Effect of Adding Chitosan and Oxytetracycline to the Diets of Corn in Physiological and Microbial Performance of Broiler

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Abstract. This experiment was conducted in the poultry farm of the Animal Production Department / College of Agriculture / Anbar University, from 8/12/2019 until 23/3/2019 for 6 weeks. The experiment aim to compare the addition of two different levels of chitosan and a group of antibiotics to the corn-soybean diet and their effect in physiological and microbial performance at 42 days. The experiment included T1 (control of corn diet without any addition), T2 (0.2 g oxytetracycline/kg corn diet), T3 (1 g chitosan/kg corn diet), and T4 (2 g chitosan/kg corn diet). In the experiment, 144 birds (Ross) at the age of 7 days were used with an average weight of 40 g. The chicks were distributed randomly to 4 treatments, with 3 replicates, and in each repeater, 12 birds. The results of the physiological parameters indicated that there were T4 increased significantly (P≤0.05) compared to other treatments in H / L and monocyte count at 42 days, while the results showed that there were no significant differences in the biochemical characteristics of blood. As for the tissue of Jejunum, treatment T3 was significantly (P≤0.05) superior to the rest of the treatments in the height, thickness of villi and depth of crypts over the experimental treatment. There was a significant decrease (P≤0.05) for the T3 and T4 compared to the T1 control treatment on the total bacterial count and colon bacterial count.

1. Introduction

The technological advances of the poultry industry in the application of modern nutrition programs and using genetic improvement programs allow some lines to express their adequate genetic potential. Furthermore, it has produced a generation with rapid growth and low disease resistance, which leads to excessive use of antibiotics and medicinal drugs to reduce the incidence of diseases and mortality [1]. The use of antibiotics in poultry diets as growth stimuli has become prohibited in most countries of the European Union and the USA, leading to use alternative ways to raise the immunity and reduce the infection with bacterial and fungal diseases [2]. It also impacts the bird's health, reduces its natural resistance, and the consumer health for its survival in the carcass tissues [3]. Among this addition of Chitosan as a non-food additive in poultry diets as a substance derived from Chitin, which is not harmful to animal and human health, and is a multi-unit of glucosamine and constitutes most of the external skeleton of marine organisms such as shrimp and crabs can be explored [4], and positively affects broiler chickens' productivity performance [5], and physiological performance and develops energy and protein use efficiency, and regulates the intestinal flora and creates conditions for the proliferation of beneficial microorganisms and the prevention of harmful negative and positive microorganisms of cram stain [6];[7];[8]. Additionally, Chitosan is an antifungal [9], Chitin is also an...
effective antioxidant and is added to broiler diets [10], while [11] stated that Chitosan improves the digestibility and absorption of nutrients by increasing digestive enzymes' secretion in the stomach intestine. Although using of wheat in the broiler diets of imported and local, corn it more important sources of energy in poultry diets, and it their use depends on its availability and cultivation in any city, especially with Ross strains [12]. This study was aimed to know the effect of adding the Chitosan to the corn-soybean diet on the physiological performance of broiler and determining the best level of addition.

2. Materials and methods

This experiment was conducted on the poultry farm of the Department of Animal Production at the College of Agriculture / University of Anbar, from 8/12/2019 to 23/3/2019 (42 days). The experiment aims to compare the addition of two levels of Chitosan and one level of antibiotic to the corn-soybean diet and their effect on the physiological performance of broiler at the age of 42 days. Moreover, the experimental treatments were controlled treatment T1 (without any addition), the second treatment T2 includes the addition of 0.2 g Oxytetracycline/kg feed, while the T3 includes the addition of 1 g Chitosan/kg feed and T4 includes the addition of 2 g Chitosan/kg feed. The chicks were randomly distributed to four treatments, with 3 replicates per treatment, and 12 chicks for each replicate, Total of 144 chicks, 7 days old of Ross broiler chickens, with an average weight of 168.7g were used in the experiment. Three types of diets were given during the experimental period, which included the starter diet from the one to 11days, the growth diet from 12 to 21 days and the finisher diet from 22 to 42 days as shown in Table 1. Chitosan was imported from the People's Republic of China. Blood samples of broiler were collected randomly from three birds of each treatment from jugular vein at the age of 35 days, using K-EDTA tubes to conduct tests for the number of blood cells (RBC and WBC), Packed cell volume (PCV), hemoglobin concentration (Hb) ,Hetrophil, lymphocyte, Monocyte, Basophil, Neutrophil and H/L. The blood samples were placed in the centrifuge at 3000 cycle for 15 minutes, then blood plasma was isolated and the following tests were performed: total protein, albumin, globulin, cholesterol, LDL, HDL, VLDL, glucose, Ca, P, AST, ALT,ALP and triglycerides using a kit by the Company of Spanish. The feces samples were used to perform the total bacterial count as well as the colon bacterial count by using the decimal dilutions of the samples and N.Agar and Maconnky Agar, then incubated at 37 ° C for 24 hours and the total bacterial count and colon bacterial count were estimated [13].

