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The effect of adding spirulina platensis to the high- protein diet on the tissue increase in the testes of Iraqi lambs

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Abstract: This study was conducted in the animal field - College of Veterinary Medicine / Tikrit University, and lasted for 90 days from 2/10/2022 to 31/12/2022, using two levels of Spirulina platensis (SP), 8 and 12 g/kg of concentrate feed, to raise the value of high protein feed (HP) on the other hand in lamb rations and to investigate the effect of Spirulina supplementation on testicular tissue health and reproductive outcomes in adult lambs through histological examination. 12 local lambs, purchased from Salahuddin markets / Iraq, aged 3 to 4 months, with an average initial weight of 20.5 ± 0.9 kg, were used randomly distributed into 3 treatments / 4 lambs per treatment, as follows: T1 Lamb control group- Lambs were offered the concentrate feed, designated as HP with no Spirulina. T2: Sheep were dosed 8 g Spirulina/ kg concentrate feed. T3: The lambs were supplemented with 12g Spirulina per kg concentrate feed. Light microscopic examination of testes tissues of mature lambs revealed the following differences between sections belonging to the experiment and control groups. There were signs of spermatogenesis in control lambs in the seminiferous tubules with spermatocytes and Sertoli cells, but treated groups presented histopathological alteration as necrosis; amyloid deposit; and spermatogonia degeneration in the interstitial tissue and tunica specials. Indeed, lambs receiving Spirulina experienced different levels of testicular tissue necrosis and amyloid accumulation, which can be considered deleterious effects of the alga. These results indicate that this is possible, therefore more research is necessary to fully determine the impacts of Spirulina supplementation on lamb reproductive health.

Keywords: *Amyloid deposition, Histological, Sertoli cells, Spermatogonia.*

1. Introduction

The interest in spirulina as a microalgae focuses mainly on its rich content of essential vital compounds, such as protein with all essential amino acids (60-70% of dry weight), vitamins such as B12, beta-carotene and provitamin A (-carotene), polyunsaturated fatty acids, minerals and plant phytopigments (Farag *et al.*, 2016). Spirulina algae has antioxidant, anti-tumor, anti-bacterial, anti-inflammatory, anti-viral and immunomodulatory properties. (Farag *et al.*, 2016). Compared with synthetic products, animal reproduction has been improved to a healthy state by spirulina treatment at a low cost (Shanmugapriya *et al.*, 2015).

El-Ratel,(2017) pointed out the positive effects of spirulina in enhancing the reproductive efficiency of male farm animals. Recently, oral administration of spirulina, at a level of 750 mg/male for 5 weeks as a treatment period before semen collection, has been of interest as a therapeutic strategy to improve male reproductive performance (Fouda and Ismail, 2017). It is well known that the positive effects of spirulina are in dose dependent manner and differ with treatment method, oral , administration dietary, drinking water (Bashandy *et al.*, 2016; El-Ratel, 2017).

So far, the effect of Spirulina on reproductive functions is not well known. studies conducted in have reported that increased the body and metabolic parameters, testis weights, Leydig cell number, normal seminiferous tubules, testosterone levels and steroidogenic enzymes mRNA (Nah *et al.* 2012).

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The effects of the inclusion of spirulina platensis (SP) in diets of small ruminants have been observed to be numerous. El-Deeb (2022) working with the same dobera concluded that superabundant pasture raised the production of milk, birth weight of lambs and growth rate of body of ewes and Eldaim It found that supplement improved the health of pregnant ewes, increased the weights of newborns, and enhanced the ability to survive lambs. Similar to the studies made by Holman and Aduli (2014) and Elsabagh et al. (2014) where it is evident that SP supplementation increases growth and productivity in lambs, specially the one observed by Holman & Aduli (2014) with blood health indicators. In combination, these investigations indicate that increased SP inclusion in a high protein feed for Iraqi lambs could enhance the growth of the testicular tissues. However, they have not done further research on this very issue to support this hypothesis.

