

Effect of Adding Anise and Marjoram Seed Powders and Their Mixtures to Iraqi Local Chicken Male Diets on Seminal Plasma Traits

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Abstract

This study was conducted to determine the effectiveness of three levels of anise and marjoram seed powders and their mixture added to the diets of male Iraqi local chickens in the traits of sperm plasma, the males used in the trial were divided into four treatments (12 male per treatment) and by three replicates per treatment (four males per repeater) as the division of the trial treatments was as follows: the first treatment T1 (a control treatment fed on a basic diet without any addition), the second treatment T2 was fed on a basic diet added to 1.5 g of anise seed powder/kg feed, third treatment T3 fed on a base diet with 1.5 g of marjoram seed powder/kg feed, the fourth treatment T4 fed on a base diet with 1.5 g of anise seed powder + 1.5 g of marjoram seed powder/kg feed, the study period lasted 3 months from 16/2/2021 to 3/6/2021 (a period of two weeks was allocated for the purpose of preparing birds and responding to the collection process), collecting semen with special collection tubes and transferring it directly to the centrifuge (borrowed from a private laboratory) for the purpose of obtaining sperm plasma, then the sperm plasma was transferred to a specialized laboratory for the purpose of studying the traits that included the concentration of (total cholesterol, total protein, triglycerides, MDA enzyme activity, SOD enzyme activity, GSH-px), these traits were calculated every 22 days. The results obtained from the current study show the effective effect of anise and marjoram in reducing the concentration of total protein, cholesterol, and MDA activity, while the effect was evident in the activity of SOD and GSH-px enzymes in sperm plasma.

Keywords: anise seeds, marjoram seeds, male chickens, bird's semen.

Introduction: Sperm are characterized by their high content of long-chain unsaturated fatty acids, the presence of these acids makes the sperm more vulnerable to oxidative damage that may affect it, whether inside or outside the living body (freezing processes of semen), which leads to the aging of the sperm and the shortening of its life period, and any side effect can cause changes in the environment of the sperm, especially the peripheral body, and its loss of mobility, in addition to the loss of fertility capacity and low reproductive efficiency of the bird (13), the factors that were mentioned prompted researchers to find food additives added to

bird diets with the aim of reducing oxidative damage that may affect sperm and preserving it as much as possible, of these additives, which are widely used is anise, anise is one of the aromatic plants that grow in many countries of the world, the oil extracted from the seeds of the anise plant contains many active compounds such as anethol, methylchavicol methylcaffecul, ecugenol, estragole and other substances that have an antibacterial activity, antioxidant activity as well as its general stimulating properties for the immune system (24, 18), anise can also be used in human food as a delicacy, enhancer of digestion and enhanced digestive function due

to its delicious and spicy taste (22), and contains furano-comarins, flavonoid glycosides, fatty acids, phytoestrogens, starch, protein, choline, mucilage, B vitamins, calcium, magnesium, potassium and iron (21, 16). As for marjoram is a herbaceous plant known scientifically as *Origanum Vulgar* similar to mint is limited to content many minerals, vitamins and glycosides in addition to plant estrogens as well as thymol and cavacrol, the part used by its leaves, flowers and oil (1), and contains phenolic compounds, gallic acid, polyphenols, rosmarinic acid and quercetin as rosmarin and quercetin are the two most important compounds found in the plant have antioxidant properties (15), and due to the lack of targeted studies of male Iraqi local chickens and the importance of anise and marjoram in poultry nutrition and their clear physiological effects, the present study resorted to evaluating the effectiveness of anise and marjoram on the biochemical traits of sperm plasma.

