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Correlation between reproductive hormones and physical traits of the testicular, seminal structures, and scrotal in awassi lambs fed spirulina

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Abstract
This study was conducted in Tikrit, Iraq, to evaluate the effects of Spirulina on growth and reproductive traits in 24 local awassi lambs (3-4 months, 20.5±0.9 kg). The lambs were randomly assigned to six groups, each receiving high- or low-protein feed with or without Spirulina (8 or 12 g/kg) Treatments: T_1: (high protein, no spirulina), T_2: (High protein + 8 g spirulina/kg), T_3: (high protein + 12 g spirulina/kg), T_4: (Low protein, no spirulina), T_5 (Low protein + 8 g spirulina/kg), and T_6 (low protein + 12 g spirulina/kg). Blood samples were collected on days 45 and 90 to measure serum testosterone and ICSH levels using a Minividus device. Scrotal circumference, testicular volume, and histomorphological characteristics of the testicles were recorded biweekly. After slaughter, epididymal and testicular measurements were taken, including testicular weight, length, and thickness. Testosterone strongly correlates with testicular circumference (0.993) in control lambs, but shows negative correlations in Spirulina-supplemented groups, e.g., T_5 with epididymal length (-0.826). ICSH strongly negatively correlates with epididymal length (-0.998) and testicular width (-0.971) in T_6, highlighting Spirulina's impact. In Treatment 3, ICSH negatively correlates with lumen diameter (-0.853) and positively with epithelial thickness (0.046). At day 90, testosterone negatively correlated with most testicular dimensions (e.g., width: -0.958), showing a strong overall negative impact. At day 90, ICSH had strong negative correlations with most testicular dimensions (e.g., thickness: -0.867), indicating a general negative impact. Testosterone positively correlates with testicular circumference in controls but negatively in Spirulina groups. ICSH negatively impacts testicular dimensions, especially in T_6.

Keywords: Spirulina, reproductive hormones, testicular morphology, protein

Introduction
Nutritional management of sheep is critical to improving reproductive performance, and protein intake plays a significant role in this process. Protein is a key component of animal diets, influencing growth, reproductive health, and overall productivity. In ruminants, including sheep, dietary protein influences reproductive hormone synthesis and reproductive tissue development. Adequate protein intake promotes fertility by supporting hormonal balance and physiological functions necessary for successful reproduction (Gilbreath *et al*., 2021) [9]. Research has demonstrated that protein supplementation can improve reproductive outcomes in sheep. For example, increased dietary protein levels have been linked to improved ovulation rates and improved overall reproductive performance. Furthermore, high-protein diets are essential for the growth and maintenance of male reproductive organs, including the testes and scrotum, which are integral to optimal sperm production (Ma *et al*., 2022) [19]. Higher protein intake has been shown to enhance testicular size and function, which are important indicators of fertility (Crean *et al*., 2023) [7].

Spirulina, a cyanobacterium rich in proteins, vitamins, and essential fatty acids, has emerged as a valuable nutritional supplement in livestock nutrition. Spirulina contains high levels of essential amino acids, which can supplement protein intake and potentially enhance reproductive performance in sheep (Moghanlo & Shariatzadeh, 2022) [20]. Its inclusion in the diet has been reported to positively affect levels of reproductive hormones, including testosterone and luteinizing hormone (LH), which play critical roles in regulating spermatogenesis and reproductive function (Li *et al*., 2024) [17]. The effect of Spirulina on hormonal balance and testicular development has been documented in various studies, highlighting its potential to improve reproductive parameters in male sheep (Khudair and Hamad, 2024; Khair al., 2021) [16, 14].

The relationship between sex hormones and the development of testicular and scrotal structures is well established. For example, testosterone is essential for testicular growth and maintenance of scrotal circumference, which is associated with sperm production and fertility (Lostroh, 1969) [18]. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) also play important roles in regulating spermatogenesis and overall seminiferous tubule health (Reiter, 1980) [22]. These hormones influence the growth and morphology of the testes and seminiferous tubules, which impact sperm production and fertility outcomes (Kameni *et al*., 2022) [13]. In summary, the combination of high-protein diets and spirulina supplements represents a promising approach to promoting reproductive health in sheep. By influencing hormonal balance and supporting reproductive tissue growth, such nutritional strategies could contribute to improved fertility and overall reproductive efficiency (Han *et al*., 2022; Davis *et al*., 2023; Khalaf and Ory, 2021) [10, 23, 15]. Understanding the interactions between diet, hormones and reproductive traits is essential for developing effective strategies to improve sheep production and fertility.

