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Histological changes in the testes of male Awassi sheep fed a diet containing *spirulina platensis*

Aslam Saud Alwan Hamad[1] , Nada Aziz Khalid [2] ,Shaymaa Ali Hassan [3]

[1]Department of Animal Production, College of Agriculture, Tikrit University, Iraq

[2]Department of Biotechnology, College of Applied Sciences, Samarra University, Iraq

[3]Department of Biology, College of Education for Women, Tikrit University, Iraq

Corresponding author: E-mail: Aslam.alwan@tu.edu.iq

Abstract

Effect of feeding *spirulina platensis (Sp. Platensis*) in the diets of male Awassi sheep on some histological characteristics of the testicles, using 12 males distributed into three groups (4 males/group) distributed randomly, the feed was provided without additives to the first group and was considered a control group (T1), while the 2nd group (T2) was given concentrated feed with the addition of (80 g/kg) of concentrated feed, while the 3rd group (T3) was fed concentrated feed with the addition of 120 g/kg of concentrated feed. In order to achieve the benefit of adding *Sp. platensis* to the feed, histological sections of the testicles were studied after slaughtering the animals. The results showed, that feeding with *Sp. platensis* showed significant differences between the control group and the other two groups. Necrosis was observed in and the deposition of amyloid fluids with damage to the STs in the two treatment groups compared to the control, with the occurrence of degeneration of the interstitial tissue as well as the tunica propria. This may explain that feeding with *Sp. platensis* at these rates under study has It results in potential damage to the testicular tissue. To gain more knowledge, this aspect must be studied and more experiments must be conducted at different ages on male sheep.

Introduction

Sp. platensis has been reported in several studies as an effective nutritional supplement to enhance the reproductive performance of animals (Kistanova et al.,2009 ; Iatrou et al.,2022). This algae represents an excellent source of protein which it contains 60-70% calculated on dry matter basis in addition to containing almost all amino acids AA and vitamins fatty acids FA and essential minerals EM (Farag et al.,2016 ; Gargouri et al.,2019). The inclusion of *Sp. platensis* in the nutrition of farm animals such as (sheep and goats and cattle) has led to increased fertility and production profit and improved health (Madeira et al.,2017). Furthermore, Holman et al.,(2014) investigated the effect of *Sp. platensis* on sheep and goats and found that *Sp. platensis* has great potential for economic value and profitability as a nutritional supplement for lambs. Furthermore, *Sp. platensis* is believed reduce and protecte various organs from toxic chemicals improve taste and digestibility (Abdel-Daim et al.,2013). There have been previous studies, such as those conducted by (Kistanova et al.,2009), Granaci, (2007). Positive results have been reported for the supplementation of Sp. platensis with regard to sperm motility (cells with half the number of chromosomes) and their viability (Abdel-Khalek etal.,2023). Also reported that increasing the intake of Sp. platensis in the diet improves (semen quality and sperm motility) despite the available research on the potential benefts of Sp. platensis supplementation for various aspects of animal health and reproduction there still paucity of studies investigating effects on the reproductive parameters of domestic male farm animals abundance of nutrients in *Sp. platensis* makes it aninvaluable food source especially in areas with limited traditional feed resources (Al-Yahyaey etal,2023 ;Podgórska, 2024). Research has shown that *Sp. platensis* can signficantly enhance the health and productivity of livestock (Becker, 2007 ; Holman and Malau-Aduli,2013). *Sp. platensis* algae has shown great potential in ruminants nutrition such as cattle and sheep (El Deeb et al.,2022) . It is easily digestible and serves as a source of high quality protien which essential for the growth

and development of these animals (Thompson,2008). Furthermore, *Sp. platensis* contains bioactive compounds can enhance rumen function and improve nutrient absorption (Marjanović et al.,2024). This leads to better overall health increased ruminants productivity (Joseph McGrath et al.,2018). Studies suggest that *Sp. platensis* supplementation in diets of ruminant can enhance milk production (improve weight gain) and enhance nutritional quality of animal products (Khan et al., 2013). *Sp. platensis* highly valued for its protein content which rivals traditional animal based protein sources (Zhou et al.,2025). With protein content of up to 70% *Sp. platensis* provides all essential amino acids making an excellent alternative to conventional protein sources in feeds of animal (Podgórska-Kryszczuk,2024). This is particularly useful area where protein rich feed ingredients are scarce (Luqman et al.,2023). Use of *Sp. platensis* as protein source supports animal growth and health (Lestingi et al.,2024) while contributing more sustainable livestock production by reducing reliance on conventional sources protein such as soybean and fishmeal which have significant environmental impacts (Pillai et al., 1993).

