Effect of Adding Caper (*Capparis spinosa* L.) Fruit Powder and Magnesium Oxide to the Diet on Rumen Fermentation Characteristics and Serum Testosterone Concentration in Local Male Lambs

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Abstract

This study was conducted in sheep farms belonging to the College of Agriculture / University of Anbar during the period from February 1 to May 1, 2025, and it aims to determining the effects of adding caper (*Capparis spinosa*) fruit powder and magnesium oxide on the Rumen Fermentation Characteristics and the testosterone hormone levels in the local male lamb. The lambs were fed a basic diet consisting of (Maize 20%, wheat bran 22%, barley 25%, soybean meal 10%, alfalfa hay 20%, limestone 1%, table salt 1%, antitoxin 0.2%, and premix 0.8%). In the experiment, 30 lambs aged 3.5–4.5 months and with an average initial weight of 25.20 ± 1.29 kg were used, Animals were randomly allocated under completely randomized design (CRD) to six treatments (05 lambs/treatment). The control treatment without the addition of magnesium oxide and caper fruit powder (Control) (T1); the treatment with the addition of magnesium oxide at 7 mg/kg dry matter (MgO 7 mg/kg DM) (T2); the treatment with the addition of caper fruit powder at 2.5 g/kg dry matter (Caper 2.5 g/kg DM) (T3); the treatment with the addition of a mixture of caper at 2.5 g/kg dry matter + magnesium oxide at 7 mg/kg dry matter (Caper 2.5 g/kg DM + MgO 7 mg/kg DM) (T4); the treatment with the addition of caper fruit powder at 4.5 g/kg dry matter (Caper 4.5 g/kg DM) (T5); and the treatment with the addition of a mixture of caper at 4.5 g/kg dry matter + magnesium oxide at 7 mg/kg dry matter (Caper 4.5 g/kg DM + MgO 7 mg/kg DM) (T6). The feeding trial period was 90 days. During day 0 and 90 of the experiment, samples of blood were taken at 7:00 am before feeding, and rumen fluid samples were made on day 90. The outcomes proved statistical significance of affected treatment with powdered caper fruit, magnesium oxide, and their combinations compared to that one of the control group. The experimental treatments did not cause any alteration in the rumen fluid properties that would fall out of the physiological normal range that includes volatile fatty acids, ruminal pH, ammonia levels and the concentration of testosterone hormones. Introduction of caper fruit powder into the diet or magnesium oxide into the diet or a combination of both did not cause any adverse effects on the rumen fluid characteristics and animal health. These results show that magnesium oxide and caper fruit powder can be used safely as food additives in the diets of local sheep.

Keywords: Male Lambs; powdered caper fruit; magnesium oxide; rumen milk; testosterone hormone

Introduction

The nature and quality of nutrition taken up by the animals will either have a positive or negative effect on their performance [22]. Thus, scientists and experts have insisted on the need to offer proper and balanced nutrients in the diets of animals depending on the productive nature of the animal [26]. This is due to the fact that the development and

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productivity of rumen fluid is directly influenced by the development and composition of the diets as well as the kind of feed material fed [25]. Moreover, the use of dietary supplements and plants containing antioxidant and antimicrobial properties in improving rumen environment, animal growth and development has been given growing scientific attention [21].

Such factors prompted the researchers to pay attention to natural plants which can be found in the Iraqi setting, including the most significant caper (*Capparis spinosa*) with its antioxidative properties and the abundance of bioactive substances[5]. In this regard, the current research sought to examine the influence of caper fruit powder and magnesium oxide on the rumen fluid parameters and the development and maturity in the animal body by enhancing the quality of the rumens and promoting the effectiveness of the production of testosterone hormone.

One of the plants that have potential biological activity and which are naturally available in the Iraqi environment is caper fruit that is regarded as a safe substitute to synthetic feed additives. It is considered a valuable medicinal plant as it contains high amounts of phenolic compounds and flavonoids including rutin and quercetin, which have a high antioxidant ability and contribute to stabilizing rumen pH and increasing the generation of volatile fatty acids [24]. Moreover, caper fruits possess the nutritional quality, as they do include proteins, fats, carbohydrates, dietary fiber and vitamin C [16].

Past researchers suggest that caper plants have polyphenols that enhance microbial ecology and ruminal fermentation efficiency. These are due to their effects on the rumen fluid properties that are realized by modulation of microbial action and fermentation patterns resulting in increased production of volatile fatty acids, lowered ammonia level, and stabilization of ruminal PH [35].

