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Mustafa Saleh Khodair
Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil, Iraq

Mohammed KA Altamimi
Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil, Iraq

Emad Abdulgabber Ali
Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil, Iraq

Corresponding Author:
Mustafa Saleh Khodair
Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil, Iraq

Effect of adding different levels of alcoholic extract of soursop leaf powder to drinking water on sex hormones and antioxidant enzymes in male broiler chickens Roos308

Mustafa Saleh Khodair, Mohammed KA Altamimi and Emad Abdulgabber Ali

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Abstract
This experiment was conducted at the poultry research farms of the College of Agriculture, Department of Animal Production, Al-Qasim Green University, from November 1, 2024, to January 28, 2025, to determine the effect of an alcoholic extract of powdered graviola (*Annona muricata*) leaves on sex hormones (testosterone and estrogen) and antioxidant enzymes in Ross 308 broiler chickens. Sixteen Ross 308 roosters were used and randomly assigned to four experimental groups, with four replicates per group (one rooster per replicate). The experimental groups were as follows: Group 1 (T_1)-control group (no supplement); Group 2 (T_2)-alcoholic extract of powdered graviola (*Annona muricata*) leaves added to drinking water at a concentration of 10 ml/L; Group 3 (T_3)-15 ml/L; and Group 4 (T_4)-20 ml/L. The main objective was to evaluate the effect of the alcoholic extract of the graviola plant (*Annona muricata*) on hormone and enzyme properties. The extract was prepared in the laboratories of the College of Science/University of Babylon according to the methods described in the Materials and Methods section. The results showed that treatments T_4 and T_3 significantly improved sex hormone levels, with treatments T_4 and T_3 showing an increase in testosterone and a decrease in estrogen in male broiler breeder hens**.** The fourth treatment also recorded a clear improvement in the concentration of the glutathione enzyme (GSH) compared to the control treatment, and a decrease in the concentration of the MDA enzyme in the fourth treatment compared to the control treatment.

Keywords: Broiler, breeder males, graviola powder, alcoholic extract, Antioxidants, testosterone hormone, estrogen hormone

Introduction
Poultry is one of the most important food sources for humans worldwide, providing high-quality animal protein in the form of meat and eggs. It also contributes to the national economy by enhancing food security and increasing national income (FAO, 2022) [1]. With the growing demand for poultry products, improving bird health and production efficiency has become a key objective in animal research, particularly concerning the reproductive performance of broiler worms, whose hormonal and physiological health directly affects insemination quality and flock efficiency (Adeimi *et al.*, 2021) [2]. A recent trend in improving poultry health is the use of medicinal plants rich in bioactive compounds as natural feed additives. Among these plants, soursop (*Annona muricata* L.) stands out. This tropical plant grows in South America, Africa, and Southeast Asia, and is known for its leaves and other parts, which are rich in flavonoids, acetogenins, alkaloids, and phenols—powerful natural antioxidants (Oulabe *et al.*, 2020; Artawegona *et al.*, 2023) [4, 3]. It also contains a wide range of active ingredients, such as flavonoids, tannins, and phenolic compounds, in addition to acetogenins, which are the main components of the graviola plant, as well as a rich mix of amino acids and minerals (Yang et al., 2015; Mohammed, Abbas 2016) [5, 6]. Graviola is important in providing antioxidants and protecting against bacteria, parasites, and free radicals (Orak et al., 2019; Abbas, Ali 2023) [7, 8]. Recent studies have also shown that graviola has numerous medicinal properties and is beneficial in treating certain types of cancer by extracting different parts of the plant (roots, leaves, fruits) as aqueous or

alcoholic extracts (Maesaroh, Dono 2022) [9]. Therefore, this study aimed to demonstrate the effect of the alcoholic extract of Graviola leaf powder on hormones and enzymes, and to identify the optimal concentrations for use.

Materials and Methods

This study was conducted at the Poultry Research Farms of the Faculty of Agriculture, Department of Animal Production, Al-Qasim Green University, to evaluate the expected effects of adding an alcoholic extract of soursop (*Annona muricata*) leaf powder to the drinking water of Roos 308 broiler chickens on sex hormones (testosterone, estrogen) and antioxidant enzymes (MDA, GSH, CAT). Sixteen Roos 308 roosters, with an average weight of 2.6 kg and an age of 55 weeks, were used in the experiment. The birds were raised in a floor system within cages and then divided into four experimental groups (four replicates per group), with one rooster per replicate. An alcoholic extract of soursop (*Annona muricata*) leaf powder was added to the drinking water daily. In the first group, no alcoholic extract was added. In the second group, 10 ml of the alcoholic extract of soursop leaf powder was added to the drinking water. In the third group, 15 ml of the alcoholic extract of soursop leaf powder was added to the drinking water. In the fourth group, 20 ml of the alcoholic extract of soursop leaf powder was added to the drinking water. A 10 cm layer of coarse wood shavings was used as bedding. Lighting was provided for 14 hours daily, followed by 10 hours of darkness. Feeders were hung at chest level inside each cage, and water was provided via inverted drinkers. Each rooster received 120 g of feed once daily throughout the study period. The feed contained 14.5% crude protein and 2800 kcal/kg (Ross, 2022) [10].