Materials and Methods

In this study, 24 local Awassi lambs (3-4 months, 20.5±0.9 kg) were purchased from Salahuddin markets in Iraq. They were transported to the animal laboratories in Tikrit and housed in new cages. Spirulina was obtained from Etuoke Banner Kangsheng Algae Industry Company, China. The lambs were sampled into six groups and fed on high-protein concentrate with 8 or 12 g/kg of spirulina per lamb. Six treatments were analyzed: Control (T_1) consisted of high-protein feed without spirulina, while Treatment 2 (T_2) added high-protein feed with 8 g of spirulina powder /kg concentrate. Treatment 3 (T_3) included high-protein feed with 12 g of spirulina powder /kg concentrate. Compared to 4 (T_4) low-protein feed without spirulina powder, 5 (T_5) was low-protein feed with 8 g of spirulina powder /kg concentrate. Finally, 6 (T_6) protein concentrates with 12 g spirulina powder /kg concentrate.

Blood samples were taken from the lambs on days 45 and 90 of the experiment, using a syringe, blood was drawn from the jugular vein in an amount of 10 ml. The samples were left for 15 minutes, the tubes allowed the blood to clot at room temperature 25 °C. After that, for 15 minutes, a centrifuge was used at 3000/min to separate the serum from the cellular portion of the blood. After that, the isolated serum was isolated in other tubes and stored at -18 °C until the analysis of testosterone and ICSH). Every 14 days, all dimensions of the scrotum of the 24 lambs were measured using the following tools: Measuring ruler: used to measure the length of the scrotum. Verne: used to measure the thickness and width of the scrotum. Graduated container in cubic milliliters used to measure the volume of the scrotum. Measuring tape used to measure the circumference of the testicle.

After slaughtering the sheep, the testis and epididymis were carefully removed. The epididymis was separated from the testis using a dissecting scalpel to obtain separate samples of both and the following characteristics were measured (Testis weight without epididymis, testis width, testis length, testis thickness, testis volume, epididymis weight, and epididymis length). A 2 cm^3 tissue section was taken from the testes of lambs. Testicular samples were collected to explore the development of spermatogenic cells, Sertoli cells, and Leydig cells, as well as to measure the seminiferous tubule diameter, seminiferous tubule lumen diameter, and the height of the seminiferous tubule.

Results and Discussion

The table 1 details the relationship between testosterone and different testicular and epididymal characteristics in lambs under different dietary treatments. In control, testosterone shows a strong positive correlation with testicular circumference (0.993, p<0.01) and moderate correlations with testicular weight (0.592), testicular width (0.653) and testicular volume (0.783). T_2 reveals negative correlations with testicular volume (-0.781) and testicular width (-0.392). T_3 shows moderate positive correlations with testicular weight (0.446) and testicular thickness (0.599). T_4 has generally weak correlations, with slight positive correlations. T_5 showed strong negative associations with epididymal length (-0.826) and testis weight (-0.729). T_6 showed negative associations across most traits, including epididymal length (-0.707) and testis volume (-0.743).

Table 1: Correlation between testosterone and testicular and epididymal characteristics (Epididymal length, epididymis weight, testis weight, testis width, testicular thickness, Testicular circumference and testis volume) in lambs fed Spirulina

Treatments	Epididymal length/ mm	Epididymis weight/gm	Testis weight/gm	Testis width/mm	Testicular thickness/mm	Testicular circumference/mm	Testis volume/m^3
T_1	-.471-	-.278-	.592	.653	.993**	.786	.783
T_2	-.125-	-.463-	.590	-.392-	-.562-	-.295-	-.781-
T_3	.261	.479	.446	.291	.599	.351	.246
T_4	.187	-.016-	.232	.415	.232	.416	.067
T_5	-.826-	.915	-.729-	-.493-	-.526-	-.744-	-.691-
T_6	-.707-	-.592-	-.299-	-.033-	-.381-	-.558-	-.743-

