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Original article

Comparative Effects of *Balanites aegyptiaca* and *Spirulina* Supplementation on Serum Calcium, Phosphorus, and Hormonal Profiles in Male Rabbits

Fayrouz Khaled*[1], Antsar Husayn[2]

[1]*Chemistry Department, Faculty of Science, Omar Al-Mokhtar University, El-Beida, Libya*
[2]*Libyan Academy for Postgraduate Studies, Biomedicine Department, Jabal Al-Akhdar Branch, El -Libya*
Corresponding email. fayalzobair@yahoo.com

Abstract
This study evaluated the influence of *Balanites aegyptiaca* and Spirulina supplementation on mineral balance and key reproductive hormones in male rabbits. Spirulina produced a clear elevation in serum calcium, indicating enhanced mineral absorption and regulation, whereas *Balanites aegyptiaca* showed only a mild effect. In contrast, phosphorus levels remained stable across all groups, reflecting intact physiological control of phosphorus homeostasis. Hormonal analysis revealed that both supplements reduced estrogen and cortisol concentrations, suggesting a potential stress-modulating effect. Meanwhile, FSH, testosterone, and LH showed a gradual improvement, with Spirulina demonstrating the most pronounced enhancement in reproductive hormone profiles. Overall, the findings indicate that Spirulina has a stronger impact on calcium metabolism and reproductive endocrine function, while *Balanites aegyptiaca* provides moderate hormonal benefits without altering phosphorus stability. These outcomes support the possible use of both supplements, especially Spirulina, in improving mineral status and reproductive health in male rabbits.
Keywords. Balanites Aegyptiaca, Spirulina, Calcium, Phosphorus, Hormonal Profile, Male Rabbits.

Introduction

Mineral balance and hormonal regulation play essential roles in maintaining normal physiological functions, growth, and reproductive performance in mammals [1]. Calcium and phosphorus are among the most critical macrominerals involved in skeletal development, neuromuscular activity, enzymatic signaling, and energy metabolism [2]. Their homeostasis is tightly controlled by vitamin D, parathyroid hormone, and renal function, making any nutritional modulation of these minerals biologically significant [3]. In recent years, interest has grown in exploring natural supplements capable of improving mineral absorption and endocrine function in animal models [4 -11]. *Balanites aegyptiaca*, commonly known as the desert date, is a traditional medicinal plant widely used in North Africa and the Middle East. Its fruits and seeds contain saponins, flavonoids, sterols, and minerals that have been linked to antioxidant, anti-inflammatory, hepatoprotective, and fertility-enhancing activities [12]. Several studies have suggested that *Balanites aegyptiaca* may influence endocrine pathways, particularly those related to testosterone production and stress hormone modulation, although its effects on mineral metabolism remain less explored [13]. Spirulina, a nutrient-dense cyanobacterium, has attracted global attention due to its high content of bioavailable proteins, vitamins, minerals, phycocyanin, and antioxidant compounds. Its rich calcium and phosphorus composition, together with its ability to enhance gut absorption, make it a promising candidate for improving mineral status in animals [14]. Recent findings also highlight Spirulina's positive influence on reproductive hormones, stress biomarkers, immune responses, and oxidative balance [15]. These bioactive properties suggest that Spirulina could play a dual role in modulating mineral levels and supporting hormonal homeostasis. Rabbits represent a valuable model for studying nutritional interventions due to their sensitivity to dietary changes and well-characterized reproductive physiology. Understanding how natural supplements such as *Balanites aegyptiaca* and Spirulina affect mineral and hormonal balance in male rabbits can provide insights relevant to animal production, reproductive health, and clinical research. Given these considerations, this study aimed to compare the effects of *Balanites aegyptiaca* and Spirulina on serum calcium, phosphorus, and hormonal parameters, including estrogen, cortisol, FSH, LH, and testosterone in male rabbits. By integrating biochemical and endocrine outcomes, the research provides a clearer understanding of how these two natural supplements influence physiological homeostasis.