2. Materials and Methods

This study was conducted in the animal field of the College of Veterinary Medicine, Tikrit University, and it was completed during 90 days from 10/2/2022 to 12/31/2022.The experiment was started after the 14 days' acclimatization period to regulate the animals' weights.All the animals used were sourced randomly from the local markets of Saladin Governorate- Iraq and the species used were local lambs.a 14-day lead-in period to condition the animals and stabilize their weights.Twelve local lambs were used, purchased from the markets of Saladin Governorate - Iraq. The kids were between 3 and 4 months of age and their mean initial weight was 20.5 ± 0.9 kg. The animals were transported to the animal field of the College of Veterinary Medicine, Tikrit University. Spirulina was bought from Etuoke Banner Kangsheng Algae Industry Co., Ltd Ltd in the Mongolia region in China, known to be among the largest organisations in the production and selling of algae in general and spirulina in particular. The lambs were offered the concentrated 1 diet and spirulina powder was inclusion rate of 8000mg and 12000mg per kilogram of concentrate feed respectively; The total diet offered was 3% of BW in two portions in the morning and evening.Concentrate diet was provided Dry matter 95.81% Crude protein 15.01% Ash 4.74% Crude fat 3.36% Crude fibre 6.16% Energy 2810 kcal / kg of concentrate feed.o 12/31/2022. The study was initiated after a 14-day lead-in period to condition the animals and stabilize their weights.Twelve local lambs were used, purchased from the markets of Saladin Governorate - Iraq. They were between 3 and 4 months old, and their average starting weight was 20.5 ± 0.9 kg. The animals were transferred to an animal field at the College of Veterinary Medicine at Tikrit University. Spirulina was purchased from Etuoke Banner Kangsheng Algae Industry Co., Ltd. Ltd in the Mongolia region of China, which is considered one of the leading companies in the production and distribution of algae in general and spirulina in particular. The lambs were fed the concentrated1 diet, and spirulina powder was added at an amount of 8 and 12 g/kg concentrated feed, and the diet was provided at 3% of body weight in two meals in the morning and evening. Concentrate diet was offered Dry matter 95.81% Crude protein 15.01% Ash 4.74% Crude fat 3.36% Crude fibre 6.16% Energy 2810 kcal/kg concentrated feed. Experimental treatments are illustrated below: T1: A concentrate diet with no spirulina was provided to lambs. T2: Sheep were offered 8 gm of spirulina per kg of concentrate diet. T2: Spirulina was provided to sheep at the rate of 12 g of spirulina per kilogram of concentrate diet. A sample of the lamb testes tissue measured 2 cm3 was extracted. The testicular samples were taken for the purpose of understanding the growth of spermatogenic cells, Sertoli, and Leydig cells, in addition to the ability to quantify the diameter of seminiferous tubule, diameter of lumen of seminiferous tubule, and height of seminiferous tubule biofilm. The tissue samples were collected through removing small chunk of tissue from the lamb testes using clean and sharp tool, surgical scissor. The risk to cause injury to the tissues were minimized to the highest level and the samples were handled in the best way possible. The collected tissue pieces which were cubic in shape with a dimension of (2×3) cm were directly immersed in 10 ml of 10% of formalin solution in 50 ml plastic vial.The tissue samples were well fixed and preserved because the whole samples was submerged in the formalin solution. As we know that fixing aid in maintaining the tissue morphology and formallyin is one of the best fixing agent. Due to the necessity of preserving the samples' original samples' composition, the glass vials were closed as tightly as possible to avoid formalin leakage and possible external contamination. Using indelible ink, each vial was appropriately marked with information such as; type of

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tissue, date when taken, mode of preservation, & percentage of formalin. Consequently, this precise labeling method improves the trackability of the samples and makes it easy to determine their origin in cases of necessity. The sample vials were stored properly, away from moisture to maintain tissue quality and no alteration of formalin concentration. Because of the close adherence to these standard operating procedures and protocols, it was made possible to effectively prepare and conserve the tissue samples analyzed in this particular MSc research study and analyses. Learn lab assignment of preparation of histological slides stained with hematoxylin and eosin. Histology sections of testis were developed from the testis of twenty lambs so that the investigators are able to determine the cellular makeup and roles in the tissue section. This was made possible though a surgical biopsy where by fixing the affected tissue sample by using a sharp instrument, and fixing with formalin. The sample was next prepared to get sections of thin tissues and stained with hematoxylin and eosin to differentiate the tissues and the cells. The obtained slides were then mounted under a special microscope and the structural parameters were evaluated in terms of their dimension.

3. Results and Discussion

From Table 1, it is evident that third group, which received 12 grams of spirulina, had a significantly low value of seminal tubule diameter of 62.53 µm than the control group, and the second treatment, which received8 grams of spirulina, had a significantly low value of seminal tubule diameter of 83.46 µm than the control group. From the table and observing the values I have given some conclusion can be made and this shows that at a high dose the spirulina can have some adverse effects on the seminal tubule diameter.

Table 1.
Effect of spirolina supplementation on testicular tissue health and reproductive outcomes in adult lambs through histological examination.

Treatments	**Tubules µm/ Somniferous**	**Lumen µm / diameter**	**Thickness of µm / Epithelial**
T1 : Control	a107.16±9.51	50.80±15.29	3.01±0.50
T2 : Spirolina 8gm	ab83.46±12.41	43.07±0.96	3.19±0.39
T3 : Spirolina 12gm	b62.53±8.33	41.00±4.00	2.63±0.10

Note: Different letters within column indicating of significant differences ($p<0.05$).

