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### Efficiency of the Effect of Adding Anise and Marjoram Powders and their Mixture to the Feeds of Local Male Roosters on Semen Characteristics

<sup>1</sup>Nashwan Majeed Ali Al-Gburi, <sup>2</sup>Mohammed Rasoul Mahdi Jasim

<sup>1</sup>DNA Research Center, University of Babylon, Hillah-Najaf Street, Babylon State, 51001, Iraq

<sup>2</sup>Department of Animal Production, Faculty of Agriculture, University of Al-Qadisiyah, 58002, Iraq

Corresponding Author: **Nashwan Majeed Ali Al-Gburi**

#### Abstract

48 sexually mature males from Iraqi local chickens (24 weeks old) were divided into four treatments (12 males/treatment, 4 males/replicated), the experimental treatments were divided into four treatments, and the percentages of addition were as follows: Control treatment (T1) was fed on a regular feed without any supplement, the second treatment (T2) was fed on a regular feed with 1.5 g/kg of anise seed powder added to it, the third treatment (T3) was fed on a regular feed with 1.5 g/kg of marjoram seed powder added to it, the fourth treatment (T4) was fed on a regular feed with a mixture of anise and marjoram seeds powder added to it, 1.5 gm anise + 1.5 marjoram / kg feed, the study included estimating the quantitative characteristics of semen such as: (ejaculate volume, sperm concentration, compressed sperm volume, semen quality factor, collective motility, individual motility, dead sperm

percentage, deformed sperm percentage), the results of the current study indicated that the addition of anise and marjoram seed powder, individually or synergistically, significantly improved the quantitative characteristics of the semen of local male chickens such as (ejaculate volume, sperm concentration, collective and individual movement of sperm) The mixture also achieved tangible results in reducing the percentage of dead and deformed sperm, it is concluded from the current study that the addition of anise and marjoram powders to the diets of Iraqi local male chickens significantly improved the semen characteristics of males when compared to the control treatment, which leads to raising the reproductive efficiency and productivity of the bird, so it can be introduced as food additives to improve the reproductive efficiency of the bird.

**Keywords:** Anise Seeds, Marjoram Seeds, Male Chickens, Bird's Semen

#### Introduction

Anise plant (*Pimpinella anisum* L) is one of the plants with medicinal and therapeutic effect, this plant belongs to the Apiaceae family (Carrot Family), the Arabic name of the anise plant is derived from the origin of the word anisum, this plant is known by several names, including Nikon, Taqdah, sweet cumin, and the sweet seed, the Middle East is the original home of the plant's growth, appearance description of the plant, it is a herbaceous plant consisting of a stem that is half a meter high and thin with serrated leaves, as for its flowers, they are usually small, oval in shape, which, after maturity, turn into small brown fruits (El-Hady, 2005; Chevallier, 1996) [18, 15]. The seeds and oils are the most used basic component of this plant, as they contain anethole, the active substance from this plant, which acts as sex hormones, especially estrogens, this plant can be used to enhance the function of the digestive system and has an effective role in improving the proportion of feed consumption due to its acceptable taste in addition to containing multiple compounds that act as antioxidants and stimulate immunity (Cabuk *et al.*, 2003; Osman *et al.*, 2005; Muzahim, 2009) [13, 29, 27]. As for marjoram, it is a perennial herbaceous plant whose scientific name is *Origanum vulgare*, which is similar to mint, as the most important active compounds found in the main parts of this plant are thymol and carvicol, which are abundant in flowers and the oil extracted from it, which is used in the manufacture of many food preservatives as an antioxidant, the active compounds in the marjoram plant usually act synergistically, as it has been noted that the marjoram plant has a high ability to enhance the functioning of the immune system (Fotea *et al.*, 2008) [19], and due to the lack of targeted studies on male Iraqi local chickens and the low reproductive capacity of this bird, this study aimed to show the single and synergistic effect of anise and marjoram powders in the diets of local male chickens on the quantitative characteristics of semen.

