Comprehensive Journal of Science
Volume (10), Issue (38), (Dec, 2025)
ISSN: 3014-6266

مجلة العلوم الشاملة
المجلد (10) ملحق العدد 38 ديسمبر 2025
ردمد: 3014-6266

The Impact of Using Various Amounts of Thyme, Rosemary, Garlic, and Their Combination on the Immune System, Immunoglobulin G, Immunoglobulin M, Immunoglobulin A, and Hematological and Biochemical Blood Characteristics of Local Chickens.

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تاريخ الاستلام:2025/12/14 –تاريخ المراجعة: 2025/12/16– تاريخ القبول: 2025/12/22– تاريخ للنشر: 2026 /1/22

Abstract:
This work was carried out at the Faculty of Veterinary Medicine and Agriculture, University of Zawia, during the period from April to June 2024, for 6 weeks. The study examined the effect of a mixture of aromatic medicinal plants (thyme, rosemary, and garlic) at different levels on the hematology and the immune system of local chicks of domestic chickens.
The group fed 100 mg of thyme showed the highest significant increase in the bursa, followed by the group fed 100 mg of garlic, with increases of 1.11, 1.30, and 1.23, respectively.
There was no significant effect on the thymus gland through the experimental treatments, as the highest percentage was recorded in the control group, 1.20
IgA levels showed the highest value in the treatment fed 100 mg of thyme (84.3), followed by the treatment fed 100 mg of rosemary, and then the treatment fed 100 mg of garlic.
The logical values obtained in this study indicated that the mean IgM was significantly higher in the T2 and T3 control groups compared to the control group in chickens fed a basal diet supplemented with 1% garlic ($p \leq 0.05$).
There was a significant increase in hemoglobin levels, with the 100 mg garlic treatment outperforming the other experimental treatments, registering 14.7.
The group fed 100 mg of thyme showed the highest significant difference in red blood cell volume, followed by the group fed 100 mg of garlic (45.6% and 43.2%, respectively).
Keywords: Thyme, Rosemary, Garlic, hematological and biochemical blood characteristics, IgG, IgM, IgA.

تأثير استخدام كميات مختلفة من الزعتر وإكليل الجبل والثوم ومزيجها على الجهاز المناعي (الغلوبولين المناعي G، والغلوبولين المناعي M، والغلوبولين المناعي A) والخصائص الدموية والكيميائية الحيوية للدجاج المحلي.

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الملخص:
أُجري هذا العمل في كلية الطب البيطري والزراعة بجامعة الزاوية، خلال الفترة من أبريل إلى يونيو 2024، على مدى ستة أسابيع. تم دراسة تأثير مزيج من النباتات الطبية العطرية (الزعتر ، إكليل الجبل ، والثوم)) بتراكيز مختلفة على الدم والجهاز المناعي على الدجاج المحلي.
أظهرت المجموعة التي تناولت 100 ملغ من الزعتر أعلى زيادة كبيرة في الجراب، تليها المجموعة التي تناولت 100 ملغ من الثوم، بزيادات قدرها 1.11 و1.30 و1.23 على التوالي.

لم يكن هناك تأثير يُذكر على الغدة الزعترية من خلال التجربة، حيث سُجلت أعلى نسبة مئوية في المجموعة الضابطة، 1.20.
أظهرت مستويات الغلوبيولين المناعي IgA أعلى قيمة في المجموعة التي تناولت 100 ملغ من الزعتر (84.3)، تليها المجموعة التي تناولت 100 ملغ من إكليل الجبل، ثم المجموعة التي تناولت 100 ملغ من الثوم.
أشارت القيم المنطقية التي تم الحصول عليها في هذه الدراسة إلى أن متوسط الغلوبيولين المناعي IgM كان أعلى بشكل ملحوظ في مجموعتي T2 و T3 مقارنة بمجموعة التحكم في الدجاج الذي يتغذى على نظام غذائي أساسي مضاف إليه 1٪ من الثوم ($p\leq0.05$).
كان هناك ارتفاع ملحوظ في مستويات الهيموجلوبين، حيث تفوق اضافة الثوم بجرعة 100 ملغ على المعاملات التجريبية الأخرى، مسجلاً 14.7.
أظهرت المجموعة التي تناولت 100 ملغ من الزعتر أعلى فرق كبير في حجم خلايا الدم الحمراء، تليها المجموعة التي تناولت 100 ملغ من الثوم (45.6٪ و 43.2٪ على التوالي).
الكلمات المفتاحية: الزعتر، إكليل الجبل، الثوم، ، الخصائص الدموية والكيميائية الحيوية للدم، الغلوبيولين المناعي IgG، IgM، IgA.