Materials and methods

A total of fifteen healthy adult male rabbits were housed under controlled temperature, proper ventilation, and a 12:12 h light–dark cycle. Animals were acclimatized for one week and provided with standard pellet feed and water ad libitum throughout the experiment. Spirulina powder was obtained from NOW Foods and purchased from Belgray Pharmacy in Al-Bayda City, Libya. *Balanites aegyptiaca* fruits were collected from Sebha, authenticated by a specialist from Sebha University, air-dried, powdered, and stored under controlled conditions until use. Rabbits were divided into three groups for six weeks: a control group, a *B. aegyptiaca* group receiving 100 mg/kg extract daily, and a Spirulina group receiving 400 mg/kg powder daily. All treatments were given orally once per day. At the end of the experimental period, blood samples were collected by heart puncture, allowed to clot, and centrifuged at 3000 rpm for 15 minutes. The resulting

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serum was carefully separated, labeled, and stored at –20°C until biochemical analysis. Serum calcium and phosphorus were measured using standard colorimetric methods following the manufacturer's protocols. Hormonal parameters, estrogen, cortisol, FSH, LH, and testosterone, were quantified using validated ELISA kits appropriate for rabbit serum. All assays were performed in duplicate to ensure analytical accuracy. The ethical approval for this study was obtained from the Libyan Academy, Al-Jabal Al-Akhdar Branch. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test, with significance accepted at $p < 0.05$.

Results

The results presented in (Table 1) show distinct effects of *Balanites aegyptiaca* and Spirulina supplementation on serum calcium levels in male rabbits. The Spirulina-treated group demonstrated a significantly higher calcium concentration of 6.97 ± 0.361 mg/dl, compared with the control (5.18 ± 0.284 mg/dl) and the *Balanites aegyptiaca* group (5.32 ± 0.159 mg/dl). The differing superscripts confirm a statistically significant increase ($p < 0.05$), indicating that Spirulina has a stronger regulatory influence on calcium metabolism. This effect may be attributed to Spirulina's rich mineral composition and presence of bioavailable calcium-binding proteins, which enhance intestinal absorption and retention. In contrast, *Balanites aegyptiaca* did not significantly elevate calcium levels relative to the control, suggesting a milder influence on mineral homeostasis. In contrast to calcium, phosphorus levels did not show significant differences among treatment groups. The control, *Balanites aegyptiaca*, and Spirulina groups recorded 6.05 ± 0.276, 6.25 ± 0.293, and 6.38 ± 0.276 mg/dl, respectively, all sharing the same superscript letter. These findings indicate that neither treatment produced a statistically meaningful alteration in serum phosphorus. The stability of phosphorus concentrations may reflect tight physiological regulation, as phosphorus homeostasis is strongly controlled by renal excretion and endocrine pathways involving parathyroid hormone and vitamin D. (Figures 1and 2) visually support these findings, demonstrating a pronounced elevation in calcium exclusively in the Spirulina group, while phosphorus values remain consistent across treatments. Overall, the results suggest that Spirulina has a marked enhancing effect on serum calcium, whereas *Balanites aegyptiaca* exerts minimal influence on mineral balance. The unchanged phosphorus levels across groups confirm that supplementation did not disrupt phosphorus regulatory mechanisms.

Table 1. Change of plasma calcium and phosphorus of male rabbits treated with Balanites aegyptiaca and Spirulina

Parameter	Experimental groups		
	Control	*Balanites aegyptiaca*	Spirulina
Calcium	5.18 ± 0.284^{b}	5.32 ± 0.159^{b}	6.97 ± 0.361^{a}
Phosphorus	6.05 ± 0.276^{a}	6.25 ± 0.293^{a}	6.38 ± 0.276^{a}