Materials and methods

The study was conducted at Tikrit University within the Animal Production Field of the College of Veterinary Medicine, for the period from late fall 2021 to mid-winter 2022. The study aimed to measure the extent to which sexual characteristics (testicles from a histological point of view) were affected by two levels of Spirulina. Because Sp. is characterized by high protein, the goal of adding it to raise the value of concentrated feed (its chemical analysis is shown in Table 1)* provided to animals from protein. Twelve males from the local Iraqi sheep breed were distributed into three groups (each group 4 males) distributed randomly. The feed was provided without additives to the first group and was considered a control group (T1), while the second group (T2) was given concentrated feed by adding 80 g/kg of concentrated feed, while the third group (T3) was given Concentrated feed by adding 120 g/kg of concentrated feed to achieve the benefit of adding Sp. platensis to the feed, the histological sections of the testicles were studied after slaughtering the animals.

Table 1: Chemical composition of concentrated feed in the study area.

Dry matter	95.71%
Crude protein	11.11%
Crude fat	3.37%
Crude fibre	6.17%
Ash	4.73%
Energy	2820 kcal/kg

The testes of male sheep were collected after slaughter, and a tissue section of 2 cm^3 was taken from them, with the aim of collecting samples for studying the tissue sections and detecting the development status of spermatozoa, Leydig cells(LDs), and Sertoli cells(SCs), and on the other hand to identify the biofilm of the seminiferous tubule ST, the height of the cell membrane, and the development of the ST. The testicles of male sheep were collected after slaughter, and a tissue section of 2 cm3 was taken from them, with the aim of collecting samples to study the tissue sections and detect the status of (sperm development, LDs and SCs) and from another side to identify (the biomembrane of the ST, the height of the cell membrane and the development of the ST), and to obtain the tissue samples using a sharp sterile surgical instrument,

formalin was used at a concentration of 10% and placed in plastic containers with a capacity of 50 ml for the purpose of immersing the tissue pieces in it to be preserved and to ensure that their morphological characteristics do not change and that there are no undesirable characteristics for the purpose of making tissue slides and reading them by storing them in a dry, cool and dark environment. To maintain the quality of the tissues and to ensure that the formalin does not change, the containers were tightly closed and numbered for each animal and the date was written on them. The numbering method facilitates the method of tracking them and for us to identify them easily. Following the standard critical protocols, tissue samples were successfully prepared and preserved. The tissue slides were stained with eosin and hematoxylin and then read under a microscope and the dimensions were carefully measured.

Results and discussion

Feeding male sheep to a concentrated diet containing 80 g/kg of *Sp. platensis* as a concentrate feed or 120 g/kg of *Sp. platensis* as a concentrate feed is what the results of Table 2 indicate. By reviewing the results of the statistical analysis, it becomes clear to us that the differences between the groups treated with *Sp. platensis* and the control group were not statistically significant in the diameter of the seminiferous tubules, but in terms of the arithmetic difference, it is noted that the second group was less than the control group by 9%. On the other hand, we note that the second group had decreased in the lumen diameter of the seminiferous tubule compared to the control group by a statistically significant difference, while feeding the lambs with 120 g of *Sp. platensis* did not affect the lumen of the tubule compared to the control group.

Table (1) Effect of *Sp. platensis* on seminiferous tubule diameter, tubule lumen diameter and tubule epithelial thickness in male sheep testes

T.	tubules Somniferous/µm	Lumen diameter/µm	thickness of Epithelial /µm
T1	78.12±10.36	a56.21±5.72	2.72±0.23
T2	71.51±9.78	b38.14±2.18	2.81±0.21
T3	78.08±23.29	42.52ab ±6.18	3.42±0.43

Treatments:T. , T1 : Control , T2 : Spirolina 80gm/kg , T3 : Spirolina 120gm/kg , Micrometer: µm

When viewing the microscopic examination of the tissue sections of the testes of the control group (Figures 1 and 2), the tissue structure is normal and has a functionally active structure. The sperm and Sertoli cells in the seminiferous tubule indicate that the process of spermatogenesis is proceeding normally, which is an indication that the testes have the ability to produce sperm efficiently.

