



Evaluation of Humoral Immunity to Infectious Bronchitis and Newcastle Disease Vaccines and Received *Artemisia herba alba* Extract in layer hens

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Abstract

The present study aims to evaluate the effect vaccination and oral administration of aqueous extract of *artemisia herba alba* on the immune status. forty laying hens (Lohman Brown, 1 year-old, body weight 1.8 kg) were divided into four groups (ten) individuals. First one is the control; the second group treated with vaccine against NDV and IBV; third group received 2ml/bird/day 5% extracts of *artemisia herba alba*; fourth group treated with 2ml/bird/day 5% extracts of *artemisia herba alba* and vaccinated against NDV and IBV. Antibody titers against NDV and IBV were measured after 14 and 28 days. Results present significant increase in the globulin of the fourth group as compared to the others. The level of NDV antibodies increases significantly in this group as compared with control and third groups after 14 days. The same group shows a significant increase at 28 days as compared with the control one only. On the other hand, antibody titer of IBV increased significantly in the second and the fourth groups compared to the control group at 28 days, but no significant differences among all groups after 14 days of the experiment.

In conclusion, this study indicates a limited positive effect of the herb extract on humoral immunity of layer chickens.

Keyword: extract, antibody titer, globulin, NDV and IBV.

تقييم المناعة الخلطية للقاحي التهاب الشعبتي المعدي ومرض نيوكاسل والتجريب بمستخلص نبات الشيش في الدجاج البياض

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الخلاصة:

تهدف الدراسة الحالية لتقييم تأثير التمنيع ضد نيوكاسل والالتهاب الشعبتي المعدي وكذلك مستخلص نبات الشيش المائي على الحالة المناعة في الدجاج البياض. تم استخدام 40 دجاجة (سلالة لوهمان البني بعمر 1 سنة، معدل وزن الجسم 1.8 كغم)، قسمت عشوائياً على اربع مجاميع بالتساوي (10 طير لكل مجموعة). المجموعة الاولى اعتبرت سيطرة. المجموعة الثانية لقحت بلقاحي ND+IB، المجموعة الثالثة جرعت ب2مل/ طير يومياً تركيز 5% مستخلص نبات الشيش المائي والمجموعة الرابعة لقحت بلقاحي ND+IB و جرعت ب2مل/ طير يومياً تركيز 5% مستخلص نبات الشيش المائي. قيس المعيار الحجمي لأضداد المرضين بعد 14 و28 يوم.

أظهرت النتائج ارتفاع معنوي لكلوبيولينات المجموعة الرابعة مقارنة ببقية مجاميع التجربة. وارتفعت معنويًا أعداد مرض نيوكاسل في المجموعة الرابعة مقارنة بالمجموعتين الثالثة والسيطرة بعد 14 يومًا للتجربة. أيضًا، هناك ارتفاع معنوي للمجموعة الرابعة مقارنة مع مجموعة السيطرة عند 28 يومًا. من ناحية أخرى، المعيار الحجمي لأعداد الالتهاب الشعبي المعدي يرتفع معنويًا في المجموعتين الثانية والرابعة مقارنة بمجموعة السيطرة عند 28 يومًا، ولكن لا توجد فروقات معنوي بين جميع المجاميع بعد 14 يومًا للتجربة.

الاستنتاج: تشير النتائج إلى تأثير إيجابي محدود لمستخلص العشب على المناعة الخلطية في الدجاج البياض.
الكلمات المفتاحية: مستخلص، المعيار الحجمي، كلوبيولين، نيوكاسل

Introduction

Infectious bronchitis and Newcastle disease are most common respiratory diseases in chickens, (1). Infectious bronchitis virus [IBV] is separated world widely virus and very difficult to control since it has a short time of generation, extensive genetic diversity and high rate of mutation, (2). IBV belongs to the Coronaviridae family, which causes a very infectious respiratory disease, (3). Some IBV strains cause infections in other body organs, (4). Chickens of all ages are susceptible to the IBV virus, (5). The morbidity rate could reach 100% infection level, but the mortality rate relies on the secondary infection, immunity status, flock age, field management and environmental factors, (6).

Newcastle disease is a viral infection that is very contagious of avian species caused by avian Paramyxovirus serotype-1. This disease causes significant economic losses in poultry production, (7). The prevalence rate is higher in younger chickens, (8). The variability of clinical signs depends on the variability of virus strains, (9). Newcastle outbreaks are highly spread in north part of Iraq (10). No significant differences of among chickens that were vaccinated with commercial mixed vaccine (IBV+NDV) or single ND, and IB vaccines, (11). Evaluation of humoral immunity is the best index to determine resistance in chickens against Newcastle disease virus, (12). *Artemisia herba-alba* is one of the medicinal herbs. It usually grows

in arid areas, (13). The powder and extract of the *Artemisia (A annua)* increased the chicken performance, the weight of the bursa of fabricius and thymus, the IgG titer and cellular immunity of broilers, (14). Oral intake of levels of artemisinin (50 and 500 ppm) were found to be immunotolerant for humoral responses and low level of (5 ppm) was immunostimulatory as compared to untreated chickens, (15).