How to make an alcoholic extract of powdered soursop leaves

The alcoholic extract of the leaves of the Graviola plant was prepared at the University of Babylon/College of Science Laboratory according to Ladd *et al.* (1978) [11]. 50 grams of Graviola leaves were placed in a porous bag (thimble) made of strong filter paper, and placed in the incubator E of the Soxhlet apparatus. 250 ml of 90% methanol alcohol was heated in flask A at a temperature of 80 °C, and its vapors condensed in the condenser D. The condensed extract was dripped into the thimble containing the raw plant and extracted by contact. When the liquid level in the incubator rose to the top of the siphon tube C, the liquid contents in the incubator flowed down to flask A. The process continued until not a drop of methanol solvent was left in the siphon tube. The soaking process lasted for approximately 6 hours. One of the advantages of this method compared to other extraction methods is the possibility of extracting a large quantity of the plant with a much smaller quantity of solvent, thus saving time and energy, and therefore economic feasibility and applicability. The crude alcoholic extract was separated by filtration, and the filtered material was then concentrated using a rotary evaporator until it dried in a hot water bath at 40 °C and stored in a refrigerator.

Measuring the levels of sex hormones in blood serum.

In the last week of the experiment, blood samples were drawn from the pterygoid vein using a 5 ml syringe with a blood-drawing needle, and then placed in tubes as described by Al-Daraji *et al.* (2008) [12]. These tubes were then centrifuged at 6000 rpm for 15 minutes to separate the serum from the cellular component, and then the samples were frozen at-20 °C. Measurements were performed at the laboratories of Ghaya Al-Maarefa Company, located in Babylon Governorate, Iraq, where testosterone and estrogen levels were measured using specialized equipment.

Measuring certain antioxidant enzymes present in seminal plasma.

In the final week of the experiment, seminal fluid was collected for seminal plasma separation, followed by measurement of oxidative enzymes: glutathione peptide, malon di aldehyde, and catalase (Deelles *et al.*, 2014) [13]. The measurements were performed at the Advanced Laboratories in Babylon Governorate using a Smart space analyzer manufactured by BioRad, USA. The measurements were conducted according to the manufacturer's instructions and using their specific testing kit.

Table 1: Shows the components of the feed used in feeding males.

Feed Ingredients (%)	Males
Yellow corn	58.8
Soybean meal (48%)	12.7
Wheat bran	22.1
Vegetable oil (sunflower)	2.3
Salt	0.1
Limestone	1.5
Premix	2.5
Total	100
Calculated Chemical Composition	
Crude protein (%)	14.5
Metabolizable energy (kcal/kg feed)	2800

***It consists of methionine 0.29%, lysine 0.61%, choline chloride 0.1%, and sodium bicarbonate 0.3%.

* *The chemical analysis of the feed was calculated according to the UFFDA 2020 program.

* **Energy and protein content were calculated according to Ross 2022 [10] guidelines.

Table 2: Chemical analysis of graviola leaf powder: The chemical composition of the leaves was determined according to the standard method followed, and this analysis was carried out in the Environment and Water Laboratory at the Ministry of Science and Technology in Baghdad.

Material	%
Crude protein	22.56
Moisture	19.50
Ash	3.60
Crude fiber	2.44
Fat	2.40
NFE	55.60

Statistical Analysis

The data were analyzed using a completely randomized design (CRD) to examine the impact of the studied parameters on the various traits. Significant differences between the means were assessed using Duncan's test (1955) [14].

Results and Discussion

Table (3) shows the effect of adding the alcoholic extract of the graviola plant on the sex hormones testosterone and estrogen. With regard to testosterone, the third treatment (T_3) and the fourth treatment (T_4) were significantly superior

to the rest of the treatments ($p \leq 0.01$). The second treatment was also superior to the first treatment. As for estrogen, the results showed a significant difference ($p \leq 0.01$) between the treatments, with the first treatment (T_1) being superior to the second treatment (T_2) and the fourth treatment (T_4). However, there was no significant difference between the first treatment (T_1) and the third treatment (T_3), or between the second treatment (T_2) and the third treatment (T_3).

