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Impact of seaweed on growth performance, sperm quality, and testicular histomorphology of ram

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ABSTRACT

Sperm quality is critical for male fertility, which is largely dependent on testicular development; however, nutrition is critical for optimal growth, gonadal development, and reproductive functions. This study aimed to investigate the effects of *Gracilaria parvispora*, a red seaweed on growth performance, testicular physiology, and semen quality in ram. Fifteen indigenous healthy rams, weighing 10-11 kg and aged 12-13 months, were randomly allocated into three groups. One group was considered as control and supplied with a German grass and concentrate mixture (control diet). The other two groups were provided with a control diet plus seaweed mixture at 7 g/d or 14 g/d. The starting body weight, scrotal circumferences, and semen quality of rams were similar for all groups. However, post-treatment, both the seaweed-treated groups showed significantly higher body weight, total weight gain, daily weight gain, and increased scrotal circumference and testicular dimensions, compared to the control group. Moreover, the semen parameters, including sperm concentration, live sperm percentage, mass motility, and progressive motility were observed to be significantly increased, while decreasing abnormal sperm percentage in the seaweed-treated group. Furthermore, DPPH and ABTS scavenging abilities of sperm and *in vitro* fertilization rate were increased dramatically in seaweed-treated groups. Additionally, the testicular histology of seaweed-treated groups revealed a thinner tunica albuginea with expanded seminiferous tubules containing a higher number of spermatogenic cells inside. These findings suggest that seaweed *G. parvispora* could be a valuable dietary supplement for enhancing the growth and reproductive performance of ram.

INTRODUCTION

Sheep farming has been impacted greatly by its expanded contributions to milk, meat, and wool production in the socio-economic condition of small-scale farmers, largely in arid and semi-arid regions across the world [1]. Sheep farming is a crucial part of animal agriculture in Bangladesh and ranked third, next to the cattle and goat population [2]. Besides the promising dwarf indigenous sheep distributed throughout the country [3], the Garole, an Indo-Bangla crossbred sheep, is raised in northwestern Bangladesh for its medium wool and higher meat yield [4]. Sheep, unlike other livestock species, possess an extraordinary ability to adapt to various production systems, ranging from extensive to intensive farming, and can survive in different climatic conditions, including extreme arid to humid environments [5]. Moreover, sheep exhibit high prolificacy, early sexual maturity, and the ability to thrive consuming low-quality roughage [6,7]. Therefore, sheep farming is popular among poor and marginal farmers in rural areas of developing and least-developed countries [8]. Despite the beneficial aspects, the acute scarcity of genetically superior stud rams is

one of the primary obstacles to sheep production across rural settings [9,10]. Most farmers are not interested in rearing breeding rams due to their musky smell which hinders sheep reproduction through natural mating [11]. Therefore, artificial insemination (AI) might be a better option, where the quality of rams and their sperm are crucial factors [12]. Moreover, AI in sheep is more difficult due to the unique anatomy of the cervix having numerous folds and twists, making it challenging to navigate with an insemination gun [13]. Nonetheless, detecting estrus in sheep can be more complicated than in other species because of their silent estrus and poor external signs of heat [14]. As a result, breeders recommended more advanced AI technologies, including laparoscopic AI (LAI) and transcervical AI (TCAI) [15], which are not feasible in the rural developing world. Therefore, natural mating with genetically superior high-quality ram might be the best option for the sustainable sheep farming business. The quality of the breeding male is primarily dependent on the quality and fertilizing ability of sperm, which solely relies on testicular development and spermatogenesis within the testis [16]. In contrast, nutrition is critical in supporting optimal growth, testicular development, sperm output, and reproductive functions of breeding males [17]. A reduction of semen volume and the quality of spermatozoa by undernutrition has been reported in adult ram [18]. In addition, micronutrients, especially antioxidants, are essential to neutralize harmful free radicals and protect sperm cells from oxidative damage, thus promoting healthy sperm function [19]. Therefore, it is indispensable to focus on dietary interventions besides genetic potentials to enhance male fertility for sustainable sheep reproduction.

Seaweed, an autotrophic plant accumulated in specific coastal zones, holds promise as a potential food source for human consumption [20]. It is recognized for its significant carbohydrate, protein, fiber, vitamin, and mineral content while being low in fat [21]. Moreover, seaweeds contain bioactive compounds with antioxidant, immunomodulatory, and anti-inflammatory properties that can protect cells from oxidative damage [22]. Red seaweeds (macroalgae) are generally rich in protein [23] and special polysaccharide, Floridian starch [24]. Various studies reported the potential of seaweed to be used as an unconventional feed ingredient to improve digestion, nutrient utilization, and immune function in different livestock species [25,26]. Moreover, seaweeds are the source of chelated micro-minerals and omega-3 fatty acids, whose incorporation into the diet has shown positive effects on semen quality and fertility traits in ruminants [26]. Additionally, the polysaccharides and polyphenols of seaweed have been shown to have anti-inflammatory and protective effects on testicular tissue [27]. Therefore, utilizing seaweed as an alternative sheep feed supplement could address the health benefits and increase the sperm quality and fertility of rams. Although various studies have reported the impact of seaweed supplementation on the growth performance of various livestock species [28,29], research focusing on the reproductive parameters of rams is limited. In our previous study, we observed that *Gracilaria parvispora*, a red seaweed found in St. Martin's Island, having high CP content (12.2%) and DM degradability (43.3%), positively impacted the growth of lambs in an *in vivo* trial [30]. Therefore, the present study was encountered to elucidate the beneficial effects of *G. parvispora* on the growth performance, sperm quality, and testicular morphology of rams.