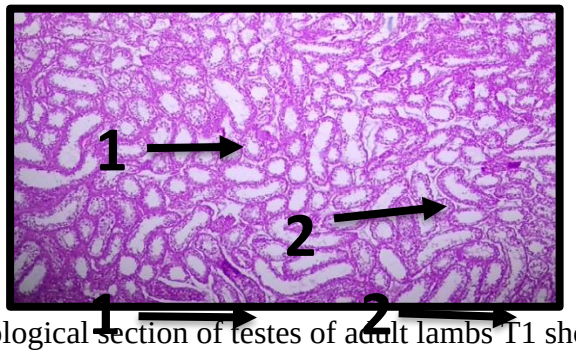

Figure 1 : Histological section of testes of adult lambs T1 shows: (1) Seminiferous tubules with spermatozoa and sertoli cells, (1) Interstitium, (H and E 10X).

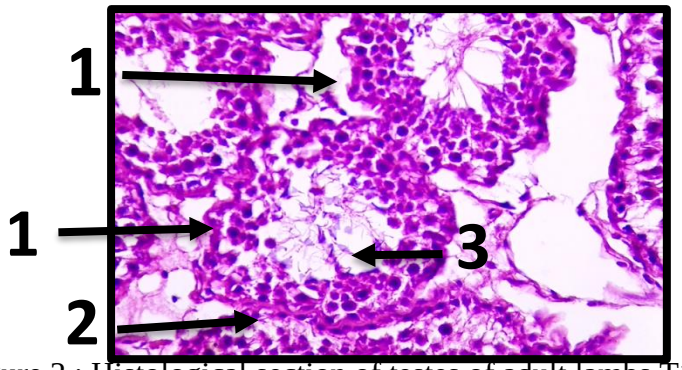

Figure 2 : Histological section of testes of adult lambs T1 shows: 1-spermatozoa (seminiferous tubule) and 2- sertoli cells, 3- Interstitium, (H and E 40X).

Histological examination at 10X and 40X magnification indicates the presence of sperm inside the seminiferous tubules. This indicates the activity of sperm formation, which means that the testicles are mature and ready for ejaculation, producing sperm cells. It also indicates the presence of Sertoli cells, whose primary function is to support and nourish the sperm cells in the growth phase. We conclude that the sperm formation environment is normal. It is noteworthy that the interstitial tissue and amyloid fluid show the presence of a small amount of protein material from the amyloid fluid inside the interstitial tissue. This may indicate early signs of protein accumulation. One of the characteristics that are linked to age is the occurrence of amyloid fluid deposition, which increases as the animal ages. However, it may not result in pathological effects if the deposition is not in large quantities. In general, the histological examination indicates that the testicles are functioning normally, with active sperm production and likely minor pathological changes. The slow deposition of amyloid substances may not cause significant damage with age.

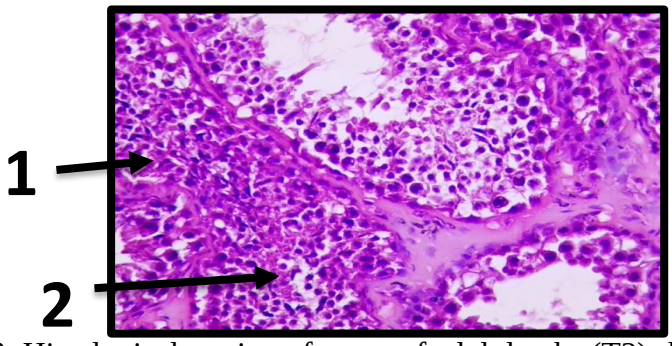

Figure 3: Histological section of testes of adult lambs (T2) shows: 1- spermatozoa and sertoli cells in seminiferous tubule and 2- Amyloid fluid in Interstitium, (H and E 40X).

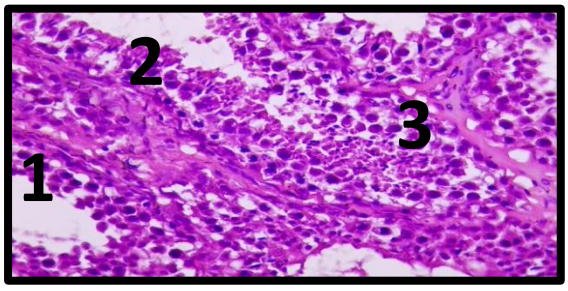

Figure 4: Histological section of testes of adult lambs (T2) shows: 1- Seminiferous tubules with spermatozoa and sertoli cells, 2- necrosis of Interstitium, and 3- deposition of amyloids fluid: (H and E 40X).