Treatments: T_1: (High protein, no spirulina), T_2: (High protein + 8 g spirulina/kg), T_3: (High protein + 12 g spirulina/kg), T_4: (Low protein, no spirulina), T_5 (low protein + 8 g spirulina/kg), and T_6 (Low protein + 12 g spirulina/kg). Correlation is significant at the 0.05 level (2-tailed).*Correlation is significant at the 0.01 level (2-tailed) **Spirulina, rich in antioxidants and nutrients, has been shown to improve reproductive parameters, as evidenced by Abdel-Daim *et al*. (2013) [1], who found that Spirulina enhanced sperm quality and testosterone levels in rats. This supports the positive correlations seen in T_3, where testosterone correlated moderately with testicular traits. High-protein diets also promote testosterone production, aligning with Joshi, (2022) [11] who highlighted the importance of protein for maintaining hormonal balance, which is reflected in the positive correlations of Control. However, excessive Spirulina can lead to nutrient imbalances and negatively impact testosterone, as noted by

Belay *et al*. (1996) [5], which contrasts with the negative correlations in Treatments 5 and 6 (Low protein + Spirulina). Additionally, Kabir *et al*. (2002) [12] found that low protein impairs reproductive function, consistent with the weak correlations in low-protein treatments. Thus, balancing Spirulina and protein intake is crucial for optimizing reproductive health.

Table 2: Correlation between ICSH and testicular and epididymal characteristics (Testicular weight, testicular width, testicular length, testicular thickness, testicular volume, epididymal weight, epididymal length) in lambs fed Spirulina

Treatments	Epididymal length/ mm	Epididymis weight/gm	Testis weight/gm	Testis width/mm	Testicular thickness/mm	Testicular circumference/mm	Testis volume/m^3
T_1	-.677-	-.839-	-.385-	-.229-	.370	.118	.796
T_2	.941	.745	.912	.015	-.827-	-.347-	.203
T_3	-.046-	.149	-.377-	-.815-	-.783-	-.966-*	-.631-
T_4	-.332-	-.503-	-.450-	-.473-	-.570-	-.451-	-.722-
T_5	.661	-.365-	-.162-	-.373-	-.428-	.498	-.082-
T_6	-.473-	-.971-*	-.820-	-.660-	-.748-	-.741-	-.998-**

Treatments: T_1: (High protein, no spirulina), T_2: (high protein + 8 g spirulina/kg), T_3: (High protein + 12 g spirulina/kg), T_4: (Low protein, no spirulina), T_5 (Low protein + 8 g spirulina/kg), and T_6 (Low protein + 12 g spirulina/kg). Correlation is significant at the 0.05 level (2-tailed).* Correlation is significant at the 0.01 level (2-tailed)

The Table 2 presents the correlation between ICSH (Interstitial Cell Stimulating Hormone) and testicular and epididymal characteristics in lambs under different treatments. In Control, ICSH was negatively correlated with testicular weight (-0.677), width (-0.839), and length (-0.385), but positively correlated with epididymal length (0.796). T_2 showed strong positive correlations with testicular weight (0.941), width (0.745), and length (0.912), though a negative correlation was observed with testicular volume (-0.827). T_3 had mostly negative correlations, particularly with epididymal weight (-0.966) and testicular thickness (-0.815). T_4 showed consistently negative correlations, while T_5 had mixed results. T_6 displayed strong negative correlations, especially with epididymal length (-0.998) and testicular width (-0.971). Spirulina's antioxidant and nutritional properties positively impact reproductive health by modulating hormone levels such as ICSH. Abdel-Daim *et al*. (2013) [1] found that Spirulina enhanced reproductive health in rats, correlating with the strong positive relationships observed in Treatment 2, where ICSH showed positive associations with testicular weight (0.941) and length (0.912). This supports the beneficial effects of moderate Spirulina supplementation. Additionally, high-protein diets improve reproductive performance and hormone production. Zebari *et al*. (2019) demonstrated that high-protein diets led to better reproductive health and higher ICSH levels, reflected in Treatments 1 and 2 with positive correlations between ICSH and traits like epididymal length (0.796) and testicular weight. Conversely, Belay *et al*. (1996) [5] cautioned against excessive Spirulina due to potential nutrient imbalances that could impair ICSH function, as seen in Treatment 6 with strong negative correlations. Similarly, Morgan *et al*. (2020) [21] linked low protein with reduced hormone levels and impaired testicular growth, consistent with negative correlations in low-protein treatments. Thus, moderate Spirulina with high protein benefits reproductive health, while high Spirulina doses in low-protein diets may be detrimental.

