



Effect of *Ziziphus Spina-Christi* Leaves on the Level of Testosterone Hormone and Semen Parameters of Iraqi Bucks Semen

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A B S T R A C T

The study aimed to assess the effects of different concentrations of *Ziziphus spina-Christi* (ZSC) leaves on the fertility of buck goats. Twelve buck goats aged 8-12 months with an average body weight of 22 ± 2 kg was divided randomly into three dietary treatments: T1, T2, and T3, all animals were fed a uniform diet in addition to feeding with 20 g/kg (2%) of ZSC for T1, 40 g/kg (4%) of ZSC for T2; the T3 was used as a control that consumed a basal diet only. The experiment lasted for three months. On day 45 and 90, blood samples were collected for plasma testosterone measurement using an immunochromatographic assay. Additionally, semen samples were collected, at the same time of blood collection, for macroscopic (volume, colour, and pH) and microscopic (semen concentration, mass and individual motility percentage, and live-dead sperm percentages evaluations). The results showed that ZSC at 4% significantly increased the testosterone hormone concentration and all physical parameters at 45 and 90 days compared to 2% ZSC and control groups. Additionally, ZSC at 4% significantly improved the microscopic parameters at both periods compared to 2% ZSC and control groups. The present findings revealed that ZSC at 4% diet could enhance goat semen processing and enhanced significantly the testosterone hormone that had higher values compared to (2%) of the ZSC control group. Similarly, (4%) of ZSC affected the physical assessment of semen, such as the volume, colour, and pH values. Thus, adding ZSC with a high concentration (4%) could enhance the goat semen parameters.

Keywords: *Ziziphus Spina-Christi*, buck goats, testosterone, semen parameter

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INTRODUCTION

The idea of herbal medicine is outdated; however, people may consume any of these ingredients, as well as any vitamins, minerals, herbs, or by-products of these ingredients. According to Abdoul-Azize's (1) description, these are "plants or plant parts are used for their flavor, scent, or medicinal properties." Herbs have a long history in populated areas, used in food, medicine, flavoring, beverages, and cosmetics (2). Many individuals increasingly use herbal medicines in medical settings throughout various cultures to address various health concerns (3).

Medicinal herbs attracted researchers due to their antioxidant properties, bioactive compounds, and lower toxicity. Plants are protected from harm and disease by these substances (i.e. plant secondary metabolites), which also contribute to the scent, flavor, and color of plants. The term "phytochemicals" refers to a group of substances categorized into different classes, including saponins, flavonoids, glycosides, tannins, alkaloids, and terpenoids (4). Many medicinal plants help treat a variety of ailments because they contain pharmaceutical chemicals, such as *Ziziphus spina-Christi* (Sidr), which is a multipurpose tree in the Rhamnaceae family that is a native of Arabia and is used in herbal medicinal pain relievers (5). The natural

antioxidants found in *Ziziphus spina-Christi* can be used in lower levels of total phenolic compounds, lessening lipid peroxidation and increasing the activity of the endogenous antioxidant enzyme (6). Because *Ziziphus spina-Christi* contains many polyphenols such as flavonoids, it was widely used as an anticancer, antimicrobial, antiviral, and antibacterial agent. On the other hand, abundant, low-cost (7).

Livestock is a crucial income source. Goats are important animals in producing meat and milk, especially given the country's predominant agricultural systems. Goats can adapt to their environment and survive in challenging conditions, especially in dry areas. Many studies aim to improve goat reproductive performance using hormonal, nutritional, and management tools. Goats excel in adapting to harsh environments, utilizing low-value food, and grazing in underdeveloped areas. Livestock can be increased using the available feed to meet energy needs for basic life functions, protein production, and multifactorial disorders that affect infertility (8). Adulthood in males is characterized by general nature symbols, and most goats go through puberty at a young age despite the wide variations in genotypes. Goats can mature sexually as early as 3–4 months. According to (9), the discovery of viable sperm in the epididymis of dairy goats at 110 days found that male goat sperm was viable at 120 days old, and those male buck goats reached sexual maturity at 509 days. The body produces testosterone, one of the primary sex hormones, in both men and women. In contrast to the ovaries and placenta in women, it is primarily created by the Leydig cells of the testes in men. It is also released by the adrenal cortexes of both sexes (10). ZSC contains minerals and vitamins, such as vitamin C, which is critical in improving nutrient production in bull semen. It was reported that a natural flavonoid was used to increase blood testosterone levels.