The experiment was of one-way analysis and the trend included the effect of the treatments using the General Linear Model and SAS statistical software version 9.1 [14], was used in addition, a significant difference between mean values was tested using the Duncan test [15] at a significance level of 0.05.
Table 1 Diets used in the experiment and the calculated chemical composition

| Components            | Starter diet (1-11 days) | Growth diet (12-21 days) | Finisher diet (22-42 days) |
|-----------------------|--------------------------|--------------------------|---------------------------|
| Corn                  | 56                       | 60                       | 64.8                      |
| Wheat                 | 0                        | 0                        | 0                         |
| Soya bean meal        | 38.4                     | 34.1                     | 29                        |
| Premix*               | 2.5                      | 2.5                      | 2.5                       |
| Oil                   | 1.8                      | 2.5                      | 3                         |
| Limestone             | 0.7                      | 0                        | 0                         |
| CaHPO₄                | 0.8                      | 0.8                      | 0.7                       |
| DL-Methionine         | 0.1                      | 0.1                      | 0                         |
| L-Lysine              | 0                        | 0                        | 0                         |

**Calculated chemical composition**

| Components            | Starter diet (1-11 days) | Growth diet (12-21 days) | Finisher diet (22-42 days) |
|-----------------------|--------------------------|--------------------------|---------------------------|
| Crude protein         | 23                       | 21                       | 19                        |
| Metabolizable energy  | 3013                     | 3103                     | 3185                      |
| (Kcal/kg feed)        |                           |                          |                           |
| Fibers                | 2.72                     | 2.64                     | 2.55                      |
| Fat                   | 4.31                     | 5.12                     | 5.75                      |
| Lysine                | 1.41                     | 1.29                     | 1.15                      |
| Methionine            | 0.66                     | 0.64                     | 0.51                      |
| Cysteine              | 0.38                     | 0.36                     | 0.33                      |
| Ca                    | 0.99                     | 0.91                     | 0.87                      |
| P Available           | 0.47                     | 0.45                     | 0.43                      |

*premix supplied per kg of diet: vitamin A: 10000 IU, vitamin D₃: 2500 IU, vitamin E: 40 mg, vitamin K: 2 mg, vitamin B₁: 2 mg, vitamin B₂: 7.5 mg, vitamin B₆: 3 mg, vitamin B₁₂: 0.025 mg, pantothenic acid: 10 mg, niacin: 35 mg, folic acid: 1 mg, biotin: 0.05 mg, betaine: 100 mg, Fe: 70 mg, I: 2 mg, Cu: 15 mg, Mn: 70 mg, Zn: 60 mg, Se: 0.25 mg and its provide: 10.3%, CP: 6.1 MJ, lysine: 5.2%, methionine: 8.2%, cystine: 0.5%, thrreonine: 0.5%, tryptophan: 0.2%, calcium: 24.8% and phosphorus: 7.7%