## Materials and Methods

This study was conducted in one of the fields belonging to chicken breeders in Jableh District - 65 km north of Babylon Governorate / City Center for the purpose of verifying the addition of anise and marjoram powder individually and synergistically to the diets of Iraqi local male chickens in the qualitative characteristics of semen for the period from 2/16/2021 to 3/6/2021. The trial treatments were divided into four treatments as follows:

The first treatment (T1) is a control transaction without any addition.

The second treatment (T2) added anise seed powder to its diet at a rate of 1.5g/kg feed.

The third treatment (T3) added powdered marjoram seeds to its diet at a rate of 1.5g/kg feed.

The fourth treatment (T4) added powdered anise and marjoram seeds to its diet at a rate of 1.5 gm anise + 1.5 marjoram / kg of feed.

In this study, 48 mature male Iraqi domestic chickens, 24 weeks old, were used, these males were placed in a hall dedicated to breeding for the purpose of preparing them to adapt to the hall, the diet, and training to respond to the semen collection process (a two-week period was allocated for the purpose of adapting the birds, and thus the start of the semen collection process will be from the date of 3/3/2021), the birds were fed on a uniform diet as shown in Table 1, the birds were distributed in cages designated for breeding, as they were divided randomly into 4 treatments (12 males for each treatment, 4 males for the repeated) the process of collecting semen from birds took place at 1 o'clock in the afternoon, preceded by a process of cutting off the feed and water, in order to avoid contamination of the collected semen with excrement, followed the method indicated by Al-Daraji (2013) [5] for the purpose of semen collection, as this method is summarized by the presence of two people for the collection process, the first person holding the bird between his thighs and pulling the bird's head back and the collector forward, while the task of the second person carrying out the collection process is limited to massaging the dorsal-abdominal region lightly until the nipple becomes erect and clearly visible, holding the collection tube with his second hand, after completing the collection process with special tubes, the semen is transferred to a cork container in order to avoid the influence of sunlight when moving from the experimental field to the specialized laboratory for the purpose of studying the characteristics under study, according to the average volume of the ejaculate immediately after the completion of the collection process for each male separately after weighing the tubes while they are empty and then weighing them after collection, as the difference in weight represents the volume of the ejecta, as for the individual and group motility of sperm, it was estimated in the laboratory according to the method of Al-Daraji (2013) [5]. As a drop of semen is placed on a warm glass slide, the movement is estimated using a microscope with 40x magnification according to a scale of evaluation prepared for measurement, ranging from zero to percent, as for the concentration of sperms only by using a blood cell counting slide, as the sperms are counted in five squares according to the method of Allen and Champion (1955) [10], while the percentage of dead sperm was calculated using a mixture of

two dyes eosin-necrocin, where a drop of semen is placed on a glass slide, then a drop of the dye is placed on the same slide and mixed by the tip of another glass slide according to the method of Lake and Sterwart (1978) [24], as for the percentage of deformed sperm, it was estimated according to the method reported by Al-Daraji and his group (2002) [3], using a mixture of Eosin bluish-fast green. As for the volume of compressed sperm, it was calculated according to the method reported by Al-Daraji (2002) [3] using capillary tubes, which were filled with three-quarters of their volume and placed in the special device, and then read by a special ruler prepared for this purpose. The data of the study were analyzed statistically by the SPSS program using the complete random design (C.R.D) to show the effect of different treatments on the studied traits, and for the purpose of obtaining significant differences between the averages of the studied traits, Duncan (1955) [17] multinomial test was used.

**Table 1:** Composition of the diet used in feeding birds

Fodder material	Usage rate
yellow corn	60
Barley	7
Soybean meal (40% protein)	23
limestone	6.7
table salt	0.3
Mixture of vitamins and minerals	3
the total	100
Chemical composition	
Crude protein	16
Representative energy (kWh/kg suspension)	2708
Lysine	0.75
Methionine	0.36
Methionine and cysteine	0.64
Calcium	3.36
Available phosphorous	0.41

\*The values of the chemical composition of the fodder materials included in the composition of the feed were calculated according to the reports of the US National Research Council (N.R.C., 1994). (Al-Daraji and Razuki, 2012).

