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# Effect of Iraqi probiotic and Antibiotics neomycin on the intestinal flora, humoral and cellular immunity of broiler chicks

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**Abstract:**

*The present study is aimed effect of using Iraqi probiotic and antibiotic (Neomycin) on immunity and microbiological traits in broiler. It is includes four treatments by rearing 240 chicks of Ross broiler 308 in cages so that each one consists of four floors and each floor has a cage with dimension of 1.5 × 1 m2 . The chicks are randomly divided to groups; each group contains 60 chicks for each treatment that contain three groups in each treatment (20 chicks for each group). The work has been done in the poultry farm of the College of Agriculture in Al Muthanna University from 27-9-2014 till 1-11-2014 as followed:*

1. *First treatment: control treatment without adding.*
2. *The second treatment: Provide Iraqi probiotics by drinking water at a dose of 1 g / liter of water.*
3. *Third treatment: Provide antibiotic (neomycin) at a dose of 1 g / 10 liters of water.*
4. *Fourth treatment: Provide Iraqi probiotics at a dose of 0.5 g / 1 liter of water with the submission of an antibiotic (neomycin) at a dose of 0.5 g / 10 liters of water.*

*The results show that appearance a significant value ($p \leq 0.05$) in immunity when adding the Iraqi probiotic in drinking water compared to all the experimental treatments with a significant decrease ($p \leq 0.05$) in logarithmic numbers of total aerobic bacteria and coliform bacteria. A significant increase ($p \leq 0.05$) in numbers for the*

*logarithmic lactobacillus bacteria in the contents of duodenum and ceca in the treatment of Iraqi probiotic compared with other treatments.*

**Key words**: Probiotics, Antibiotic, neomycine, intestinal flora, humoral, cellular immunity, broiler.

## Introduction

The poultry of the best animals in the rapid processing of animal protein, Concerned with global companies have been set up poultry industry (Billard, 2004). As a result, productivity has improved strains, especially strains of broilers, Characterized by such a high efficiency in faster growth, and increase high weighted, and high efficiency on feed conversion (Jackie, 2003). These specifications are increased food requirements and administrative and health (Naji, 2006).

As well as the negative effects of antibiotic residues in meat of broiler (Burgat, 1999), be a case of non-bacterial balance between harmful and beneficial bacteria in the intestinal tract causing an increase in pathogenic bacteria that have the ability to resist antibiotics which leads to the emergence of disease states as a result of suppressed immune systems (Andermont, 2000). Which made the researchers looking for ways to raise the body immunity to reduce the chances of bacterial infections disease in broilers, one of these methods the use of probiotics, which are used to reduce the negative effects of antibiotics, as a result of the intensive use of antibiotics, That this approach can lead to increased somatic immune, improved health and activity as well as improve the productive performance of poultry (Naji et al., 2011).

The internationally endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Rachmilewitz et

al., 2004). Other definitions advanced through the years have been restrictive by specification of mechanisms, site of action, delivery format, method, or host, Probiotics have been shown to exert a wide range of effects (Vaillancourt, 2006). The mechanism of action of probiotics (e.g., having an impact on the intestinal microbiota or enhancing immune function) was dropped from the definition to encompass health effects due to novel mechanisms and to allow application of the term before the mechanism is confirmed (Sanders, 2006). Physiologic benefits have been attributed to dead microorganisms (Al-Gharawi, 2012). Furthermore, certain mechanisms of action (such as delivery of certain enzymes to the intestine) may not require live cells. However, regardless of functionality, dead microbes are not probiotics (Lee et al., 2007).

The antibiotics are a group of drugs that kill bacteria or lead to slow growth and is used as a treatment for various diseases in the first antibiotic produced commercially for the first time in 1939 since there are differences between antibiotics and chemotherapy drugs (Nair, 2012), one of these antibiotic is neomycin, It is an antibiotic of the total amino Glycoside, and it creates from a fungal species Streptomyces fradiae isolated from organisms present in the soil (Waksman and Lechevalier, 1949). This antibiotic is a broad spectrum, stance and fought for the growth of bacteria, directly affects in negative bacteria gram, which include Salmonella, E. coli and Klebsiella and Enterobactor (B.P, 1988).

The present study aims to statement impact of the use of Iraqi probiotics and Neomycin antibiotic in microbial and immunological characteristics of broiler chicks Ross308.

## Materials and Methods

### Preparation of fermented feed:

A commercial broiler starter and finisher diet (Table 1) were purchased from local market. Chicks were fed on starter diet

during the first three weeks, and then transferred to finisher diet were used for the reminder of the experimental period which was lasted for 6 weeks.

