



Spirulina and Vitamin E Supplementation at Sustainable Feeding Levels Enhance the Reproductive Performance and Health of Iraqi Awassi Rams

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Abstract

This study evaluated the individual effects of Spirulina (*Arthrospira platensis*) and Vitamin E supplementation on reproductive performance, antioxidant status, inflammatory markers, hormonal profiles, and serum biochemical parameters in Iraqi Awassi rams. Twenty-four mature rams were randomly assigned to four groups: control (G1), Spirulina at 1 g/kg (G2), Spirulina at 5 g/kg (G3), and Vitamin E at 400 IU/kg (G4). Over a 90-day trial, blood and seminal samples were collected to assess key physiological indicators. Rams in the high Spirulina group (G3) exhibited significantly elevated levels of testosterone (5.3 ± 0.4 ng/mL, $P < 0.05$), luteinizing hormone (2.9 ± 0.2 mIU/mL, $P < 0.05$), and follicle-stimulating hormone (2.0 ± 0.2 mIU/mL, $P < 0.05$), alongside improved semen quality (sperm concentration: $3.6 \pm 0.2 \times 10^6$ /mL; motility: $82.6 \pm 2.7\%$, $P < 0.05$). Antioxidant biomarkers (SOD: 15.2 ± 0.4 U/mL; CAT: 11.5 ± 0.3 U/mL; GPx: 9.2 ± 0.2 U/mL) were highest in G3, with a marked reduction in malondialdehyde (3.8 ± 0.1 nmol/mL, $P < 0.05$). Inflammatory markers (TNF- α : 30.4 ± 1.2 pg/mL; IL-6: 20.7 ± 1.0 pg/mL; CRP: 4.1 ± 0.2 mg/L) were significantly lower in G3 and G4 ($P < 0.05$). Serum biochemical analysis revealed increased total protein (8.0 ± 0.3 g/dl) and glucose (98.4 ± 2.5 mg/dl), and reduced cholesterol (100.3 ± 2.9 mg/dl), triglycerides (68.5 ± 1.8 mg/dl), urea (28.3 ± 0.9 mg/dl), and creatinine (1.20 ± 0.03 mg/dl) in supplemented groups, particularly G3 ($P < 0.05$). These findings demonstrate that Spirulina and Vitamin E, when used separately, can enhance reproductive and metabolic health in rams, with Spirulina showing dose-dependent superiority. The results support the use of Spirulina—especially at 5 g/kg—as a functional feed additive to improve physiological resilience and reproductive efficiency in ruminants.

Keywords: Spirulina, Reproductive performance, Oxidative stress, Inflammatory Mediators, Awassi Rams

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Introduction

Among the most important factors affecting productivity and genetic advancement in livestock, particularly in sheep breeding under a well-organized system, reproductive performance takes center stage. Among indigenous breeds, the Iraqi Awassi ram has an important economic and genetic value for its tenacity and hardness to cope with severe environments and contribute toward food security in the region. However, reproduction incompetence among this breed is

increasingly blamed on oxidative stress, nutritional deficiencies, and underlying genetic factors, which compromise semen quality and fertility outcomes (5,10). Oxidative stress, an imbalance between the ROS and the body antioxidant machinery, plays a major role in male reproductive dysfunction. Excessive ROS may cause damage to spermatozoan integrity, impair motility, and induce morphological abnormalities. All these aspects collectively impede fertilizing potential and are considered causative factors of subfertility in rams. Oxidative stress is one condition, therefore, whose

improvement by targeted nutritional strategies is important for the improvement of reproductive health among those breeds vulnerable to it, such as the Awassi (1, 21).

Dietary antioxidants have emerged as promising interventions to reduce the oxidative burden and improve semen quality and immune profile. Spirulina is a nutrient-dense cyanobacterium, *Arthrospira platensis*, that harbors all the basic nutritional requirements and has a rich profile of bioactive compounds such as phycocyanin, β -carotene, and γ -linolenic acid. These molecules have potent antioxidant and anti-inflammatory activity. Thus, several studies have proved their efficacy in improving sperm motility, viability, enzymatic antioxidant activity in rams, and other livestock species. Their multifaceted benefits make spirulina a promising candidate for reproductive improvement, especially in nutritionally stressful regions of the world (9, 28).

Vitamin E (α -tocopherol) is a well-known, fat-soluble antioxidant widely applied in animal nutrition for the purpose of protecting sperm membranes against lipid peroxidation. The addition of this compound to diets has been associated with an improvement in sperm motility and a decrease in morphological defects, apart from enhancing the activities of endogenous antioxidants such as superoxide dismutase (SOD) (6, 29). Besides, Vitamin E has also been found to exert immunomodulatory activities by inhibiting pro-inflammatory cytokines like TNF- α and IL-6, implicated in reproductive disorders (31). These properties make it useful for addressing oxidative and inflammatory stressors relevant to male fertility.

