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Influence of addition of *Thymus vulgaris L.*, *Origanum vulgare L.* and *Tribulus terrestris* in the ration of some indices of ram's semen – Review

Natalya Miteva
Agricultural Academy, Agricultural Institute-Stara Zagora, Bulgaria
Corresponding author: nataliya.z.miteva@abv.bg

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Abstract: The reproductive ability of male breeding animals is of great importance for increasing productivity and production efficiency in sheep farming. Improving the quantitative and qualitative indicators of semen, especially when applying artificial insemination, is of great importance for production results on a sheep farm. For years, has been studied on the possibility of improving the quality of the obtained seed material and its storage through the use of various additives of biologically active substances of plant origin.
The aim of this study is to review the possibilities for using some bioactive plants in sheep breeding to improve reproductive performance in male animals. Since the number of plants containing biologically active substances is very large, this review pays special attention to medicinal plants that are widespread and freely growing in Bulgaria, with proven therapeutic effects and have been used for many years in traditional medicine. In our opinion, the most promising in this regard are common thyme (*Thymus vulgaris L.*), oregano (*Origanum vulgare L.*) and *Tribulus terrestris*.

Keywords: *Thymus vulgaris L.*; *Origanum vulgare L.*; *Tribulus terrestris*; semen; rams

INTRODUCTION

In search of natural alternatives to synthetic chemical compounds used in animal husbandry, researchers are turning to bioactive compounds and metabolites of plant origin. These plant bioactive substances, included as feed additives or other food components, have a wide range of effects on the animal body - from improving health and productivity, to toxic and lethal outcomes. The interest in using plant bioactive substances is also due to the fact that they are cheap as a resource and are safe for the environment. At the same time, it is necessary to clarify all information about their concentration, structure, as well as knowledge of their absorption, metabolism and biological effects, in order to fully assess their potential effect of their application in animal husbandry (Durmich and Blache, 2012, Mahfoudhi, et al., 2016).

Plant bioactive substances positively or negatively affect almost all stages of the male and female reproductive cycle. They can affect both reproductive behavior (courtship, mating) and increase sensitivity to sex steroids (Gauthaman and Adikan, 2006; Gauthaman and Ganeson, 2008; Patel et al., 2011).

Some plant extracts reduce sperm production (Randel et al., 1992), while others increase their number (Kistanova, 2005), or change the components of sperm plasma (Yousef, 2005).

Biologically active substances contained in plants have the potential to modulate many vital functions in the animal body. What will be the effect of the application of plant bioactive substances depends on many factors - source, chemical structure, concentration, dose, and interaction with the animal body.

The presence of biologically active substances in plants has been known and used in folk medi-

cine for centuries, but only in recent decades have larger-scale studies begun on the development of technologies for their production and distribution, as part of efforts to achieve clean, ecological, ethical and economically viable animal husbandry systems.

The aim of the present study is to determine the influence of biologically active substances contained in three plant species (thyme - *Thymus vulgaris L.*, oregano - *Origanum vulgare L.* and tribulus terrestris - *Tribulus terrestris*), added to the ration of rams on reproduction and basic indicators of the semen.

***Thymus vulgaris L. (*thyme)**

Common thyme (*Thymus vulgaris L.*) belongs to the genus Thymus (Thymus L.), family Lamiaceae. The genus includes over 350 different species. They are aromatic perennial plants and shrubs with a height of 5 to 40 cm.

The use of thyme for medicinal purposes was first documented in 1589. For many decades, the essential oil of *Thymus vulgaris L.* has been included in pharmacopoeias and standard textbooks on phytotherapy (Martindale, 1972). Thyme oil contains 46% phenols, of which 44% thymol and 3.6% carvacrol, as well as other important components. Various studies confirm that thyme oil contains polyphenolic acid and other components, such as borneol, geraniol, pinene, linalool, cineole, sabinene, myrcene, limonene and cymene (Rizk, 1986). Thyme is a good source of vitamins. The plant is particularly rich in vitamin A and vitamin C. Vitamin A is an antioxidant, important for maintaining healthy mucous membranes and skin, as well as for good vision (Vineetha, 2014). Vitamin C provides resistance against infectious diseases and successfully copes with free radicals. The plant is a source of vitamin B6 or pyridoxine, with 100 grams of the plant providing about 0.35 mg or 27% of the daily recommended intake of this vitamin (National Institutes of Heath, 2016). Other vitamins found in this herb include vitamin K, vitamin E, and folic acid (Vineetha, 2014). Thyme is also a source of minerals that are vital for optimal health. Thyme leaves are an excellent source of potassium, calcium, iron, manganese, magnesium, and selenium (Sharangi et al., 2013).