**Table1. Composition of basal diet.**

| Items | Basal Diet | |
|---|---|---|
| | 1 to 22 d | 23 to 35 d |
| Corn | 44.9 | 53.10 |
| Wheat | 18.0 | 15 |
| Soybean meal (45%) | 33 | 27 |
| Mineral and vitamin premix | 1 | 1 |
| Oil | 2 | 3 |
| Limestone | 0.8 | 0.6 |
| Dicalcium phosphate | 0.3 | 0.3 |
| Total | % 100 | % 100 |
| **Calculated analysis** | | |
| Crude protein (%) | 21.92 | 19.70 |
| Metabolism energy (kilo calorie per kg. Diet) | 2990 | 3100 |
| Calcium (%) | 0.93 | 0.85 |
| Phosphorus (%) | 0.48 | 0.45 |
| Methionine (%) | 0.55 | 0.50 |
| Lysine (%) | 1.35 | 1.25 |
| Methionine + Cysteine (%) | 0.85 | 0.91 |
| Folic acid | 1.1 | 1.2 |

* produced by Ghadeer Babylon, calculated analysis according to NRC (1984).

## Broiler husbandry and experimental design:

The experiment was carried out at poultry research farm-faculty of agriculture- university of Al-Mothanna, Iraq, during the period from from 27-9-2014 till 1-11-2014 and aimed to effect of using Iraqi probiotic and antibiotic (Neomycin) on immunity and microbiological traits in broiler. A total of 240 one day old Ross308 broiler chicks were randomly assigned (CRD) chicks in the six experimental groups were fed as follow:

1. First treatment: control treatment without adding.
2. The second treatment: Provide Iraqi probiotics by drinking water at a dose of 1 g / liter of water.

3. Third treatment: Provide antibiotic (neomycin) at a dose of 1 g / 10 liters of water.
4. Fourth treatment: Provide Iraqi probiotics at a dose of 0.5 g / 1 liter of water with the submission of an antibiotic (neomycin) at a dose of 0.5 g / 10 liters of water.

Each treatment group was replicated three times with 20 chicks per replicate. Chicks were reared in battery cages (1.5 × 1.0 m) with four tears. Chicks were raised in a temperature and humidity controlled room with a 24-h. constant light schedule and ad. libitum access to water and feed throughout the experiment.

**Sampling procedure and analytic methods:**

At the end of the experimental period, two birds per replicate were slaughtered and blood collected in heparinized tube and centrifuged at 2000 round per minute for 15 min. serum samples were then isolated and stored at 20 ºC until use for analysis. Serum titer against NDV were determined by using an indirect enzyme-linked immunosorbent assay (ELISA) as described by Feng et. al. (2009).

Additionally, the bursa of fabricus of these chickens were removed and weighted. Relative bursa weight to body weight and bursa index were calculated. One gram from duodenum and cecum contents were diluted 10-fold with buffered peptone water, and then one hundred microliters of supernatant was pour on to MacConkey agar and Lactobacilli MRS agar and incubated at 37 ºC with 13% CO2 for 48 h. in order to plate count for coliform and Lactobacilli respectively. Total plate count for aerobic bacteria on nutrient agar was also performed by using pour plate count procedure according to APHA (1978). Saccharomyces cervisia yeast were enumerated

on potato dextrose agar after aerobic incubation at 37 ºC for 24 h. bacterial and yeast colonies were counted and expressed as colony forming units per gram (cfu/ g).

## Statistical analysis:

Data generated from the present experiment was subjected to statistical analysis using the GLM procedure of SAS (2001) statistical software package. When significant differences were noted, mean were compared using Duncan's multiple range test (1955).

## Results and discussion:

Bursa of fabricius weight, bursa relative weight and bursa index data are shown in table 2. Birds drink water with Iraqi probiotics had higher ($P \leq 0.05$) bursa weight, bursa relative weight and bursa index compared with other treatments. Measurement of immune organ weight is a common method for evaluation of immune status. Stringfellow et. al. (2011) and Bai et. al. (2013) demonstrated that bird drink water containing probiotics could influence the immune organ weights.

**Table 2. Effect of Iraqi probiotic and Antibiotics neomycin on bursa relative weight and bursa index.**

| Treatment | relative weight of bursa | bursa index |
|---|---|---|
| $T_1$ | 0.066±0.002 b | 1.000±0 bc |
| $T_2$ | 1.803±0.011 a | 0.119±0.001 a |
| $T_3$ | 0.955±0.013 c | 0.063±0.002 b |
| $T_4$ | 1.076±0.013 b | 0.071±0.001 b |

T1: Control group without adding. T2: fe Provide Iraqi probiotics by drinking water at a dose of 1 g / liter of water.T3: Provide antibiotic (neomycin) at a dose of 1 g / 10 liters of water. T4: Provide Iraqi probiotics at a dose of 0.5 g / 1 liter of water with the

submission of an antibiotic (neomycin) at a dose of 0.5 g / 10 liters of water. a,b Means within columns with no common superscript differ significantly (P < 0.05).

Effect of Iraqi probiotic and Antibiotics neomycin on both humeral and CMI are shown in table 4. Blood serum ELISA titer against NDV and CMI were significantly (P≤0.05) higher in birds drink water with Iraqi probiotics.