Although Spirulina and Vitamin E have their own merits, few studies have compared their reproductive effects on local breeds, particularly in the Iraqi Awassi ram. The development of nutritional, evidence-based protocols requires relative efficacy, considering regional breeding systems. The derived knowledge would provide a focused reproductive management that enhances semen quality, elevates antioxidant defenses, and minimizes inflammatory mediators under field conditions.

The present study is designed to investigate the effects of supplementation of Spirulina and Vitamin E on the main reproductive parameters in Iraqi Awassi rams.

Materials and Methods

Experimental Design

This study was conducted under the prevailing semi-intensively managed conditions in western Iraq during the late summer season (August/September) in which the ambient temperature fluctuated between 38°C to 44°C with relative humidity averaging between 35 and 45%. Using this data, a THI calculated on the same parameters remained above 80, indicating that ruminants were in moderate and severe heat stress conditions. This study was designed to investigate the comparison and evaluation of Spirulina *Arthrospira platensis* and Vitamin E supplementation on semen quality, antioxidative status, inflammatory mediators, seminal plasma biochemistry, and hormonal profile of Iraqi Awassi rams. Twenty-four Iraqi Awassi rams ($n = 8$ in each group) aged 10 months old were randomly allotted to four different treatment groups (G1-G4). The experiment was conducted at a private farm in Al-Anbar Governorate, Iraq, during its late summer and under regular management practices. The ethical clearance was approved from the Faculty of Agriculture, University of Anbar.

Prior to the start of the experiment, all rams underwent a 14-day acclimatization period under the same housing and feeding conditions as used during the trial. This allowed animals to adapt to handling and diet before data collection began.

All animals were fed a standard concentrate feed mixture (CFM) formulated to meet maintenance and reproductive requirements for 90 days. Water was provided *ad libitum* entire the trial. The experimental groups and feed compositions are detailed below in (Table 1). All rams were clinically examined and confirmed free of reproductive tract abnormalities or infectious diseases before enrollment. Routine veterinary checks and semen evaluations were performed to exclude animals with pre-existing reproductive disorders.

Table 1. Experimental Groups and Feed Composition

Group	Description	Supplement Level	Feed Composition (% of CFM)
G1	Control group	0 g/kg Spirulina, no Vit E	Alfalfa meal (35%), Wheat bran (30%), Corn grain (15%), Cottonseed meal (10%), Molasses (3%), Limestone (2%), Common salt (1%)
G2	Low Spirulina supplementation	1 g/kg Spirulina	Same as G1 + <i>Spirulina platensis</i> at 1 g/kg of CFM
G3	High Spirulina supplementation	5 g/kg Spirulina	Same as G1 + <i>Spirulina platensis</i> at 5 g/kg of CFM
G4	Vitamin E supplementation	400 IU/kg Vitamin E	Same as G1 + Vitamin E (α -tocopherol) at 400 IU/kg of CFM

Supplementation Details

- *Spirulina platensis* powder was procured from a certified source and tested for purity and nutritional content, following procedures described by Abdel-Khalek et al. (1).
- Vitamin E (α -tocopherol) was provided as a powder mixed into the concentrate feed at a rate of 400 IU/kg, following previous work demonstrating efficacy in rams (30, 16).
- *Spirulina* powder and Vitamin E capsules were mixed thoroughly into the daily concentrate ration to ensure uniform intake. Feed was offered individually, and refusals were recorded to confirm that each ram consumed the full intended dose.
- Daily feed intake was monitored, and supplement consumption was verified to ensure that animals received the complete assigned dose throughout the experimental period.

Data Collection

Semen Quality

Semen samples were collected twice weekly for 90 days. For each ram, mean values of sperm motility, viability, morphology, and concentration were calculated across all time points and used for statistical comparison among treatment groups. Semen samples were collected with an artificial vagina based on the protocols of G ng r, I H et al. (20). Immediately after collection, semen samples were diluted 1:1 with a Tris–egg yolk–glycerol extender (composition: Tris 3.63 g, citric acid 1.99 g, fructose 0.5 g, egg yolk 20%, glycerol 7%, distilled water to 100 mL). Dilution was performed to standardize sperm concentration and facilitate microscopic evaluation. All samples were processed within 30 minutes of collection to minimize variability. Sperm motility and morphology were assessed using a

phase-contrast microscope ($\times 400$ magnification, $40\times$ objective lens). For each sample, at least five non-overlapping microscopic fields were examined, and a minimum of 200 spermatozoa were evaluated per slide. Motility was expressed as the percentage of progressively motile sperm, while morphology was recorded as the proportion of normal versus abnormal forms.

