Han SB, Yoon YD, Ahn HJ, Lee HS, Lee CW, Yoon WK, Park SK, Kim HM (2003) Toll-like receptor-mediated activation of B cells and macrophages by polysaccharide isolated from cell culture of *Acanthopanax senticosus*. *International Immunopharmacology* 3, 1301-1312.
- 16-Redmond SB, Chuammitri P, Andreassen CB, Palic D, Lamont SJ (2011) Proportion of circulating chicken heterophils and CXCLi2 expression in response to *Salmonella enteritidis* are affected by genetic line and immune modulating diet. *Veterinary Immunology and Immunopathology*, 140(3-4), pp.323-328.
- 17-Park BH, Fikrig SM, Smithwick EM, (1968) Infection and Nitro blue tetrazolium reduction by neutrophils: A diagnostic aid. *The Lancet*, 2(7567), pp.532-534.
- 18-Kogut MH (2009) Impact of nutrition on the innate immune response to infection in poultry. *J of Applied Poultry Research*. 18: 111-124.