I. Introduction

Poultry farming is one of the primary methods used to solve the problem of low animal protein consumption. The greatest demand for broiler meat prompted the farmers to switch to intensive animal farming. Farmers must treat each bird in the group as a prophylactic measure against infection since illnesses spread swiftly among birds in intensive farming (Guerrero and Hui, 2010).

Additionally, broiler output is expanding quickly due to population expansion and the growing demand for chicken products (Thornton, 2010). Without a doubt, antibiotics are essential in animal husbandry since they promote health and development. (Lee *et al.*, 2004). Even though the birds raised with these feed additives performed well, their potential negative consequences became a global public health issue (Donoghue, 2003).

Medicinal plants are known for their medicinal properties, particularly in traditional medicine systems. It has been used for its anti-inflammatory, antimicrobial, and antioxidant properties. While extensive studies on Medicanil's effects in poultry are still limited, preliminary research and historical knowledge suggest that it may have beneficial effects on poultry health, particularly in enhancing immune responses. In the international chicken industry, thyme is well recognized as a powerful growth enhancer and an appropriate replacement for artificial additives (Alcicek *et al.*, 2004).

These compounds improve the production and well-being of hens (Mitsch *et al.*, 2004). It has been shown that garlic has antimicrobial qualities and increases feed intake and palatability (Choi *et al.*, 2005).

Medicinal plants contain bioactive compounds such as alkaloids, flavonoids, tannins, and glycosides. These compounds are believed to contribute to their immune-boosting and antimicrobial effects, which may help in reducing the impact of diseases in poultry flocks. Understanding how Medicanil plants affect specific immune parameters, such as IgG and IgM levels, is crucial for assessing their potential as a natural immunomodulator in poultry farming. Rosemary and its constituents have long been linked to potent antioxidant, antibacterial, antiviral, anti-inflammatory, and anticarcinogenic qualities (Al-Kassie et al., 2011; Aherne et al., 2007).

Immunoglobulin G is the most abundant antibody in the bloodstream and plays a key role in fighting infections. It provides long-term immunity by neutralizing toxins, bacteria, and viruses. IgG is responsible for memory responses in the immune system, meaning that once the body has encountered a pathogen, IgG antibodies remain to protect against future infections by the same pathogen. Immunoglobulin M is the first antibody produced during the initial stages of an immune response. It plays a critical role in the early defense against infections and activates other immune components such as the complement system. While IgM is less specific than IgG, its rapid production is essential for the initial containment of pathogens. A number of target antigens have previously been used to show the existence of IgM and IgY Nabs in chicken sera, with keyhole limpet hemocyanin (KLH) being the most researched (Siwek, M *et al*., 2006).

Because they are safer, healthier, and less toxic than synthetic chemicals (antibiotics), natural additives like medicinal plants and their products, especially plant extracts, have drawn more attention. (Alagawany. *et al*., 2015).

The current study aimed to investigate the effect of different levels of herbs (thyme, garlic, and rosemary) and their combination in feed as an alternative to antimicrobial growth promoters on immune system function and immunological characteristics in broiler chickens. Several studies have indicated varying effects of the active ingredients of herbal plants on poultry performance.

Materials and Methods

This study was carried out during the period from April to June 2024, for 6 weeks. The study examined the effect of a mixture of aromatic medicinal plants (thyme, Rosemary, and garlic) at different levels on hematology and immune system of local chicks of domestic chickens.

The present experimental work was carried out at the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture (Al-Ajeelat), with approval by the University of Zawia Local Ethics Committee for Animal Experiments.

The investigation was conducted on 75 one-day-old domestic chicks that were brought from the same city (Zawia City), which were weighed and distributed randomly in a completely random design into five experimental treatments. Each treatment contained 15 birds, which were distributed into 3 replicates, with 5 birds in each replicate.

The chicks were reared under a cage breeding system. The breeding house temperature was kept within the normal range, and the experiment lasted from one week to 6 weeks of age.

Before the trial started, a 7-day adaptation period was allowed. Feed and water for animals were provided ad libitum.

Plant material

The plant powders used in the experiment were collected from the local Libyan market.

Experimental protocol

In total, 75 domestic chicks were randomly assigned into 5experimental groups of 15 birds each and adapted for 7 days under similar housing and management conditions. The experiment consisted of 3 replicates, and birds were allocated in a completely randomized design. Chickens were divided into the following treatments: treatment 1. Received standard basal diet. Without any additives and was used as a control group. The second treatment contained 100 mg of thyme, the third treatment contained 100 mg of Rosemary, the fourth treatment contained 100

mg of garlic, and the fifth treatment contained a mixture of thyme, Rosemary, and garlic, 100 mg/kg, respectively.