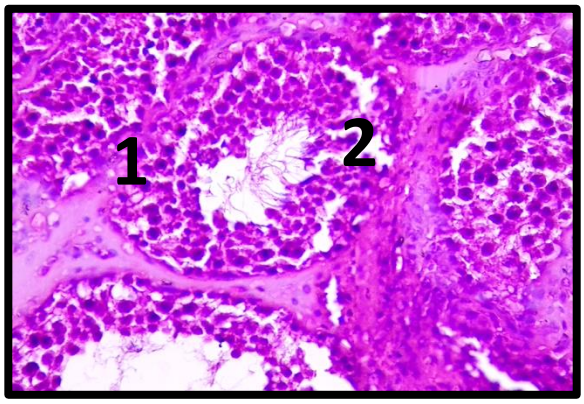

Figure 5: Histological section of testes of adult lambs (T2) shows: 1- Spermatozoa, 2- necrosis of Interstitium, and 3- deposition of amyloids fluid in tunica propria: (H and E 40X).

When studying the tissue sections of the second treatment T2 male sheep fed 80 g/kg of concentrated feed in Figures 3, 4 and 5, changes are observed in the testicular tissue that may appear pathological, such as the presence of amyloid fluid, which we show as follows in Figures 3 and 4, as the deposition of amyloid fluid in the interstitial tissue of the testicle indicates an abnormal accumulation of protein materials in its tissues. The presence of amyloid fluid is accompanied by a direct effect on the normal functioning of the testicle, which may result in a deficiency in responding to hormone activity and its role in sperm formation. Sometimes, a link can be made between the occurrence of a state of aging or tissue damage and the presence of amyloid fluid deposition. In Figure 5, necrosis is observed in the interstitial tissue of the testicle, and one of the most prominent symptoms of this condition is that what it indicates is the occurrence of a state of cell death resulting from a serious injury, and it certainly has many other causes such as exposure to toxins, lack of oxygen or inflammation in general.

One of the most prominent symptoms that accompany the presence of interstitial tissue necrosis is the observation of weak growth of Leydig cells, which in turn is reflected in reproductive performance in general as a result of the effect on the production of testosterone produced by these cells (Monageng et al,2023). The connective tissue surrounding the seminiferous tubules shown in Figure 5 indicates the deposition of starchy fluids in the outer sheath, which is the widespread spread of starchy deposits outside the interstitial space. This may lead to negative results and changes in the seminiferous tubules, resulting in a defect in the process of sperm formation Keable et al,2016).In general, sperm function and endocrine activity in the testicles were shown by the results of the second treatment to the effect that the lambs suffered from obvious testicular damage including amyloidosis and necrosis.Many studies have indicated that amyloid deposition in testicular tissue may occur with age or in response to a chronic inflammatory condition, and that such pathological manifestations may be due to the negative effects of the treatment used, environmental conditions, or genetic predisposition amyloid deposition in the testicles is associated with normal tissue function, resulting in decreased fertility (Westermark et al., 2005).Many causes such as oxidative stress, toxins, decreased blood supply, and various factors may affect the appearance of necrosis in the interstitial tissue (Pizzino et al.,2017). This is what Zhang et al., (2011) indicated that testicular necrosis is associated with significant disturbances in spermatogenesis and this may lead to infertility. Pepys,(2001) has suggested that it may not lead to significant impairment of testicular function in older animals with amyloid deposits in testicular tissue unless they are widespread or accompanied by other pathological changes.

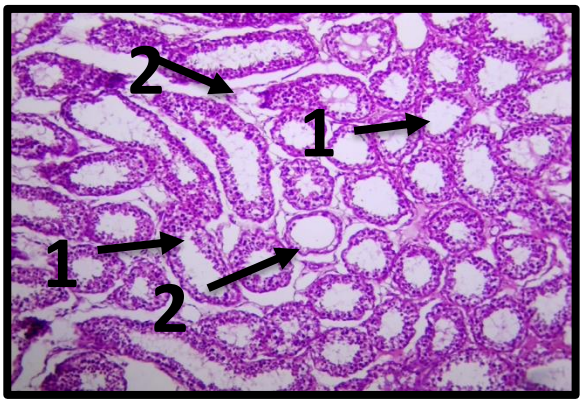

Fig 6 : Histological section of testes of adult lambs (T3) shows: 1- Spermatozoa, 2- necrosis of Interstitium: (H and E 10X).

