

Effect of Adding Different Levels of Aqueous Extract of Lemongrass Leaves on The Hormonal, Physiological, And Immune Traits for Rooster of Breeder Broiler (ROSS 308)

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ABSTRACT: *This study was conducted in the field of domestic birds / Department of Animal Production / College of Agriculture - Al-Qasim Green University for a period of 6 weeks from 1/11/2021 - 15/12/2021, preceded by two weeks to train roosters to collect semen, to study the effect of adding the aqueous extract to lemongrass leaves in different levels on the reproductive characteristics of males of meat broiler mothers (Ros 308) at the age (50) weeks and by 16 roosters, the roosters were randomly divided into 4 treatments with 4 replications, each replicate included only one rooster. The treatments were as follows: The first treatment was the control group without adding, the second treatment was adding 200 ml of aqueous extract of lemongrass leaves/L of water, and the third treatment was adding 300 ml of aqueous extract of lemongrass leaves/L of water, the fourth treatment was adding 400 ml of aqueous extract For lemongrass leaves/L of water. The following results were: T3 treatment was significantly ($P < 0.05$) excelled in red blood cells over T1 and T4 treatments. T2 and T3 were significantly excelled in haemoglobin, hematocrit, testosterone, LH and white blood cell counts significantly ($P < 0.01$) compared to the T1 and T4, a significant improvement ($P < 0.05$) for T3 treatment increase in the albumin compared to T1 and T4 treatments. The treatments (T2, T3 and T4) showed a significant ($P < 0.01$) excellency in the percentage of globulin compared with the control treatment. A significant improvement ($P < 0.05$) in the percentage of total protein in the T2, T3 and T4 treatments compared to the control treatment.*

Keywords: *Aqueous extract of lemongrass leaves, hormones, immune traits, male broiler mothers.*

1. INTRODUCTION

The fertility of broiler mothers is critical for increasing the efficiency of poultry production. This fertility decreases at the age of 50 weeks (Remero-Sanche et al., 2008) due to a decrease in the male hormone testosterone responsible for the fertility of roosters. The decrease is back to the conversion of testosterone to estrogen by the aromatase enzyme, which inhibits the secretion of the reproductive hormones LH and FSH (Thibier and Wagner, 2002), with the ageing of male mothers of broilers has several negative effects on semen quality, as well as significantly reduces the activity of antioxidant enzymes in epididymal sperm and thus increases the production of Reactive Oxygen Specie (ROS) which leads to cell death and reduced semen quality (Ahsan et al., 2014). These problems prompted the use of medicinal plants that are safe, cheap, reduce mortality and able to maintain the optimum

growth of sperm (fondant et al., 2010), these plants can exert antimicrobial, antioxidant and immune-enhancing activities (Hashemi et al., 2009), antioxidants of natural origin were preferred over those of natural origin that could be used instead of synthetic chemical materials (Grmski et al., 2019). In recent years, the demand for natural sources increased such as plant-derived antioxidants from essential oils in poultry feed due to fewer side effects (Alcicek et al., 2003; Attia et al., 2017). Botanical treatments seem to be an alternative approach due to the adverse effects associated with the use of synthetic drugs (Manviha and Bidya, 2014).

One of these perennial medicinal plants used is lemongrass (*Cymbopogon citratus* L.), this plant contains steroids, alkaloids, saponins, tannins, anthraquinones, phenols and flavonoids, which make these chemical compounds act as antioxidants, anti-fungals and anti-bacteria (Thorat et al., 2017), lemongrass leaves contain 71.03% moisture, 3.83% crude protein, 2.94% ash, 4.76% crude fat, 9.30% crude fiber, 20.73% carbohydrates. At squeezed, Lemongrass leaves produce a yellow and an essential oil such as citral, citrulline, nerol geraniol, geranyl acetate, myrcene and terbitol (Adejuwon and Ester, 2007), aqueous extracts of dried leaves are used to treat many diseases and infections (Shah et al., 2011) because lemongrass leaves are rich in important nutrients. There are no local studies on reproductive characteristics, Therefore, this study was conducted to find out the effect of adding different levels of aqueous extract of lemongrass leaves and to determine the best ratios as antioxidants and aromatase inhibitors and their effect on the reproductive characteristics of male broiler mothers.