Caper plants and magnesium oxide are also discussed as the natural feed additives, which have become a topic of growing interest recently because of their possible positive impact on the welfare, productivity, and quality of livestock feed [33]. Magnesium is important in animal metabolism as it relates to better physiological functioning and reduced stress on the metabolic processes by its impact on rumen fluid environment. It is involved in the stabilization of ruminal pH, decreased excessive acidity, increased microbial fermentation efficiency and augmented production and ratio of volatile fatty acids. These effects were positively reflected in the overall health status of the animals. and the efficiency of nutrient utilization especially in arid and semi-arid areas where the natural forage of food and the lack of the necessary minerals are deficient in nutritional value [1]

As such, the objective of the study was to determine how the addition of caper fruit powder and magnesium oxide influences the concentration of testosterone hormone, as well as rumen characteristics such as volatile fatty acids, ammonia concentration and ruminal pH in local male lambs.

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Material and Methods

Prepare samples:

The study was conducted in the sheep fields of the College of Agriculture / University of Anbar, after obtaining approval from the Scientific Research Ethics Committee / University of Anbar, numbered 306 and dated 26/4/2024, during the period from February 1 to May 1, 2025. A total of 30 local male lambs aged 3.5–4.5 months, with an average body weight of 25.20 ± 1.29 kg, were used in the experiment. All necessary requirements to complete the research were available in the fields. The lambs were fed a concentrated diet at 3% of body weight, containing 13.6% crude protein, 5.89% ash, 11.56% moisture, 2.77% fat, 11.11% fiber, and 55.07% carbohydrates, with free access to water. The feed additives were provided by thoroughly mixing them with the daily ration throughout the experimental period, and the experiment lasted for 90 days.

Experimental Design

The animals were randomly divided according to a completely randomized design (CRD) into six treatments (5 lambs per treatment), as follows: The control treatment without the addition of magnesium oxide and caper fruit powder (T1); the treatment with the addition of magnesium oxide at 7 mg/kg dry matter (T2); the treatment with the addition of caper fruit powder at 2.5 g/kg dry matter (T3); the treatment with the addition of a mixture of caper at 2.5 g/kg dry matter + magnesium oxide at 7 mg/kg dry matter (T4); the treatment with the addition of caper fruit powder at 4.5 g/kg dry matter (T5); and the treatment with the addition of a mixture of caper at 4.5 g/kg dry matter + magnesium oxide at 7 mg/kg dry matter (T6). The feed additives were provided by thoroughly mixing them with the daily ration throughout the experimental period.

Blood Sampling and Hormonal Analysis.

To read serum testosterone levels, Blood samples were collected via the jugular vein at seven o'clock in the morning before feeding, and at Two time intervals on days (0, 90) A commercial kit (Testosterone Gamma 2) was used to measure serum testosterone. The test was performed using the equipment provided by the company and a normal curve was plotted to determine the levels of the hormones [32].

Rumen Fluid Testing and Analysis.

Rumen fluid of every lamb was collected two hours after feeding on the day 90 of the experiment. Fluid aspiration was conducted using a custom-made device, which consisted of a rubber tube, which was introduced orally and pushed to the rumen, with a suction device connected to the tip of the distal end of the tube. This procedure went according to [29] report.

The pH of the ruminal was determined right after the collection with the help of a portable pH meter (Chinese manufacture). Then, the fluid was filtered through a series of gauze and it was moved to sterile plastic containers, where further analyses were going to be conducted. The concentration of volatile fatty acids was measured using the gas chromatography (Shimadzu, Japan), and the concentration of ammonia was measured by the Kjeldahl technique.

Statistical Analysis

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Statistical analysis was performed using one-way analysis of variance (ANOVA) with the SAS program, version 9.1 (30). Differences between treatment means were evaluated using Duncan's multiple range test (10) at a significance level of ($P < 0.05$).

Chlorophyll content

using a graduated cylinder contained water to determine the berries volume, and berries total contents of sugar (%): Taking 1ml from pure juice, add 1ml of phenol 5% then 18ml of distilled water and then 5ml of H2SO4 30 min in boiling water at 60 ∘C

Results and Discussion

1. Effect of Adding Caper Fruit and Magnesium Oxide on Testosterone hormone level.

As the statistics in Table 1 shows, the testosterone level at the end of the experiment (day 90) and at the beginning of the study showed statistically significant difference (P 0.05). The testosterone levels were found to be very high in all the experimental treatments as compared to the control group.

