



Study the Effect of Protexin® as a Probiotic and levamisole on The Immunity of Broilers

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Abstract

This study was conducted to evaluate the effect of Protexin® and levamisole as an immune modulator. A total of 125 unsexed (Ross 308) broiler chicks with an average initial body weight of 43g to all chicks at the age of one day were randomly divided into five equally treated groups (25 chicks per pen). Group 1 was considered as control group fed with commercial ration without any additives, group 2 was fed with commercial ration with the addition (daily) of 1g protexin® /liter of drinking water 1- 10 days, 1g protexin /2 liters of drinking water 10-20 days and 1g protexin® /4 liters of drinking water 20 day- end of the study, group 3 was fed with commercial ration with the addition (each 3 days a week) of 1g protexin® /liter of drinking water 1- 10 days, 1g protexin® /2 liters of drinking water 10-20 days and 1g protexin® /4 liters of drinking water 20- end of the study, group 4 was fed with commercial ration with the addition of levamisole 10 µg/kg B.w. daily, group 5 was fed with commercial ration with the addition of levamisole 10 µg/kg b.w. 3 days a week. The experiment lasted for five weeks (35 days). Chicks were vaccinated against New castle disease (ND) on age (10,20,30) days , by drinking water method, also vaccinated against Infectious Bursal Disease (IBD) on age (14) days by drinking water method. At days 1, 12, 22 and 35 blood samples were collected to estimate the immune response by ELISA test for ND and at 35 day for IBD. The titer of antibody (Ab) increased significantly ($P \leq 0.05$) against NDV and IBD in G2 (Protexin® daily) as compared with G1(control group) at the age of 22 and 35days.

Key words: : probiotic (Protexin®), levamisole, broilers, ELISA , immune response

دراسة تأثير البروتوكسين كمعزز حيوي والليفاميزول على المناعة في فروج اللحم

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الخلاصة

أجريت هذه الدراسة لمعرفة تأثير إضافة المعزز الحيوي التجاري (البروتوكسين) والليفاميزول في ماء الشرب على المناعة لدجاج اللحم. استخدم 125 فرخ غير مجنس من نوع (روز 308) وزعت عشوائيا بعمر يوم واحد إلى خمسة معاملات متساوية كل مجموعة تحتوي على 25 طير وكان معدل وزن الطير الواحد 43 غرام بعمر يوم واحد, وكما يلي: المعاملة الأولى/غذيت عليقة أساسية بدون أن تجهز بالبروتوكسين او الليفاميزول , إما المجموعة الثانية فقد أعطيت العلف بالإضافة الى البروتوكسين بجرعة 1 غرام من البروتوكسين /لتر من ماء الشرب ولمدة 10 ايام الاولى من التجربة , و بجرعة 1 غرام من البروتوكسين /2 لتر من ماء الشرب ولمدة 10 ايام الثانية من التجربة , و بجرعة 1 غرام من البروتوكسين /4 لتر من ماء الشرب ولمدة 10 ايام الثالثة من التجربة والى نهاية التجربة, وأعطيت المجموعة الثالثة العلف بالإضافة الى البروتوكسين بجرعة 1 غرام من البروتوكسين /لتر من ماء الشرب ولمدة 10 ايام الاولى من التجربة (3أيام اسبوعيا) , و بجرعة 1 غرام من البروتوكسين /2 لتر من ماء الشرب ولمدة 10 ايام الثانية من التجربة (3أيام اسبوعيا), , و بجرعة 1 غرام من البروتوكسين /4 لتر من ماء الشرب ولمدة 10 ايام الثالثة من التجربة والى نهاية التجربة(3أيام اسبوعيا), وأعطيت المجموعة الرابعة عقار الليفاميزول بجرعة 10ملغم /كغم من وزن الجسم في ماء الشرب يوميا , وأعطيت المجموعة الخامسة عقار الليفاميزول بجرعة 10ملغم /كغم من وزن الجسم في ماء الشرب 3 ايام اسبوعيا استمرت فترة التجربة لمدة 5 اسابيع (35 يوم) .