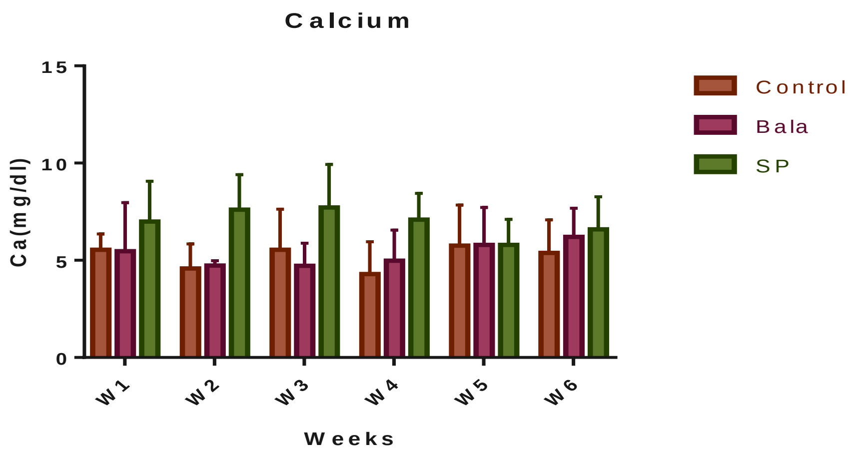

Figure 1. Changes in blood plasma of calcium during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

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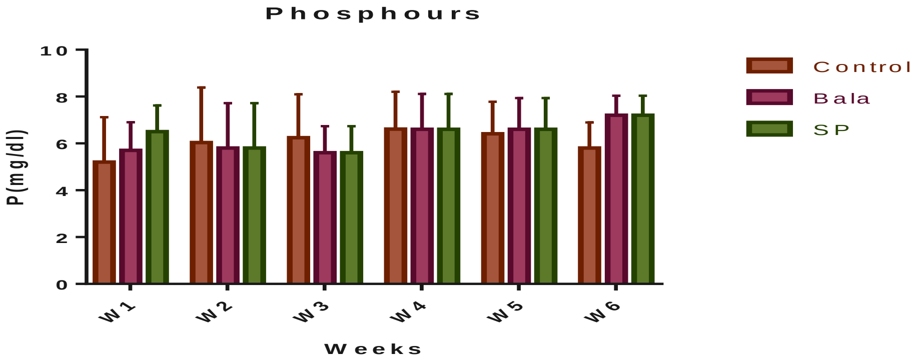

Figure 2. Changes in blood plasma of phosphorusduring treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

(Table 2) Plasma estrogen in the control group measured 29.054 ± 0.153 pg/mL, while rabbits treated with *Balanites aegyptiaca* showed a reduction to 28.028 ± 0.185 pg/mL, and the lowest level was recorded in the Spirulina group at 27.890 ± 0.102 pg/mL. FSH was 0.874 ± 0.015 mIU/mL in the control animals and increased to 1.006 ± 0.011 mIU/mL with *Balanites aegyptiaca* and 0.990 ± 0.011 mIU/mL with Spirulina. Cortisol concentrations decreased from 60.520 ± 0.954 ng/mL in the control group to 52.400 ± 2.526 ng/mL in *Balanites aegyptiaca*-treated rabbits, with the lowest value observed in the Spirulina group at 50.033 ± 3.270 ng/mL. Testosterone increased from 1.772 ± 0.035 ng/mL in the control to 1.973 ± 0.085 ng/mL in the *Balanites aegyptiaca* group and 2.083 ± 0.067 ng/mL in Spirulina-treated rabbits. LH levels were 0.771 ± 0.014 mIU/mL in the control group, rising to 0.799 ± 0.019 mIU/mL with *Balanites aegyptiaca*, and reaching 0.838 ± 0.011 mIU/mL in Spirulina-treated animals.