Antioxidant Biomarkers

Superoxide dismutase (SOD) (MBS1602165, MyBioSource.com, USA), catalase (CAT), glutathione peroxidase (GPx) (MBS 1607434, MyBioSource.com, USA), and malondialdehyde (MDA) (#EU2577, Finetest, China) were measured in blood samples using ELISA kits which were validated to be ovine species-specific (32, 31).

Inflammatory Mediators

Plasma levels of tumor necrosis factor- α (TNF- α) (ESH0025, Fine test, China), interleukin-6 (IL-6) (ESH0019, Fine test, China), and C-reactive protein (CRP) (MBS738645, MyBiosource, USA) were measured using commercial ELISA kits, as described by El-Ratel and Gabr (12).

Seminal Plasma Biochemistry

Following the experimental period, semen was collected from all the rams through an artificial vagina. Seminal plasma was centrifuged at 3000 rpm for 15 minutes and stored at -20°C until biochemistry analysis. Citrate concentration was also measured spectrophotometrically using a citrate lyase commercial enzymatic kit (BioSystems, Spain) based on citrate conversion to oxaloacetate and acetate, followed by NADH production measured at 340 nm a method previously confirmed in ruminant seminal plasma (12). Zinc and iron concentrations were determined by atomic absorption spectrophotometry (AAS) after wet digestion

of seminal plasma samples in nitric and perchloric acids according to methods described by the Association of Official Analytical Chemists (6) adapted for application in ovine seminal plasma (2). The samples were analyzed in a flame AAS (Shimadzu AA-7000, Japan) with 213.9 nm and 248.3 nm wavelength settings for zinc and iron, respectively. All data analyses were conducted in duplicate for accuracy, and quality control was ensured with the assistance of certified reference materials and blank samples. Results were expressed as mean $\pm$ standard error (SE).

Hormonal Profile

Serum testosterone levels, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were determined by using ovine-specific ELISA kits (Biolab, Italy). The hormonal assessments were conducted to investigate the endocrine response to supplementation, and guidelines by Espina-Ávila et al. (16) and Salah et al. (41).

Serum Biochemical Analysis

The serum samples were collected at the end of experimental duration for biochemical estimation. Albumin and total protein levels were measured using Henry et al. (24), and globulins were found as a difference between total protein and albumin. Triglyceride and total cholesterol of serum were estimated by Richmond's method (39) and high-density lipoproteins (HDL) by Abell et al. (4). Very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) concentrations were calculated by the formula of Friedewald et al. (19). Serum urea and creatinine were measured by Henry et al. (24) and Fabiny and Eringhausen (17), respectively. The activities of AST and

ALT were estimated as provided by Reitman and Frankel (37). All serum biochemical analyses were performed by a spectrophotometer and a diagnostic chemical kit (Bio Diagnostics, USA), previously utilized by Abdelnaby et al. (2) and Baiomy et al. (8).

Statistical analysis

Normality of residuals was assessed with the Shapiro–Wilk test. The values were expressed as mean $\pm$ standard error (SE). One-way analysis of variance (ANOVA) was used to assess the impact of feeding treatment on all the parameters measured, including semen quality, antioxidant biomarkers, inflammatory mediators, hormonal profile, seminal plasma biochemistry, and serum biochemical characteristics. Statistical analysis was carried out using SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA) (42). Substantial differences between means of treatments were ascertained through Duncan's Multiple Range Test (11), and statistical significance was established at $P < 0.05$.

Result and discussions

Semen Quality

The results showed that sperm concentration, progressive motility, viability, and normal morphology were maximum in G3, receiving the high dose of Spirulina, with significant differences to all other groups, $P < 0.05$. The lowest semen quality parameters were observed in G2 (Vitamin E). While intermediate improvements in both G2 and combined treatment group G4 were detected, the improvement exhibited by the two groups was highly significant over G1 (control), yet less than that manifested in G3, as shown in **Table 2**.

Table 2. Effect of Spirulina and Vitamin E Supplementation on Semen Quality Parameters in Iraqi Awassi Rams (Mean $\pm$ SE)

Parameter	G1	G2	G3	G4
Sperm Concentration ($\times 10^6/\text{mL}$)	2.1 $\pm$ 0.1 ^d	2.8 $\pm$ 0.1 ^c	3.6 $\pm$ 0.2 ^a	3.2 $\pm$ 0.1 ^b
Progressive Motility (%)	68.2 $\pm$ 2.3 ^d	75.4 $\pm$ 2.5 ^c	82.6 $\pm$ 2.7 ^a	78.9 $\pm$ 2.4 ^b
Viability (%)	71.5 $\pm$ 2.1 ^d	78.3 $\pm$ 2.3 ^c	85.2 $\pm$ 2.5 ^a	81.4 $\pm$ 2.2 ^b
Normal Morphology (%)	76.8 $\pm$ 2.0 ^d	82.6 $\pm$ 2.2 ^c	88.3 $\pm$ 2.4 ^a	85.1 $\pm$ 2.1 ^b

G1: Control, G2: 1 g/kg Spirulina, G3: 5 g/kg Spirulina, G4: Vitamin E 400 IU/kg. Different superscript letters (^a, ^b, ^c, ^d) within the same row indicate statistically significant differences ($P < 0.05$) according to Duncan's Multiple Range Test.