** The chemical composition values were calculated according to N.R.C. [16].

3. Results and Discussion

3.1 Cellular traits of blood

Table (2) includes the effect of adding chitosan and oxytetracycline to the corn ration on the cellular blood traits of broiler. There were no significant differences between the treatments in the numbers of red blood cells, white cells, lymphocytes, and heterophile cells. Packed cell volume, and hemoglobin concentration, while there was a significant superiority (P≤0.05) in the proportion of heterophile cells to lymphocytes in favor of the T4 it compared with the rest of the treatments, as well as the same superiority. The significance of T4 in the numbers of monocytes compared to the rest of the treatments except for T2, which did not differ significantly with T4 and the reason behind the high ratio of L/H is that chitosan in the intestine decomposes and leads to the disintegrate of the N-acetyl complex with the formation of -D-glucose Amin because it easy of decompose [17]. Chitosan also increases the number of white blood cells, contributing to improving immunity [18]. These results were in agreement with [19], who did not find any significant differences between treatments in the numbers of white and red blood cells and lymphocytes when chitosan was added to the basic diet at level of 200 and 400 mg/kg feed compared with the control and the treatment of antibiotic (Avilamycin) addition at a rate of 44 mg/kg feed for brown Hy-Line laying hens at 28 weeks.
Table (2) Effect of adding Chitosan and Oxytetracycline to the corn-soybean diet on cellular traits of blood for broiler

| Trait            | Treatments | Average | SEM* | Significance |
|------------------|------------|---------|------|--------------|
|                  | T1         | T2      | T3   | T4           |              |
| RBC x10^6 cells /mm^3 | 3.360      | 3.073   | 2.886 | 2.453        | 2.943        | 0.615 | N.S** |
| PVC%             | 34.3       | 33.3    | 31.0  | 29.3         | 32.0         | 3.50  | N.S   |
| Hb mg / 100ml WBC| 10.4       | 10.5    | 10.8  | 10.7         | 10.6         | 0.779 | N.S   |
| RBC x10^3 cells /mm^3 | 18033      | 16833   | 20233 | 14500        | 17400        | 4186  | N.S   |
| heterophile %    | 56.6       | 57.3    | 54.0  | 64.3         | 58.0         | 6.38  | N.S   |
| Lymphocyte%      | 36.0       | 33.6    | 39.0  | 26.3         | 33.7         | 5.71  | N.S   |
| H/L              | 1.37       | 1.70    | 1.38  | 2.45         | 1.81         | 0.387 | 0.0576 |
| Monocyte %       | b          | b       | b     | a            | 6.25         | 1.08  | 0.0332 |
| Basocyte %       | b          | ab      | b     | a            |              |       | N.S   |

* SEM: standard error of the mean.
** N.S.: Not significant at significant level (P≤0.05).

a, b, c: The different letters within a single row indicate a significant difference between the treatments.
T1: control, T2: 0.2 g Oxytetracycline / kg, T3: 1 g Chitosan / kg, T4: 2 g Chitosan / kg.

3.2 Biochemical traits

Table (3) includes the effect of adding chitosan to the corn ration on the biochemical traits of the broiler. There were no significant differences between the treatments in the concentration of blood plasma glucose, cholesterol, triglycerides, high, low, and very-low-density proteins, protein, albumin, globulin, calcium, phosphorus, enzymes transporting the amino group, (ALT and AST) and alkaline phosphatase (ALP). These results agreed with [20]. They did not notice any significant differences when adding chitosan at 0.025% to broiler diets in the serum concentration of cholesterol, triglycerides, and HDLC level at 42-days.

The results also agreed with [21], who did not notice any significant differences in the concentration of triglycerides, cholesterol, alkaline phosphatase enzyme, phosphorous, and calcium when adding chitosan at 0.08% for seven weeks to broiler diet. The results agreed with [22], who did not notice any significant differences in the level of calcium, phosphorous, amino group transfer enzymes (ALT and AST), and alkaline phosphatase (ALP) in the blood serum between the addition of the treatment of copper-loaded chitosan at levels of 50, 100 and 150 mg/kg feed to Broiler diet compared to the control treatment at 42 days of age. While the results did not agree with the same researcher, was found a significant increase (P≤0.05) in the level of total protein and albumin in the blood serum of broiler when adding 100 mg of copper-loaded chitosan/kg feed, compared to the control treatment at 42 days.
Table (3) Effect of adding Chitosan and Oxytetracycline to the corn-soybean diet on biochemical traits of blood for broiler