Table 2. Changes in plasma Hormonal of male rabbits treated with Balanites aegyptiaca and Spirulina

Parameter	**Experimental groups**		
	Control	***Balanites aegyptiaca***	**Spirulina**
Estrogen	29.05 ± 0.153^{a}	28.02 ± 0.185^{b}	27.89 ± 0.102^{b}
FSH	0.87 ± 0.015^{b}	1.00 ± 0.011^{a}	0.99 ± 0.011^{a}
Cortisol	60.52 ± 0.954^{a}	52.40 ± 2.526^{a}	50.03 ± 3.270^{b}
Testosterone	1.77 ± 0.035^{a}	1.97 ± 0.85^{a}	2.08 ± 0.067^{a}
LH	0.77 ± 0.014^{b}	0.79 ± 0.019^{ab}	0.83 ± 0.011^{a}

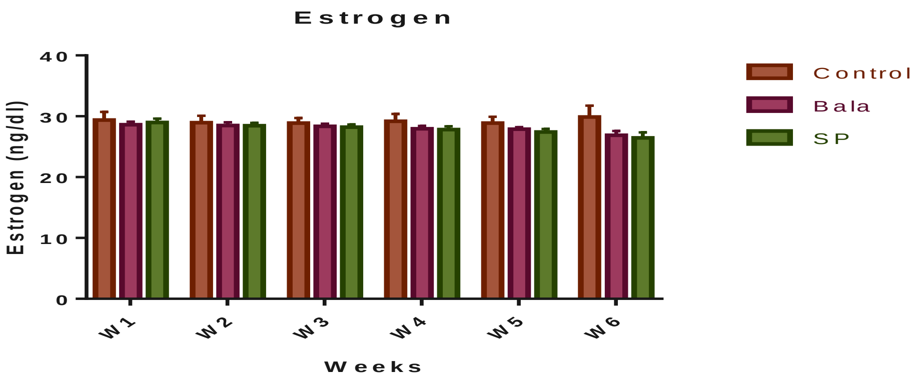

Figure 3. Changes in blood plasma of estrogen hormone during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

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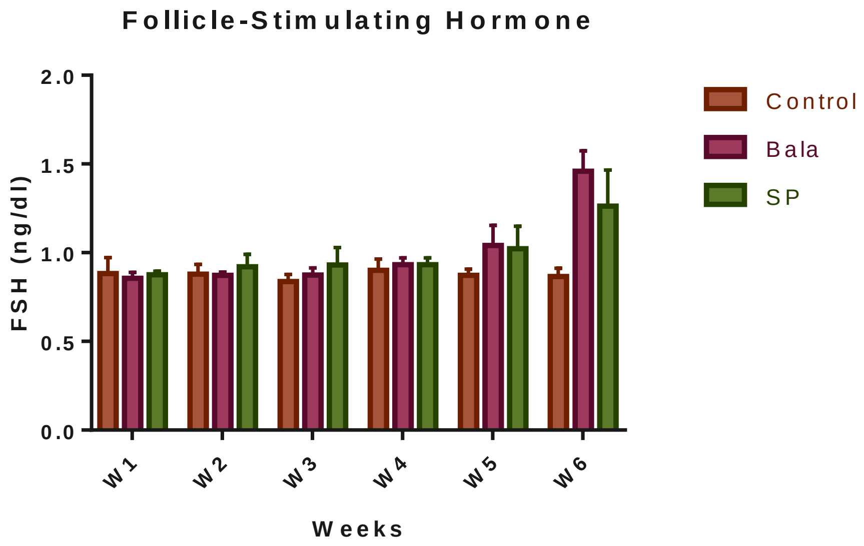

Figure 4. Changes in blood plasma of follicle-stimulating hormone (FSH) during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