Table 3: Correlation ratio of testosterone and ICSH hormones with tubules Somniferous/µm, Lumen diameter ⁄µm and thickness of Epithelial ⁄µm in lambs fed Spirulina

Treatments	Testosterone			ICSH hormones		
	Tubules Somniferous µm	Lumen diameter µm	Thickness of epithelial µm	Tubules Somniferous µm	Lumen diameter µm	Thickness of Epithelial µm
T_1	-.932-	.889	-.686-	-.508-	-.044-	.201
T_2	-.952-*	-.702-	-.317-	-.281-	-.430-	.206
T_3	.974*	-.199-	.803	.044	-.853-	-.046-
T_4	.320	.896	.574	.159	.706	-.438-
T_5	.774	-.389-	.189	-.852-	.315	.331
T_6	.848	.572	.871	.346	-.626-	-.215-

Treatments: T_1: (High protein, no spirulina), T_2: (High protein + 8 g spirulina/kg), T_3: (High protein + 12 g spirulina/kg), T_4: (Low protein, no spirulina), T_5 (Low protein + 8 g spirulina/kg), and T_6 (Low protein + 12 g spirulina/kg). Correlation is significant at the 0.05 level (2-tailed).*

The Table 3 illustrates the relationships between testosterone and ICSH (Interstitial Cell Stimulating Hormone) with seminiferous tubule thickness, lumen diameter, and epithelial thickness in sheep fed Spirulina under different dietary treatments. In Control, testosterone negatively correlates with seminiferous tubule thickness (-0.932) and epithelial thickness (-0.686), but positively with lumen diameter (0.889), indicating higher testosterone is associated with a larger lumen but thinner epithelium. T_2 shows a strong negative correlation with seminiferous tubule thickness (-0.952, $p<0.05$) and lumen diameter (-0.702), suggesting that higher testosterone reduces both tubule and lumen size. T_3 reveals a strong positive correlation with tubule thickness (0.974, $p<0.05$) and epithelial thickness (0.803), but a negative correlation with lumen diameter (-0.199), indicating that higher testosterone is linked to increased tubule and epithelium size. T_4 shows moderate positive correlations with lumen diameter (0.896) and epithelial thickness (0.574), indicating an overall positive effect of testosterone. In T_5, testosterone positively correlates with tubule thickness (0.774) but negatively with lumen diameter (-0.389). Finally, T_6 shows strong positive correlations with tubule thickness (0.848) and epithelial thickness (0.871), and moderate with lumen diameter (0.572).

In Control, ICSH negatively correlates with tubule thickness (-0.508) and lumen diameter (-0.044), and positively with epithelial thickness (0.201), suggesting ICSH is associated with smaller tubules and lumen. Treatment 2 shows weak to moderate negative correlations with tubule thickness (-0.281) and lumen diameter (-0.430), with a weak positive correlation with epithelial thickness (0.206). In Treatment 3, ICSH negatively correlates with lumen diameter (-0.853) and weakly with epithelial thickness (-0.046), while being near zero with tubule thickness (0.044). Treatment 4 shows a positive correlation with lumen diameter (0.706) but negative with epithelial thickness (-0.438). In Treatment 5, ICSH negatively correlates with tubule thickness (-0.852) and positively with epithelial thickness (0.331), while in Treatment 6, ICSH shows positive correlation with tubule thickness (0.346) but negative correlations with lumen diameter (-0.626) and epithelial thickness (-0.215). These results are consistent with studies showing Spirulina's complex effects on reproductive health. For instance, El-Abo El-Ela *et al*. (2024) [2] found Spirulina improves reproductive tissue health and testosterone levels, aligning with results from Treatments 1 and 2. Al-Yahyaey *et al*. (2023) [3] supported the positive impact of Spirulina on testosterone and tissue characteristics observed in Treatment 3. Conversely, high Spirulina doses might increase oxidative stress, as noted by Belay *et al*. (1996) [5], correlating with negative outcomes in Treatment 6. Additionally, Kabir *et al*. (2002) [12] highlighted the adverse effects of low protein on ICSH levels, explaining the negative correlations observed in low protein treatments.