This means that at 8 g/kg, spirulina does not appear to affect the diameter of seminal tubules than when it is given at 12 g/kg spirulina. From this point, it is possible to make some conclusions: moderate portion of spirulina, 8 g seems to be less dangerous for seminal tubule diameter, but 12 g, clearly, can negatively affect the diameter. A rather suggestive study of the effects of spirulina on the male human reproductive system shows that spirulina may influence seminal tubule diameter. The health of reproduction was known to be improved by spirulina treatment, therefore, the seminal tubule diameter could be raised (Al-Yahyaey et al.,2023). Another study by the research showed that at this energy intake, the spirulina could protect the Leydig cells from damage and increase the volume of these cells and the diameter of seminal tubules even under the conditions of physical fatigue (Lazuardi et al., 2017). These findings correlate with your observations with respect to your conclusion that seminiferous tubule diameter could be improved by spirulina since perhaps high dosage might be more beneficial on those sterility markers than low dosage. As even deduced from Table 1, lumen diameter and testicular epithelium height have not been positively affected by the amount of spirulina for the second and third treatment had not differed from the control.

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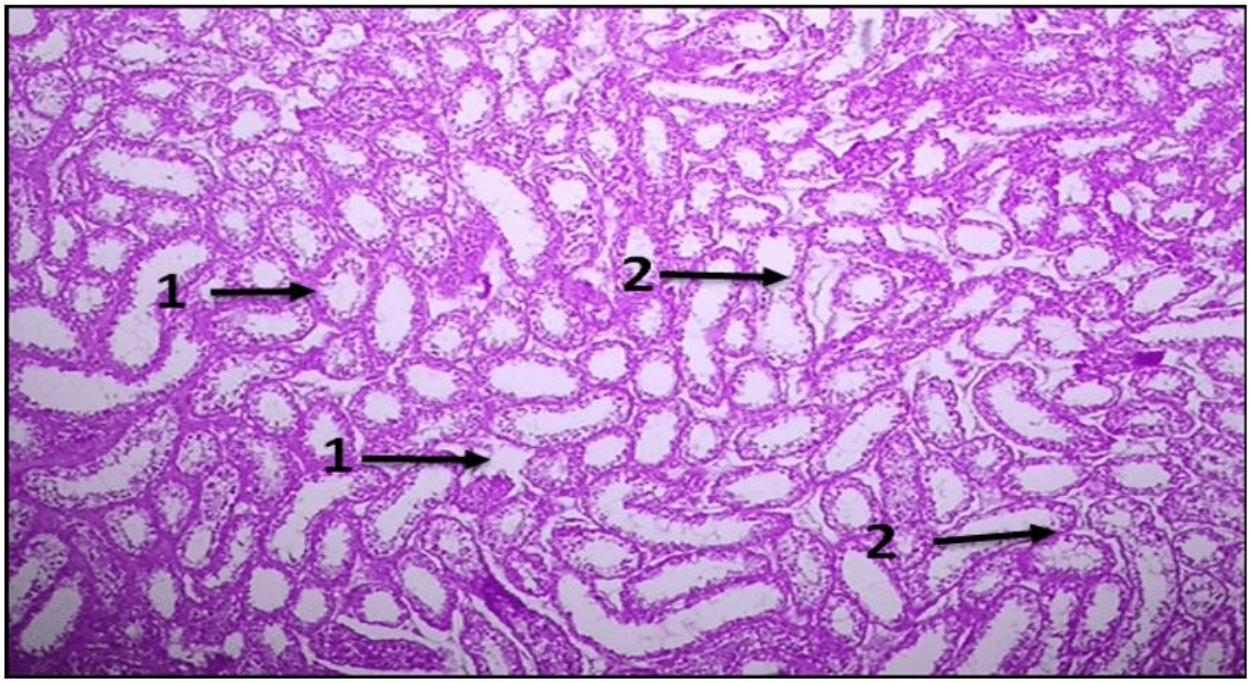

Figure 1.
Histological section of testes of adult lambs (Control Lambs) shows: 1- Seminiferous tubules with spermatozoa and sertoli cells, 2- Interstitium, (HandE 10X).

