



*Original Article*

## Evaluation of Marjoram Leaves (*Oregano vulgar*) as Feed Supplement on Quality of Semen in Awasian Pollination Rams

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Received 14 April 2022; Accepted 26 May 2022

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### Abstract

This study aimed to evaluate the effect of Marjoram leaves (*Oregano vulgar*) as a feed supplement on sexual efficiency and semen quality in Awasian pollination rams. The study was conducted on ten rams in one of the private fields in the Babylon governorate. The rams were divided randomly into two groups. First group rams are regarded as a control group (group A) that feeds on exceptional concentric food for pollination rams. The second group consists of 5 rams (group B) provided with the same concentric food plus a supplement of 3 mg/kg of body weight of *Oregano vulgar* leaves (fresh) 3 times daily for 49 days before the pollination season (April and May). Semen samples were collected at the end of 49 days by artificial vagina to evaluate the volume of ejaculate, sperm number, concentration, motility, viability, and deformities, and to measure the sperm pleomorphic parameters of the head, nucleus, and acrosome. The antioxidant status of seminal plasma was evaluated by measuring superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC). ELISA tests were used on blood serum samples to measure the hormone levels of luteinizing hormone (LH), testosterone, and estrogen. The study demonstrated a significant increase in motility, the viability of sperm, and decreasing deformities. There was no significant variation in perimorphic parameters of the head, nucleus, and acrosome sperm after using the fresh leaves of *Oregano vulgar* as supplementation to Awasian sheep. However, there was an increase in the antioxidant enzymes (SOD, GPX, and TAC) in seminal plasma and an increase in LH, testosterone, and estrogen in the blood serum of experimental group B. These findings show that using fresh leaves of *Oregano vulgar* improved the sexual efficiency of pollinated rams and maintained the physiology and perimorphic parameters of semen and sperm.

**Keywords:** Marjoram leaves, Semen quality, Pollination, Awasian rams

### 1. Introduction

*Oregano vulgar* is an aromatic flowering plant in the mint family Lamiaceae that grows in large parts of the world and Iraq. It contains secondary metabolites beneficial for animal reproduction and growth characteristics (1). *Oregano vulgar* contains phenolic compounds and essential oils, characterized by its antioxidant characteristics (2). In addition, it contains thymol as an antibacterial for gram-negative and gram-

positive bacteria, so it is considered an anti-inflammatory for pulmonary inflammation.

It was found that consuming *Oregano vulgar* leaves increased total protein and glucose in the blood. The US Ministry issued that every 100 g of the herb's leaves contains 271 calories, 7.04 fats, 60.56 carbohydrates, 12.66 proteins, and 40.3 fibers (3). Therefore, the consumption of this herb protects the animals from bloating. *Oregano vulgar* has an anti-pathogenic effect

and is considered high nutritional value (4, 5). The leaves of this herb contain about 60 compounds, the most important of which is carvacrol thymol, at about 80%. It included P-Cymene,  $\gamma$ -terpinene, Caryophytene, and B-fenchyl alcohol (3).

Previous studies have found that consumption of the herb by mice and rabbits influences sex hormones, increases sexual desire, and affects the implantation of the fetus. It is considered an active uterine stimulant (6). The importance of marjoram extract in improving the blood film characteristics and enhancing immunity in Awassian sheep has been approved previously (6).

Therefore, this study was designed to determine the effect of marjoram leaves on the reproductive efficiency of rams and their impact on the characteristics of semen and sperm when used before the pollination season to prepare rams with high reproductive efficiency.

## 2. Materials and Methods

This study was conducted in one of the private fields in Babylon governorate from 15/2/2020 until the end of the breeding season on 1/6/2020.

### 2.1. Animals

10 Awassi males, one year old, weighing 30 kg, were divided into two groups (n=5). The control group (A) was fed broths prepared for inseminated males. The treatment group was fed on diets prepared for inseminated males with the addition of minced green marjoram leaves at a rate of 3 g/kg of body weight, three times a day for 49 days.

### 2.2. Prepare Plant Leaves

The leaves were removed from the fresh plant and chopped to be added to the provender three times a day for 49 days before the breeding season in sheep.