الافراخ لقحت ضد مرض النيوكاسل بعمر (10,20,30) يوم بطريقة ماء الشرب باستعمال عترة لاسوتا, وكذلك لقحت ضد مرض الكمبورا بعمر (14) يوم في ماء الشرب.

وتم جمع عينات الدم في الاعمار 1,12,22,35 يوما لغرض اجراء فحص معدل معيار الاضداد لفابروس النيوكاسل وفابروس التهاب الجراب الخمجي (بعمر 35يوم) بطريقة الاليزا, فقد وجد ان هناك زيادة بمعيار الأجسام المضادة وبشكل معنوي ضد مرض النيوكاسل ومرض التهاب جراب فابريشيا المعدي في المجموعة الثانية المعطاة البروتوكسين بشكل يومي مقارنة مع مجموعة السيطرة.

كلمات المفتاح: المعزز الحيوي (البروتوكسين), الليفاميزول, دجاج اللحم, فحص الاليزا, الاستجابة المناعية.

Introduction:

Broilers is one of the most successful and fast growing industry that provides high quality protein at economical price. Since ancient times, different strategies have been applied to improve animal productivity and

profitability. Most important of them were always directed towards maintaining health, reducing disease

outbreak and improving general immunity (1). Antibiotics have been

successfully used in poultry production for more than fifty years for treatment of diseases. Antibiotics have also been continuously applied at sub therapeutic levels (as antibiotic growth promoters AGP) in feeds of livestock species for maintenance of health and improving performance (1). The use of AGP in animal feeds is prohibited in European Union (EU) since 2006, due to the potential risks associated with their use and development of resistant strains of bacteria, mainly in humans. Now, as a result of ban on use of antibiotics in EU, increasing demand of antibiotic free products in other parts of the world and demand for organic animal products, alternative substances are required to create a safety margin in animal production against unexpected hazards and stressful conditions. The alternatives to antibiotics should be a) safe and economical, b) have significant, sustainable and beneficial impact on animal health and production, c) be easily applied and stored, e) not contribute to environmental pollution, and f) be acceptable by consumers.

Protexin[®] is a multi-strain probiotic used in poultry feed (2). It contains naturally occurring nine different species of beneficial microflora which are generally regarded as safe by the American Food and Drug Administration (3). Probiotic (Protexin[®]) include *Lactobacillus spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Bifidobacterium spp.*, *Enterococcus spp.*, *Aspergillus spp.*, *Candida spp.*, and *Saccharomyces spp.*, improve feed

consumption in layers and broilers (4). The beneficial modes of action of probiotics include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, expression of bacteriocins (5), enzymatic activity inducing absorption and nutrition (6), immune modulatory effects (7), inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa (8).

Levamisole is a potent broad-spectrum antihelmintic, which is active against most pathologic nematodes in animals and man, levamisole a substituted thiazole, is a drug that has been intensively studied as an immunomodulating agent in humans and, levamisole restores immune response effectively in intermittent rather than continuous treatment, levamisole has been shown to increase serum antibody titers after immunization, the number of leucocytes, phagocyte activities, the expression of cytokines by monocyte/macrophages, lymphocyte proliferation and antitumor responses (9). The present study was planned to investigate the effects of supplementing diets with a multi strain commercial probiotic (Protexin[®]) and levamisole in drinking water on broiler immunity.

Materials and Methods:

Experimental design

This experiment was carried out at poultry farm, College of Veterinary

Medicine / University of Baghdad during the period from 29/12/2013 to 1/2/2014. Ten chicks at one day old were taken randomly for blood samples collected from each bird. This is achieved to determine the maternal antibody for ND virus and IBD virus by ELISA technique as well as 12, 22 and 35 days old for NDV, IBD at age 35 days only to determined the immune response . were carried out according to procedures provided by (indirect ELISA kit Synbiotics–USA).

Groups of Experiment

One hundred and twenty five day-old unsexed broilers (Ross-308) chicks were bought from a local hatchery and divided randomly and equally into five treated groups of 25 birds, as follows:

1-The first group (G1) was daily fed on basal diet without supplementation (Control).