2. MATERIALS AND METHODS

The experiment

This experiment was conducted in the poultry field of the Department of Animal Production at the College of Agriculture - Al-Qasim Green University for a period of 6 weeks from 1/11/2021 - 15/12/2021. Before starting the experiment, the roosters were trained to give semen for a period of only two weeks, the experiment included the effect of adding different levels of aqueous extract of lemongrass leaves on reproductive characteristics, testicle size and tissues was studied. In this experiment, 16 roosters of ROS 308 breed mothers of broilers were used, and roosters were prepared from the company - Taq Taq in Dohuk, with an average weight of 5.5 kg and at the age of 50 weeks, the roosters were raised in the hall of the Animal Production Department, prepared and divided into reservations, the dimensions of each reservation (1.5 x 1) m², each reservation divided into three sections according to the terrestrial breeding system, the lighting system was followed (14 days/10 dark hours).

The treatments

Roosters were randomly distributed among the reservations by 4 treatments, each treatment included 4 replications and one rooster for each replication.

1. The first treatment (T1): without addition.
2. The second treatment (T2): 200 ml/L aqueous extracts of lemongrass leaves.
3. The third treatment (T3): 300 ml/L aqueous extracts of lemongrass leaves.
4. Fourth treatment (T4): 400 ml/L aqueous extracts of lemongrass leaves.

The data were analyzed using a completely random design (CRD) to study the effect of the studied treatments on different traits, and the significant differences between the means were

compared using Duncan's (1955) polynomial test. The program SAS (2012) was used in the statistical analysis according to the following mathematical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

The diet used in the study

Roosters were fed on a diet containing 15% crude protein and energy represented by 2715 kilocalories/kg of feed, it was prepared in the Jawhara Factory – Babylon, the diet was provided at 110 gm of feed/birds/day, the feed was provided at fixed times, feed contents were in table 1.

Table 1. The diet contents in the experiment and its chemical composition

Feed		Chemical content**	
Contents	Percent	Contents	Percent
Corn	37	Representative energy (kilo calories / kg feed)	2715
Wheat	15	Crude protein	15.93
Barley	12	Crude fibers	3.45
Soybean meal (44% protein)	18	Calcium	1.26
Wheat bran	12	Phosphor	0.75
Mixtures of vitamins and minerals(Premix)	2	Methionine + Sistine NE	0.76
Limestone powder	3	Lysine	0.85
Vegetable oil	1		
Total	100%		

** Chemical analysis calculated according to the NRC (1994).

Preparation of the aqueous extract of lemongrass leaves and use

Lemongrass leaf powder material was prepared from Baghdad local markets, and the dried leaves were placed 20 gm in 1 litre of distilled water to obtain 200 ml (concentrated extract)/litre of drinking water and 30 gm to obtain 300 ml (concentrated extract)/litre of drinking water and 40 gm to obtain 400 ml (concentrated extract) / litre of drinking water and placed at a temperature 60 °C for 48 hours, then it was filtered with a filter paper (Hernandez *et al.*, 1994).

Measurement of hormones and immune traits

Blood samples were drawn from the pterygoid vein by using a 5 ml syringe equipped with a Needle according to Al-Darraji *et al.* (2008), these tubes are placed in a centrifuge at 6000 rpm for 15 minutes to separate the serum from the cellular fraction. After separation, the serum samples were transferred to other plastic tubes and kept at a temperature of -20°C. Hormonal and immunological tests (albumin, globin, total protein) were performed.

Measurement of sex hormones

The level of luteinizing (LH) and testosterone hormone in the blood serum was measured using a special detection kit from the Chinese company (Bioassay Technology Laboratory) by using ELISA technology, it was analyzed at the college of veterinary medicine - University of Kufa.

Cellular characteristics of blood

Blood tests were conducted in the Analytics Laboratory (College of Science / University of Babylon), the blood picture was measured using the (Mythic 18 ret) device by taking (50 microns) of the blood sample and the analysis was done automatically by using the detection kit of the device, the following tests were performed:

1. red blood cells RBC.
2. Hemoglobin Hb.
3. PCV bound blood cell volume.
4. White blood cells WBC.