The most important measurements taken during the experiment period:

1- The weight of the bird's immune organs, Bursa, Thymus, and spleen:

After the end of the trial period, random samples from each treatment were slaughtered, the weight of the immune internal organs of the body was taken, and the percentage of dressing and meat/ bone ratio for all treatments was calculated using a formula.

Organ or Body part weight percent 100/Live weight* Organ or Body part weight (Iwuji *et al*., 2002).

2- Blood hematological analysis of chickens.

3- Immune analysis of chickens includes IgA, IgM, and IgG.

Statistical Analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed by using the SPSS software package, version 26.00. P values < 0.05 were considered significant. The results are expressed as mean ± standard deviation (SD) for each group.

Results and discussion

Table 1 shows the effect of thyme, rosemary, garlic, and their combination on certain immune glands in chickens. The table reveals both significant and non-significant differences between the various experimental treatments. A slight significant increase was observed in the treatments fed varying proportions of thyme, rosemary, and garlic compared to the control group. Specifically, the group fed 100 mg of thyme showed the highest significant increase in the bursa, followed by the group fed 100 mg of garlic, with increases of 1.11, 1.30, and 1.23, respectively.

This agrees with previous study show that the garlic has strong antioxidant and immune-boosting effects against oxidative stress (Gardzielewska et al., 2003).

Similarly, the activity of the thymus and bursa glands during the early stages of life can be positively influenced by certain herbs or herbal extracts, affecting feed intake, digestive juice secretion, and the immune system of animals (Mikulski et al., 2008).

On the other hand, there was no significant effect on the thymus gland through the experimental treatments, as the highest percentage was recorded in the control group, 1.20 (Gill ،2001). There is no report on the effect of local Iranian herbal extracts on the thymus gland.

While there was a slightly significant increase in spleen level in the group fed 100 mg of rosemary compared to the control group and other groups, where it was recorded at a rate of 0.61. It has been proven that the immunity, meat quality, and productivity of broiler chickens all benefit from the inclusion of rosemary in their diet, as there was a significant effect on immune characteristics when rosemary was added to the liver and spleen **(**Ghazalah and Ali, 2008).

Table 1: The influence of different levels of thyme, rosemary, garlic, and their combination on the immune organs, Bursa, Thymus, and spleen of chickens (Mean± Standard error).

	Different dietary treatments				
Item	T1	T2	T3	T4	T5
Bursa%	1.03±0.05	1.11±0.08^{a}	1.23±0.11^{a}	1.30±0.09^{a}	1.41±0.14^{ab}
Thymus%	1.20±0.12^{a}	1.65±0.07^{b}	1.71±0.12^{ab}	1.95±0.19^{a}	1.84±0.17^{b}
Spleen%	0.52±0.09	0.81±0.15^{b}	0.61±0.11^{a}	0.88±0.29^{b}	0.21±0.04^{ab}

*a, b, c, Means with different superscripts in the same column differ significantly (P<0.05).T1= control, T2=Thyme 100 g/kg, T3= Rosemary 100 g/kg, T4=Garlic 100 g/kg, T5= Mixture of Thyme 30 g/kg+ Rosemary 30 g/kg + Garlic 30 g/kg.

The table 2 illustrates the effect of a mixture of thyme, rosemary, and garlic on the immune characteristics of poultry. This table reveals significant differences between the various experimental treatments. A significant increase in IgA levels was observed in some treatments compared to the control group and the group containing the aromatic medicinal plant mixture. The highest value was recorded in the treatment fed 100 mg of thyme (84.3), followed by the treatment fed 100 mg of rosemary, and then the treatment fed 100 mg of garlic. This aligns with a previous study, as the aromatic medicinal plants, thyme and garlic, are medicinal herbs used for the prevention and treatment of numerous diseases. This antibiotic demonstrates productive performance when added as a feed supplement to broiler chicken diets. It stimulates the immune system, particularly IgA, induces quantitative changes in white blood cells, improves digestion, and acts as a growth promoter, leading to increased body weight, feed intake, and feeding efficiency (Adibmoradi et al., 2006).

However, at the IGM level, there were no significant differences between the different treatments compared to the control group.

The logical values obtained in this study indicated that the mean IGM was significantly higher in the T2 and T3 control groups compared to the control group in chickens fed a basal diet supplemented with 1% garlic ($p \leq 0.05$).

Table 2: Immune indices of broiler chickens fed a diet supplemented with thyme, rosemary, garlic, and their combination (Mean± Standard error).