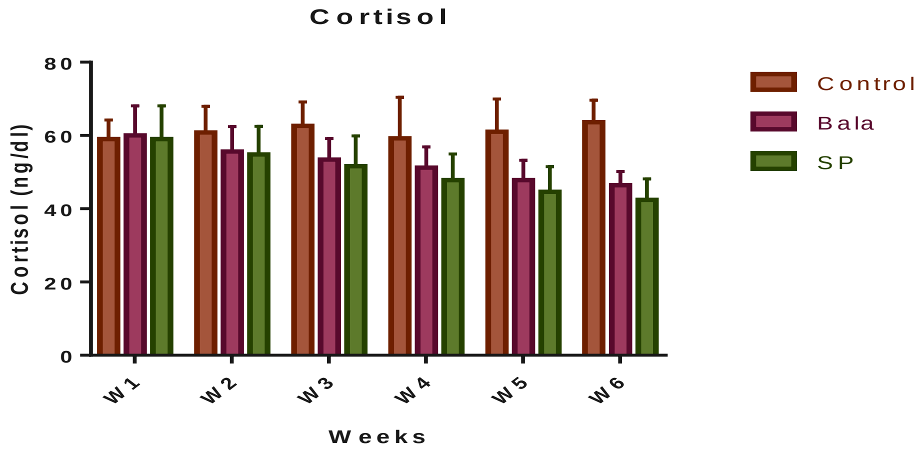

Figure 5. Changes in blood plasma of Cortisol hormone during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

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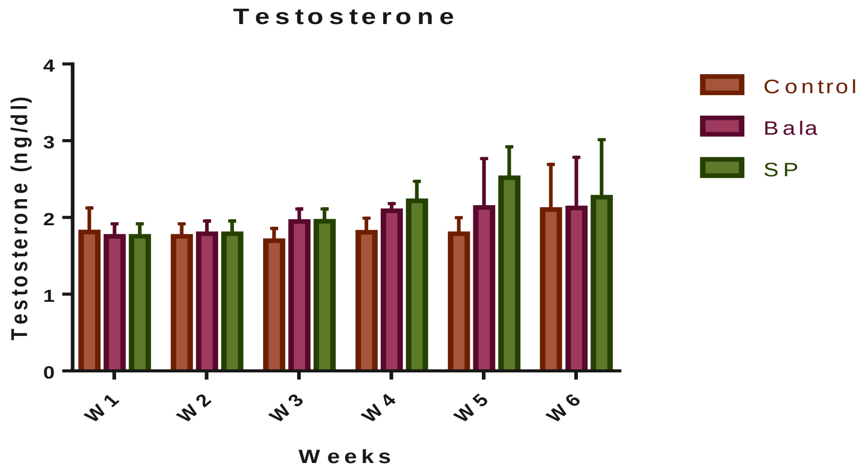

Figure 6. Changes in blood plasma of testosterone hormone during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

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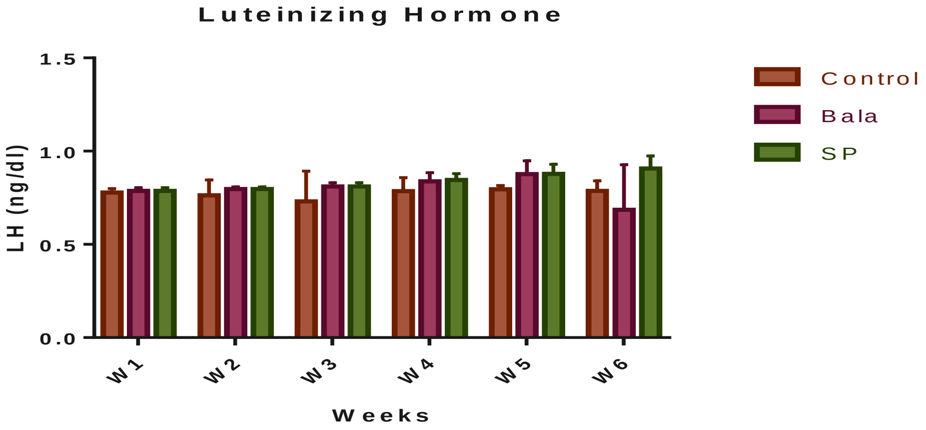

Figure 7. Changes in blood plasma of luteinizinghormone (LH) during treatment of male rabbits with Balanites aegyptiaca (Bala) and Spirulina (SP)