The flavonoids and the newly identified flavonoid's steroidogenic effects significantly increased steroidogenesis in Leydig cells, primarily by increasing the gene's expression (11). A natural flavonoid's effects on the reproductive system of rats were looked into because of its significant biological activities (12). Since the hormone responsible for spermatogenesis and sexual behavior is testosterone, the seasonal pattern of testosterone secretion may reduce the effectiveness of male reproduction at certain times of the year (13). Thus, the study aimed to investigate the effects of ZSC leaves as a natural feed additive on productivity functions such as semen evaluation and measured Testosterone level.

MATERIALS AND METHODS

Ethical Approval

The study was conducted based on the ethical approval received from the local Research Ethics Committee, College of Veterinary Medicine, University of Baghdad.

Plant Materials

The leaves of *Ziziphus spina-Christi* were collected from gardens at the College of Veterinary Medicine, University of Baghdad; afterward, they were sent to the Directorate of Seed Testing and Certification in Abu Ghraib City to identify and authenticate the plants on the 27th of October 2022. Afterward, The Ministry of Science and Technology and the Department of Environment and Water laboratories received the samples to determine total phenolic content on 7th November 2022, and the result was 50.12 mg/1 g. The collected leaves were dried under shade, and then ground in a blender with a size of 1.00 mm in diameter and put in the oven at 70 °C for 3 h to remove moisture. After that, they were weighed into two groups of 40 g and 20 g (14) with some modifications in the amount of *Ziziphus spina-Christi*.

Animals and Experimental Design

Twelve buck goats, aged 8 to 12 months old and weighing an average of 22 ± 2.0 kg, were brought from the neighborhood market in Baghdad, Iraq. These bucks were kept at the animal farm of the College of Veterinary Medicine, University of Baghdad. These animals were allowed to graze freely on the college fields for 1-2 hours daily while fed basic diets (roughages and concentrates) 3-4% of body weight, kind of concentrate % (Corn 25, wheat 20, Wheat bran 20, soybeans 30, premix 2, lipid 1.5, calcium 1, salt 500). Also, it was maintained on the farm for two weeks to allow for adaptation.

Three groups containing four animals were formed: The first group (T1) was fed daily rations of 20 g/kg (2%) of *Ziziphus spina-Christi* alongside a basic diet. The second group (T2) was applied 40 g/kg (4%) of *Ziziphus spina-Christi* plus a daily diet. While the third group was the control (T3) only had a basic diet. The experiment was conducted in the winter from January to March.

Blood Sample

On day 45 and 90 after treatment, three animals from each group were randomly chosen for blood sample collection. Blood samples (about 5 mL) were obtained from the jugular vein by using sterilized needles. The sample was placed in the anticoagulant tube (EDTA).

Testosterone Measurement

The testosterone ELISA DSL (Diagnostic System Laboratories Inc. Texas, USA) kits were utilized in testosterone analyses of goats, according to (15). All samples were kept in sterilized tubes free from the anticoagulant substance and separated by centrifuge 4000 rpm for 10 min; the test steps are as follows: First, add 70 μ L sample without air bubbles to the diluent. Then Place the test card into the MF-C100 incubator for 15 min. After that, As directed in the analyzer's instruction manual, place the test card into the analyzer's cardholder and begin the test. Finally, A few seconds later, the outcome will be automatically displayed on the screen.

Semen Collection

Semen was collected from Male Goats at ages (9-11 months), they were trained for a month using a goat artificial vagina (AV) at 37 °C. The age of the female goats used was 9-12 months. They were not housed with the males throughout the experiment but were brought together only during semen collection, their non-pregnant status was confirmed via ultrasound examination. The goats were sexually excited using females which induced estrus by injecting intramuscularly E2 (0.2%) 2cc/animal 24 hours from semen collection to increase the goat's sexual activity.

