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# The Effect of Adding Different Levels of Aqueous Extract of Wormwood Plant and Comparing with the Antibiotic Tylosin in Drinking Water on some Immune and Biochemical Characteristics of Broiler Chickens

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# تأثير إضافة مستويات مختلفة من المستخلص المائي لنبات الشيح ومقارنته بالمضاد الحيوي التايلوسين في مياه الشرب على بعض الصفات المناعية والكيموحيوية لفروج اللحم

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## ABSTRACT:

**Introduction:**

This study was conducted to know the effect of adding wormwood extract to drinking water and comparing it with the antibiotic tylosin on some immune and biochemical characteristics of broiler chickens.

**Methods:**

525 broiler chickens of the Rose 308 strain used in this experiment, divided into 6 treatments with 3 replicates for each treatment. The treatments were as follows: (T1) drinking water without addition (control treatment), (T2), (T3) 0.25, 0.50 gm tylosin / liter of water. (T4), (T5), (T6) 0.25, 0.50, 1 ml wormwood extract / liter of water. Some blood biochemical parameters were measured like total protein, glucose, cholesterol, triglycerides and immune parameters to determine the amount of antibodies against Newcastle disease, Gumboro, infectious bronchitis and influenza in broiler chickens.

**Results:**

The results showed no significant differences in the percentage of glucose and triglycerides, while there significant differences (P≤0.01) in the percentage of cholesterol and total protein, in which the wormwood treatments were superior compared to the tylosin treatments and control. In the immune characteristics, there significant differences (P≤0.01) in the size of antibodies directed against Newcastle disease and influenza, in favor of the tylosin treatments compared to the wormwood treatments, while for gumboro and infectious bronchitis, there were no significant differences.

**Conclusion:**

The aqueous extract of wormwood gave a similar effect to the antibiotic tylosin with regard to the biochemical characteristics of blood, there was no significant difference between the treatments with regard to antibodies directed against the Newcastle, gumboro , infectious bronchitis and influenza.

**Keywords**: Artemisia; Tylosine; broiler chicken; biochemical; immune.

## INTRODUCTION:

The indiscriminate use of antibiotics in animal production has accelerated the development of many pathogens as well as harmful commensal microorganisms, which can lead to treatment failure and economic losses and can serve as a source of genes that can be transferred to humans. In addition, there are also concerns about human health regarding the presence of antimicrobial residues in meat [ 1]. Tylosin is an important antibiotic in the field of broiler farming, as it contributes significantly to the prevention and treatment of bacterial diseases that may affect the health and productivity of birds. Despite the high effectiveness of tylosin, it has some side effects that must be taken into account when given in high doses, including the risk of developing bacterial resistance if used excessively or unhealthily [2]. Wormwood is one of the most famous species that have been widely used as natural materials all over the world for many centuries, as it has been used mainly to treat gastrointestinal diseases [3]. Recently, research has shown that wormwood has antioxidant, hepatoprotective, antibacterial, antifungal, and bronchial soothing effects [4]. The various applications of this plant species are due to its chemical composition, which contains particularly flavonoids, essential oils, phenolic acids and coumarins [5]. Poultry in general and broilers in particular are susceptible to viruses at all ages, the most important of which are Newcastle virus, infectious bronchial virus, influenza and Gumporo [6]. These diseases cause significant economic losses in poultry production and the prevalence of these viruses is higher in younger chickens [7]. Clinical signs vary according to the severity of the virus infection [8]. The aim of the research was as a result of the development of multiple strains of pathogenic bacteria. It was necessary to search for medicinal plants that have the ability to inhibit or kill these bacteria, so that they may in the future constitute natural alternatives to antibiotics one of these medicinal plants is wormwood.

## MATERIALS AND METHODS:

This study was conducted to compare the addition of different levels of Artemisia herbal extract and tylosin antibiotic to drinking water on some blood biochemical parameters including estimation of total protein, glucose, cholesterol, and triglycerides, and immunological parameters including estimation of the level of the volumetric standard of antibodies directed against Newcastle disease, Gumboro disease, infectious bronchitis, and avian influenza for Ross 308 broiler chickens. In the experiment, 525 one-day-old broiler chicks of Ross 308 strain with an average weight of (42±2 g/chick) were used, unsexed and the chicks were distributed randomly after weighing into six equal treatments, each treatment contained 75 chicks with three replicates in each replicate of 25 chicks. The experimental treatments were as follows: Treatment 1 (T1): Standard drinking water without addition (control treatment), Treatment 2 (T2): Standard drinking water + 0.25 gm tylosine/liter of water, Treatment 3 (T3): Standard drinking water + 0.50 gm tylosine/liter of water, Treatment 4 (T4): Standard drinking water + 0.25 ml wormwood extract/liter of water, Treatment 5 (T5): Standard drinking water + 0.50 ml wormwood extract/liter of water, Treatment 6 (T6): Standard drinking water + 1 ml wormwood extract/liter