Table 1. Effect of adding different levels of caper fruit powder and magnesium oxide on testosterone level (ng/dL) of local male lambs (Mean ± SEM)

Significant level	testosterone level (ng/dL)						Days
	T6	T5	T4	T3	T2	T1	
N.S	0.066±1.26 a	0.305±1.300 a	0.100±1.000 ab	0.25±1.40 a	0.176±1.23 a	0.145±1.43 b	Zero
0.0002	0.284±6.33 ab	0.284±6.93 a	0.115±5.70 b	0.115±5.90 b	0.290 ± 4.90 b	0.152 ± 3.60 c	90

Data are presented as Mean ± SEM (n = 5).
Means within the same row with different superscripts differ significantly ($P \leq 0.05$).

The superiority of all the treatments as compared to the control group could also be explained by the antioxidant quality of all the constituents of caper plant and magnesium oxide which can be classified as natural and synthetic radical scavengers. These derivatives have a high potential of neutralizing reactive oxygen species (ROS) and prevent various free radicals. ROS have been reported to affect the spermatogenesis and decrease the level of testosterone secretion [12]. Additionally, ROS have been suggested to cause damage to the Leydig cells, which is the principal area of testosterone synthesis, reducing the level of hormones in the circulation [9].

Antioxidant supplements have been found to reduce intratesticular concentrations of ROS and improve total sperm reserves with administration to rams, thus promoting continuous spermatogenesis and raising the concentration of testosterone. Antioxidants also counter oxidative stress in spermatozoa

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and testicular tissues [11]. Moreover, there are some antioxidants that play a direct role in steroidogenesis [31]. In line with the current results, research on intramuscular injection of magnesium oxide in the different concentrations have shown positive effects in the testicular measures and semen quality with a significant rise in the levels of testosterone in the rams [8].

2. Effect of Adding Caper Fruit and Magnesium Oxide on Volatile Fatty Acids.

Table 2 indicates the existence of statistically significant differences ($P \leq 0.05$) between treatments regarding the concentration of propionic, butyric, and acetic acids.

Table 2. Effect of adding different levels of caper fruit powder and magnesium oxide on the major volatile fatty acids concentration (mmol/L) in rumen fluid of local male lambs (Mean ± SEM)

Moral e level	Treatments						FFA
	T6	T5	T4	T3	T2	T1	
<.0001	0.380± 5.15 b	0.076± 5.39 b	0.470± 9.92 a	0.386± 5.73 b	0.565± 9.01 a	0.123 ± 10.06 a	propionic
<.0001	0.501± 15.85 c	0.360± 19.42 b	0.548± 24.80 a	0.307± 18.19 bc	2.03± 20.41 b	0.449± 25.20 a	butyric
<.0001	0.760± 10.52 d	0.604± 12.14 cd	0.039± 15.96 a	0.144± 12.37 cd	1.12± 13.77 bc	0.096 ± 15.3 ab	Acetic

Data are presented as Mean ± SEM (n = 5).

Means within the same row with different superscripts differ significantly ($P \leq 0.05$).

Natural and synthetic antioxidants have strong effects on the nature of rumen fluid by altering fermentation dynamics, microbial populations, and the rumen health. These effects increase the functionality of rumen, neutralize the detrimental metabolic pathways, and promote nutrient absorption, which enhanced growth performance and physiological status of lambs. [19].

It has been evidenced that the caper plant contains active secondary metabolites, which include flavonoid, glucosinolates and volatile aromatic compounds like camphor. These components influence the rumen microbial community and change fermentation patterns and volatile fatty acid profiles depending on the dosage and the part of the plant used [15]. Moreover, addition of plant extracts that are rich in flavonoids, phenolic compounds and essential oils has been shown to control microbial growth leading to significant change in ruminal fermentation with minimal effect to rumen pH. This highlights the fact that the efficacy of plant additives in promoting fermentation and the state of microorganisms, and not necessarily acidity per se) [18].

The above effects could also be attributed to the fact that magnesium oxide facilitates the

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action of fiber-degrading bacteria and counteracts the negative effects of acids produced in the rapid fermentation of carbohydrates. The incorporation of magnesium oxide together with the feed additives like monensin is reported to alter rumen fermentation profiles by stabilizing pH, decreasing ammonia nitrogen (NH3-N) concentration and increasing nitrogen utilization efficiency with substantial differences observed across dosage level and diet compositions [7]. Also, the joint use of sodium bicarbonate and magnesium oxide has been reported to increase rumen pH significantly as compared to the control group because they have a high buffering capacity against acid generated during fermentation.[14]

3. Effect of Adding Caper Fruit and Magnesium Oxide on to Ruminal PH.

Table 3 shows that the level of pH in rumen at the end of the experiment (day 90) significantly differed ($P \leq 0.05$).