Semen Evaluation

For mass activity (%) A drop of semen was taken immediately after sample collection on a glass slide at 37 °C, and it was then inspected under a microscope with a power of 40× to determine the proportion of moving sperm. Depending on the speed and intensity of the waves created by the sperm's collective movement, they were recorded depending percentage: (0) means no perceptible motion, (10-20) limited movement that does not produce waves, (30-40) Slower motion and disappear wave motion, (50-60) Slow-moving waves with found individual mobility, (70-80) motion that is vigorous and has waves and eddies moving at a normal speed, and (90-100) rapid-moving eddies and waves. Whereas individual motion (%) A drop of semen is placed on a warm glass slide, which is then covered and examined under 40× magnification. Three to four drops of sodium citrate at a concentration of 2.9% were then placed on the slide as a diluent they were recorded depending on percentage: (0) Completely disappear movement, (10 – 20) Few motile sperm, (30-40) Simple chaotic movement, (50-60) Half of the sperm is motile (70-80) Some sperm move straight while others move around, and (90-100) All sperms are motile in a rowing posture (7). Sperm concentration was calculated according to (16) by using the Neubauer hemocytometer chamber. Nonetheless, the dilution of these samples was (1:200), and after five minutes, sperm were counted and calculated according to the following formula: $(N/80 \times 400 \times 10 \times 200 \times 1000)$.

Finally, Sperm viability includes the live and dead measured by 1:4 eosin nigrosine dilution rate (5 µL raw semen: 20 µL eosin nigrosine) was used to test sperm vitality (17). The semen was equally distributed on the first slide surface and allowed to dry at room temperature for 45-60 seconds. Before testing, the samples were examined under a light microscope to determine the percentage of living or dead sperms (typically, living sperms glow, whereas dead absorb eosin stain, resulting in pink posterior and white anterior c). (17) At the same time, volume was measured by reading the measurements on the collection tube depending on when transferred immediately in a water bath at 37 °C to evaluate the physical semen characteristics. The examination was conducted in the field laboratory of the College of Veterinary Medicine. The pH was determined by using an indicator paper (phenolphthalein paper) and a colorimeter

according to (18). Semen color was determined according to (17, 27). Where each number indicates categorization. Several colors mean (1) cloudy, (2) milky, (3) thin and creamy, and (4) creamy

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA with interaction and least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P \leq 0.05$ is considered statistically significant (19).

RESULTS AND DISCUSSION

Measuring of Testosterone Hormone Levels

According to Table 1, the current research demonstrated that there was a significant rise ($P < 0.001$) in testosterone levels for group T2 during the first (1.58 ± 0.07) and second (1.79 ± 0.08) period in comparison to T1 (0.67 ± 0.03 and 0.95 ± 0.03) and control (0.83 ± 0.08 and 0.92 ± 0.09) groups.

Table 1. Effects of different concentrations of *Ziziphus spina-Christi* (ZSC) on testosterone hormone (ng/mL)

Periods	1st period (45 day)	2nd Period (90 day)
Control	0.83±0.08 Ab	0.92±0.09 Ab
2%-T1	0.67±0.03 Bb	0.95±0.03 Ab
4%-T2	1.58±0.07 Ba	1.79±0.08 Aa
P-value	$P < 0.001$	$P < 0.001$

Values are the mean±SEM, n= 4. ^{A-B} means with a lacking capital superscript in the same row significantly differ ($P \leq 0.05$). ^{a-b} means that a lacking small superscript in the same column significantly differs ($P \leq 0.05$)

ZSC may positively impact fertility, as various parameters studied have shown favorable results. This could be because the level of testosterone hormone has increased significantly in the group (T2) during this study, as reported by (20). Furthermore, another study by (21) suggests that antioxidants present in ZSC can stimulate the normal functioning of Leydig cells. Testosterone significantly impacts male sexual behavior and spermatogenesis (22). Furthermore, using vitamins C and E significantly impacts the treatment of male infertility. The beneficial effects of ZSC on the male reproductive system may be due to the actions of ZSC components, specifically vitamins C and E, flavonoids, and antioxidants, according to (23). Antioxidants have been found in studies to enhance the normal function of Leydig cells. Vitamins C and E also treat male infertility (23). Vitamin E stimulates the pituitary gland, producing ICSH and testosterone, accelerating sperm maturation by increasing seminiferous tubule sperm synthesis (24). Flavonoids improve gene expression, Leydig cell viability, testosterone production, and oxidative damage. Vitamin E, often known as tocopherol, is a phenolic molecule. The chemical analysis revealed a single peak corresponding to tocopherol with a total value of 32.9 mg/kg. The ZSC includes just γ -tocopherol, a vitamin E with anti-inflammatory and antioxidant properties (25, 26).