## Results and Discussion

The results shown in Table (2) show the effect of adding different levels of anise and marjoram powders and the synergy between them on the average ejection volume (ml), as it is noted from the presented results that the treatments T4 and T3 achieved a highly significant superiority ( $P \leq 0.01$ ) in the average ejection volume for durations 1 and 2 compared to the rest of the coefficients of the experiment, during period 3, treatment T4 recorded a significant ( $P \leq 0.05$ ) superiority over the rest of the study treatments, while in period 4 a highly significant superiority ( $P \leq 0.01$ ) was observed for treatment T4 over treatments T2 and T1, as for the sperm concentration rate, the addition treatment T4 recorded a significant superiority for periods 1 and 2, while the treatment T3 recorded a significant superiority for the period 3, while no treatment recorded any significant improvement for the 4th period of the study, the volume of compressed sperms had a clear effect for the T4 synergistic mixture treatment for periods 1, 2 and 3, while no treatment recorded a significant superiority between its averages for the 4 periods of the experiment for the volume of compressed sperm.

**Table 2:** The effect of adding powdered anise and marjoram seeds and their mixture to the diets of Iraqi local male chickens on the average ejaculate volume (ml), sperm concentration (cm<sup>3</sup>), and compressed sperm volume (%) (Average± standard error)

Treatments	Ejaculate volume (ml)				Sperm concentration (cm <sup>3</sup> )				Compressed sperm volume			
	1	2	3	4	1	2	3	4	1	2	3	4
T1	0.42±0.001 <sup>b</sup>	0.38±0.01 <sup>b</sup>	0.46±0.00 <sup>c</sup>	0.55±0.00 <sup>b</sup>	1.44±1.01 <sup>b</sup>	1.48±0.14 <sup>c</sup>	1.69±0.55 <sup>c</sup>	1.73±1.11 <sup>b</sup>	10.83±0.22 <sup>ab</sup>	11.22±1.32 <sup>b</sup>	13.22±1.43 <sup>b</sup>	14.27±1.66 <sup>c</sup>
T2	0.41±0.001 <sup>b</sup>	0.38±0.01 <sup>b</sup>	0.47±0.01 <sup>c</sup>	0.53±0.01 <sup>b</sup>	1.44±0.24 <sup>b</sup>	1.65±0.22 <sup>b</sup>	1.96±0.52 <sup>b</sup>	1.72±1.23 <sup>b</sup>	10.88±1.10 <sup>a</sup>	11.78±1.54 <sup>b</sup>	13.88±1.25 <sup>b</sup>	14.11±1.45 <sup>b</sup>
T3	0.52±0.00 <sup>a</sup>	0.43±0.00 <sup>a</sup>	0.51±0.01 <sup>b</sup>	0.58±0.00 <sup>ab</sup>	1.67±0.11 <sup>a</sup>	2.01±1.23 <sup>ab</sup>	2.14±1.32 <sup>a</sup>	1.73±1.44 <sup>b</sup>	10.83±1.14 <sup>ab</sup>	13.14±1.57 <sup>ab</sup>	14.07±1.11 <sup>ab</sup>	14.55±1.25 <sup>a</sup>
T4	0.58±0.001 <sup>a</sup>	0.48±0.01 <sup>a</sup>	0.55±0.01 <sup>a</sup>	0.63±0.01 <sup>a</sup>	1.73±1.71 <sup>a</sup>	2.11±0.87 <sup>a</sup>	2.00±1.97 <sup>ab</sup>	1.73±1.13 <sup>b</sup>	10.92±1.22 <sup>a</sup>	13.85±2.97 <sup>a</sup>	14.55±2.41 <sup>a</sup>	14.66±1.85 <sup>a</sup>
Significance level	0.01	0.01	0.05	0.01	0.05	0.01	0.01	N.S	0.05	0.05	0.05	N.S

\*The different letters within one column indicate significant differences between the averages of the treatments at the level of significance (P≤0.01) and (P≤0.05), N.S is not significant, the numbers from 1 to 4 indicate the study periods (each number represents a period of three weeks), the treatments T1, the first treatment (control), T2, the second treatment, was fed on a diet with 1.5 g/kg of anise seed powder added, T3, the third treatment, was fed on a diet with 1.5 g/kg of marjoram seed powder added, T4 The fourth treatment was fed on an added diet To it 1.5 gm anise seed powder + 1.5 gm marjoram seed powder / kg feed.