### 2.3. Semen Collection

The artificial vagina was used for semen collection from sheep. When the sheep approached the estrus at the end of the period of puberty, it was kept in numbered tubes to measure the volume of the ejaculate and to conduct physiological tests on it for comparison between the control group and the experimental group.

The individual motility of sperms was measured using a light microscope under the magnification power of 40 $\times$  according to the method mentioned in (7). Live sperms' percentage was measured using the technique that Khan and Ijaz (8) mentioned. The percentage of abnormalities was detected using Eosin and Nicrocin dyes examined under the oil lens with a magnification of 1000 $\times$  (7). The total number of sperms was counted using a Hemocytometer. Sperm concentration was measured using a Spectrophotometer by measuring the amount of light passing through the sample according to den Daas (9). The shape and dimensions of the sperms were also measured using the ISAS R method, where Area, Length, and Width were measured by using moving sperms in diluted semen samples at a concentration of 100  $\times$  10 sperm/ml in a warm slide (37 m) as mentioned in Yániz, Vicente-Fiel (10). Male reproductive hormones were also measured using the ELISA technique according to the commercial kit in the seminal plasma (11).

### 2.4. Biochemical Tests

1- SOD was measured in seminal plasma according to the instructions in the commercial Superoxide dismutase Elisa kit (USA) (12).

2- Gpx was measured in the seminal plasma of rams according to the method previously described by Kasimanickam, Pelzer (13). The TAC was measured in the seminal plasma according to the instructions mentioned in the Total Antioxidant Capacity Elisa Kit (abcam, ab65329).

### 2.5. Statistical Analysis

The statistical program SAS (SAS, 2012) was used for data analysis to study the effect of marjoram leaves on the studied traits according to the complete random design (CRD). Significant differences between the means were compared with the t-test to compare the means in this study.

## 3. Results and Discussion

The study showed that the use of fresh marjoram leaves as a fodder addition to Awassi rams prepared for pollination in the breeding season is of better value than dosing animals with water or alcoholic extracts of this plant because of

drying the material in terms of quantity and distribution of volatile compounds using high temperatures and length of drying time. The study showed that the intake of inseminated rams 3 g / kg of body weight three times a day for 49 days led to an improvement in the physiological characteristics of the semen (Table 1).

The individual movement and vitality were affected at a significant level of  $P<0.05$ , and it also decreased sperm deformations at a significant level of  $P<0.05$ . There was no significant effect on ejaculation volume, number, and concentration of sperm. The morphological characteristics of the sperm (Table 2) did not show any defect or negative results of marjoram

leaves as a feed additive. The measurements of the sperm's head showed fundamental differences in the head's area, circumference, length, and width from the standard measurements. Also, no changes occurred in the nucleus's area, circumference, length, and width (Table 3).

As for the acrosome, consumption of marjoram leaves did not show any effect on it, which indicates that the plant's containment of flavonoids, terpenes, and other antioxidants had a significant role in repairing, restoring, and perpetuating testicular cells and sperm and protecting them from the influence of oxidative factors (Table 4).

**Table 1.** Effect of using marjoram leaves on semen characteristics of rams

characteristics of semen	Control group	Treatment group	T-test
Ejaculate volume (ml)	1.1±0.1	1.5±0.2	0.469 NS
Sperm concentration (10x/ml)	2989.41±11.24	3010±10.41	58.06 NS
Sperm number (10x/ml)	3000±7.341	3014±10.56	37.92 NS
Viability %	51.13±2.71	60.14±2.11	5.635 *
Abnormalities %	20.23±1.37	10.21±1.37	4.711 *
Individual motility %	48.60±1.43	60.84±1.21	7.514 *

\* ( $P<0.05$ ), NS: Non-Significant

**Table 2.** Effect of using marjoram leaves as a feed additive on sperm head shapes

Sperm head measurements Mm <sup>2</sup>	Control group	Treatment group	T-test
Headspace	38.23±1.14	39.23±1.13	4.263 NS
Head circumference	25.41±1.21	25.61±1.31	3.096 NS
head length	9.27±0.30	9.23±0.40	1.268 NS
head width	5.44±0.31	5.31±0.41	0.883 NS