These findings are in accordance with previous studies that have reported the antioxidative and reproductive-enhancing effects of Spirulina and Vitamin E supplementation (40). The marked improvement in sperm concentration, motility, viability, and morphology

in G3 agrees with findings by El-Ratel and Gabr (12), who showed improved semen parameters in heat-stressed rabbits after spirulina administration. Similarly,

Abdelnaby et al. (2) reported that Spirulina supplementation enhances spermatogenesis and reduces oxidative damage to sperm cells.

The moderate improvements in G2 and G4 further confirm the observations made by El-Sheshtawy et al. (15), showing that Vitamin E enhances sperm motility and viability through stabilization of membrane integrity and reduction in lipid peroxidation. Collectively, these results support the use of Spirulina—particularly at higher doses—as a potent natural additive for improving reproductive performance in rams exposed to nutritional and environmental stressors.

Antioxidant Biomarkers

At the end of the experimental period, antioxidant biomarkers showed significant differences among treatment groups: enzymatic antioxidant activity was enhanced, and lipid peroxidation was reduced, especially in rams receiving Spirulina at 5 g/kg/day or Vitamin E during supplementation, as compared to controls. Super oxide dismutase, catalase, and glutathione peroxidase activities were higher in G3 (the high Spirulina group) than in other treatment groups, but MDA levels were lowest in G3 and G4—the combined treatment group—in comparison to the other treatment groups, indicating a positive impact on maintaining oxidative balance and reducing cellular damage (Table 3).

Table 3. Effect of Spirulina and Vitamin E Supplementation on Antioxidant Biomarkers in the serum of Iraqi Awassi Rams (Mean $\pm$ SE)

Parameter	G1 (Control)	G2 (1 g/kg Spirulina)	G3 (5 g/kg Spirulina)	G4 (Vitamin E 400 IU/kg)
SOD (U/mL)	12.4 $\pm$ 0.5 ^b	14.8 $\pm$ 0.6 ^a	15.2 $\pm$ 0.4 ^a	14.6 $\pm$ 0.5 ^a
CAT (U/mL)	8.9 $\pm$ 0.3 ^c	10.2 $\pm$ 0.4 ^b	11.5 $\pm$ 0.3 ^a	10.8 $\pm$ 0.4 ^b
GPx (U/mL)	7.1 $\pm$ 0.2 ^c	8.4 $\pm$ 0.3 ^b	9.2 $\pm$ 0.2 ^a	8.9 $\pm$ 0.3 ^b
MDA (nmol/mL)	5.6 $\pm$ 0.2 ^a	4.3 $\pm$ 0.2 ^b	3.8 $\pm$ 0.1 ^c	4.0 $\pm$ 0.2 ^c

Different superscript letters (^a, ^b, ^c) within the same row indicate statistically significant differences ($P < 0.05$) according to Duncan's Multiple Range Test.

These antioxidant enzyme activity elevations and lipid peroxidation reductions are in agreement with recent findings in animal nutrition studies. The significant increase in the levels of SOD, CAT, and GPx, concurrently with a decrease in MDA in G3, can be interpreted as evidence of improved oxidative protection and cellular membrane stability. Salah et al. (41) found that Spirulina supplementation had significantly enhanced antioxidant defenses while also diminishing many of the pathological changes in broilers challenged with ochratoxin A, underscoring its systemic protective effects. El-Sayed et al. (14) observed that Spirulina supplementation combined with Vitamin E in transport-stressed calves caused a significant decrease in MDA and an increase in SOD and CAT activities, thus supporting the synergistic effectiveness against oxidative stress.

Overall, these findings support Spirulina and Vitamin E as antioxidants, Spirulina at a high dose being the most effective. Thus, the strategic inclusion of Spirulina, especially at elevated levels, as a potent natural antioxidant in ruminant diets, may be supported when the animals are challenged nutritionally or environmentally.

Inflammatory Mediators

Markers of inflammation varied significantly among the treatment groups. The treated rams with Spirulina and Vitamin E contained minimum concentrations of pro-inflammatory cytokines compared to the control group. The group with high Spirulina (G3) and the group with Vitamin E (G4) contained the lowest levels of TNF- α , IL-6, and CRP, indicating a high anti-inflammatory response as in (Table 4).