Table 3: Shows the effect of adding the alcoholic extract of the graviola plant on the sex hormones testosterone and estrogen.

Mean ± Standard Error (%)		Treatment
Estrogen (pg/ml)	Testosterone (ng/ml)	
631.67±4.41 a	15.67±0.20 c	T_1
563.33±14.52 b	19.23±1.92 b	T_2
615.00±18.03 ab	22.70±0.66 a	T_3
486.67±14.52 c	25.10±0.11 a	T_4
**	**	Significance Level
Means with different letters within the same column differ significantly from each other. ** ($p \leq 0.01$).		

The use of an alcoholic extract of the graviola plant, containing a mixture of flavonoids, saponins, and steroids (biologically active compounds), has been shown to enhance testosterone production and thus improve fertility. This is achieved by promoting the expression of steroid proteins necessary for cholesterol to enter mitochondria, which in turn leads to increased testosterone production (Martin and Touaibia, 2020) [15]. The active compounds in graviola contribute to the regulation of sex hormone secretion by controlling the activity of the hypothalamic-pituitary-gonadal axis, leading to increased secretion of gonadotropins (FSH) and luteinizing hormone (LH). Elevated LH levels in male blood increase testosterone levels by stimulating Leydeck cells. This, in turn, inhibits the function of Sertoli cells, which support germ cell development by providing a suitable environment for spermatogenesis and its release into the testicular tubules (Sturkie, 2000) [17]. These results demonstrate the efficacy of Herbal extracts inhibit the aromatase enzyme, which leads to increased testosterone levels in older males and enhances their fertility (Adeldust *et al.*, 2017) [18].

Effect of alcoholic extract of the graviola plant on antioxidant enzymes:

Table (4) shows the effect of adding the alcoholic extract of the graviola plant on the antioxidant enzymes catalase, glutathione peptide and malon di aldehyde. When measuring the catalase enzyme, we find no significant differences between the experimental treatments. As for the glutathione enzyme (GSH), the second treatment (T_2), the third treatment (T_3), and the fourth treatment (T_4) significantly outperformed the first treatment ($p \leq 0.01$). As for the malon di aldehyde compound, the first treatment (T) significantly outperformed the second, third, and fourth treatments ($p \leq 0.01$), and there is no significant difference between the second and third treatments. The second and third treatments outperformed the fourth treatment.

Table 4: Effect of adding different levels of alcoholic extract of soursop on the antioxidant enzymes of male broiler breeder hens.

Mean±Standard Error(%)			Treatment
MDA	Glutathione	Catalase	
32.90±0.36 a	29.33±0.64 b	30.10±0.55	T_1
30.60±0.26 b	32.43±0.54 a	29.96±0.14	T_2
29.90±0.32 b	32.43±1.15 a	30.63±1.44	T_3
25.67±0.17 c	34.70±0.26 a	30.17±0.44	T_4
**	**	N.S	Significance Level
Means with different letters within the same column differ significantly from each other. ** ($p \leq 0.01$). S.N			

Graviola fruit contains compounds and minerals that act as antioxidants, such as iron, phosphorus, zinc, calcium, copper, magnesium, manganese, potassium, selenium, and sodium, as well as phenols, carotenoids, and flavonoids. These substances play a role in reducing reactive oxygen species (ROS) by inhibiting free radical activity and contribute to the donation of hydrogen ions to lipids, thus reducing oxidation. Antioxidant enzymes perform numerous functions in reducing oxidative stress. The enzyme catalase breaks down hydrogen peroxide into water and oxygen. When catalase activity decreases, the concentration of hydrogen peroxide (H_2O_2) increases. Therefore, the enzyme glutathione peroxidase (PX_GSH) converts glutathione with reduced reactive potential to its oxidatively inactive form (GSSG). As a result of this reaction, PX_GSH is converted to its active reduced form by removing a selenium atom. The reducing enzyme then becomes capable of reacting with hydrogen peroxide and other free radicals (Sturty and Alvarez, 1983) [19]. Graviola contains a compound known as quercetin, which belongs to a chemical group that includes compounds that contribute to the activity of antioxidant enzymes, in addition to its potential role in improving stomach function (Nguyen *et al.*, 2020) [21].

Conclusion

The fourth treatment (20 ml) and the third treatment (15 ml) achieved the best results compared to the control treatment. The percentage of testosterone improved, and the percentage of estrogen decreased. As for the antioxidant enzymes, with regard to glutathione (GSH), its concentration improved in the fourth treatment (20 ml) and the third treatment (15 ml). There was also an improvement in the concentration of MDA, as its concentration decreased in the fourth treatment (20 ml) and the third treatment (15 ml) compared to the control treatment.

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