Table 4: Correlation ratio of testosterone with the dimensions of the scrotum of Awassi lambs at day 45 and 90

	Day 45					Day 90				
Treatments	**Thickness/cm**	**Circumference/cm**	**Height/cm**	**Width/cm**	**Size/cm³**	**Thickness/cm**	**Circumference/cm**	**Height/cm**	**Width/cm**	**Size/cm³**
T_1	-.278-	-.120-	-.422-	-.551-	-.422-	-.960-*	-.960-*	.478	-.940-	-.708-
T_2	-.318-	-.612-	.692	-.402-	-.251-	-.878-	-.187-	-.681-	-.958-*	.354
T_3	.329	-.005-	.783	.158	-.017-	-.916-	-.951-*	-.465-	-.992-**	-.923-
T_4	.273	.406	.561	.273	.500	-.666-	.151	-.294-	-.032-	-.360-
T_5	-.184-	-.152-	.070	-.858-	-.404-	.198	.368	-.698-	-.184-	-.152-
T_6	.837	.462	-.251-	.583	-.647-	.756	.863	.693	.993**	.979*

Treatments: T_1: (high protein, no spirulina), T_2: (high protein + 8 g spirulina/kg), T_3: (high protein + 12 g spirulina/kg), T_4: (low protein, no spirulina), T_5 (low protein + 8 g spirulina/kg), and T_6 (low protein + 12 g spirulina/kg). Correlation is significant at the 0.05 level (2-tailed).*

The table 4 examines the relationship between testosterone levels and testicular dimensions in Awassi sheep at days 45 and 90 of the experiment. At day 45, in the high-protein, no Spirulina group (Control), testosterone negatively correlated with testicular thickness (-0.278), circumference (-0.120), height (-0.422), width (-0.551), and volume (-0.422), indicating smaller testicular dimensions with higher testosterone. In the high-protein + 8 g Spirulina/kg group (T_2), testosterone had a negative correlation with thickness (-0.318) and circumference (-0.612), but a positive one with height (+0.692), suggesting variable effects of Spirulina. The high-protein + 12 g Spirulina/kg group (T_3) showed positive correlations with thickness (+0.329) and height (+0.783), but minimal impact on circumference, width, and volume, reflecting less consistent effects. In the low-protein, no Spirulina group (T_4), testosterone positively correlated with circumference (+0.406), height (+0.561), width (+0.273), and volume (+0.500), indicating beneficial effects on testicular dimensions under low-protein conditions. The low-protein + 8 g Spirulina/kg group (T_5) showed negative correlations with thickness (-0.184), circumference (-0.152), width (-0.858), and volume (-0.404), indicating varied effects. The low-protein + 12 g Spirulina/kg group (T_6) had positive correlations with thickness (+0.837), circumference (+0.462), and width (+0.583), but negative ones with height (-0.251) and volume (-0.647), showing significant positive effects on certain dimensions.

At day 90, testosterone negatively correlated with thickness (-0.878), circumference (-0.187), height (-0.681), width (-0.958), and volume (+0.354), demonstrating a strong negative impact on most dimensions. This aligns with studies like Al-Yahyaey *et al*. (2023) [3], showing how testosterone affects testicular development. Treatment 6's results, with improvements in testicular dimensions, support findings from Abo El-Ela *et al*. (2024) [2], which reported Spirulina's beneficial effects on reproductive health.

Table 5: Correlation ratio of ICSH hormones with the dimensions of the scrotum of male Awassi sheep at day 45 and day 90

	Day 45					Day 90				
Treatments	**Thickness/cm**	**Circumference/cm**	**Height/cm**	**Width/cm**	**Size/cm³**	**Thickness/cm**	**Circumference/cm**	**Height/cm**	**Width/cm**	**Size/cm³**
T_1	-.839-	-.517-	.535	.000	.535	-.599-	-.599-	.535	-.131-	-.841-
T_2	.851	.310	.722	.119	.331	-.656-	.867	.146	.070	.446
T_3	-.156-	-.024-	.101	-.128-	.047	.355	-.040-	-.088-	.286	.068
T_4	-.676-	-.679-	-.577-	-.676-	-.566-	-.867-	-.015-	-.653-	-.423-	-.864-
T_5	.419	.773	.445	.632	.516	.211	.335	.838	.419	.773
T_6	-.175-	-.201-	-.263-	-.425-	.843	.158	-.162-	.065	-.198-	-.361-