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of water. Feed was provided freely during the experimental period and distributed to replicates with calculated and recorded weights. Feed was provided for each age stage, which included two stages: the starter stage, in which feed was used from one day old to 21 days old, and the finisher stage from 22 to 35 days old. According to the requirements and needs of the bird for compounds and nutrients and according to the Ross 308 broiler production guide for 2023 and in accordance with [9]. Blood samples were collected from the brachial vein at the age of 35 days of birds, with two birds from each replicate selected randomly, and blood was collected directly in tubes that did not contain an anticoagulant, as these tubes were placed in a centrifuge at a speed of 3000 rpm for 15 minutes, after which the serum was separated and stored directly at a temperature of -20 °C until tests related to the biochemical and immunological characteristics of the blood serum were performed. The volumetric standard of antibodies in the blood serum directed against Newcastle disease, Gumboro disease, infectious bronchitis IB and avian influenza were also measured at the age of 35 days using the indirect enzyme immunoassay (ELISA) technique. The statistical program [10] was used to analyze the data to study the effect of different treatments on the studied traits according to the Completely Randomized Design (CRD), and the significant differences between the means were compared using the [11] multinomial test based on the following mathematical model: $Yij = \mu + Ti + eij$

## Preparation of Experimental Materials:

A quantity of wormwood powder was purchased, prepared by grinding the stem and leaves of the wild wormwood plant, and the dose was 10 g per 200 ml of distilled water to prepare the wormwood aqueous extract in the following manner: A quantity of 250 g of the prepared wormwood powder was taken for a quantity of water of 5 liters, depending on the previously mentioned doses. This quantity (250) g was soaked in the calculated quantity of water (5) liters for 5 hours, after which the water was heated to a temperature of 80 Celsius with the prepared material (wormwood powder) on the fire (before boiling), then the material was left for another four hours to cool, after which the extract was filtered from the impurities of the wormwood plant using a light piece of cloth to finally produce a pure solution of wormwood extract to be used later with the three wormwood extract treatments and at different concentrations. Tylosine is an antibiotic belonging to the macrolide group and is widely used in the poultry industry, especially in raising broilers to combat bacterial infections. In the experiment, the antibiotic tylosin (500 gm/box) was used. It is a light yellow to brown powder that is soluble in water and produced by the Dutch company (Interchemie) (Tylosine tartrate 100%).

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## RESULTS AND DISCUSSION

### Biochemical blood characteristics:

The results of the statistical analysis in Table (1) indicated that there were no significant differences between the treatments in glucose values, but arithmetic differences appeared, as the highest glucose value was found in the third treatment, which reached 281 mg/100 ml of plasma, while no significant differences were observed between the first (control) and the second, fourth and fifth treatments, which recorded values of 250.33, 256.66, 239.00, 218.00 mg/100 ml of plasma, respectively, and the sixth treatment recorded the lowest value in glucose concentration, which reached 205.00 mg/100 ml of blood plasma. The table shows that the percentage of triglycerides in the blood did not record significant differences between the six treatments, while the fifth treatment recorded the highest arithmetic rate among the treatments, which amounted to 226.00 mg/100 ml of plasma. The control treatment had the lowest level of triglycerides among the treatments, as it recorded 180.00 mg/100 ml of plasma. The results of the statistical analysis in Table (1) indicated that the fourth, and sixth treatments recorded a significant superiority ($P<0.01$) over the rest of the treatments and were significantly similar to the control treatment, the second treatment and the fifth treatment. These three treatments were significantly similar to the third treatment, which recorded the lowest value for the six experimental treatments in the level of cholesterol in the blood. It was noted from the table that the percentage of total protein in the blood increased in the second and third treatments ($P<0.01$) over the rest of the experimental treatments, which were significantly similar to the fourth, fifth and sixth treatments. On the other hand, these three treatments were similar to the control treatment, which recorded the lowest value for total protein concentration in the blood. The addition of medicinal plants represented by wormwood oil during 42 days of rearing at a rate of 200 and 300 ppm to the broiler feed led to the best results by noting a decrease in the rate of cholesterol, triglycerides and glucose and an increase in the rate of total protein compared to the control treatment [12]. This is contrary to what was found in our current study. While the current study agreed with what [13] found in an experiment conducted to evaluate the effect of two medicinal plants, fenugreek and wormwood, on the performance of biochemical changes in the blood of broilers, as it was found that the treatment of wormwood extract 0.5 ml/L is within the usual values suggested by some, and the usual glucose values in the blood of broilers are about 2.27 to 3 g/L. The results of the current study were similar to what [14] observed giving the antibiotic tylosin at a rate of 4 g/10 L of drinking water to broilers at the age of 19 days for five days did not observe any significant differences in the levels of glucose and phosphorus in the blood plasma of broilers compared to the control treatment. There were no significant differences in a study conducted by [15] between the experimental treatments (23.53), (35.29), (47.06) and (11.76)/wormwood and the control treatment in cholesterol, while there were significant differences ($p<0.01$) between the treatments in the percentage of triglycerides compared to the control treatment for broilers, which may be similar to our current study. The results of the current study were consistent with what was shown by [16] with a significant increase in the percentage of both cholesterol and