As for the results presented in Table (3), the effect of the treatment on both the semen quality factor, the collective and individual movement of the sperm, during periods 1, 2 and 3, the averages of the semen quality factor recorded a clear superiority when compared with the rest of the experimental treatments, while no treatment recorded any over the course of 4, as for the mass movement of sperm, treatment T4 recorded a highly significant (P≤0.01) superiority over the rest of the experimental treatments during the first period, as for periods 2 and 4, the addition

treatments T4 and T3 recorded a significant superiority (P≤0.05) over the rest of the treatments, while the treatment recorded T2 clear superiority during the period 3, as for the individual movement estimate, treatment T4 recorded a clear superiority during the first period when compared with the experimental treatments, while it was noticed during periods 2 and 4 that there was no effect of the additive, and for the period 3, treatments T4, T2 and T1 recorded superiority compared to treatment T3.

**Table 3:** Effect of adding powdered anise and marjoram seeds and their mixture to the diets of Iraqi local male chickens on semen quality factor, Collective and individual movement ratio (%), (average ± standard error)

Treatments	Semen quality factor				Collective movement %				Individual movement %			
	1	2	3	4	1	2	3	4	1	2	3	4
T1	1.36±0.12 <sup>a</sup>	0.97±0.48 <sup>d</sup>	1.56±1.22 <sup>c</sup>	1.78±1.10 <sup>a</sup>	63.22±1.33 <sup>d</sup>	73.25±1.66 <sup>b</sup>	83.22±1.44 <sup>b</sup>	80.32±2.14 <sup>b</sup>	70.12±1.11 <sup>d</sup>	86.20±1.22 <sup>a</sup>	93.14±1.18 <sup>a</sup>	88.55±2.23 <sup>a</sup>
T2	1.12±0.44 <sup>c</sup>	1.33±0.95 <sup>c</sup>	1.58±1.12 <sup>c</sup>	1.76±1.14 <sup>a</sup>	65.33±1.44 <sup>c</sup>	73.14±1.96 <sup>b</sup>	88.21±1.20 <sup>a</sup>	82.69±1.4 <sup>b</sup>	72.14±2.04 <sup>c</sup>	82.14±1.02 <sup>a</sup>	91.01±2.10 <sup>a</sup>	86.14±1.24 <sup>a</sup>
T3	1.66±1.11 <sup>b</sup>	1.67±1.11 <sup>b</sup>	1.92±0.77 <sup>b</sup>	1.80±1.25 <sup>a</sup>	70.45±1.99 <sup>b</sup>	75.91±1.30 <sup>a</sup>	78.33±1.01 <sup>b</sup>	86.33±2.66 <sup>a</sup>	77.21±2.11 <sup>b</sup>	83.47±2.54 <sup>a</sup>	78.14±1.22 <sup>b</sup>	88.77±2.13 <sup>a</sup>
T4	1.97±1.14 <sup>a</sup>	1.88±1.01 <sup>a</sup>	2.11±1.74 <sup>a</sup>	1.80±1.47 <sup>a</sup>	75.65±2.71 <sup>a</sup>	78.22±2.14 <sup>a</sup>	79.61±2.04 <sup>b</sup>	88.36±2.98 <sup>a</sup>	81.14±0.14 <sup>a</sup>	83.47±2.03 <sup>a</sup>	83.01±1.66 <sup>a</sup>	88.69±2.97 <sup>a</sup>
Significance level	0.01	0.01	0.05	N.S	0.01	0.05	0.05	0.05	0.01	N.S	0.01	N.S