3. RESULTS AND DISCUSSION

Level of sex hormones

The results of table 2 appeared variations in the level of sex hormones, T2 and T3 treatments recorded the highest significant ($P < 0.01$) in the level of testosterone and LH hormone over the T1 and T4 treatments that recorded the least significant decrease, there were no significant differences between the T1 and T4 treatments.

Table 2. Effect of adding an aqueous extract of lemongrass at different levels of roosters' hormones ROSS 308

Treatments	mean \pm standard error	
	Testosterone (Ng/dl)	LH (Mm/ml)
T1	0.226 \pm 0.02 b	0.580 \pm 0.32 b
T2	1.213 \pm 0.006 a	2.62 \pm 0.40 a
T3	1.456 \pm 0.06 a	3.09 \pm 0.33 a
T4	0.450 \pm 0.33 b	1.25 \pm 0.03 b
Significant level	**	**

The averages with different letters within the same column differ significantly between them. ** ($P < 0.01$).

The moral improvement of the sex hormones testosterone and LH is due to medicinal plants that contain active compounds that play an important role in fertility, especially the production of testosterone by increasing the expression of steroid protein is important for the entry of cholesterol into the mitochondria, which leads to an increase in testosterone in testicular cells (Martin and Touaibia, 2020), the use of the herbal extract improved the characteristics of the semen, as well as the histological analysis of the testis has more sperm than the control group, this indicates the improvement of sex hormones. The increase in the secretion of LH hormone in male blood leads to the stimulation of Leydig cells, thus leading to an increase in the secretion of the male hormone testosterone in the blood and an increase in FSH receptors in Sertoli cells. Sertoli cells lead to the formation and support of spermatogenesis by providing the necessary and necessary materials for the formation of sperm. There is a strong relationship between Sertoli cells and the development of germ cells in birds. So, Sertoli cells which help to regulate the development of germ cells by providing the appropriate environment for sperm, work to release sperm regularly in the testicular tubules (Sturkie, 2000). These results supported the use of herbal extracts as aromatase inhibitors in raising the male hormone testosterone in aged roosters to improve testis (Adeldust *et al.*, 2017), this study also agreed with (Weinbauer and Nieshlag 1991). The increase in testosterone increased the sexual desire of birds, increased semen volume and

improved semen quality through the effect of testosterone directly on the process of sperm production. The flavonoids can increase the level of LH by inhibiting Aromatase enzymes in the anterior pituitary gland (Wang *et al.*, 1994), aromatase inhibition inhibits estrogen biosynthesis and thus releases the pituitary axis from the effect of estrogen negative reactions for LH and FSH. Also, in agreement with Ali 2017, the use of Anastrozole acted as an aromatase inhibitor which raised LH levels in aged roosters even at low levels, the addition of medicinal plants led to an increase in the hormone LH, and a decrease in estrogen, which rises in the Males in old age (Ganber and Spiteller, 1995).

Cellular traits of blood

There are variations in table 3 by adding aqueous extract of lemongrass leaves on the numbers of RBC red blood cells, haemoglobin HB concentration, PCV hematocrit ratio, and the numbers of WBC white blood cells of ROSS308. The number of red blood cells ($10^6 / \text{mm}^3$ of blood), T3 treatment recorded the highest number of red blood cells with a significant difference ($P < 0.05$) compared to the T1 and T4 treatments, The second did no significant differences with T3 on the one hand and T1, T4. The concentration of haemoglobin Hb (gm / 100 ml of blood and PCV (%)) that T2 and T3 treatments recorded the highest percentage in haemoglobin concentration and haemoglobin recipe significant excellently with a level ($P < 0.01$) compared to the T1 and T4 treatments, which recorded the least significant difference with a level ($P < 0.01$), as for the number of white cells ($10^3/\text{mm}^3$ blood) T2 and T3 treatments at a significant level ($P < 0.01$) were excelled to the first treatment, which recorded a decrease in the number of white blood cells, there were no significant differences between T2, T3, and T4 treatments on the one hand, and between the T4 and T1 on the other hand..

Table 3. Effect of aqueous extract of lemongrass leaves at different levels on blood cellular traits of ROSS308 roosters.