Table 3. Effect of adding different levels of caper fruit powder and magnesium oxide on on ruminal pH in the presence of magnesium oxide (Mean ± SEM)

Moral e level	Treatments						Days
	T6	T5	T4	T3	T2	T1	
0.0003	0.085± 5.81 c	0.124± 6.41 ab	0.063± 6.36 ab	0.035± 6.19 b	0.106 ± 6.53 a	0.065± 6.62 a	90

Data are presented as Mean ± SEM (n = 5).

Means within the same row with different superscripts differ significantly ($P \leq 0.05$).

Caper fruits are rich in bioactive compounds such as flavonoids, alkaloids, and glucosinolates which possess antioxidant and anti-inflammatory effects and have been used traditionally as therapy and preventive agent [4]. The presence of flavonoids in the ruminant diets has been linked to favorable impact on productive performance and improved digestive efficiency and antioxidant capacity. This corresponds to the increases in ruminal fermentation markers, which include a rise in propionate production, and a decrease in parasitic activity, resulting in the reduction of ammonia and methane emissions, and the optimization of the microbial environment[27].

The caper plants are also characterized by high fermentability and gas production potential when compared to conventional roughages which makes them to be a promising alternative feed source that would supplement the nutrient absorption efficiency [3]. In past experiments, it was found that addition of forage leaves to rice straw diets positively impacted rumen pH and fermentation efficiency and the importance of plant additives in strengthening microbial balance in rumen[17]. On the other hand, rumen pH reduction caused by high-concentrate dieting negatively affects the activity of fiber rumen degrading bacteria [20].

Natural or artificial antioxidants have a significant impact on the rumen pH by controlling the oxidative stress and activities of microorganisms. The natural antioxidants, including plant extract and vitamins,

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mitigate the oxidative stress through the decrease in the ROS formation, and thus inhibit the additional decline in rumen pH [23]. Additionally, plant extracts contain biologically active compounds such as flavonoids and phenolic acids, which help to create a healthier rumen microbial environment, reducing oxidative stress and overall metabolic activity and digestibility of feed [13]. Magnesium oxide also enhances further growth of the cellulose decomposing bacteria and the generation of acid. Nevertheless, when consumed in high amounts, fast fermentable carbohydrates, in particular grains, reduce ruminal pH and inhibit the activity of rumen microorganisms, especially those related to fiber degradation [32].

4. Effect of Adding Caper Fruit and Magnesium Oxide on Ammonia Concentration.

Table 4. Influence of varying amounts of caper fruit powder and magnesium oxide on ammonia (mg/dL) concentration in local male lamb rumen fluid (Mean ± SEM)

Moral e level	Treatments						Days
	T6	T5	T4	T3	T2	T1	
0.0005	0.26± 24.33 c	0.27± 25.85 a	0.19± 23.99 b	0.18± 25.42 ab	0.53 ± 25.65 a	1.03± 21.08 c	90

Data are presented as Mean ± SEM (n = 5).
Means within the same row with different superscripts differ significantly ($P \leq 0.05$).

Ammonia nitrogen (NH3 N) concentrations in rumen are a significant measure of proteins degradation by ruminal microorganisms and use of non-protein nitrogen compounds in the diet. Ruminal ammonia can be elevated leading to health complications in ruminants [34]. Surveys of plant-based feed additives that are high in polyphenols and tannins have shown their ability to change the microbial set up of rumen and to enhance fermentation processes, which consequently improved nutrient digestibility and growth performance. These additives also inhibit total biohydrogenation of unsaturated fatty acids, which increases their absorption into the small intestine [5]. The present findings are close to the previous studies ‘which reported that nutritional and Physiological parameters were significantly affected ($P \leq 0.05$) by the dietary treatments. on the endocrine balance and metabolic responses in ovine subjects thus supporting the positive association between nutritional treatments, rumen performance and the endocrine system [36, 37]. The transformation and use of dietary protein by the rumen in the breakdown of nitrogen in lambs is directly related to ruminal ammonia synthesis. The MgO can regulate the nitrogen metabolism by increasing their enzyme activity, gene expression and other processes that take place in the body and thus influence the ammonia levels in the rumen water[6].

Conclusion

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The results reveal that the integration of caper fruits, magnesium oxide, or both of them do not have any harmful effect on rumen fluid characteristics. On the contrary, these additive interventions developed a significant improvement of ruminal fermentation processes in the diet of local Awassi lambs. This enhancement was in the form of enhanced physiological efficiency hence supporting high digestive functions and enhancing the well-being of the animalThe combination of girdling and 10 $g.L^{-1}$ with either cultivar was superior treatment with most of parameters compared to the control of both cultivars.

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