Table 4. Effect of Spirulina and Vitamin E Supplementation on Inflammatory markers in the serum of Iraqi Awassi Rams (Mean $\pm$ SE)

Parameter	G1 (Control)	G2 (1 g/kg Spirulina)	G3 (5 g/kg Spirulina)	G4 (Vitamin E 400 IU/kg)
TNF- α (pg/mL)	42.6 $\pm$ 1.8 ^a	36.2 $\pm$ 1.5 ^b	30.4 $\pm$ 1.2 ^c	31.1 $\pm$ 1.3 ^c
IL-6 (pg/mL)	28.9 $\pm$ 1.4 ^a	24.3 $\pm$ 1.2 ^b	20.7 $\pm$ 1.0 ^c	21.5 $\pm$ 1.1 ^c
CRP (mg/L)	6.8 $\pm$ 0.3 ^a	5.2 $\pm$ 0.2 ^b	4.1 $\pm$ 0.2 ^c	4.3 $\pm$ 0.2 ^c

Different superscript letters (^a, ^b, ^c) within the same row indicate statistically significant differences ($P < 0.05$) according to Duncan's Multiple Range Test.

The significant decrease in the inflammatory biomarkers TNF- α , IL-6, and CRP in Spirulina and Vitamin E-supplemented rams shows their potential immunoregulatory activities. The lowest values recorded in high Spirulina (G3) and Vitamin E (G4) groups show a synergic effect of inhibiting systemic inflammation and immune resistance. These findings were supported by Yadav and Kumar (46), where they deduced Spirulina improved antioxidant activity and reduced inflammatory cytokines in Barbari goats. Similarly, Momo et al. (34) reported Spirulina supplementation enhanced reproductive performance and inhibited stress-induced inflammation in doe rabbits. El-Sayed et al. (14) demonstrated co-administration of Spirulina with Vitamin E caused considerable inhibition of TNF- α and IL-6 in transport-stressed calves. In chickens, Spirulina was found by Salah et al. (41) to successfully reverse

mycotoxin-induced inflammatory reaction, and El-Sheshtawy et al. (15) established that Vitamin E lowered CRP and preserved membrane stability in rams. Generally, the above findings further validate the current study and advocate the strategic use of Spirulina particularly at the higher levels of inclusion—natural anti-inflammatory treatment in ruminant medicine.

Concentration of Seminal Plasma Citrate and Trace elements

Rams in G3 (high Spirulina group) possessed the highest levels of citrate and zinc, implying increased accessory gland activity and mineral availability. Supplemented groups were lowest for iron compared to control; the reason not being known but a possible rationale being reduced oxidative requirement. Improved biochemical status is quite clearly shown by the graph in G3 followed by G2, G4, and G1 (Figure 1).

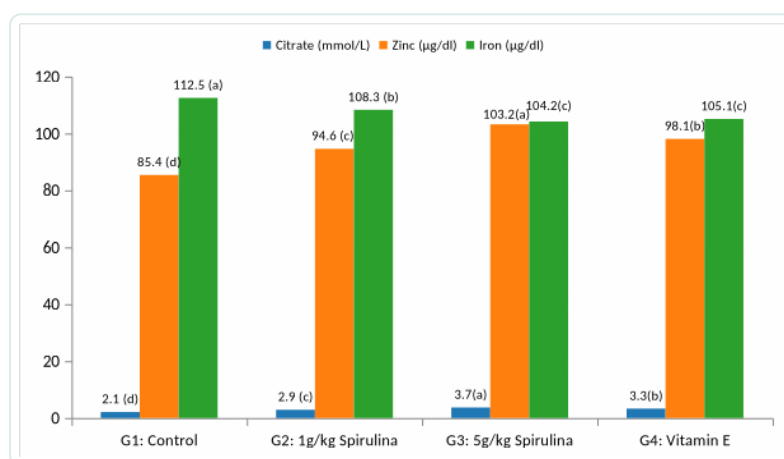


Figure 1. Effect of Spirulina and Vitamin E Supplementation on Seminal Plasma Citrate and Trace Elements in Iraqi Awassi Rams.