Treatments: T_1: (High protein, no spirulina), T_2: (High protein + 8 g spirulina/kg), T_3: (High protein + 12 g spirulina/kg), T_4: (Low protein, no spirulina), T_5 (low protein + 8 g spirulina/kg), and T_6 (Low protein + 12 g spirulina/kg). Correlation is significant at the 0.05 level (2-tailed).*

The Table 5 presents the relationship between ICSH levels and testicular dimensions in Awassi rams at days 45 and 90 of the experiment, including testicular thickness, circumference, height, width, and volume. At day 45, in Control (High-protein, no Spirulina), ICSH levels negatively correlated with testicular thickness (-0.839) and circumference (-0.517), but positively with height (+0.535) and volume (+0.535). This suggests that higher ICSH levels may be linked to smaller testicular thickness and circumference, yet larger height and volume. In T_2, ICSH positively correlated with testicular thickness (+0.851) and height (+0.722), but had weaker correlations with circumference (+0.310), width (+0.119), and volume (+0.331). This indicates a positive impact of ICSH on thickness and height, with less clarity on other dimensions. T_3 showed negative correlations with thickness (-0.156) and circumference (-0.024), minimal positive correlations with height (+0.101) and width (-0.128), and a non-significant correlation with volume (+0.047), reflecting variable effects. Treatment 4 (low-protein, no Spirulina) had negative correlations with all dimensions: thickness (-0.676), circumference (-0.679), height (-0.577), width (-0.676), and volume (-0.566), indicating a general negative effect. In T5, ICSH positively correlated with all dimensions: thickness (+0.419), circumference (+0.773), height (+0.445), width (+0.632), and volume (+0.516), suggesting beneficial effects. In Treatment 6 (low-protein + 12 g Spirulina/kg), ICSH negatively correlated with thickness (-0.175), circumference (-0.201), height (-0.263), and width (-0.425), but positively with volume (+0.843), indicating varied effects with a notable positive impact on volume.

At day 90, Control showed negative correlations with thickness and circumference (-0.599), while positively correlating with height (+0.535). The width correlation was weak (-0.131) and volume had a significant negative correlation (-0.841), suggesting a general negative effect of ICSH on most dimensions except height. T_2 had a negative correlation with thickness (-0.656) and a positive one with circumference (+0.867), with weak correlations with height (+0.146) and width (+0.070), and a notable positive correlation with volume (+0.446). This reflects diverse effects of ICSH, with a clear positive impact on circumference. T_3 had positive correlations with thickness (+0.355) and width (+0.286), but negative with circumference (-0.040) and height (-0.088), with a non-significant correlation with volume (+0.068), indicating inconsistent effects. T_4 had strong negative correlations with thickness (-0.867), height (-0.653), width (-0.423), and volume (-0.864), with an insignificant correlation with circumference (-0.015), reflecting a clear negative impact. T_5 showed positive correlations with all dimensions: thickness (+0.211), circumference (+0.335), height (+0.838), width (+0.419), and volume (+0.773), indicating a notable positive effect. In T_6, ICSH had weak positive correlations with thickness (+0.158) and height (+0.065), but negative correlations with circumference (-0.162), width (-0.198), and volume (-0.361), showing varied effects with minimal positive impacts.

The reviewed studies collectively highlight the multifaceted impact of *Spirulina platensis* on reproductive health, particularly in terms of hormonal regulation and testicular development. Spirulina, rich in proteins, vitamins, and antioxidants, enhances reproductive tissue health by mitigating oxidative stress and promoting hormonal balance, notably improving testosterone levels and sperm quality Abo El-Ela *et al*. (2024) [2]. The effects of *Spirulina* on testicular dimensions, however, appear to be dose-dependent and influenced by baseline dietary conditions. Lower doses of Spirulina, especially in conjunction with low-protein diets, can positively affect the correlation between interstitial-cell stimulating hormone (ICSH) and reproductive organ dimensions, particularly in enhancing circumference and volume. This suggests that Spirulina compensates for protein deficiencies, optimizing hormonal function (El-Ratel *et al*. 2023) [8]. However, higher doses or imbalanced diets may lead to variable outcomes, reflecting the complexity of nutrient-hormone interactions (Asín *et al*. 2021) [4].

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