Materials and Methods:

This study was conducted for the purpose of knowing the effect of adding anise and marjoram seed powder individually and attributable to the diets of male Iraqi local chickens on sperm plasma traits, the study continued from 16/2/2021 to 3/6/2021 (the period from 16/2 to 1/3, which amounted to two weeks allocated for the purpose of preparing birds for the hall and their response to training for the purpose of collecting semen), the study treatments were divided into four treatments and by three replicates for each treatment (12 birds per treatment / 4 birds per repeater) and the addition ratios were distributed as follows: (The first treatment T1 is a control treatment without any addition, the second treatment T2 is added to its diet 1.5 of anise seed powder / kg of feed, the third treatment T3 is added to its diet 1.5 g of marjoram seed powder / kg of fodder, the fourth treatment T4 is added to its diet 1.5 g anise powder + 1.5 g marjoram powder / kg feed), the process of collecting the fluid was

carried out at the end of every 22 days of the study period of 95 days by taking two males from each repeater / six males for each treatment, the collection process was carried out after one o'clock in the afternoon preceded by a cutting of water and feed to ensure that the collected semen is not contaminated with waste and feces, I followed the method referred to by (4) for the purpose of collecting semen, as this method is summarized in the presence of two people for the first collection process that holds the bird between its thighs and pulls the bird's head back And the complex is forward, while the task of the second person in charge of the collection process is limited to massaging the dorsal abdominal region lightly until the nipple is erect and protrudes clearly, holding with his second hand the collection tube, after completing the process, the semen is diluted with a diluted solution Normal saline 9% and transferred to the centrifuge directly for the purpose of obtaining sperm plasma and conducting study tests, which included estimating total cholesterol according to the instructions of the manufacturer of the kit, according to the method indicated by (9), which depends on the interaction of cholesterol with ferric chloride and sulfuric acid to give a pink color, the protein concentration was estimated according to the method indicated by (26), the estimation of the effectiveness of the SOD enzyme was measured using a measurement kit produced by GMBH according to the method indicated by (14), the enzyme (GSH-px) was estimated according to the method mentioned by (25), while the MDA concentration was estimated According to the (23) method, which is based on the estimation of the value of thiobarbitric acid, the determination of triglyceride followed the instructions supplied with the Kit manufactured by Randox and according to the method of (10), study data were analyzed statistically by SPSS 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 using (8) polynomial test.

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 (20). (5).

Results and Discussion:

The results presented in Table 2 show the effect of treatment with anise and marjoram and their mixture on the diets of Iraqi domestic chicken roosters, as it is clear from the table that the concentration of cholesterol (Mg/dl) achieved a significant decrease ($P \leq 0.01$) in the treatment of addition T4 (1.5 g of anise seed powder + 1.5 g of marjoram seed powder) and the treatment of addition T3 (1.5 g of marjoram seed powder) when compared with the treatment of addition T2 (1.5 g of anise seed powder) and control treatment T1 and for breeding periods First, third and fourth, while during the second

period of rearing, the T4 treatment achieved a significant decrease ($P \leq 0.01$) when compared with the rest of the trial treatments, as for the protein concentration (Mg/dl), the T4 treatment achieved a high significant decrease ($P \leq 0.01$) when compared with the rest of the trial parameters and for all trial periods in sequence, while the results presented in the same table show the concentration of triglycerides (Mmol/l) in the seminal plasma, as the T3 treatment recorded a high significance superiority ($P \leq 0.01$) over the rest of the trial parameters during the first period, while the T4 treatment recorded a high significance superiority ($P \leq 0.01$) over the rest of the trial treatments during the second period, and during the third and fourth periods, the T4 and T3 treatments achieved a significant high superiority ($P \leq 0.01$) over the T2 and T1 treatments respectively.

Table 2: Effect of adding anise and marjoram seed powder and their mixture to local male chicken diets on some sperm plasma chemical properties (average± standard error).