The increased seminal plasma citrate and zinc levels in Spirulina high dose-treated rams (G3) reflect enhanced accessory gland activity and bioavailability of minerals. Citrate, the key indicator of prostate and seminal vesicle functions, was largest in G3 and reflects increased glandular secretion and metabolic conservation of spermatozoa. Zinc, a key factor in sperm membrane stability and motility, was also the highest in G3, as corroborated by Mansour and Zeitoun (33) that Spirulina supplementation in Barki lambs significantly increased seminal plasma zinc and semen quality. Firdaus et al. (18) also confirmed through meta-analysis that Spirulina increases the intake of trace elements as well as antioxidant enzyme activity in small ruminants. Iron was modestly lowered in supplemented groups, perhaps due to reduced oxidative stress and reduced iron-dependent catalysis demand, according to Murali Mohan et al. (35)

in Nellore ram lambs. These results altogether support the improved biochemical status in G3, followed by G2, G4, and G1, and validate the place of Spirulina in optimizing reproductive biochemistry.

Effect of Spirulina and Vitamin E Supplementation on Serum Hormonal Profile in Iraqi Awassi Rams

Figure 2 describes the treatment groups for testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels. Rams treated with 5 g/kg Spirulina (G3) exhibited the highest value for the three hormones, indicative of enhanced testicular and endocrine function. The treatment group supplemented with 1 g/kg Spirulina (G2) and the Vitamin E-supplemented group (G4) also showed improvement over the control (G1), though to a reduced extent. These

results suggest that Spirulina, particularly at higher doses, may influence the hypothalamic-pituitary-gonadal axis and increase reproductive hormone secretion.

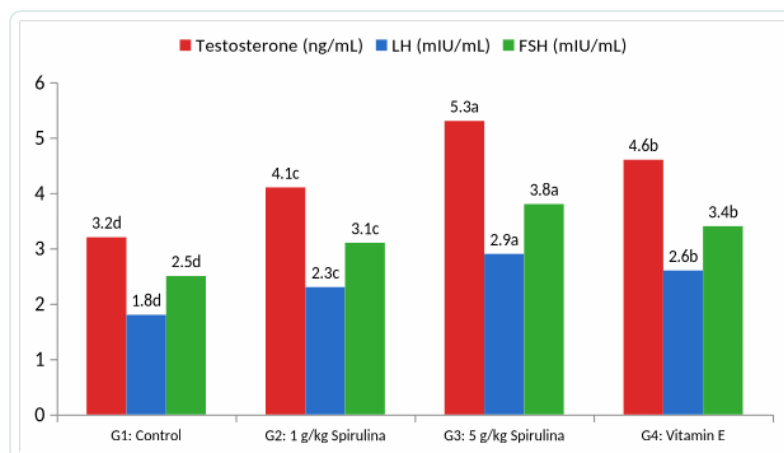


Figure 2. Concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) across the four treatment groups.

The increased levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in Spirulina-Vitamin E-supplemented rams particularly in the high Spirulina group (G3) reflect greater testicular function and stimulation of the hypothalamic-pituitary-gonadal axis. These hormone increases accord with El-Ratel and Gabr (12), which demonstrated Spirulina and Vitamin E improved reproductive hormone levels and embryo quality in heat-stressed rabbits. Hamad and Hassan (23) confirmed that Spirulina supplementation in Awassi lambs affected serum testosterone and ICSH levels, an effect that was in correlation with testicular morphology and reproductive traits. Rengaraj and Hong (38) explored the role of Vitamin E in poultry and outlined its potential to protect sperm quality and endocrine homeostasis against oxidative damage. Abdelnour et al. (3) highlighted Spirulina's bioactive phycocyanin and polyunsaturated fatty acids as the key

modulators of reproductive hormones and fertility of livestock. All these findings collectively confirm the findings here and suggest that Spirulina, especially at higher inclusion rates, holds great promise as an organic reproductive endocrine function stimulant in ruminants.

Effect of Spirulina and Vitamin E Supplementation on Serum Biochemical Parameters

According to Table 5, Spirulina and Vitamin E supplementation significantly increased serum biochemical values in Iraqi Awassi rams. The level of total protein and glucose concentration was significantly higher with higher doses of Spirulina, peaking in G3. The cholesterol, triglycerides, urea, and creatinine level decreased significantly in G2, G3, and G4 when compared to the control. The highest Spirulina group, 5 g/kg (G3), gave the best biochemical improvement. Superscript letters show statistically significant differences between groups at $P < 0.05$.