These results are in agreement with that of Alkhalf et. al. (2010) who showed that a significant increase in serum immunoglobulin M, immunoglobulin G and complement C4 level when compared with broiler fed control soy-corn diet. Feeding fermented soybean meal (FSBM) were also significantly increased the level of IgA and IgG than birds fed entire soybean (Arun et al. , 2006; Cetin et. al.,2005).

**Table 3. Effect of Iraqi probiotic and Antibiotics neomycin on Cell mediated immunity (CMI) and Blood serum ELISA titer against NDV.**

| Treatment | Cell mediated immunity (CMI) | Blood serum ELISA titer against NDV |
|---|---|---|
| $T_1$ | 0.169±0.015 b | 2730.4±302.4 bc |
| $T_2$ | 0.281±0.011 a | 3070.6±273.1 a |
| $T_3$ | 0.165±0.014 b | 2721.4±298.4 c |
| $T_4$ | 0.173±0.013 b | 2743.5±292.6 b |

T1: Control group without adding. T2: fe Provide Iraqi probiotics by drinking water at a dose of 1 g / liter of water.T3: Provide antibiotic (neomycin) at a dose of 1 g / 10 liters of water. T4: Provide Iraqi probiotics at a dose of 0.5 g / 1 liter of water with the submission of an antibiotic (neomycin) at a dose of 0.5 g / 10 liters of water. a,b Means within columns with no common superscript differ significantly (P < 0.05).

These results suggest that the probiotics have an immune modulating impact on broiler chicks both in standard production situations and during challenge (El-Husseiny et. al., 2008; Gao et. al, 2008, 2009). This positive effect on the immune system may be partially responsible for the improved broiler

performance (Naji et. al., 2014) and turkey live weight at marketing age (Firman et. al., 2013).

Previous studies indicated that the probiotics can increase the content of small size peptides, which may improve the immune function of animal (Feng et. al.,2007; Chen et. al., 2009). Wang et. al. (2003) stated that piglets increased the concentration of immunoglobulin by adding 3 g/kg small peptides in the basal diets. Gao et. al. (2009) isolated anti-oxidant peptides from cottonseed protein hydrolysates which may improve the immune function as well. Thus, the increase in the serum ELISA titer against NDV may be attributed to small peptides formed during the probiotics given (Feng et. al., 2007).

The effect of Iraqi probiotic and antibiotics neomycin on microbial counts is shown in table 4. The total number of aerobic and coliform bacteria in duodenum and cecum were significantly ($P \leq 0.05$) decreased in birds drink probiotics than those of bird drink antibiotic neomycin or control. Drinking probiotics was significantly ($P \leq 0.05$) increased the total number of Lactobacilli in duodenum and cecum of broiler chicks.

These findings were in agreement with that reported by Abdel-Raheem et. al. (2012); Li et. al. (2008) and Mountzouris et. al. (2007).

Primarily probiotics cause a reduction of pathogenic bacteria including Salmonella and Campylobacter in the digestive tract. The lactic and acetic acid produced by the bacteria in the probiotics creates an acidic environment with a PH about 4. At this level of acidity, molecules of acid can enter the bacteria through their cell membranes, and the increased acidity within the cells interferes with enzymatic processes, killing the bacteria (Heres et. al., 2003). Probiotic is somewhat more effective against Salmonella and Campylobacter because Lactobacillus also outcompetes the Salmonella for nutrients in the feed itself (Heres et. al., 2002).

**Table 4. Effect of Iraqi probiotic and Antibiotics neomycin on aerobic, coliform bacteria and Lactobacilli in duodenum and cecum**

| Treatment | Duodenum | | | Cecum | | |
|---|---|---|---|---|---|---|
| | aerobic | Coliforms | Lactobacilli | aerobic | Coliforms | Lactobacilli |
| $T_1$ | 5.22 ±0.05 a | 11.30 ±0.13 a | 4.29 ±0.05 b | 4.19 ±0.04 a | 8.07 ±0.07 a | 4.47 ±0.06 b |
| $T_2$ | 3.95 ±0.04 b | 9.81 ±0.08 b | 7.58 ±0.03 a | 2.37 ±0.05 b | 5.36 ±0.04 b | 6.63 ±0.06 a |
| $T_3$ | 2.68 ±0.06 c | 8.76 ±0.11 c | 4.01 ±0.04 c | 2.03 ±0.04 c | 4.88 ±0.09 c | 4.13 ±0.08 c |
| $T_4$ | 2.72 ±0.05 c | 8.85 ±0.12 c | 4.12 ±0.06 c | 2.10 ±0.05 c | 4.96 ±0.06 c | 4.20 ±0.07 c |

T1: Control group without adding. T2: fe Provide Iraqi probiotics by drinking water at a dose of 1 g / liter of water.T3: Provide antibiotic (neomycin) at a dose of 1 g / 10 liters of water. T4: Provide Iraqi probiotics at a dose of 0.5 g / 1 liter of water with the submission of an antibiotic (neomycin) at a dose of 0.5 g / 10 liters of water. a,b Means within columns with no common superscript differ significantly ($P < 0.05$).

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