Studied traits / Study periods	Treatments	Cholesterol concentration Mg/dl	Protein concentration Mg/dl	Triglyceride concentration Mmol/l
First period	T1	28.33±1.05 a	3.22±0.88 a	1.23±0.83 c
	T2	28.08±2.13 a	2.14±1.22 b	1.25±1.03 c
	T3	27.33±1.22 b	2.04±1.01 b	2.04±1.33 a
	T4	26.41±1.08 b	1.98±0.83 c	1.95±1.22 b
significance level		**	**	**
Second period	T1	26.22±2.07 a	2.44±1.98 a	2.43±1.22 c
	T2	25.38±1.18 b	2.14±1.33 b	2.45±0.91 c
	T3	25.02±1.41 b	1.88±1.14 c	2.83±1.93 b
	T4	24.81±2.01 c	1.22±0.66 d	3.01±1.02 a
significance level		**	**	**
Third period	T1	21.33±2.03 a	2.98±1.65 a	2.65±1.41 d
	T2	21.04±2.33 a	2.43±0.88 b	2.74±1.99 c
	T3	20.35±2.66 b	2.01±1.03 c	2.80±1.93 b
	T4	20.20±2.44 b	1.33±1.06 d	3.12±1.55 a
significance level		*	**	**
Fourth period	T1	18.92±1.22 a	1.98±0.87 a	3.11±2.04 d
	T2	18.33±2.01 a	1.63±1.02 b	3.44±1.04 c
	T3	16.33±1.02 b	1.44±0.78 c	3.58±1.55 b
	T4	16.04±1.88 b	1.14±0.88 d	3.88±1.95 a
significance level		**	**	**

* The different letters within one column indicate that there are significant differences between the trial treatments and each month.

*Each of the four periods represents a period of 22 days, the treatments T1, T2, T3, T4 ate a control diet with anise and marjoram added in proportions (0, 1.5 g / kg anise seed powder feed, 1.5 g / kg marjoram seed powder feed, 1.5 + 1.5 g / kg mixed powder feed). (**) Represents the differences between the treatments of the trial at the level of significance $P \leq 0.01$. (*) Represents significant differences between the treatments of the trial at the level of significance $P \leq 0.05$.

The results presented in Table 3, which show the effect of adding anise and marjoram

powders and their mixtures to the diets of Iraqi local chicken roosters on the concentrations of some oxidation enzymes, it is clear from the table that the treatment with powder and mixture T2, T3 and T4 had the clear effect of reducing the activity of MDA concentration ($\mu\text{mol/ml}$) when comparing these treatments with the control treatment T1, which was fed on a normal diet without any addition, while the additive treatments T4, T3 and T2 and the additive anise and marjoram seed powder and their mixture in equal proportions recorded a clear activity and a significant superiority of $P \leq 0.05$ in raising the concentrations of oxidation enzymes represented by SOD and

GSH-px and sequentially when compared with the control treatment T1.

Table 3: Effect of adding anise and marjoram seed powder and their mixtures to the diets of local male chickens on some oxidation enzymes in sperm plasma (mean \pm standard error).

Studied traits / Study periods	Treatments	MDA Mmol/ml	SOD IU/ml	GSH-px IU/ml
First period	T1	2.44 \pm 1.63 a	1.78 \pm 0.012 d	0.78 \pm 0.01 d
	T2	2.14 \pm 1.88 b	2.13 \pm 0.023 c	0.92 \pm 0.023 c
	T3	1.90 \pm 1.53 c	2.84 \pm 0.018 b	1.23 \pm 0.054 b
	T4	1.44 \pm 0.97 d	3.02 \pm 0.35 a	1.48 \pm 0.065 a
Significance level		*	*	*
Second period	T1	1.58 \pm 1.01 a	2.85 \pm 1.01 d	1.18 \pm 0.92 d
	T2	1.47 \pm 1.02 b	3.12 \pm 0.85 c	1.32 \pm 1.02 c
	T3	1.33 \pm 1.0 c	3.54 \pm 0.045 b	1.44 \pm 0.82 b
	T4	1.14 \pm 0.97 d	3.82 \pm 0.11 a	1.59 \pm 1.14 a
Significance level		*	*	*
Third period	T1	1.76 \pm 0.08 a	2.92 \pm 1.01 d	0.82 \pm 0.88 d
	T2	1.58 \pm 0.95 b	3.44 \pm 0.036 c	0.98 \pm 1.04 c
	T3	1.45 \pm 0.09 c	3.78 \pm 0.034 b	1.28 \pm 1.22 b
	T4	1.22 \pm 0.12 d	4.02 \pm 0.025 a	1.36 \pm 1.18 a
Significance level		*	*	*
Fourth period	T1	1.82 \pm 1.06 a	3.55 \pm 1.12 d	0.87 \pm 0.75 d
	T2	1.60 \pm 1.07 b	3.96 \pm 0.95 c	0.90 \pm 0.96 c
	T3	1.30 \pm 1.02 c	4.25 \pm 1.05 b	1.36 \pm 1.05 b
	T4	1.12 \pm 1.08 d	4.88 \pm 0.048 a	1.68 \pm 0.88 a
Significance level		*	*	*