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triglycerides in the blood plasma of broilers fed (50 mg) tylosin per kilogram of feed compared to other additives for both cinnamon powder and ginger.

**Table (1) The effect of adding the aqueous extract of wormwood and the antibiotic Tylosine to drinking water on the biochemical characteristics of the blood of broiler chicks (mean ± standard error).**

| Treatments | Biochemical properties | | | |
|---|---|---|---|---|
| | Glucose (mg/100ml) | Triglycerides (mg/100ml) | Cholesterol (mg/100ml) | Total Protein (mg/100ml) |
| T1 | 250.333 ±12.454 A | 180.000 ±15.307 A | 180.666 ±20.464 AB | 19.333 ±2.333 B |
| T2 | 256.666 ±14.992 A | 205.000 ±3.785 A | 183.333 ±19.666 AB | 29.333 ±1.855 A |
| T3 | 281.000 ±24.006 A | 209.333 ± 8.987 A | 188.333 ±3.527 B | 27.000 ±2.081 A |
| T4 | 239.000 ±2.516 A | 217.333 ±10.989 A | 213.333 ±7.688 A | 24.666 ±0.881 AB |
| T5 | 218.000 ±44.298 A | 229.666 ± 35.144 A | 185.333 ±5.364 AB | 23.666 ± 3.382 AB |
| T6 | 205.000 ± 15.502 A | 226.000 ± 8.962 A | 196.333 ± 25.899 A | 22.333 ±2.185 AB |
| Significant | N.S. | N.S. | ** | ** |

G1= Control treatment, G2= Addition of 0.25 g tylosine/L of drinking water, G3= Addition of 0.5 g tylosine/L of drinking water, G4= Addition of 0.25 ml artemisia extract/L of drinking water G5= Addition of 0.5 ml artemisia extract/L of drinking water G6= Addition of 1 ml Artemisia extract/L of drinking water. N. S.: not significant. ** Different letters within the same column mean significant differences between the means of the coefficients at the level ($P<0.01$).

The results of [17] differed from our results, which revealed that when adding wormwood extract at a rate of 4% - 8% to broiler feed during 6 weeks of rearing, there was a decrease in the level of cholesterol in the blood compared to control treatment chicks. The results of the current study did not agree with what [18] showed that adding the antibiotic tylosin by oral dose at a dose of 25 mg/kg of body weight at the age of three weeks increased the level of cholesterol concentration in the blood of broiler chickens. The results of our current study did not agree with what [12] found, as medicinal plants represented by wormwood and cumin oil were added during 42 days of rearing at a rate of 100, 200, and 300 parts per million to the broiler feed, and the two



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rates of 200 and 300 parts per million gave the best results by observing the presence and increase in the total protein rate compared to the control treatment.