Table 5. Effect of Spirulina and Vitamin E Supplementation on Serum Biochemical Parameters in Iraqi Awassi Rams (Mean ± SE)

Parameter	G1 (Control)	G2 (1 g/kg Spirulina)	G3 (5 g/kg Spirulina)	G4 (Vitamin E 400 IU/kg)
Total Protein (g/dl)	6.5 ± 0.2 ^d	7.3 ± 0.2 ^c	8.0 ± 0.3 ^a	7.6 ± 0.2 ^b
Glucose (mg/dl)	84.2 ± 2.1 ^d	91.8 ± 2.3 ^c	98.4 ± 2.5 ^a	94.7 ± 2.2 ^b
Cholesterol (mg/dl)	115.6 ± 3.4 ^a	107.2 ± 3.1 ^b	100.3 ± 2.9 ^d	102.8 ± 3.0 ^c
Triglycerides (mg/dl)	80.4 ± 2.2 ^a	74.1 ± 2.0 ^b	68.5 ± 1.8 ^d	70.9 ± 1.9 ^c
Urea (mg/dl)	35.1 ± 1.1 ^a	31.4 ± 1.0 ^b	28.3 ± 0.9 ^d	29.7 ± 0.8 ^c
Creatinine (mg/dl)	1.45 ± 0.05 ^a	1.33 ± 0.04 ^b	1.20 ± 0.03 ^d	1.26 ± 0.03 ^c

Different superscript letters (^a, ^b, ^c, ^d) within the same row indicate statistically significant differences ($P < 0.05$) according to Duncan's Multiple Range Test.

The rise in serum biochemical changes in Spirulina and Vitamin E-supplemented rams indicates higher metabolic activity and overall physiological well-being. The highest rises in the total protein and glucose, with related drastic falls in cholesterol, triglycerides, urea, and creatinine, in the high Spirulina-fed rams (G3) indicate improved hepatic function, use of energy, and renal efficiency. These results support Wang et al. (46), which indicated that Spirulina supplementation of lambs enhanced glucose and immunoglobulin G levels but reduced triacylglycerol and liver enzymes, reflecting enhanced metabolic and immune status. Firdaus et al. (18) also supported by meta-analysis that Spirulina enhances antioxidant enzyme activity and stability of serum biochemical markers in small ruminants. In the case of Vitamin E, Hakeem et al. (22) concluded that

graded doses in Kail sheep significantly increased serum albumin, globulin, and total protein, verifying its role in protein synthesis and antioxidant defense. Irmak et al. (25) showed that Vitamin E and β -carotene increased concentrations of follicle-stimulating hormone and triglycerides in nutritionally-flushed sheep, verifying its endocrine and metabolic activity. Apart from this, Musa et al. (36) found Vitamin E and selenium supplementation to increase the immunoglobulins and lipid profile of Yankasa sheep, justifying its systemic protective functions. All these studies, therefore, justify the biochemical improvements in G3, followed by G2, G4, and G1, and justify the strategic use of Spirulina and Vitamin E as functional feed supplements in ruminant nutrition.

Table 6. Body Weight of Awassi Rams (kg) before and after treatment

group	initial body weight (mean $\pm$ sd)	final body weight at 90 days (mean $\pm$ sd)
control	26.2 $\pm$ 1.1 ^a	35.5 $\pm$ 1.4 ^a
spirulina low	26.3 $\pm$ 1.0 ^a	36.2 $\pm$ 1.3 ^{ab}
spirulina high	26.4 $\pm$ 1.2 ^a	37.1 $\pm$ 1.5 ^b
vitamin E	26.1 $\pm$ 1.1 ^a	36.0 $\pm$ 1.2 ^{ab}

Body weight

The initial body weights of Awassi rams were comparable across all groups (~26 kg), confirming baseline homogeneity. The present study demonstrated that all Awassi rams gained weight during the 90-day supplementation period, with final body weights increasing from approximately 26 kg at baseline to 35–37 kg. It is notable that rams fed with the high dose group of Spirulina had the greatest gain in weight at 37.1 $\pm$ 1.5 kg, significantly more than the differences between the control and treated groups (Table 6). This indicates that Spirulina may serve as an excellent appetite stimulant in small ruminants. The nutrient composition of Spirulina supplementation and the nutrient density of Spirulina to Sheep may work synergistically. The high-quality protein, essential amino acids, and vitamins found in Spirulina, with phycocyanin being one of the active ingredients, allow for the increased digestibility of nutrients and efficiency of nitrogen use, and offer antioxidant defense mechanisms (14). These effects may have led to the reduced metabolic stress and increased efficiency of body growth that resulted in higher body weight gain and carcass characteristics similar to the data obtained in the present study. There is evidence from

previous research with Nellore ram lambs and the use of Spirulina that provides confirmation of its effectiveness as a functional feed additive in the production of Sheep Systems.

According to a study on Najdi lambs in a semi-arid environment (7), supplementing their diet with spirulina helped improve the ability to digest nutrients and positively impacted their growth performance. Spirulina also improves the biochemistry of growing male lambs and helps support their growth through the maintenance of metabolic equilibrium by influencing their body's antioxidant status (1). On the other hand, the addition of vitamin E to the diet produced moderate improvement in lambs' body weight. Due to its role as a lipid soluble antioxidant, vitamin E stabilizes the cell membrane. Although it has been shown to play an important role in reproductive and immune health, it did not appear to directly affect petroleum jelly production.