Physical Parameters of semen

Table 2 shows that ZSC levels significantly affect the physical assessment of semen, particularly volume, color, and pH values. Compared to the control animals, semen volume and color significantly rose ($P<0.05$) over time with varying ZSC doses, especially in T2 (0.60 0.04, 0.80 0.04) throughout the second phase. While pH maintained unimportant over both groups and times, as well as the color likewise exhibited a substantial rise (3.75 ± 0.25 , 4.75 ± 0.25).

Table 2. Effects of different concentrations of *Ziziphus spina-Christi* (ZSC) on physical evaluation of semen

Traits	Period (day)	Groups			LSD
		Control	ZSC-2%	ZSC-4%	
Volume	45	0.57±0.02 ^{Ba}	0.42±0.02 ^{Bb}	0.60±0.04 ^{Ba}	0.090
	90	0.67±0.02 ^{Ab}	0.57±0.02 ^{Ab}	0.80±0.04 ^{Aa}	
Color	45	1.75±0.25 ^{Bb}	2.50±0.28 ^{Bb}	3.75±0.25 ^{Ba}	0.078
	90	2.75±0.25 ^{Ab}	3.50±0.28 ^{Ab}	4.75±0.25 ^{Aa}	
pH	45	6.42±0.04 ^{Ba}	6.30±0.02 ^{Ba}	6.45±0.02 ^{Ba}	0.100
	90	6.55±0.02 ^{Aa}	6.57±0.04 ^{Aa}	6.62±0.02 ^{Aa}	

Values are the mean ± SEM, n= 4. A-B means that a lacking capital superscript in the same column of each trait significantly differs ($P\leq 0.05$). a-c Means with a small superscript in the same row of each trait significantly differ ($P\leq 0.05$). Number of colors means (1) cloudy, (2) milky, (3) thin creamy, and (4) creamy

The study found that goats accumulate new sperm with a minimum capacity of 3 mL, and current findings were supported by (27). The sperm volume, color, and pH were measured using an Artificial Vagina (AV) setup. There were no significant differences ($P>0.05$) in semen volume for the AV technique, but a trend was observed with higher color scores indicating a higher concentration of sperm, which is

Table 3. Effects of different concentrations of *Ziziphus spina-Christi* (ZSC) on microscopic evaluation of semen

Traits	Period (day)	Groups			LSD
		Control	ZSC-2%	ZSC-4%	
Concentration (106)	45	1238.7±2.65 ^{Aa}	1221±4.20 ^{Bb}	1355±5.40 ^{Ba}	11.79
	90	1246.5±2.50 ^{Ab}	1249±2.75 ^{Ab}	1374±5.13 ^{Aa}	
Mass motility (%)	45	32.250±2.17 ^{Bb}	31.00±2.48 ^{Bb}	41.00±1.47 ^{Ba}	5.88
	90	40.500±1.48 ^{Ab}	41.50±2.21 ^{Ab}	51.00±1.47 ^{Aa}	
Individual motility (%)	45	30.750±2.01 ^{Bb}	26.00±2.73 ^{Bb}	40.50±1.84 ^{Ba}	6.80
	90	39.500±2.02 ^{Ab}	36.00±2.04 ^{Ab}	51.25±2.86 ^{Aa}	
Live (%)	45	49.000±2.94 ^{Ab}	53.20±2.95 ^{Bb}	76.75±2.01 ^{Ba}	7.26
	90	54.250±2.32 ^{Ab}	61.25±2.46 ^{Ab}	87.50±1.70 ^{Aa}	
Dead (%)	45	51.000±2.94 ^{Aa}	46.73±2.95 ^{Aa}	23.25±2.01 ^{Ab}	6.20
	90	45.750±2.32 ^{Aa}	38.75±2.46 ^{Bb}	12.50±1.70 ^{Bc}	