| Trait          | Treatments | Average | SEM* | Significance level |
|----------------|------------|---------|------|-------------------|
| Glucose mg/100ml | T1         | 227     |      |                   |
|                | T2         | 222     |      |                   |
|                | T3         | 203     |      |                   |
|                | T4         | 227     |      |                   |
|                | T1         | 220     | 17.1 | N.S**             |
| Cholesterol mg/100ml | T1         | 189     |      |                   |
|                | T2         | 197     |      |                   |
|                | T3         | 195     |      |                   |
|                | T4         | 202     |      |                   |
|                | T1         | 196     | 9.86 | N.S               |
| Triglyceride mg/100ml | T1         | 173     |      |                   |
|                | T2         | 173     |      |                   |
|                | T3         | 179     |      |                   |
|                | T4         | 175     |      |                   |
|                | T1         | 175     | 8.63 | N.S               |
| HDL mg/100ml   | T1         | 51.0    |      |                   |
|                | T2         | 50.3    |      |                   |
|                | T3         | 47.6    |      |                   |
|                | T4         | 49.6    |      |                   |
|                | T1         | 49.6    | 3.90 | N.S               |
| LDL mg/100ml   | T1         | 103     |      |                   |
|                | T2         | 112     |      |                   |
|                | T3         | 112     |      |                   |
|                | T4         | 118     |      |                   |
|                | T1         | 111     | 12.1 | N.S               |
| VLDL mg/100ml  | T1         | 34.6    |      |                   |
|                | T2         | 34.6    |      |                   |
|                | T3         | 35.8    |      |                   |
|                | T4         | 35.0    |      |                   |
|                | T1         | 35.0    | 1.72 | N.S               |
| Protein g/100ml| T1         | 5.50    |      |                   |
|                | T2         | 5.23    |      |                   |
|                | T3         | 5.40    |      |                   |
|                | T4         | 5.53    |      |                   |
|                | T1         | 5.41    | 0.599| N.S               |
| albumin g/100ml| T1         | 3.96    |      |                   |
|                | T2         | 3.73    |      |                   |
|                | T3         | 3.16    |      |                   |
|                | T4         | 3.46    |      |                   |
|                | T1         | 3.58    | 0.413| N.S               |
| Globulin g/100ml| T1         | 1.53    |      |                   |
|                | T2         | 1.50    |      |                   |
|                | T3         | 2.23    |      |                   |
|                | T4         | 2.06    |      |                   |
|                | T1         | 1.83    | 0.447| N.S               |
| Ca mg/100ml    | T1         | 11.5    |      |                   |
|                | T2         | 11.4    |      |                   |
|                | T3         | 9.60    |      |                   |
|                | T4         | 10.4    |      |                   |
|                | T1         | 10.7    | 1.14 | N.S               |
| P mg/100ml     | T1         | 5.76    |      |                   |
|                | T2         | 5.40    |      |                   |
|                | T3         | 5.26    |      |                   |
|                | T4         | 5.93    |      |                   |
|                | T1         | 5.59    | 0.705| N.S               |
| ALP IU     | T1         | 8.33    |      |                   |
|         | T2         | 8.00    |      |                   |
|         | T3         | 7.00    |      |                   |
|         | T4         | 10.6    |      |                   |
|         | T1         | 8.50    | 3.73 | N.S               |
| AST IU     | T1         | 11.0    |      |                   |
|         | T2         | 9.00    |      |                   |
|         | T3         | 8.00    |      |                   |
|         | T4         | 13.0    |      |                   |
|         | T1         | 10.2    | 4.52 | N.S               |
| ALT IU    | T1         | 73.3    |      |                   |
|         | T2         | 66.3    |      |                   |
|         | T3         | 85.3    |      |                   |
|         | T4         | 82.0    |      |                   |
|         | T1         | 76.7    | 12.4 | N.S               |

* SEM: standard error of the mean.
** N.S.: Not significant at significant level (P≤0.05).

a, b, c: The different letters within a single row indicate a significant difference between the treatments at a significant level (P≤0.05). T1: control, T2: 0.2 g Oxytetracycline / kg, T3: 1 g Chitosan / kg, T4: 2 g Chitosan / kg.