Therefore, spirulina, especially in greater quantities, provides both nutritional enhancement and antioxidant protection, which is why they produced superior growth performance than the Awassi breed. Therefore, it would be wise to consider feeding spirulina to locally raised

breeds, particularly in areas with challenging semi-arid conditions where preserving nutrient uptake and antioxidant balance is essential for max productivity.

Conclusion

Dietary supplementation of Spirulina or Vitamin E improved reproductive, biochemical, body weight, and antioxidant parameters in Iraqi Awassi rams. Spirulina (5 g/kg) was particularly effective, enhancing reproductive hormones, semen quality, antioxidant activity, and metabolic indicators, thereby supporting testicular function and oxidative balance. Vitamin E also showed benefits, mainly in reducing lipid peroxidation and inflammatory markers, though less pronounced than Spirulina. Overall, both supplements may serve as nutritional strategies to enhance reproductive performance and resilience under stress conditions.

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مقالة أصيلة

تحسين الأداء التناسلي وصحة كباش العواسي العراقية من خلال تناول مكملات السبيرولينا وفيتامين هـ بمستويات تغذية مستدامة

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الخلاصة

هدفت هذه الدراسة إلى تقييم التأثيرات الفردية لمكملات السبيرولينا (*Arthrospira platensis*) وفيتامين E على الأداء التناسلي، حالة مضادات الأكسدة، مؤشرات الالتهاب، مستويات الهرمونات، والمعايير الكيميائية الحيوية في مصل دم كباش العواسي العراقية. تم توزيع أربعة وعشرين كبشاً ناضجاً عشوائياً على أربع مجموعات: المجموعة الضابطة (G1)، سبيرولينا بتركيز 1 غم/كغم (G2)، سبيرولينا بتركيز 5 غم/كغم (G3)، وفيتامين E بتركيز 400 وحدة دولية/كغم (G4). خلال فترة تجربة استمرت 90 يوماً، تم جمع عينات الدم والسائل المنوي لتقييم المؤشرات الفسيولوجية الرئيسية. أظهرت الكباش في مجموعة السبيرولينا العالية (G3) ارتفاعاً معنوياً في مستويات التستوستيرون (0.4 ± 5.3 نانوغرام/مل، $P < 0.05$)، الهرمون اللوتيني LH (2.9 ± 0.2 وحدة دولية/مل، $P < 0.05$)، والهرمون المنشط للجريب FSH (2.0 ± 0.2 وحدة دولية/مل، $P < 0.05$)، إلى جانب تحسن في جودة السائل المنوي (تركيز الحيوانات المنوية: $0.2 \pm 3.6 \times 10^6$ مل؛ الحركة: $2.7 \pm 82.6\%$ ، $P < 0.05$). كانت مؤشرات مضادات الأكسدة الأعلى في G3 (15.2 ± 0.4 SOD؛ وحدة/مل؛ 11.5 ± 0.3 CAT؛ وحدة/مل؛ 9.2 ± 0.2 GPx؛ وحدة/مل)، مع انخفاض ملحوظ في المالونديالدهيد MDA (3.8 ± 0.1 نانومول/مل، $P < 0.05$). كانت مؤشرات الالتهاب (30.4 ± 1.2 TNF- α بيكوغرام/مل؛ 20.7 ± 1.0 IL-6؛ بيكوغرام/مل؛ 4.1 ± 0.2 CRP ملغم/لتر) أقل بشكل معنوي في G3 و G4 ($P < 0.05$). أظهر التحليل الكيميائي الحيوي للمصل زيادة في البروتين الكلي (0.3 ± 8.0 غم/دل) والجلوكوز (2.5 ± 98.4 ملغم/دل)، وانخفاضاً في الكوليسترول (2.9 ± 100.3 ملغم/دل)، الدهون الثلاثية (68.5 ± 1.8 ملغم/دل)، اليوريا (0.9 ± 28.3 ملغم/دل)، والكرياتينين (0.03 ± 1.20 ملغم/دل) في المجموعات المكملية، خصوصاً G3 ($P < 0.05$). تؤكد هذه النتائج أن السبيرولينا وفيتامين E، عند استخدامها بشكل منفصل، يمكن أن يعززا الصحة التناسلية والتمثيل الغذائي في الكباش، مع تفوق واضح للسبيرولينا بجرعات عالية. وتدعم النتائج استخدام السبيرولينا، خاصة بتركيز 5 غم/كغم، كمضاف غذائي وظيفي لتحسين الكفاءة الفسيولوجية والتناسلية في المجترات.

الكلمات المفتاحية: السبيرولينا، الأداء التناسلي، الإجهاد التأكسدي، الوسائط الالتهابية، كباش العواس