In recent decades, scientific research has paid serious attention to the benefits of the biologically active substances contained in thyme. A variety of methods of application are used - from including the dried whole plant in the ration (Gauthaman and Ganesan, 2008; Patel et al., 2011), through extracts added to concentrated feed (Al-Zubaidy et al., 2013; Khnissi et al., 2024), to direct use of the extract as a cryopreservation solution (Vahedi et al., 2018). A limited number of authors have conducted studies related to various indicators of fertility of male animals from different breeds of sheep and monitored their change when using the plant biologically active substances of thyme.

The effect of the application of extract in different concentrations, obtained from two plants (*Thymus vulgaris L.* and *Ziziphusspina Christi*) on sperm activity in Awassi rams was studied by Al-Zubaidy et al. (2013). Three concentrations were tested (0.001, 0.05 and 0.5 mg), and sperm activity was assessed at the first, 24th, 48th, 72nd hour of the experiment. The results showed a significant decrease in sperm activity when treated with concentrations of 0.005 and 0.01 mg. Only when treated with extract of *Thymus vulgaris L.* at a concentration of 0.001 mg was a significant increase in sperm motility observed. According to the authors, the positive results using a concentration of 0.001 mg of thyme can be considered encouraging for subsequent studies with other concentrations, and place thyme on the list of plants that have been found to increase male fertility (Mbongue et al., 2005).

Khnissi et al. (2024) studied the effect of thyme essential oil supplementation under heat stress conditions on testicular size, rectal temperature, testicular temperature, ejaculate quantity and density, sperm motility and kinematic parameters, etc. The authors noted that rams that received essential oil during the stress period had significantly lower temperatures (rectal and testicular) than those in the control group. According to them, thyme essential oil supplementation improved the stability of homeostasis in stressed animals. The essential oil supplementation did not

affect the blood parameters except for the mean hemoglobin concentration in erythrocytes, which was positively affected. The testes circumference showed a significant increase in the group treated with thyme essential oil. The normal morphology of spermatozoa is improved significantly in all treated groups. In conclusion, the authors summarize that rams treated with thyme essential oil fertilized more ewes at first estrus and noted an improvement in fertility.

Vahedi et al. (2018) evaluated the effect of using *Thymus vulgaris L.* extract as a natural antioxidant on the performance of ram semen during cryopreservation and after defrosting. The data showed that the quality of frozen and then defrosted ram semen was significantly improved by adding 4 mL/dL of thyme extract to the dilution solution, an effect that is likely due to the polyphenolic compounds in the extract that have antioxidant activity. The authors recommend more research to determine the appropriate doses of thyme extract to be added to sperm diluters. Similar results, but with semen of male goats, have been noted by other authors (Ismail et al., 2020; Kchikich et al., 2024).

Khnissi et al. (2025) evaluated the protective effect of a thyme essential oil nanoemulsion during preservation of ram semen. Three preservation media were studied – control, with thyme essential oil and with essential oil nanoemulsion. The results showed that the addition of *Thymus vulgaris L.* essential oil and its nanoemulsion to the cryopreservation medium reduced the stress induced by cooling, leading to increased sperm motility (Gumus et al., 2017). This study also highlighted the progressive improvement of sperm motility in the essential oil nanoemulsion at 4°C after 24 hours and sustained motility at 15°C even after 5 hours, suggesting its potential for use as a cryoprotectant.

***Origanum vulgare L.* (oregano)**

The Lamiaceae family is characterized by great diversity and distribution throughout the world. Oregano (*Origanum vulgare L.*) is one of the aromatic perennial herbs of the Lamiaceae family, which is known by various names around the world. Oregano is native to West Asia and the Mediterranean region, but is now naturalized almost everywhere (Gaur, 1999; Chauhan and Nautiyal, 2007). It is one of the most traded culinary herbs in the world (Chalchat and Pasquier, 1998; D'Antuono et al., 2000). Oregano essential oil contains carvacrol and/or thymol as the main component(s) and other minor constituents such as c-terpinene, p-cymene, linalool, etc. Carvacrol is the main active compound of oregano and is present in the highest amount (Baranauskiene et al., 2006; Bansleben et al., 2009). Biologically active substances are contained in the highest concentration in fresh and dried plants and exhibit antioxidant, anti-inflammatory, antimicrobial, antitumor, etc. effects (Baricevic et al., 2001; Fei et al., 2017; Leyva-Lopez et al., 2017; Gayoso et al., 2018).