3.3 Tissue of Jejunum

Table (4) includes the effect of adding chitosan and oxytetracycline to the corn diet on the height and thickness of the villi and the depth of the crypts. Significant superiority appeared in favor of the T3 treatment compared to the rest of the experimental treatments. Also, the treatment T4 was significantly superior to both T1 and T2 in this trait, and for thickness Villi, the results showed significant superiority of the T3 treatment compared to the T4 treatment, but it did not differ significantly from both T1 and T2, and these two treatments did not differ significantly from T4, the reason may be due to the increase in the number of beneficial bacteria and the decrease in the number of harmful bacteria, and the preservation of the mucous membrane of the small intestine, and it is a prebiotic [23], and these results were in agreement with [18] who noticed it adding chitosan to broiler diets at levels of 0, 0.2, 0.1 and 0.3, 0.5% it is work to increase the height and density of villi and improve the internal environment of the small intestine, which leads to increased absorption and utilization of Nutrientsat at 42 days .The results also it agreed with [6] and [22]. The results also did not agree with [24], who noticed significant differences between treatments when adding chitosan to the diet at a level of 0.25, 0.50, 0.75, and 1 g/kg of feed compared to the control diet for broilers at 35 days.
Table (4) Effect of adding Chitosan and Oxytetracycline to the corn-soybean diet in the tissue of Jejunum for broiler.

| Trait                | Treatments | Average | SEM* | Significance level |
|----------------------|------------|---------|------|-------------------|
| Villus height        | T1: 467    | 475     | 43.1 | 0.0017            |
|                      | T2: 457    | b       | b    |                   |
|                      | T3: 514    | a       |      |                   |
|                      | T4: 461    | b       |      |                   |
| Villus thickness     | T1: 57.3   | 56.9    | 10.4 | 0.0173            |
|                      | T2: 55.8   | a       |      |                   |
|                      | T3: 63.6   | b       |      |                   |
|                      | T4: 51.1   | b       |      |                   |
| crypts Depth         | T1: 51.6   | 58.6    | 8.94 | 0.0001            |
|                      | T2: 48.3   | a       |      |                   |
|                      | T3: 71.7   | c       |      |                   |
|                      | T4: 62.9   | b       |      |                   |

* SEM: standard error of the mean.

3.4 Microbial content

Table (5) includes the effect of adding chitosan and antibiotic to the corn diet on the total bacterial count and colon bacterial count for broilers, and we notice a significant decrease (P≤0.05) on the total bacterial count and colon bacterial count for the two treatments T3 and T4 compared to the control treatment (T1) and it did not significantly different from the positive control treatment (T2), and the reason is that adding chitosan to broiler diets reduced the harmful microbial content in the intestine, and these results agreed with [18] and [22].

Table (5) Effect of adding Chitosan and Oxytetracycline to the corn-soybean diet in the microbial content of the intestine of broiler

| Trait             | Treatments | Average | SEM* | Significance level |
|-------------------|------------|---------|------|-------------------|
| total bacterial count | T1: 8.18   | 6.74    | 0.0111 | 0.0017          |
|                    | T2: 7.10   | a       | ab   |                   |
|                    | T3: 6.26   | b       |      |                   |
|                    | T4: 5.42   | c       |      |                   |
| Ecoli bacteria     | T1: 6.83   | 5.19    | 0.0424 | 0.0012           |
|                    | T2: 5.61   | a       | ab   |                   |
|                    | T3: 4.07   | b       |      |                   |
|                    | T4: 4.28   | b       |      |                   |

* SEM: standard error of the mean.

4. Conclusion

The results indicated there were added 2 g chitosan/kg corn diet increased significantly compared to other treatments in H / L and monocyte count at 42 days, while the results showed that were no significant differences in the biochemical characteristics of blood. As for the tissue of Jejunum, treatment 1 g chitosan/kg corn diet was significantly superior to the rest of the treatments in height, the thickness of villi, and depth of crypts. There was a significant decrease in the diet with 1 and 2 g of chitosan/kg compared to the comparison treatment on the total bacterial count and colon bacterial count.
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