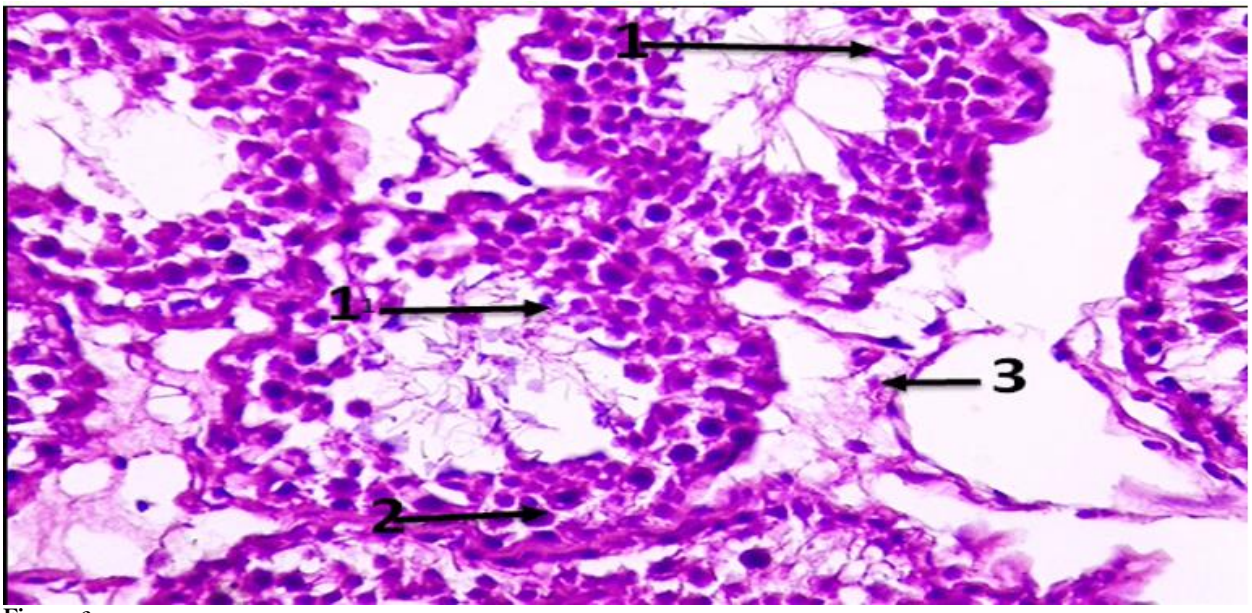

Figure 2.
Histological section of testes of adult lambs (Control Lambs) shows: 1-spermatozoa (seminiferous tubule) and 2- sertoli cells, 3- Interstitium, (HandE 40X).

In figures 1 and 2, the histological examination results of the control lambs that were not treated with spirulina indicate that the testicular tissues show a normal and healthy structure. The normal arrangement of the seminiferous tubules, spermatogonia, Sertoli cells, and interstitial tissue indicates that there were no pathological changes or abnormalities in the testes of these animals. This is

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important because it establishes a baseline or control condition, indicating that under standard, untreated conditions, the testes maintain a normal histology. This is essential for comparison in studies where interventions or treatments are applied, as any deviations from this normal structure in the treated groups can be attributed to treatment effects. In short, the normal histological structure in the control animals confirms that the testicular tissues are healthy and functioning properly, and is a critical reference point for assessing the effect of any experimental treatments on the testes.

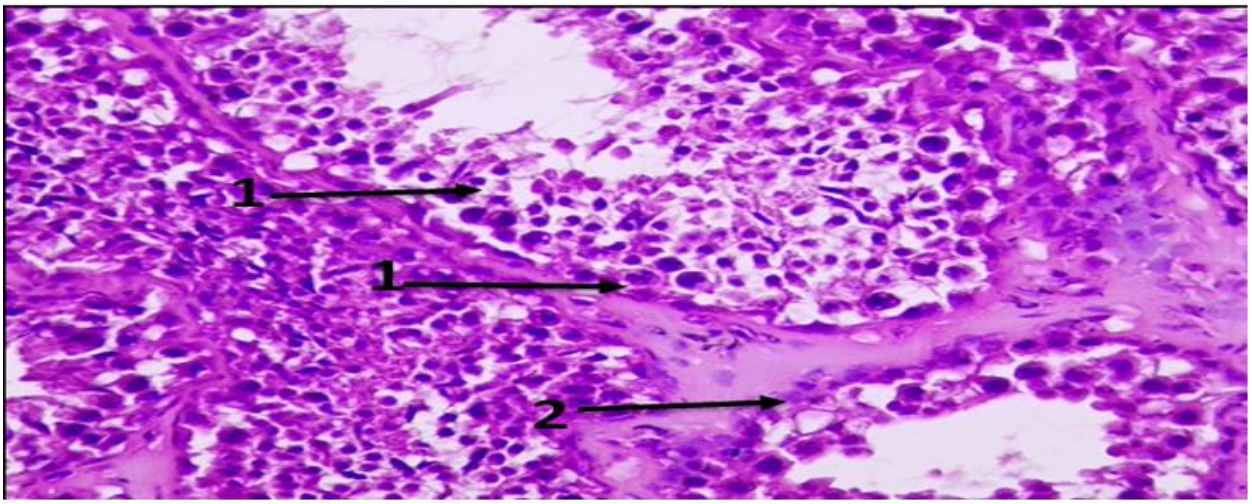

Figure 3.
Histological section of testes of adult lambs (T2- spirolina 8gm) shows: 1- spermatozoa and sertoli cells in seminiferous tubule and 2- Amyloid fluid in Interstitium, (HandE 40X).