Alenezy et al. (2019), conducted a study evaluating the effect of adding oregano extract to an *in vitro* sperm dilution medium. This study investigated the antioxidant and antimicrobial effects of oregano extracts at different concentrations (0.3, 0.6, 1.2 μg/ml and 25.0, 50.0, 100.0 μg/ml, respectively), in the dilution medium. The starting oregano solution was diluted with double distilled water (1 mg/ml) and stored at 4˚C until use. The authors found a significant improvement in sperm quality parameters such as total motility, live spermatozoa count when oregano extract was added at a concentration of 1.2 μg/ml. Treatment of semen with a higher concentration of oregano extract (100 μg/ml) for 2 hours without additional antibiotic addition improved sperm characteristics. According to the authors, incubation of sperm in a medium containing oregano extracts has a beneficial effect on the characteristics of ram's sperm.

The results of Alenezy et al. (2019) are in line with the study conducted by Ahlam et al. (2024) in other animal species. Ahlam et al (2024) studied the effect of oregano essential oil (*O. vulgare L.*) supplementation on sperm motility in three different animal species over a 24-hour period and found that this supplementation significantly improved sperm motility in bulls, dogs and rabbits. This improvement was observed within the first 10 minutes, characterized by a decrease in

the number of immobile spermatozoa and an increase in their progressive motility. These positive effects persisted even after 24 hours, and the spermatozoa motility remaining very good in all studied groups.

Another in-depth study found that oregano essential oil supplementation improved the antioxidant activity of sperm. These improvements may be a major contributing factor to increased sperm motility (Zarhouti et al., 2023). Increased sperm motility through oregano essential oil supplementation may effectively promote their ability to reach and penetrate the oocyte. This improvement is critically important in natural and assisted reproduction, where progressive sperm motility remains a determining factor (Dcunha et al., 2024).

Unlike other authors, Al-Najar et al. (2022) studied the effect of using fresh oregano leaves as a feed supplement in the diet of Awassi rams on the indicators that determine sexual activity and sperm quality. The study was conducted on ten animals, divided into two groups: control and experimental. The experimental group received an identical ration to the control plus the addition of fresh oregano leaves (3 mg/kg live weight) three times daily for 49 days before the mating season (April and May). The indicators of the obtained sperm that the authors studied were ejaculate volume, sperm count, concentration, motility, viability and deformations of the head, nucleus and acrosome of the spermatozoa.

The authors evaluated the antioxidant properties of seminal plasma by measuring the values of superoxide dismutase, glutathione peroxidase and total antioxidant capacity. Standard ELISA tests were used to determine the levels of luteinizing hormone, testosterone and estrogen. The results showed a significant increase in sperm motility and viability. No significant changes were reported in the indicators related to sperm deformation. An increase in the values of antioxidant enzymes in seminal plasma and an increase in the studied hormones were reported. In conclusion, the authors believe that the use of fresh oregano leaves improves the sexual activity of rams and improves the parameters of sperm.

Tribulus terrestris

The species *Tribulus terrestris*, is an annual herbaceous plant and belongs to the genus Tribulus, family Zygopgyllaceae. It is widespread in sandy and stony places, along roads and as a weed in vineyards and crop fields. The plant is thermophilic and compact deposits are found in Southern Bulgaria and along the Black Sea coast (Semerdjieva, 2013).

The plant's root extract contains resins, alkaloids, nitrates, and oils. Various compounds have been identified in the plant, including steroids, vitamins, alkaloids, unsaturated fatty acids, aspartic acid, saponins, tannins, flavonoids, resins, potassium nitrate, and glutamic acid. The fruits of the plant contain small amounts of essential oil, resin, and traces of alkaloids (0.001%). They also contain fats and resin with a sweet odor when burned, as well as a rich mineral content (Meena et al., 2010). *Tribulus terrestris* contain about 70 different chemical compounds (Stefanescu et al. 2020). It is a plant of important medicinal importance, used in natural medicine, and can be found all over the world (Kostova and Dinchev, 2005).

In Bulgaria, the research on the chemical composition of *Tribulus terrestris* began in the late 1960s. A scientific team analyzed the phytochemical composition of Bulgarian populations of the plant (Tomova and Panova, 1965; Panova and Tomova, 1970). They created a drug for use in veterinary medicine that increased sperm quality in rabbits (Tomova et al., 1966; Tomova and Gyulemetova, 1978). The authors developed a drug called Tribestan, which was later standardized in a clinical trial (Tomova et al., 1978).

An experiment was conducted to determine the stimulating effect of Tribestan on rams intended for breeding, as well as on those that showed sexual impotence and impaired sperm quality. The drug was administered orally according to a scheme developed by the authors (Dimitrov et al., 1987). According to the authors, the applied therapy leads to an extension of the period of sexual activity and to an improvement in sperm production by healthy rams during the mating period. The results of the therapy of rams with impaired sexual libido show that the animals recover by

the 7-8th day without significant morphological changes in the structure of both the testicles and the epididymis. The use of the drug led to a significant effect on the endocrine activity of the testicles, an increase in the level of testosterone and normalization of the sexual activity of rams affected by coital impotence.