Values are mean±SEM, n= 4. A-B means with a lacking capital superscript in the same column of each trait significantly differ ($P\leq 0.05$). a-c Means that a lacking small superscript in the same row significantly differs ($P\leq 0.05$)

A common belief is that the movement of waves in semen indicates the motility and viability of sperm. Ejaculate motility is crucial for semen assessment (27). Table (3) shows an important variance in ZSC levels, with the 40 g amount leading to a more powerful semen wave. A study showed the percentage of sperm motility in bulls with the addition of *Ziziphus*, in which these findings indicate that the initial average motility value before equilibration was 83.8%, which is a feasible condition for further processing. Researchers consider high sperm motility to indicate high sperm quality with appropriate fertilizing potential and fertility (29). The movement of sperm could be utilized to calculate the number of dosages delivered per

a significant ($P<0.05$) difference in color scores between groups. The semen ejaculate volume was 0.8-0.5 mL, with the normal color being milky or creamy; this coincided with (27). The normal color of semen, according to (16), usually had a milky or creamy tint, whereas the color of semen was within the normal similar to the results of (28). The pH of semen in this study was in the normal range of 6.9-7.2, according to (28).

Microscopic Analysis of Semen Parameters

The groups of sperm concentrations are increasing over time, as demonstrated in (Table 3). In particular, the current analysis has linked increased ZSC levels with increased T1 and T2 values. With comparable ZSC concentrations of 51.00 ± 1.47 and 51.25 ± 2.86 million sperm, respectively, throughout the second timing, T2 demonstrated both high mass and individual motility rates. The difference between this and the control is considerable. Additionally, mass motility significantly improved during the second session with a rise in ZSC concentration that peaked at 51.00 ± 1.47 . Finally, with increased ZSC level, individual motility was enhanced with time. On the other hand, the live sperm percentage of the T1 and T2 grew significantly ($P<0.05$) as well as in the control; at the same time, improvement in the group receiving treatment was more significant with an increased amount of ZSC in the second phase was recorded (61.25 ± 2.46 , 87.50 ± 1.70). In contrast, the dead sperm percentage of T1 and T2 was significantly ($P<0.05$) lower in the final period, with a change in concentration and period throughout the study

ejaculation. During the winter period of the trial, sperm motility was significantly ($P<0.05$) lower in both treatment groups. These findings coincide with (9) but contradict those of (7). The quantity of sperm cells per unit volume (mL) of seminal plasma is commonly indicated by sperm cell concentration or sperm density.

T2 male goats were 8-12 months old. As they develop, goats undergo physiological changes that affect their reproductive abilities. Abah et al. (34) report that young male semen parameters, including motility, fluctuate. Variations are normal as the reproductive system matures. Even though the semen was visually good, age-related variability likely lowered motility rates in our study. Semen

quality is also affected by collection stress. Stress, especially from handling and environmental changes, can lower livestock semen quality, according to Kumar et al. (35). We collected goat semen outside the mating season when their reproductive physiology is not optimal for semen production. This disruption in the reproductive cycle can stress animals, affecting semen quality and motility. The observed poor motility results may be due to semen collection stress and being done outside of mating season.

The ability of ZSC to maintain sperm motility is due to flavonoids, which act as antioxidants and reduce membrane destruction during semen processing (22). High motilities in ZSC may be due to iron, calcium, phosphorus, vitamins, and flavonoids (16). Sperm motility, viability, and fertility are elevated in mammalian species with minimal ROS levels (30). Consuming antioxidant-rich diets may boost sperm fertility. Over 70% of breeding goat spermatozoa should be morphologically normal (31).