* The different letters within one column indicate that there are significant differences between the trial treatments and each month
 Each of the four periods represents a period of 22 days, the treatments T1, T2, T3, T4 ate a control diet with anise and marjoram added in proportions (0, 1.5 g / kg anise seed powder feed, 1.5 g / kg marjoram seed powder feed, 1.5 + 1.5 g / kg mixed powder feed). () Represents significant differences between the treatments of the trial at the level of significance $P \leq 0.05$.

The results shown in Tables 1 and 2, which represent a decrease in some criteria and indicators that indicate the effect of the general structure of the sperm, such as the

decrease in the concentration of cholesterol and protein and the activity of the enzyme malondialdehyde, may be explained because the addition of anise and marjoram to the diets of male Iraqi domestic chickens has significantly improved the reproductive performance of the bird in general, especially that the high concentration of cholesterol in the seminal plasma gives clear evidence of an increase in the proportion of dead and deformed sperm, which causes an increase in the concentration of cholesterol (3), this may explain why cholesterol and protein are low because anise and marjoram possess active substances such as anethole and polyphenol in anise (11), which is considered one of the most important natural substances that have antioxidant

activity, especially since there is a clear relationship between the increase in sperm activity and vitality on the one hand and the decrease in fat peroxide indicators and low cholesterol and protein on the other hand, as the more sperm activity requires energy for the sustainability of its vitality and movement, as well as the decrease may give clear evidence of a decrease in the percentage of dead and deformed sperm (4), so there is a link between the increase in the activity of glutathione peroxidase and SOD and the decrease in MDA activity, as the rise of the latter is a clear indication of lipid peroxide in the living cell and the formation of free radicals, which causes damage to the plasma membrane forming the sperm, the results presented in Table 2 show a decrease in MDA activity in the treatments added to their diets anise and marjoram powders containing polyphenol, which is an antioxidant (12), antioxidants such as phenolic compounds, flavonoids, rosmarinic acid, quercetin and campherol found in marjoram are instrumental in inhibiting the formation of free radicals as they bind the hydroxyl group to the metal ion and prevent its formation (6), this gives a clear reason to explain the results obtained in the treatment of marjoram, previous studies have shown that there is a relationship between oxidation indicators and decreased sperm motility, as the formation of free radicals and increased fat peroxide activity limits sperm activity due to the damage to the sperm cell due to free radicals that can penetrate through the membrane of the sperm cell, causing inhibition of the enzyme glucose-6- phosphate dehydrogenase (2), this enzyme controls the pathways of reactions within the living glucose cell and the activity of NADPH, which has a major role in ATP energy production and sperm motility, therefore, the decrease in the concentration of naturally occurring antioxidants in sperm plasma increases oxidative stress that causes damage to the sperm, especially since bird sperm is characterized by its vitality and high activity (19), another reason that gives accuracy to the

results obtained in the current study is that the concentration of phenolic substances in general increases the integrity of the cell membrane by preventing the arrival of harmful molecules into the living cell, which can damage the stability of the cell membrane (17) ‘SOD and GSH-px enzymes are biological antioxidants found naturally in the body and in high-activity living cells, as they synthesize free radicals and protect the living cell from oxidative stress, especially sperm cells (7).

Conclusions: Based on the results obtained from the current study, which is the improvement of the morphological traits of the 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 qualitative traits of the sperm plasma, which requires adding them to poultry diets as well as improving most of the studied traits and thus can be used as food additives that contribute to improving the reproductive performance of the bird.

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