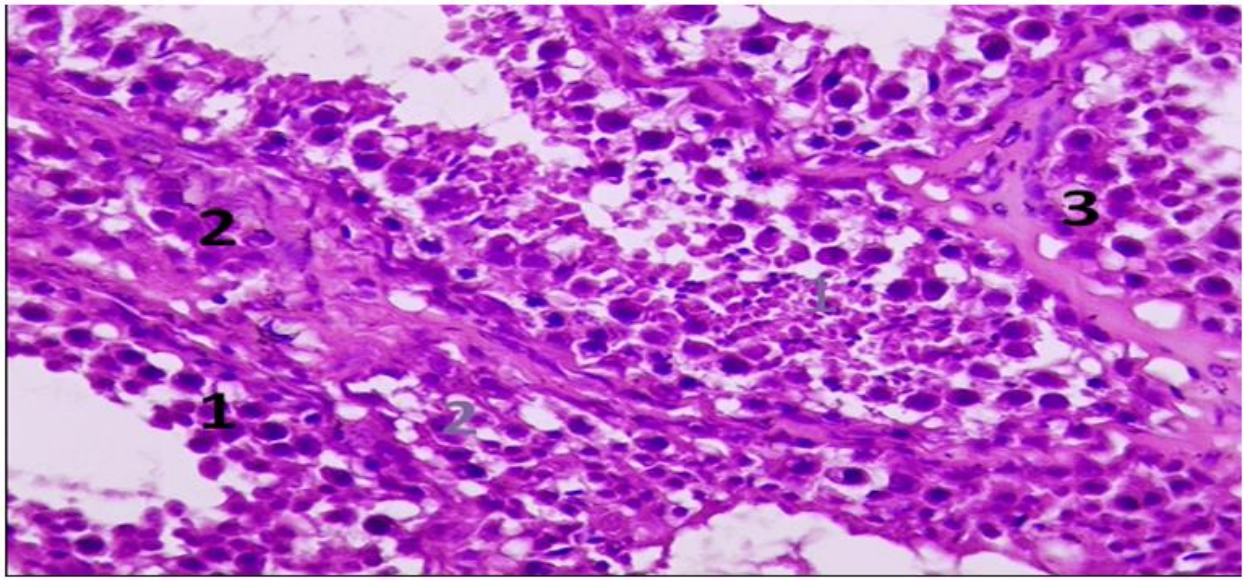

Figure 4.
Histological section of testes of adult lambs (T2- spirolina 8gm) shows: 1- Seminiferous tubules with spermatozoa and sertoli cells, 2- necrosis of Interstitium, and 3- deposition of amyloids fluid: (HandE 40X).

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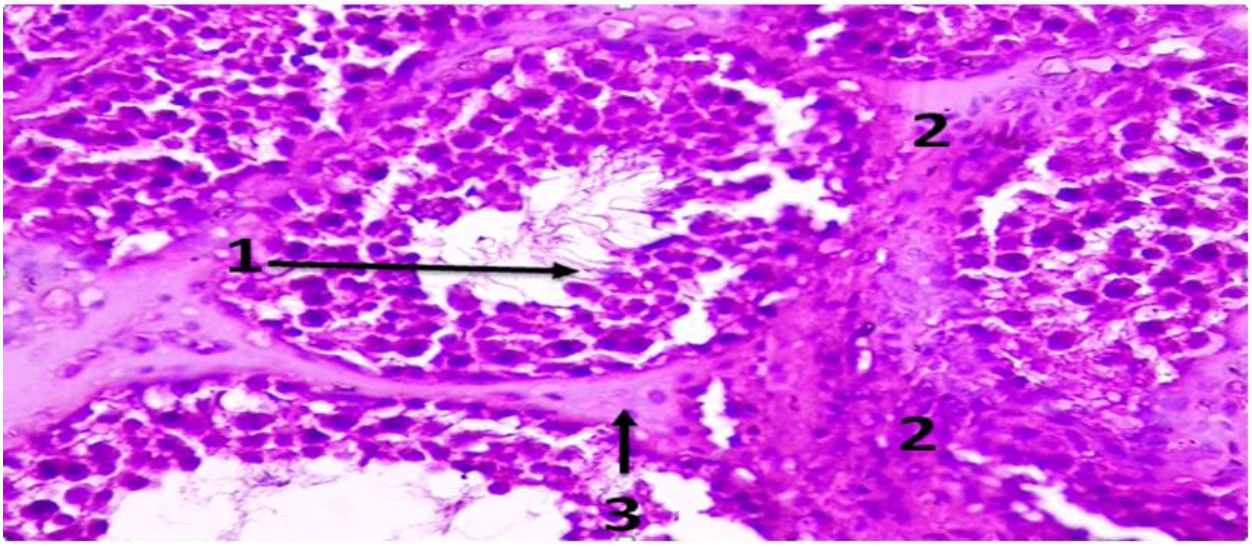

Figure 5.
Histological section of testes of adult lambs (T2- spirolina 8gm) shows: 1- Spermatozoa, 2- necrosis of Interstitium, and 3- deposition of amyloids fluid in tunica propria: (HandE 40X).