This research aims to study aqueous extract of *Artemisia herba alba* effect on titer of antibodies to ND and IB virus and measure blood proteins as indicator of humoral immunity.

Materials and Methods

This poultry field trial was carried out in the College of Agriculture / University of AL- Qadisiyah during period of 1/11/2017 to 28/11/2017. Forty chickens of (1 year-old, average body weight 1.8 kg) were distributed randomly into four groups, equally. The first group was considered as a control. Starting of the first day of the experiment, the third and the fourth groups were dosaged with 2ml/bird/day concentrations of 5% extract of *artemisia herba alba*. After 7 days, the second and the fourth groups were vaccinated with Newcastle disease virus, LaSota strain: 105.5 EID50 and infectious bronchitis, massachusetts serotype: 102.5 EID50 (Volvac® company) with drinking water. Humoral immunity was measured at 14 and 28 days.

The study samples were withdrawal from the brachial vein and serum were

separated. These serum samples were preserved under -20 Celsius till laboratory analysis.

Humoral immunity against Newcastle and infectious bronchitis were detected by ELISA test. Biotic 8800 XL was used to measure antibody titers.

Blood proteins: total protein were determined using Biuret colorimetric method according to Spinreact Company. Method of Bromo cresol green (BCG) was used to measure serum albumin. Serum globulin was measured through serum albumin subtracting of total protein.

The preparation of the aqueous extract of *Artemisia herba-alba* done according to procedure of Hernandez et al. 1994, (16).

Data were analyzed by SPSS program. The Completely Randomized Design (C.R.D) and means were compared according Duncan's multiple range test at $P \leq 0.05$.

Results and discussion

Table (1) shows that there is non-significant differences in both total protein and albumin among groups after 28 days of the experiment. There is a significant increase in globulin of the fourth group as compared to the other groups. This result partially agree with the results that obtained by (17), who reported that total proteins, globulin and albumin showed no significant differences between different dietary treatments.

According to table (2), significant increase in antibodies levels NDV ($p < 0.05$) in group 4 that was vaccinated and received 5% *artemisia herba alba* extract as compared with the control and third group that was received 5% *artemisia herba alba* extract.

The difference between the fourth and the second groups is not significant after 14 days for the experiment. Significant increase in antibodies levels of NDV in groups 4 as compared to the first group. Numerical increase is existed in the fourth group as compared with second and third groups after 28 days of experiment.

On the other hand, there is no significant difference in the antibody titer of infectious bronchitis among groups after 14 days of the experiment. The antibody titer increased significantly in the second and the fourth groups as compared to the control, but this increase is not significant as compared with the third group after 28 days of experiment, table (2). There is statistical increase in the level of antibodies against Newcastle disease in the fourth group as compared to second groups. The same relation is between levels of antibodies (Newcastle and infectious bronchitis) of the third as compared and control at 28 day of the experiment, table (2). These results agree with the findings of (14), who reported that powder and the extract of another type of *Artemisia* (*A. annua*) increases the IgG titer. Increasing antibodies titer levels might due to the artemisinin contained in *Artemisia* (15).

Conclusion

There is a statistical increase in the level of antibodies against Newcastle disease in the fourth group as compared to the second one. Levels of antibodies (Newcastle and infectious bronchitis) of the third group as compared to control at 28 day of the experiment also present statistical increase. this result indicates a limited positive effect of the herb extract on humoral immunity of layer chickens.

Table (1): Effect of vaccination and oral administration *Artemisia herba alba* extract on some blood parameters of layer chickens.

Groups	Mean ± Standard. Error Total Protein (gm/dl)	Mean ± Standard. Error Albumin (gm/dl)	Mean ± Standard. Error Globulin (gm/dl)
G1	4.260±0.15	1.740±0.07	2.520±0.09B
G2	4.460±0.20	1.440±0.12	2.820±0.07B
G3	4.300±0.15	1.680±0.10	2.620±0.11B
G4	4.560±0.14	1.560±0.19	3.000±0.07A

Similar letters denote to non-significantly differ at [p<0.05]. Different letters denote to significantly differ at [p<0.05].

Table (2): Effect of vaccination and oral administration *Artemisia herba alba* extract on antibody titers against NDV and IBV.

Groups	Mean± Std. Error antibody titer of ND		Mean± Std. Error antibody titer of IB	
	after 14 days	after 28 days	after 14 days	after 28 days
G1	3804.000±353.43 B	3645.250±250.35 B	6370.000±683.82 A	6271.250±707.81 B
G2	4054.250±227.60 AB	5647.500±203.99 AB	8482.500±1176.30 A	10469.750±1367.74 A
G3	3623.500±469.27 B	5213.000±380.15 AB	6765.500±1433.37 A	8986.250±1509.27 AB
G4	5384.000±763.00 A	6990.750±1422.60 A	7648.000±1039.75 A	11908.750±456.27 A

Similar letters denote to non-significantly differ at [p<0.05]. Different letters denote to significantly differ at [p<0.05].

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