**Immune characteristics:**

Table (2) shows the effect of adding the aqueous extract of wormwood and the antibiotic tylosin to drinking water on the level of the volumetric standard of antibodies directed against Newcastle disease, bronchitis, Gumboro disease, and avian influenza for broiler chicks for a period of five weeks of the experiment. Through the results, a significant difference was observed between the Newcastle disease antibody treatments, as the first treatments (control), the second and the third recorded a significant increase ($P<0.01$), while the other three treatments, the fourth, fifth and sixth, which were significantly similar, recorded a lower value than the first three treatments. As for the volumetric standard of antibodies directed against infectious bronchitis, there were no significant differences between the six experimental treatments. It was also noted through the table that no significant differences appeared regarding the volumetric standard of antibodies directed against Gumboro disease, as all six experimental treatments were significantly similar. It is clear from the table that there are significant differences between the treatments regarding the volumetric standard for antibodies directed against avian influenza, the second and third treatments recorded the highest value than the rest of the experimental treatments, which were significantly similar to each other and did not differ significantly with each of the first, fourth and sixth treatments ($P<0.01$) between them. On the other hand, these three treatments did not differ significantly from the fifth treatment, which recorded a lower standard for the volumetric antibodies directed against influenza. Wormwood compounds have antimicrobial properties against pathogens resistant to antibiotics and stimulate the immune system. The antioxidant properties of wormwood appear, including the elimination of free radicals, due to the presence of antioxidant compounds such as phenols and flavonoid groups, as these antioxidant molecules work together to neutralize harmful free radicals [19].

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**Table (2) Effect of adding the aqueous extract of wormwood and the antibiotic Tylosine to drinking water on the level of the volumetric standard of serum antibodies against viruses in broiler chickens (mean ± standard error)**

| Treatments | Volumetric standard for serum antibodies | | | |
|---|---|---|---|---|
| | Newcastle disease | Gumboro disease | Bronchitis | Influenza disease |
| T1 | 6.878<br>± 0.488<br>A | 2.167<br>± 0.286<br>A | 24.489<br>± 3.135<br>A | 17.098<br>± 1.314<br>AB |
| T2 | 6.656<br>± 1.411<br>A | 2.524<br>± 0.699<br>A | 21.150<br>± 0.717<br>A | 18.133<br>±0.409<br>A |
| T3 | 7.145<br>±0.430<br>A | 1.988<br>± 0.193<br>A | 20.968<br>± 2.522<br>A | 18.542<br>±0.00<br>A |
| T4 | 3.516<br>±0.210<br>B | 2.288<br>±0.511<br>A | 20.769<br>± 2.836<br>A | 16.400<br>± 1.433<br>AB |
| T5 | 3.833<br>± 0.597<br>B | 2.006<br>± 0.076<br>A | 24.438<br>±1.416<br>A | 14.486<br>±0.654<br>B |
| T6 | 3.097<br>±0.729<br>B | 2.158<br>±0.516<br>A | 23.564<br>±2.808<br>A | 16.873<br>±1.123<br>AB |
| Significates | ** | N.S. | N.S. | ** |

G1= Control treatment, G2= Addition of 0.25 g tylosine/L of drinking water, G3= Addition of 0.5 g tylosine/L of drinking water, G4= Addition of 0.25 ml artemisia extract/L of drinking water G5= Addition of 0.5 ml artemisia extract/L of drinking water G6= Addition of 1 ml Artemisia extract/L of drinking water. N. S.: not significant. ** Different letters within the same column mean significant differences between the means of the coefficients at the level ($P<0.01$).

The levels of the stoichiometric standard of antibodies against Newcastle disease, infectious bronchitis, Gumboro disease, and avian influenza in the blood serum of broilers in the wormwood extract treatments, which were higher than the level of protection against these diseases, may be attributed to the different concentrations of wormwood extract in the drinking water, which may play a role in stimulating the secretions of the immune system and the production of immunoglobulins A, M, and Y [20–22] stated that the use of natural plants represented by wormwood extract from one day of age until marketing age supports a strong immune system represented by the bursa of Fabricius and the thymus gland, as well as a high level of immunoglobulins in the blood serum, which is a good indicator of the immune response. [23] explained that the use of antibiotics in broilers within the permissible limits improves the performance of the immune system. Tylosine is a macrolide antibiotic. Although some antibacterial classes may negatively affect immunity by inhibiting the function of white blood