From the histological changes in Figures 3, 4 and 5 we are convinced that spirulina was actually beneficial but also toxic to the testicular tissue of lambs. Nevertheless, the fact that spermatozoa and Sertoli cells are observed within the seminiferous tubules, and the normal contour of spermatozoa implies that spermatogenesis is not exclusively affected by spirulina in the male animal. This is in concordance with prior research pointing to spirulina as having antioxidant effects, thus may help prevent oxidative stress, a contributor to testicular injury. For instance, Calella et al., (2022) stated that there was evidence that showed spirulina supplementation could improve antioxidant defense system of the body and retarded impairment of spermatogenesis imposed by oxidative stress Walke et al. (2023). At the same time we can reveal such pathophysiological changes as the presence of amyloid fluid in the interstitium and tunica propria, as well as the interstitial tissue necrosis. Amyloid deposition influences normal function of underlying tissues, and can affect testicular function and fertility. Sipe and Cohen (2000) also confirm this, pointing to the fact that the amyloid deposits within the tissue is usually linked to disturbances and damage of cells . Moreover, structural changes such as necrosis in the testicular tissue could show that spirulina possesses some pathogenic effects that are attributable to oxidative stress or inflammation. High doses, or long-term consumption of spirulina has been the concern of some authors like Assar et al. (2023) who have reported that spirulina can cause oxidative damage in specific conditions. In summary, these findings can be concluded that spirulina has ambivalent effects on the testicular tissue. However, there is necrosis and amyloid deposition making it possible to reduce normal spermatogenesis and below the normal limit fertility impact. These results identify the need for a cautious examination of spirulina dose and duration in treating infertility safely, especially when using this alga as a supplementary therapeutic agent.

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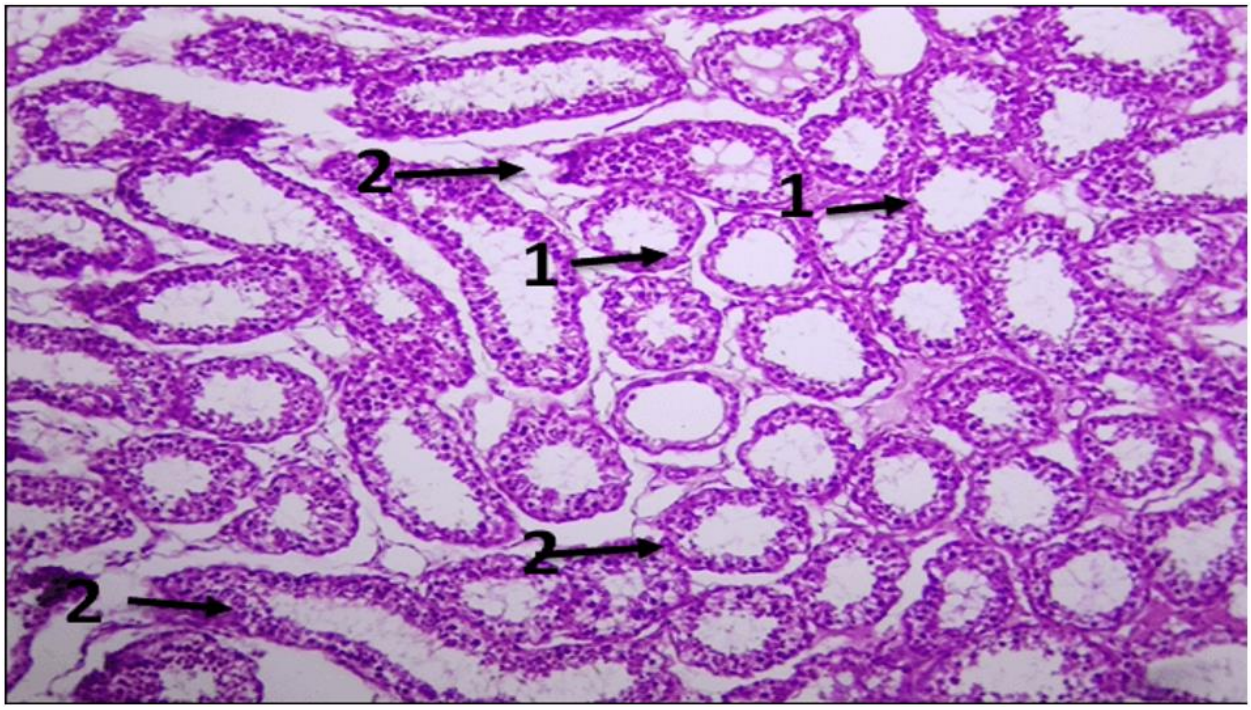

Figure 6.
Histological section of testes of adult lambs (T3- spirolina 12gm) shows: 1- Spermatozoa, 2- necrosis of Interstitium: (HandE 10X).