Treatments	mean \pm standard error			
	Red blood cells ($10^6 / \text{mm}^3$ blood)	Hemoglobin (gm/100ml blood)	PCV (%)	White blood cells ($10^3/\text{mm}^3$ blood)
T1	2.75 \pm 0.31 b	15.46 \pm 0.32 b	44.36 \pm 0.74 b	73.84 \pm 1.99 b
T2	3.15 \pm 0.42 ab	20.76 \pm 0.73 a	50.73 \pm 2.02 a	9081.28 \pm 0.75 a
T3	3.86 \pm 0.15 a	23.63 \pm 2.18 a	52.93 \pm 2.66 a	95.81 \pm 6.15 a
T4	2.48 \pm 0.31 b	15.50 \pm 1.04 b	43.90 \pm 2.07 b	88.61 \pm 3.61 ab
Significant level	**	**	**	**
The averages with different letters within the same column differ significantly between them. ** ($P < 0.01$).				

The significant improvement in the number of red blood cells in T2 and T3 is due to the role of lemongrass leaves extract because it affects the main factor which format of red blood cells in the bone marrow (Singh *et al.*, 2008), and the role of testosterone hormone which leads to increase booster of red blood cells formation (Eric *et al.*, 2014), this booster stimulates the production of red blood cells (Jelkmann, 2007). Lemongrass leaves contain iron and copper which are necessary for red blood cells (Majewska *et al.*, 2019), while blood stack and haemoglobin increase due to the increase in red blood cells (Eric *et al.*, 2014) because the blood stack represents a measurement of total blood cells, especially red cells, so the factors which affect on red blood cells also affect on other blood characteristics (Al-Husni, 2000). The white blood cells increased in T2 and T3 treatments from lemongrass

leaves extract which stimulated and activated the immune system (Toungos *et al.*, 2019). These results agreed with researchers Al-Awadi and Al-Nadawi (2020) who found significant differences in Red and white blood cells, blood stack and haemoglobin in the treatments to which lemongrass extract was added to broilers over the control treatment.

Immunomodulatory traits

Table 10 showed the effect of adding an aqueous extract of lemongrass leaves at different levels on the immunological characteristics (albumin, globulin and total protein) of ROSS 308. The results of the statistical analysis showed that there were significant differences in the experimental treatments, the concentration of albumin (100gm/ ml) recorded the highest significant level ($P<0.05$) in T2 and T3 treatments over T1 and T4 treatments which recorded the lowest concentration, while the concentration of globulin (100 gm/ml), T2, T3 and T4 treatments recorded the highest concentration with a significant difference ($P<0.01$) than T1 which recorded the lowest concentration, As for the total protein concentration (100gm/ml), T2, T3 and T4 treatments recorded the highest significant concentration ($P<0.05$) over T1 treatment which recorded the lowest concentration.

Table 4. Effect of adding an aqueous extract of lemongrass at different levels on the immune characteristics of ROSS 308 roosters.

Treatments	mean \pm standard error		
	Albumin (100gm/ ml)	Globulin (100gm/ ml)	Protein (100gm/ ml)
T1	1.246 \pm 0.03 b	2.443 \pm 0.33 b	3.689 \pm 0.35 b
T2	1.950 \pm 0.27 ab	3.843 \pm 0.06 a	5.793 \pm 0.23 a
T3	2.216 \pm 0.29 a	4.043 \pm 0.52 a	6.259 \pm 0.13 a
T4	1.258 \pm 0.18 b	4.533 \pm 0.23 a	5.791 \pm 0.56 a
Significant level	*	**	*
The averages with different letters within the same column differ significantly between them. ** ($P<0.01$).			

The significant improvement that was shown from the results of the study in the level of albumin, globulin and total protein that was shown in the blood serum is due to lemongrass leaves that enhance immunity, anti-inflammatory, anti-bacterial and antibody formation (Ntonga *et al.*, 2014). The increase in the concentrations are indicators of antibodies and total protein in the blood serum showed an increase in protein synthesis and a decrease in protein catabolism (Patterson *et al.*, 1967), the total protein is the sum of albumin and globulin concentrations, and the blood serum is affected by the age, physiological state of the bird, the environment, etc (Altman, 1979; Tumbleson *et al.*, 1976), the percentages of total protein, albumin and globulin contents were within the normal ranges that were reported in birds (Abdul Basit *et al.*, 2020), the results of this study agreed with the results that Accessed by (Olatunji *et al.*, 2015; Alagbe *et al.*, 2018; Oluwafemi *et al.*, 2020).

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