\*The different letters within one column indicate significant differences between the averages of the treatments at the level of significance (P≤0.01) and (P≤0.05), N.S is not significant, the numbers from 1 to 4 refer to the study periods (each number represents a period of three weeks), treatments T1, the first treatment (control), T2, the second treatment, fed on a diet with 1.5 g/kg of anise seed powder added, T3, the third treatment, fed on a diet with 1.5 g/kg of marjoram seed powder added, T4 The fourth treatment was fed on a diet Added to it 1.5 gm of anise seed powder + 1.5 gm of marjoram seed powder / kg of feed.

The results presented in Table No. (4) show the effect of adding the experimental treatments on the percentage of dead and deformed sperms, as it is noted that the percentage of dead sperms recorded a clear decrease when compared with the control treatment and for all study periods except for the first period, no treatment recorded any decrease in

this percentage, while the percentage of deformed sperms did not record any decrease in the experimental treatments for periods 1 and 2, while the addition treatments recorded a clear decrease for periods 3 and 4, in sequence, when compared with the control treatment.

**Table 4:** Effect of adding powdered anise and marjoram seeds and their mixture to the diets of Iraqi local male chickens on the percentage of dead and deformed sperm (%), (average ± standard error)

Treatments	%Dead sperm percentage				Deformed sperm percentage			
	1	2	3	4	1	2	3	4
T1	8.11±1.22 <sup>a</sup>	12.24±0.36 <sup>a</sup>	10.11±0.44 <sup>a</sup>	11.22±2.06 <sup>a</sup>	15.01±2.85 <sup>a</sup>	13.44±2.06 <sup>a</sup>	12.36±1.04 <sup>a</sup>	10.47±2.09 <sup>b</sup>
T2	9.22±1.11 <sup>a</sup>	10.98±2.18 <sup>b</sup>	10.0±1.66 <sup>a</sup>	8.74±2.33 <sup>b</sup>	15.11±1.22 <sup>a</sup>	13.66±2.08 <sup>a</sup>	11.88±1.03 <sup>a</sup>	12.44±1.02 <sup>a</sup>
T3	9.44±2.01 <sup>a</sup>	10.80±0.98 <sup>b</sup>	8.47±2.03 <sup>b</sup>	8.32±2.51 <sup>b</sup>	15.04±2.33 <sup>a</sup>	12.39±2.44 <sup>a</sup>	10.66±1.22 <sup>b</sup>	8.25±1.11 <sup>c</sup>
T4	9.78±2.14 <sup>a</sup>	8.44±1.66 <sup>c</sup>	8.79±1.91 <sup>b</sup>	8.16±1.02 <sup>b</sup>	14.77±2.55 <sup>a</sup>	12.66±2.14 <sup>a</sup>	8.16±1.11 <sup>c</sup>	8.55±2.08 <sup>c</sup>
Significance level	N.S	0.05	0.01	0.01	N.S	N.S	0.01	0.01

\*The different letters within one column indicate significant differences between the averages of the treatments at the level of significance (P≤0.01) and (P≤0.05), N.S is not significant, the numbers from 1 to 4 indicate the study periods (each number represents a period of three weeks), the treatments T1, the first treatment (control), T2, the second treatment, was fed on a diet with 1.5 g/kg of anise seed powder added, T3, the third treatment, was fed on a diet with 1.5 g/kg of marjoram seed powder added, T4 The fourth treatment was fed on an added diet To it 1.5 gm anise seed powder + 1.5 gm marjoram seed powder / kg feed.