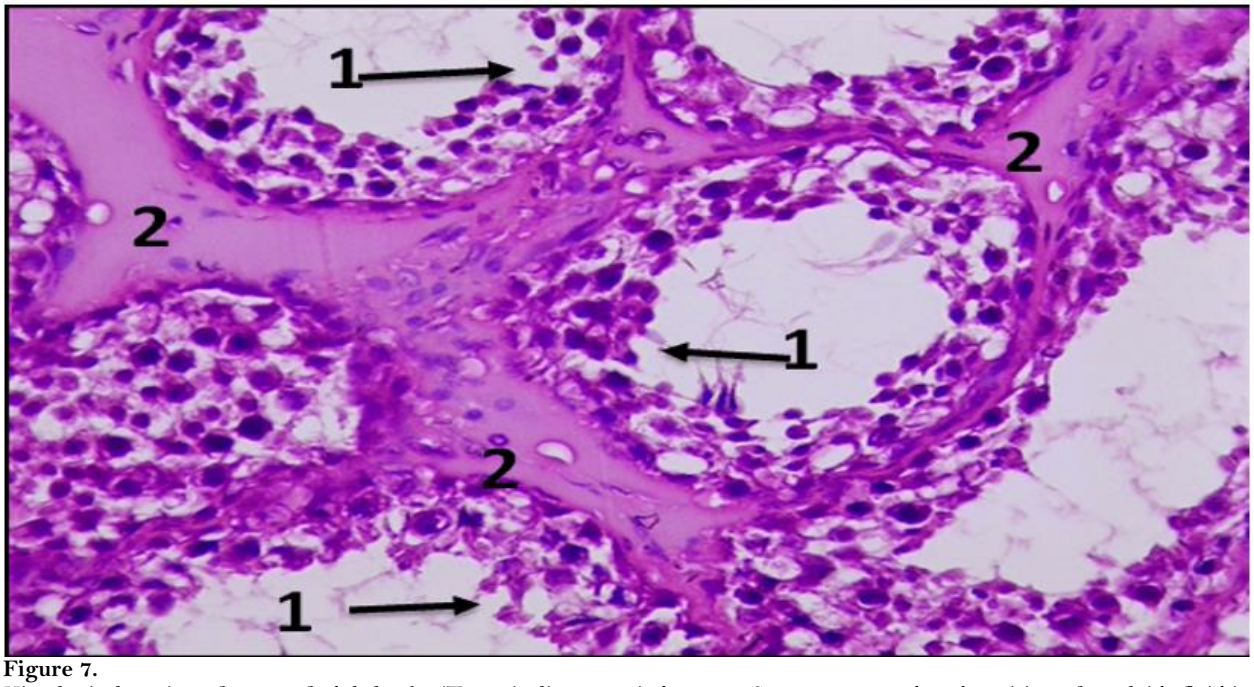

Figure 7.
Histological section of testes of adult lambs (T3- spirolina 12gm) shows: 1- Spermatozoa and 2- deposition of amyloids fluid in interstitium: (HandE 40X).