The current study assessed the proportion of live sperm in the T1 and T2 collecting groups during the natural mating season's autumn. Autumn sperm percentage assays revealed no significant ($P>0.05$) difference between groups. However, the winter proportion of sperm viability (% of live sperm) was significantly lower than that reported by (28). The current enhancement comes about because ZSC leaves are rich in vitamin E, which plays a crucial role in stimulating the pituitary gland to produce interstitial cell-stimulating hormone, which increases testosterone levels (32). ZSC leaves contain flavonoids with antioxidant activity, similar to sex hormones, which have bioflavonoids and androgenic effects in animals (1, 33). Good health and efficient food consumption improve goat sexual behaviors and reproductive health, increasing sperm production (20). Semen tests were performed in this experiment; further research is necessary to determine the ZSC impacts on further characteristics of semen and fertility.

The investigation aimed to assess how different *Ziziphus Spina-Christi* leaf concentrations affected the fertility of buck goats. According to the current research, testosterone levels increased considerably at a high concentration of ZSC (4%) compared to the ZSC control group's (2%) values. Similarly, the physical evaluation of semen, including the volume, color, and pH values, was impacted by 4% of ZSC. Likewise, an increase in concentration of ZSC level led to improved semen concentration, mass, and individual motilities with time. The ZSC concentration (4%) had a positive impact on live and dead sperm percentages. As a result, a high concentration (4%) of ZSC added could improve the testosterone hormone and some semen parameters in Iraqi bucks.

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N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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تأثير أوراق نبات السدر على مستوى هرمون التستوستيرون وخصائص السائل المنوي للماعز العراقي

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الخلاصة

هدفت الدراسة الحالية إلى تقييم تأثير تراكيز مختلفة من مسحوق أوراق نبات السدر (ZSC) *Ziziphus spina-Christi* على خصوبة ذكور الماعز المحلية. تم تقسيم اثني عشر ماعزًا تتراوح أعمارهم بين ٨-١٢ شهرًا ومتوسط وزن الجسم 22 ± 2 كجم عشوائيًا إلى ثلاث معاملات غذائية: T1 و T2 و T3، وتم إعطاء كل منها نظامًا غذائيًا أساسيًا يوميًا بالإضافة إلى علف قدره ٢٠ كجم/كجم. (٢٪) من ZSC لـ T1 و ٤٠ كجم/كجم (٤٪) من ZSC لـ T2؛ وتم استخدام T3 كعنصر تحكم يستهلك العليقة الأساسية فقط. واستمرت التجربة لمدة ٣ أشهر. في اليومين ٤٥ و ٩٠، تم جمع عينات الدم لقياس هرمون التستوستيرون في البلازما باستخدام مقاييس مناعية كروماتوغرافية. بالإضافة إلى ذلك، تم جمع عينات السائل المنوي في نفس النقاط الزمنية لإجراء التقييمات الفيزيائية (الحجم واللون ودرجة الحموضة) والمجهرية (تركيز السائل المنوي، الكتلة ونسبة الحركة الفردية، ونسب الحيوانات المنوية الحية والميتة). أظهرت النتائج أن ZSC عند ٤٪ زيادة معنوية ($P < 0.05$) في تركيز هرمون التستوستيرون وجميع المؤشرات الفيزيائية عند ٤٥ و ٩٠ يومًا مقارنة بـ ٢٪ ZSC ومجموعات السيطرة. بالإضافة إلى ذلك، تحسنت ZSC بنسبة ٤٪ بشكل ملحوظ ($P < 0.05$) في المعلمات المجهرية في كلا الفترتين الزمنيةتين مقارنة بـ ٢٪ ZSC ومجموعتي التحكم. كشفت النتائج الحالية أن تناول ZSC على نظام غذائي بنسبة ٤٪ يمكن أن يعزز عملية معالجة السائل المنوي للماعز وخصوبة الماعز ويعزز بشكل ملحوظ ($P < 0.05$) هرمون التستوستيرون الذي كان له قيم أعلى مقارنة بـ (٢٪) في مجموعة السيطرة ZSC. وبالمثل، أثر (٤٪) من ZSC على التقييم الفيزيائي للسائل المنوي، مثل الحجم واللون وقيم الأس الهيدروجيني. وبالتالي فإن إضافة ZSC بتركيز عالي (٤٪) يمكن أن يعزز معالجة السائل المنوي للماعز وخصوبة الماعز بشكل عام.

الكلمات المفاتيح: السدر، الخصوبة، الماعز، التستوستيرون، السائل المنوي