The low indicators of reproductive efficiency of male Iraqi local chickens is one of the main reasons why hybrid breeds of Iraqi local chickens did not develop, as the fertility of the chicken flocks depends mainly on the fertility of the mothers flocks, in addition, studies related to this aspect (Iraqi local chicken) are very few, so most scientific studies resorted to

the use of medicinal herbs and plants and their products such as seeds and oils as food additives, the general aim of which is to improve the productive, physiological and reproductive performance of the bird (Al-Gburi, 2017; Ali and Rebh, 2020 [7], Al-Gburi and Mahdee, 2022 [6]). The results of the current study, which show the significant

improvement in the morphological characteristics of the semen of male Iraqi local chickens, may be attributed to the fact that most of the herbal food additives work to improve the reproductive performance of the birds because they contain effective compounds that are reflected in the outcome of their final effect on the physiological and reproductive performance of the bird (Mahmood *et al.* 2014; Mohammed 2019) [25, 26]. The improvement achieved in the second treatment, T2, which added 1.5 gm of anise seed powder to its diet, indicated that one of the main components in anise is the anethole compound. This compound has a growth stimulating effect through its effect on the activity of the auxiliary glands, which interfere directly or indirectly in the digestion process in the digestive system of birds, in addition to its effective and important role in the oxidation process and curbing the formation of free radicals, so the effect may have been to reduce the formation of free radicals and lipid peroxide, because the sperm of birds has a very strong activity, so it needs an additional amount of energy for the purpose of continuing its activity, and this explains the result obtained for the second treatment (Tucker, 2002; Cabuk *et al.*, 2006; Al-Kassie, 2008) [31, 14, 9]. As Barakat and his group (2016) [12] showed that the use of 0.25 g of anise seed powder / kg of feed significantly improved the effectiveness of antioxidants due to the presence of anethole compound as an active antioxidant by 34.5%, in addition to an increase in the activity of glutathione in the blood and a decrease in the MDA index, the high of which is a dangerous indicator of high fat oxidation and the formation of free radicals. In addition, the sugars present in plant composition may be used directly for energy production and the sustainability of sperm vitality (Kang and his group, 2013; Jamshidzadeh and his group, 2015) [22, 21]. As for the improvement achieved in the third treatment, T3, in which 1.5 gm of marjoram seed powder was added to its diet, this may be due to the fact that this plant is very rich in many minerals and vitamins, especially vitamin E, which is a very effective antioxidant, in addition, marjoram contains flavonoids, glycosides, terpenes, thymol compounds, and cavaquirol, as it is known that these compounds have an important role in preserving the animal cell from any destructive effect of free radicals formed due to lipid peroxidation (Govanis and his group, 2010; Krystyna and ogonowski, 2010) [20, 23]. As Aiyelaagbe and Osamudiamen (2009) [1] indicated that the flavonoids found in marjoram are the most important effective chemical compound that contribute to limiting the formation of free radicals, thus reducing the risk of fat oxidation within the living cell, as Kia and his group (2016) [16] indicated in a study conducted to determine the effect of adding marjoram seed extract to the semen diluents of Holstein bulls stored in freeze storage, that the addition of 4 ml / dL and 8 ml / dL significantly improved sperm vitality and movement, as well as the integrity of the sperm membrane compared to the control group. In addition to a significant decrease in the concentration of MDA, an increase in the activity of the enzyme catalase and glutathione peroxidase, and a decrease in the activity of ROS, the main cause of oxidation of phospholipids and lipid peroxidation, which causes damage to the membrane and the external structure of the sperm, and the existing phenolic compounds have the ability to bind the hydroxyl group and the metal ion and terminate the activity of free radicals (Ashrafi *et al.*, 2013) [11], as for the results shown in the aforementioned tables for treatment T4, whose

birds were fed on a diet containing 1.5 gm of anise seed powder + 1.5 gm of marjoram seed powder / kg of feed, the cause may be attributed to the synergistic effect of the two powders, which results in its final outcome improving the reproductive condition of the bird.

### Conclusions

Based on the results obtained from the current study, represented by the improvement in the morphological characteristics of semen, we conclude that the addition of anise and marjoram seed powder and the mixture between them has played an important role in improving the collective and individual movement of sperm, as well as improving most of the studied characteristics, and therefore it can be used as food additives that contribute to improving the reproductive performance of the bird.

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