Discussion

The results show that plasma calcium levels increased noticeably in rabbits treated with Spirulina, demonstrating a statistically significant elevation compared with both the control group and the group treated with Balanites aegyptiaca. This rise may be attributed to the naturally high calcium content of Spirulina and its bioavailable chelated mineral complexes, which enhance intestinal absorption and reduce renal calcium loss. Spirulina is also rich in vitamin D analogues and phycocyanin, which have been reported to support calcium transport and homeostasis, contributing to improved circulating calcium status [16]. Recent studies have confirmed that Spirulina supplementation enhances mineral uptake and strengthens calcium metabolism in experimental animals [17]. In contrast, calcium levels in rabbits treated with Balanites aegyptiaca did not differ significantly from those of the control group. This suggests that Balanites does not strongly influence calcium homeostasis under normal physiological conditions. Although Balanites contains trace minerals and phytosterols, its bioactive profile is more strongly associated with glycemic control and antioxidant activity rather than mineral modulation [18]. Similar findings have been reported where Balanites supplementation produced metabolic benefits without altering serum mineral concentrations [19].

Regarding phosphorus levels, no significant differences were observed among the treatment groups, indicating that neither Spirulina nor Balanites produced a measurable effect on phosphorus balance. Phosphorus homeostasis is tightly regulated through renal filtration, parathyroid hormone signaling, and intracellular buffering systems, which may explain the stability observed across treatments [20]. Additionally, phosphorus levels tend to fluctuate only under conditions of metabolic acidosis, bone mobilization, renal dysfunction, or excessive mineral intake, none of which appear to apply in this experimental context [21]. Recent literature supports the notion that dietary supplementation in physiologically healthy animals does not typically alter circulating phosphorus status [22]. The findings reveal a noticeable reduction in estrogen levels in rabbits treated with both Balanites aegyptiaca and Spirulina compared with the control group, with the reduction being more pronounced in the Spirulina group. This decline may be linked to the phytonutrient composition of both supplements, particularly phytosterols and antioxidant constituents that can modulate aromatase activity and reduce peripheral estrogen conversion [23-28].

Recent research has shown that plant-derived bioactives can down-regulate estrogen synthesis and enhance hepatic metabolism of circulating estrogens, which supports the observed trend [29-33]. The results for FSH indicate a significant elevation following treatment with both Balanites and Spirulina. This suggests a stimulatory effect on the hypothalamic–pituitary gonadal axis, potentially due to reduced oxidative stress in reproductive tissues and improved Sertoli cell function. Spirulina in particular is known to enhance reproductive hormonal signaling through its phycocyanin content and micronutrient profile, while Balanites possesses saponins and sterols that may act as gonadotropic modulators. Similar increases in FSH have been reported in studies investigating botanical antioxidants in male reproductive models [34-44]. For cortisol, a decline was observed with both treatments, with Spirulina showing a more notable reduction. This pattern suggests an attenuation of physiological stress responses, consistent with the well-documented anti-inflammatory and antioxidant effects of Spirulina, which may decrease adrenal glucocorticoid output. Balanites also contains compounds known to mitigate oxidative and metabolic stress, which may contribute to reduced cortisol secretion [46-50]. Recent animal studies similarly report decreased cortisol following supplementation with antioxidant-rich nutraceuticals [51]. In contrast, testosterone levels showed an increasing trend in both treatment groups, suggesting enhanced Leydig cell performance, reduced lipid peroxidation in testicular tissue, and improved steroidogenic enzyme activity.

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Spirulina's essential fatty acids, zinc content, and antioxidant peptides are known to support testosterone synthesis, while Balanites has been reported to contain phytosterols that act as androgen-supportive modulators [52-60]. Recent literature confirms that antioxidant supplementation can elevate testosterone through protection of testicular steroidogenesis [61-65].

Conclusion

The study showed that Spirulina was more effective than *Balanites aegyptiaca* in increasing serum calcium, while phosphorus levels remained stable across all groups. Both supplements improved hormonal balance by reducing estrogen and cortisol and enhancing FSH, LH, and testosterone levels, with Spirulina producing the strongest effects. Overall, Spirulina demonstrated superior benefits for mineral regulation and reproductive endocrine function in male rabbits.

Conflict of interest. Nil

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