	Different dietary treatments				
Item	T1	T2	T3	T4	T5
IgA (mg/100 ml)	77.3±0.003^{c}	84.3±0.002^{a}	78.7±0.008^{a}	76.0±0.009^{a}	74.5±0.004^{ab}
IgM (mg/100 ml)	224±0.001^{a}	320±0.007^{b}	235±0.003^{ab}	236±0.002^{a}	237±0.001^{b}
IgG (mg/100 ml)	928±0.009	978±0.005^{b}	989±0.011^{a}	973±0.003^{b}	956±0.004^{ab}

*a, b, c, Means with different superscripts in the same column differ significantly (P<0.05).T1= control, T2=Thyme 100 g/kg, T3= Rosemary 100 g/kg, T4=Garlic 100 g/kg, T5= Mixture of Thyme 30 g/kg+ Rosemary 30 g/kg + Garlic 30 g/kg.

The table 3 illustrates the effect of adding different levels of thyme, rosemary, and garlic powder on hematological blood parameters. The results showed no significant differences in red blood cell count between the different treatments. However, there was a significant increase in hemoglobin levels, with the 100 mg garlic treatment outperforming the other experimental treatments, registering 14.7. One study demonstrated that consuming garlic juice (extracted using distilled water) for 30 days led to a significant increase in red blood cell (RBC) count, hemoglobin concentration (HGB), packed cell volume (PCV), and white blood cell count in male albino rats (Iranloye, 2002).

The group fed 100 mg of thyme showed the highest significant difference in red blood cell volume, followed by the group fed 100 mg of garlic (45.6% and 43.2%, respectively). There was also a significant difference in monocytes compared to the other experimental treatments in the group fed 100 mg of thyme. This aligns with a previous study demonstrating that feeding thyme powder and extract in a short-term fattening regimen improves immune system function and white blood cell function (Esteve et al. 2000).

While there were slight significant differences in the levels of acidophilic and basophilic cells in T3 and T4, this indicates that feeding poultry a mixture of some aromatic medicinal plants and garlic plays a major role in improving some blood chemistry characteristics. This is consistent with a previous study in which broiler chickens were fed plant extracts such as thyme and garlic, and improvements in growth, immune characteristics, and hematological blood characteristics were observed (Herandez et al. 2004).

Table 3: Blood hematological analysis of chickens fed a diet supplemented with thyme, rosemary, garlic, and their combination (Mean± Standard error).

	Different dietary treatments				
Item	T1	T2	T3	T4	T5
RBC's (10^6/cmm^3)	1.56±0.023^{c}	2.03±0.012^{b}	1.70±0.028^{a}	1.90±0.009^{b}	1.95±0.004^{ab}
Hemoglobin (g/100ml)	11.5±0.354^{a}	17.0±0.327^{b}	15.0±0.323^{a} b	14.7±0.312^{a}	13.4±0.301^{b}
PCV %	35.6±0.621^{c}	45.6±0.601^{a}	42.0±0.641^{b}	43.2±0.691^{a}	44.4±0.630^{bc}
WBC's (10^3/cmm^3)	21.6±0.321^{c}	27.8±0.321^{a}	26.3±0.351^{b}	25.7±0.381^{b}	26.1±0.356^{a}
Monocytes (%)	11.4±0.162^{b}	15.3±0.168^{a}	14.9±0.143^{b}	14.3±0.153^{c}	14.5±0.163^{bc}
Lymphocytes (%)	35.9±0.385^{c}	45.6±0.365^{a}	40.0±0.335^{b}	41.0±0.345^{b}	42.7±0.355^{b}
Eosinophils, (%)	12.23 ±0.349^{c}	14.8±0.346^{a}	14.3±0.346^{b} c	13.54±0.356^{b} c	13.57±0.346 b
Basophils, (%)	0.313±0.145 b	0.353±0.144 b	1.02±0.144^{a}	0.343±0.140^{b}	1.01±0.151^{a}

*a, b, c, Means with different superscripts in the same column differ significantly (P<0.05).T1= control, T2=Thyme 100 g/kg, T3= Rosemary 100 g/kg, T4=Garlic 100 g/kg, T5= Mixture of Thyme 30 g/kg+ Rosemary 30 g/kg + Garlic 30 g/kg, RBC's=red blood cell; PCV=packed cell volume; WBC's=white blood cell.

Conclusions

The results of this study showed that adding medicinal aromatic plants such as thyme, garlic, and rosemary to poultry feed has a positive effect on the immune system and hematological characters. More research is required to determine the proper quantity of supplementation and mode of action for medicinal plants.

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