Kistanova et al. (2005) studied the effect of applying *Tribulus terrestris* extract on reproductive performance in rams during the mating period. The experiment included rams of the Chernoglava Plevenska breed. The *Tribulus terrestris* extract (Vemo Ltd BG) used was administered orally, once a day with the feed at a dose of 1.5 g/animal for a period of 40 days. The most significant effect was observed in terms of sperm concentration and spermatozoa survival. The indicators of ejaculate volume and sperm motility had better values in the experimental group, but the differences with the control group were not reliable. In terms of sexual activity, all animals had a well-expressed libido. According to the authors, sexual activity in this case was influenced more by the age of the animals than by the addition of *Tribulus terrestris* extract. In conclusion, all indicators related to spermogenesis showed better values in the experimental animals compared to those in the control group.

The same year, Kistanova (2005) conducted another experiment in which she determined the effect of applying a probiotic and an extract of *Tribulus terrestris*. The author found that the probiotic had a very good effect on the volume of ejaculate and fertility of inseminated sheep, while the extract of *Tribulus terrestris* was a stronger stimulant of sexual activity and spermogenesis in rams.

Similar results were obtained by Hristov et al. (2017) for the administration of a probiotic and plant extract (Tribestan-Lactina® complex). They found that the complex increased sexual activity and improved sperm production in rams outside the mating period. An increase in ejaculate volume, improvement in sperm motility and concentration, and a reduced percentage of abnormal spermatozoa were observed.

Another experiment comparing the effect of probiotic and *Tribulus terrestris* administration on scrotal circumference, testicular volume, sperm characteristics, total cholesterol and testosterone levels in sexually mature rams was conducted by Sharaway et al. (2015). One experimental group was treated with *Tribulus terrestris* extract (1.5 g/head/day, orally in 100 ml of aqueous suspension), and the other with probiotic (10 g/head/day, orally in the form of 100 ml of aqueous suspension) for two months. The results showed that scrotal circumference, sexual desire, total sperm count increased significantly in the *Tribulus terrestris*-supplemented group compared to the probiotics and control groups. Ejaculate volume, motility, percentage of live sperm, sperm morphology, sperm concentration and percentage of acrosome integrity increased significantly in both experimental groups compared to the control group. The level of total cholesterol in serum decreased significantly in the experimental group, while the testosterone level showed a significant increase compared to the control group and the probiotic group.

Mirzadeh et al. (2019) investigated the effect of applying different amounts of *Tribulus terrestris* on the quantitative and qualitative characteristics of the sperm of mature male lambs. The study included 18 male lambs of age of three months with a live weight of about 16 kg. All lambs were fed a basic ration, and animals from the experimental groups were supplemented with *Tribulus terrestris* in an amount of 15 and 30 g/kg dry matter for 5 months. The authors evaluated the amount of ejaculate, pH, concentration, motility and morphological abnormalities in sperm. The results show that the lowest volume of ejaculate was recorded in the control group. The highest concentration, motility and survival of sperm was recorded in the experimental group that received 15 g/kg dry matter *Tribulus terrestris*. The most morphological abnormalities of sperm were recorded in the experimental group that received 30 g/kg dry matter *Tribulus terrestris*. No changes in pH were recorded in any of the groups. In conclusion, the authors recommend the use of 15

g/kg dry matter of *Tribulus terrestris* in order to obtain the best results from the use of rams.

Pour et al. (2015) consider another possibility of using an extract of the plant *Tribulus terrestris* as an improver of the medium for dilution and storage of sperm at 5^0C. The authors used seminal material from rams and a ready-made medium for dilution and storage from a commercial manufacturer (He and Hubbell, 2005). When using an extract of *Tribulus terrestris* in an amount of 5%, the best results were found in terms of sperm survival and motility up to 24 hours after receiving the ejaculates.

CONCLUSION

The reproductive ability of rams is of great importance for increasing productivity and production efficiency in sheep farming. Improving the quantitative and qualitative indicators of semen, especially when applying artificial insemination, is of great importance for the production results in the sheep farm. For years, work has been underway on the possibility of improving the quality of the obtained semen by using various additives of biologically active substances of plant origin in the rations fed to rams.

Since the number of plants containing biologically active substances is too large, we believe that special attention should be paid to medicinal plants, widely distributed and freely growing in Bulgaria, with proven therapeutic effects and used for many years in traditional medicine. The most promising in this regard, in our opinion, are common thyme (*Thymus vulgaris L.*), oregano (*Origanum vulgare L.*) and *Tribulus terrestris*.

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