2-The second group (G2) was fed on basal diet with daily supplementation of protexin[®] by dose 1g/liter for 1st-10 days,1g/2 liters for 10th-20 days and 1g/4 liters for 20th until end of the study in drinking water.

3-The third group (G3) was fed on basal diet with daily supplementation

of protexin[®] 3 days in a week by dose 1g/liter for 1st- 10 days,1g/2 liters for 10th-20 days,1g/4 liters 20 th - end of the study in drinking water.

4-The fourth group (G4) was daily fed on basal diet with supplementation of levamisole 10 µg/kg b.w. daily in drinking water.

5-The fifth group (T5) was daily fed on basal diet with supplementation of levamisole 10 µg/kg b.w. 3 days a week in drinking water.

All groups were vaccinated with Newcastle (ND) vaccine : La Sota at age 10, 20 and 30 days in drinking water and Infectious Bursal Disease (Gumboro (IBD intermediate strain)) vaccine: IBD at age 14 day in drinking water.

Dietary treatments:

The dietary treatments were basal diet, Chicks were maintained on these treatments to 5 weeks of age. All birds were fed and watered ad libitum to 35 days of age, nutrients compositions of the diets for broiler starter diet was used from 1 to 20 days of chick's age and then finisher diet was used till the end of the experiment tables (1) and (2)were based on (10).

Table (1) Feed constituents of starter ration:

Constituent	Quantity / ton	Percentage
Corn	340 Kg	34 %
Wheat	200 Kg	30%

Soy bean	300 Kg	30%
Protein	50 Kg	5%
Dicalcium	6 Kg	0.6%
Premix 1%	2 Kg	0.2%
Clopidol (anticoccidian)	0.5 Kg	0.05%
Mycovax silicate	2 Kg	0.2%

Table (2) Feed constituents of final ration:

Constituent	Quantity / ton	Percentage
Corn	490 Kg	49 %
Wheat	200 Kg	25 %
Soy bean	250 Kg	20 %
Protein	50 Kg	5 %
Dicalcium	6 Kg	0.6 %
Premix 1%	2 Kg	0.2 %
Clopidol (anticoccidian)	0.5 Kg	0.05 %
Mycovax silicate	2 Kg	0.2%

Statistical analysis

Data generated from the experiment were carried out in a complete randomized design (11). These data were subjected to ANOVA according to the general linear model procedure of (12). The significant differences among means were determined by Duncan's multiple range tests with $p \leq 0.05$ level of significance.

Results and Discussion

The results of maternal antibodies (MAb) titer of chicks at one day age and at 12 ,22 ,35 days old are presented in table (3) .The data for antibody titer against ND were recorded on 12 days, mean antibody titer values were (1685.5±91.6) for G4 (levamisole daily), 1641.6±99.4for G5 (levamisole3days), 1617.3±90.6 for G1 (control group),1606.5±88.1for G2 (Protexin[®] daily), and 1589.8±107.8

for G3 (Protexin® 3days), for groups G4,G5,G1,G2 and G3 respectively, there was no significant ($P \geq 0.05$) difference between all these groups. The results of this study at the age (12days) are homogeneous for all groups while did not record significant difference important among them, this may be the reason for high homogeneity of the serum antibody titers against the virus Newcastle to ideal health conditions that surround the experience and this was confirmed by (13). The highest mean of titer significant ($p \leq 0.05$) increase in (22days) was determined in G2 (Protexin® daily) as compared with G1 (control group), which were (4515.6 ± 349.29) and (3284.6 ± 193.99) respectively, with the demonstration of improvement for the fourth group (levamisole daily) compared with the third (protexin® 3days) and fifth (levamisole 3days) groups, which were recorded