NS: Non-Significant

**Table 3.** The effect of using marjoram leaves as a feed additive on the sperm's nucleus shape

Sperm nucleus measurements	Control group	Treatment group	T-test
nucleus space	30.70±1.9	31.70±1.6	3.072 NS
the periphery of the nucleus	32.34±0.36	32.31±0.41	2.62 NS
Nucleus length	8.30±0.19	8.10±0.19	0.747 NS
Nucleus width	4.70±0.13	4.70±0.13	0.505 NS

NS: Non-Significant

**Table 4.** Effect of using marjoram leaves on the acrosome-shape

Sperm acrosome measurements Mm <sup>2</sup>	Control group	Treatment group	T-test
Acrosome area	26.14 ± 2.41	26.00 ± 1.6	2.841 NS
circumference of acrosome	22.16 ± 1.12	22.14± 1.12	2.350 NS
Acrosome percentage	66.81± 7.66	67.31± 7.44	8.902 NS

NS: Non-Significant

Studies have shown that sperms are constantly exposed to oxidizing factors due to the low ability of antioxidants in the cytoplasm and many unsaturated fatty acids present in the sperm membrane, which increases the attack of free radicals on these acids, changing the enzymatic system inside the cell and leads to a negative impact on the quality of sperms and their ability to fertilization.

The study showed (Table 5) that the use of marjoram leaves led to an increase ( $P<0.05$ ) of the enzymatic antioxidants represented by SOD, GPX, and TAC in the seminal plasma. These antibiotics interrupt the pyro-oxidation chain of fats and reduce the secretion of toxic aldehydes (MDA), which impede sperm movement and their ability to fertilize and cause abnormalities in the head and nucleus of the acrosome of the sperm.

The study showed, as in table 6, that eating marjoram leaves in the group (B) led to a significant increase ( $P<0.05$ ) in the concentration of the ovulatory hormone, testicular fat, and estrogen in the blood because the leaves of the herb contain Diosgenine, which has an essential role in the

manufacture and construction of sex hormones as well. The plant's leaves contain a group of phytoestrogens, which stimulate the growth of genodrophic cell cells and their secretion of steroids. The phytoestrogen group also combines with estrogen receptors on cells. It increases the secretion of estrogen, which increases the sexual desire of rams; it was mentioned that marjoram has an androgenic effect that leads to an increase in sexual desire and an increase in sperm production, which is due to an increase in ovulation hormone and the testicular fat hormone. All this is because eating the plant regulates the work of the three axes (hypothalamus, pituitary, and gonad). From the results, it could be concluded that:

- 1- Marjoram regulates the axons (hypothalamus, pituitary, and gonad) and thus provides the male sexual hormonal balance.
- 2- Contains antioxidants that prevent oxidative stress fertility in sheep.
- 3- Provides a forage rich in proteins, carbohydrates, and enzymatic antioxidants.
- 4- Using marjoram as a supplement increases the sexual and reproductive efficiency of Awassi rams.

**Table 5.** The effect of using marjoram leaves on the concentration of antioxidants in the seminal plasma of fertilized rams

Antioxidants mean±standard error	Control group	Treatment group	T-test
SOD (ng/ml)	2.973±0.04	3.567±0.03	0.371 *
GPX (mv/ml)	11.43±0.54	14.33±0.4	1.632 *
TAC mM	569.40±35.01	1040±30.04	92.756 *

\* ( $P<0.05$ )

**Table 6.** The effect of adding marjoram leaves to the diet of pollinating rams on some sex hormones

Hormones mean ± standard error	Control group	Treatment group	T-test
Luteinizing hormone (mm/ml)	0.9±0.1	1.7±0.2	0.316 *
Testosterone (ng/di)	3.9±0.03	7.2±0.02	1.502 *
Estrogen (P mol/l)	37.471±3.36	55.413±4.31	7.229 *

\* ( $P<0.05$ )

### Authors' Contribution

Study concept and design: E. M. A. and A. M. A.

Acquisition of data: E. M. A. and A. M. A.

Analysis and interpretation of data: T. A. A.

Drafting of the manuscript: S. M. H.

Critical revision of the manuscript for important intellectual content: E. M. A. and T. A. A.

Statistical analysis: A. M. A.

Administrative, technical, and material support: E. M. A. and S. M. H.

### Ethics

The study protocol and all the procedures in this study were approved by the ethics committee of the , Al-Furat AL-Awsat Technical University, Najaf, Iraq.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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