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cells, macrolide antibiotics such as tylosin and erythromycin show a positive immune effect on white blood cells, especially phagocytosis, which is part of the first line of defiance against microbes in poultry. Tylosine may have contributed to enhancing the activity of natural immune cells such as T lymphocytes. Tylosine may also have affected the activation of these cells through the production of cytokines, which enhances immune activity and the ability to combat microbes and tumors. Based on these results, it can be concluded that tylosin enhances cellular immunity in poultry by increasing the proliferation of spleen cells and increasing antitumor activity, which enhances the ability of poultry to resist infectious diseases [24]. Our current study agreed with [25] that broiler diets containing the antibiotic tylosin significantly enhanced the immune response in broiler chicks infected with Newcastle disease virus through T cell proliferation and elevated immunoglobulin levels. Continuous treatment with tylosin for five weeks was found to reduce the duration of protective immunity provided by the vaccine based on the development of air sac lesions in vaccinated and treated chickens with tylosin [2], which was contrary to the results of this study. The results of the findings of this study were consistent with what was shown by [23] that the use of antibiotics in broiler chicks within the permissible limits improves the performance of the immune system and the physiological characteristics of the blood. Wormwood is one of the natural feed additives that has a great reputation for being highly effective and has the ability to treat a variety of diseases and improve antioxidant capacity and immune function [26]. The results of the study agreed with what was shown by [27] that adding Artemisia powder to broiler feed at levels of 0.5% and 1% on the immune characteristics showed that the diet containing 1% Artemisia powder led to a significant improvement in antibodies against Newcastle disease at the age of 35 days compared to the control treatment. [28] found that there was a significant increase in immunoglobulins when giving 2 ml / bird / day 5% extract of Artemisia herb compared to the control group and other groups, as the level of antibodies to Newcastle virus increased significantly in this group after 14 days of the age of the laying hens. The results of this researcher are almost similar to the results we obtained.

## CONCLUSION

the aqueous extract of the wormwood plant gave an effect similar to the antibiotic tylosin with regard to the biochemical characteristics of blood, which include glucose, triglycerides, cholesterol, and total protein. There was also no significant difference between the treatments for antibodies directed against viral diseases of Newcastle, Gumporo, infectious bronchitis and avian influenza.

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## Conflict of interests.

Non conflict of interest

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## الخلاصة

### المقدمة:

أجريت هذه الدراسة لمعرفة تأثير إضافة مستخلص عشبة الشيح الطبية إلى مياه الشرب ومقارنتها بالمضاد الحيوي التايلوسين على بعض الصفات المناعية والكيموحيوية لفروج اللحم.

### طرق العمل:

استخدم في التجربة 525 فرخ دجاج لحم من سلالة روز 308 في التجربة، مقسمة إلى 6 معاملات بواقع 3 مكررات لكل معاملة، وكانت المعاملات كالتالي: (T1)ماء شرب بدون إضافة (معاملة السيطرة)، (T2)، (T3) 0.25 ,0.50 غم تايلوسين / لتر ماء. (T4)، (T5)، (T6) 0.25 ، 0.50 ، 1 مل مستخلص الشيح / لتر ماء .تم قياس بعض المعايير الكيموحيوية للدم مثل البروتين الكلي والكلوكوز والكوليسترول والدهون الثلاثية والمعايير المناعية لتحديد كمية الأجسام المضادة ضد الامراض النيوكاسل، الكمبورو، التهاب الشعب الهوائية المعدي والأنفلونزا في فروج اللحم.

### النتائج:

أظهرت النتائج عدم وجود فروق معنوية في نسبة الكلوكوز والدهون الثلاثية، بينما كانت هناك فروق معنوية ($P\leq0.01$) في نسبة الكولسترول والبروتين الكلي، اذ كانت هناك زيادة في نسبتيهما في معاملات الشيح مقارنة بمعاملات التايلوسين والسيطرة. أما بالنسبة للصفات المناعية، فقد كانت هناك فروق معنوية ($P\leq0.01$) في حجم الأجسام المضادة الموجهة ضد مرض النيوكاسل والأنفلونزا ، اذ انخفضت في معاملات الشيح مقارنة بمعاملات التايلوسين، أما بالنسبة للكمبورو والتهاب الشعب الهوائية المعدي، فلم تكن هناك فروق معنوية بين المعاملات.

### الاستنتاج:

أن المستخلص المائي لنبات الشيح أعطى تأثيراً مماثلاً للمضاد الحيوي التايلوسين بالنسبة للخصائص الكيموحيوية للدم والتي تشمل الكلوكوز والدهون الثلاثية والكوليسترول والبروتين الكلي، ولم يكن هناك فرق معنوي بين المعاملات بالنسبة للأجسام المضادة الموجهة ضد الأمراض الفيروسية النيوكاسل والكمبورو والتهاب الشعب الهوائية المعدي وأنفلونزا الطيور.

**الكلمات المفتاحية:** الشيح; تايلوسين; فروج لحم; كيموحيوية; مناعية.

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