(4069.1 ± 333.31), (3744 ± 251.71) and (3516.3 ± 437.25) respectively. But at age 35day a significant ($p \leq 0.05$) increase of titer was found in G2 (protexin® daily, 6166.1 ± 439.35) as compared with G1 (control group, 4244.9 ± 446.63), with the demonstration of improved for the fourth group (levamisole daily, 5741.5 ± 660.33) compared with the third (protexin® 3days, 5228.6 ± 488.40) and fifth (levamisole 3days, 4596.7 ± 429.52) groups. These results may be due to use of multi strains probiotic (protexin®) which has positive effect on immune response of birds against Newcastle disease. The effects of probiotics on immune system of poultry are interesting and complicated. The direct effect might be related to stimulating the lymphatic tissue (14). And indirect effect via changing the microbial population of

the lumen of GIT (15). It is obvious that probiotics are able to enhance the immune response to different antigenic stimulants. After day 7 the maternal antibody titers in groups was below as considered appropriate for first vaccination against NDV. After vaccination the antibody response to NDV vaccine was higher in the G1 (protexin daily) than the other groups. These results are in agreements with (16) who reported that the antibody titers against ND in broilers fed with diets supplemented with probiotics containing *Bacillus subtilis* was significantly higher at 10 days post-immunization compared to the control birds. On the other hand, the effect of levamisole on the level of Ab was obvious in G4 and G5, stimulating formation of antibodies to various antigens, these results attributed to that levamisole is synthetic and helminthes agent with immune modulator properties (17). Levamisole have been used in an attempt to enhance protective immune response of chickens for prevention or control of infectious disease including Newcastle disease and infectious bursal disease (18). It's able to enhance both humoral and cellular immune response by activation of the T-cell help interferon production, activation of macrophage and act as anti stress (19). These results indicated that supplementation may help increase post vaccination humoral immune response against ND in broiler chicks.

Table (3) The results of ELISA Ab titer against Newcastle disease

	1 day	12 days	22 days	35 days
G1 Control	377.48±8545.7 A	90.6±1617.3 A	3284.6±193.99 B	4244.9±446.63 c
G2 Protexin [®] daily	377.48±8545.7 A	1606.5 ±88.1 A	4515.6±349.29 A	6166.1±439.35 a
G3 Protexin [®] 3days	377.48±8545.7 A	1589.8±107.8 A	251.71±3744 b	5228.6±488.40 b
G4 Levamisole Daily	377.48±8545.7 A	91.6±1685.5 A	333.31±4069.1 ab	5741.5±660.33 ab
G5 Levamisole 3days	377.48±8545.7 A	99.4±1641.6 A	3516.3±437.25 b	4596.7±429.52 c

Small different letters in the same column denoted that significant differences at a level ($p \leq 0.05$)

The mean values of antibody titers against IBD at 35 day of age by the indirect ELISA recorded in five groups were showed in table (4) .that significant ($P \leq 0.05$) differences were determined in G2 (protexin[®] daily) as compared with G1 (control group) at level (4664.1±326.36) and (2980.1±161.3) respectively. In this study showed improvement for the fourth group (levamisole daily) compared with the third (protexin[®] 3days) and fifth (levamisole 3days)

groups, which were recorded (3821.5±309.62),(3445.2±209.87) and(3340±249.71) respectively. After vaccination, antibody titres gradually increased in all vaccinated groups but the level was higher in the group receiving vaccine and probiotic than in the group receiving vaccine only. The results in this study agree with (20) they found that oral administration of probiotics can significantly affect both the systemic and mucosa associated immune responses resulting in disease

prevention. Probiotics also enhance the systemic antibody response to some antigens in chickens (21) and stimulate production of natural antibodies (antibodies secreted by B-1 cells and may be of isotype IgM, IgG, or IgA) in

unimmunized chickens (22), these results indicated that protexin[®] may help increase post vaccination humoral immune response against IBD in broiler chicks.

Table (4) The results of ELISA Ab titer against IBDV disease:

Days	1day	35 days
G1 Control	10737±446.90 A	2980.1±161.3 c
G2 Protexin [®] daily	10737±446.90 A	4664.1±326.36 a
G3 Protexin [®] 3days	10737±446.90 A	3445.2±209.87 b
G4 Levamisole Daily	10737±446.90 A	3821.5±309.62 ab
G5 Levamisole 3days	10737±446.90 A	3340±249.71 bc

Small different letters in the same column denoted that significant Differences at a level ($p \leq 0.05$).

As a conclusions the second group (G2) which received protexin[®] (daily) showed better results in immune response against NDV and IBDV as compared with the other groups.

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