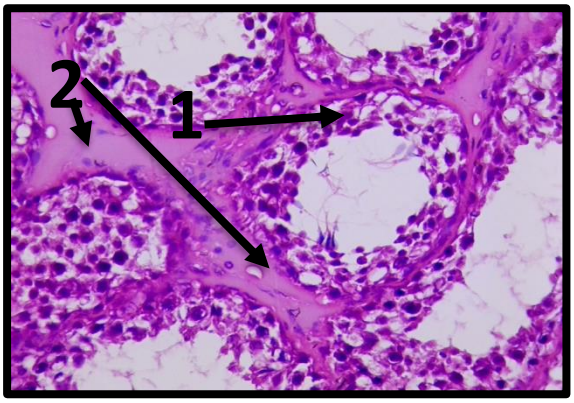

Figure 7: Histological section of testes of adult lambs (T3) shows: 1- Spermatozoa and 2- deposition of amyloids fluid in interstitium: (H&E 40X).

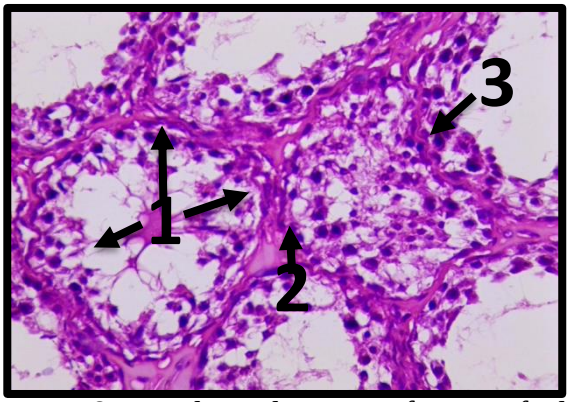

Figure 8: Histological section of testes of adult lambs T3 shows: 1- Degeneration of Spermatozoa, 2- necrosis and deposition of amyloid fluid in Interstitium, 3- necrosis of tunica propria: (H and E 40X).

As shown above in Figures 6, 7 and 8, which show the histological examination of the testicles of male sheep fed 120 gm spirulina/kg T3 concentrate feed, the testicular tissues undergo changes that appear pathological with additional signs of degeneration and more severe damage than observed in the second group. Looking at Figures 6 and 8, the histological section image showed the presence of necrosis in the interstitial tissue that may lead to cell death. With age, the testicles lose their functional ability due to what the interstitial tissue contains, such as Leydig cells, which are responsible for producing the hormone

testosterone. The presence of necrosis works primarily to disrupt hormonal balance and reduce fertility. Amyloid deposition in the interstitial tissue is a sign of abnormal protein accumulation, as we see in Figures 7 and 8, which may prevent the testicles from performing their basic function. Fibrosis occurs as a result of amyloidosis, which increases the chance of damage to the function of the testicular tissue and its structural integrity. In addition, Figure 8 shows the occurrence of sperm melting and weak formation in the seminiferous tubules. Certainly, all of this is due to amyloid deposition, which in turn creates a hostile environment that is not suitable for the development and growth of sperm.

In addition, the appearance of signs of necrosis is one of the signs of serious injury that leads to damage to the structure of the testicle, which results in the disintegration of sperm, which in turn leads to general weakness in the testicular tissues.

Westermark et al., (2005) and Zhang et al.,(2011) pointed out that observing necrosis and amyloid deposition in testicular tissue is an indicator of serious testicular diseases. These conditions are associated with exposure to toxins, systemic diseases or chronic inflammation, which negatively affect sperm production and consequently the reproductive performance of animals. Turner & Lysiak, (2008) agreed with what appears in the third treatment of the results of infection with amyloidosis and around necrosis with the presence of fibrosis causing weak sperm and a reflection of this condition leads to infertility. Pepys,(2001) reported that local or small starchy deposits may have a significant effect on testicular function unless they are associated with pathological or widespread changes. This is consistent with the results obtained indicating the presence of degeneration and necrosis along with starchy deposits, which in turn are evidence of testicular dysfunction. On the other hand, observing the third treatment shows that severe damage such as necrosis, sperm degeneration and amyloid deposition will certainly lead to poor response to endocrine stimuli and affect testicular function. The results of the microscopic examination of the testicles of male sheep treated with spirulina can be summarized as follows: The second and third treatments are described as having pathological characteristics due to the appearance of amyloid deposition, necrosis, and degeneration in sperm. These pathological variables work directly with age to weaken sperm formation and reduce fertility. When summarizing the results in the tissue slices, it becomes clear that the control group showed a normal structure, while in contrast, the second and third groups showed changes that are distinct from them and can be described as pathological, such as necrosis, amyloid deposition, and sperm degeneration. These changes are likely to lead to weakening sperm formation and reducing fertility.

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