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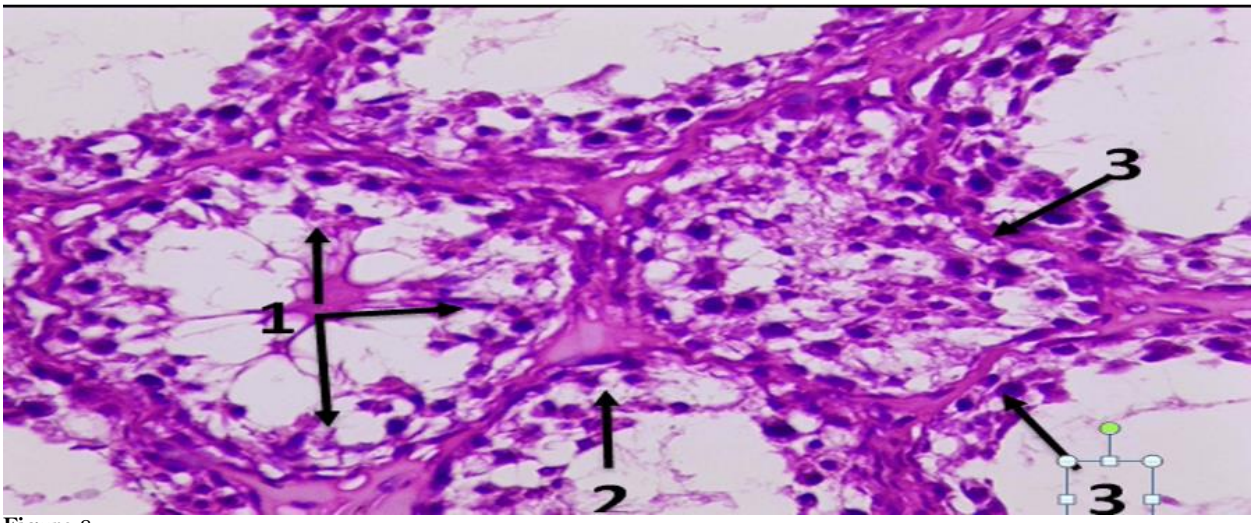

Figure 8.
Histological section of testes of adult lambs (T3- spirolina 12gm) shows: 1- Degeneration of Spermatozoa, 2- necrosis and deposition of amyloid fluid in Interstitium, 3- necrosis of tunica propria: (HandE 40X).

Figures 6 to 8 show the histopathological changes of the testicular tissue in the treated lambs, where the lambs had much serious lesion in testicular tissue than the spirulina-feed group. Figures 6 and 8: There is decrease in the number of sperms and some of the sperms appear to be abnormal in the sense they are degenerating. This implies that spermatogenesis is affected, probably resulting to low or no fertility in the sperms of these treated lambs. This observation is in conformity with studies showing that testicular toxicity and loss of spermatogenesis and sperm density are avenues of fertility dysfunctions (Boekelheide 2005; Moretti et al.,2022). Figures 6, 7, and 8: The interstitial and tunical propria are described as having necrosis; this term points to the broad-spread tissue death. Slight or severe necrosis of these areas is one of the severe pathological states that can compromise testicular physiology, hormone production, as well as support for spermatogenesis processes. The inflammation of testicular tissues resulting from necrosis is well understood, as is its impact on testicular function. Ischemia negatively alters the general microvasculature of the testes and impacts male hormone biosynthesis, Leydig cell functionality that maintains healthy testicular testosterone concentrations and total testicular health (Mealy et al., 1990; Ahmed et al., 2022) . Figures 7 and 8: There is thick accumulation of amyloid fluid (proteinaceous material) in the interstitium. This accumulation is much higher than in the spirulina treated group suggesting a more aggressive pathological reaction. Amyloid deposition may degenerate the arrangement and function of the testicular tissue and result to unending affected spermatogenic and general testicular health. The effects of amyloid deposition as a pathological feature have been widely analyzed interference with tissue architecture as well as performance. Amyloid fibrils can inhibit normal functioning of cells and tissues and enhance the tissue injury process (Pepys, 2006; Sipe and Cohen, 2000) .The observed pathological changes in this treated group are even more profound compared to those observed in the spirulina treated group. The lower count density of spermatozoa, the presence of necrotic specially in group II, and the presence of large amounts of amyloid deposition in the testes tissue call for a more aggressive or toxic treatment in this group. These results support previous investigations that postulated that extreme testicular toxicity leads to a decrease in sperm concentration and quality, as well as impaired reproductive ability (Khair et al., 2021; Boekelheide, 2005). The studies show that the treatment has toxic effect on the testicular tissue. Decrease in spermatozoa volume together with necrotic and degenerating spermatozoa suggest an impaired spermatogenetic capacity of the testes. The extensive findings of both necrosis and amyloid deposition provide additional evidence to support the idea that the general setting of the testicular tissues is becoming less hospitable to the metabolism, structure and function of normal cells and spermatogenesis. There was, in the treatment group, a more marked pathological effect on the testes

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than on the testes of the spirulina group as can be seen from figures 6 to 8. Reduced spermatozoa, necrosis, and the increased number of significant amyloid deposits point to decreased testicular function that may manifest itself in sterility or other violations. This underlines the need to evaluate toxic effects of such treatments and efforts made in this direction are still limited (Pepys, 2006;Ahmed et al., 2022) .

4. Conclusion

The normal adjacent tissue in the negative control group was compared with the changes made in the testicles after the administration of spirulina with a dosage of 12 grammes per kilogramme of diet (T3). Of these, necrosis, amyloid deposition and, spermatocyte degeneration could also be distinguished. More further research has to be undertook on spirulina following the result of this study aimed towards the possibilities of miraculous effect of spirulina in causing harm on the reproductive capacity of lambs.

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