MATERIALS AND METHODS

Materials and reagents

Unless specified, all chemicals and reagents used in the experiment were obtained from Merck KGaA (Darmstadt, Germany) and/or its affiliates. The seaweed *G. parvispora* was in kind collected from the Animal Nutrition Laboratory of BSMRAU which was randomly sampled from three different sub-locations from Saint Martin Island (latitude 20°34′ to 20°39′ N and longitude 92°18′ to 92°21′ E) in the Bay of Bengal, Bangladesh. To prepare the seaweed samples for analysis, these were thoroughly washed and cleaned using ambient seawater, followed by a series of washes with decreasing salinity, starting from 30% and ending with fresh water. After manually draining excess water, samples were analyzed.

Diets and management of animals

Fifteen indigenous rams from Animal Nutrition Field Laboratory, BSMRAU, weighing 10-11 kg and aged 12-13 months, were randomly allocated into three dietary groups of five rams in each group. The rams were reared in standard housing slatted floors. The dietary group-1 was considered as control and supplied with German grass and concentrate mixture (control diet). The other two groups were provided with seaweed mixture either at 0.7 g/kg body weight (SW 7g/d diet) or 1.4 g/kg body weight (SW 14g/d diet) in addition to the control diet. The ingredients and nutritional compositions are shown in Table 1. The rams were fed twice daily, at 06:00 and 16:00 h, and had continuous access to safe drinking water ad libitum throughout the experiment. The experimental period was continued for 28 days (4 weeks), excluding a 10-day adaptation period at the beginning. The animal study protocol was approved by the Animal Research Ethics Committee (AERC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh (Ref. No. FVMAS/AREC/2022/01).

Table 1. Ingredients and chemical composition of diets.

Ingredients (g/kg DM)	Control	SW 7 g/d	SW 14 g/d
German grass	350	350	350
Napier grass	300	300	300
Maize	100.0	100.0	100.0
Wheat bran	137.0	116.0	95.0
Til oil cake	85.0	85.0	85.0
Molasses	14.0	14.0	14.0
Seaweed	0.0	21.0	42.0
Salt	4.0	4.0	4.0
Chemical composition (g/kg DM)*			
ME (MJ/Kg DM)	9.56±0.13	9.57±0.14	9.59±0.23
DM	446.13±5.86	446.98±5.82	444.86±7.86
CP	115.54±7.38	115.03±6.19	117.61±4.58
NDF	564.54±20.91	563.74±15.01	564.19±25.22
ADF	327.71±16.66	330.43±11.43	329.19±13.92
Cfat (EE)	19.42±6.85	19.64±3.63	19.88±3.27
Ash	81.31±4.67	91.09±3.20	95.94±3.65

*Unless otherwise stated; DM- Dry matter, ME- Metabolizable energy; CP- Crude protein; NDF- Neutral detergent fiber; ADF- Acid detergent fiber; Cfat- Crude fat; EE- Ether extract.

Measurement of body weight and scrotal circumference

The body weight (kg) of each ram and their scrotal circumference (cm) were measured weekly in the morning. Weight was measured using a top-loading balance, while

scrotal circumference was assessed using a flexible metal tape (Scrotal tape; Lane Manufacturing, Co., Denver, USA) following the methodology described by Kabiraj *et al.* [31]. In brief, the testes were gently drawn into the lower part of the scrotum, and then, the thumb and fingers were placed on the scrotum to prevent the separation of the two testes. Subsequently, the tape was wrapped around the widest part of the scrotum and securely tightened to ensure full contact with the entire circumference. Repeated measurements were taken, and the mean value was recorded.

Semen collection

Semen was collected twice a week at 8:30 am from each ram using an artificial vagina, maintaining optimal pressure and temperature at around 41°C. To aid in semen collection, the rams were exposed to homosexual stimulation by a teaser male. The semen was collected after cleaning the prepuce with an antiseptic solution according to Khan *et al.* [32].

Evaluation of semen

Just after collection, each ejaculation was evaluated individually. The volume of each ejaculate was directly measured from the graduated (mm) collection vial. The color and opacity of the semen, along with the detection of any foreign substances such as pus, were examined. The motility, concentration, morphology, and viability of sperm were analyzed according to our previous study [31]. Briefly, the mass motility (%) and progressive motility (%) of raw semen were assessed by placing a 10 µl drop of fresh semen onto a clean, pre-warmed (37°C) glass slide, covering it with a 22×22 mm coverslip, under a light microscope at 40x magnification. The sperm concentration (million/ml) was assessed by the hemocytometer method. Live spermatozoa were quantified using an eosin-nigrosine stain, while normal spermatozoa were counted using a rose Bengal stain.

Assessment of antioxidant activity of fresh semen

The antioxidant activity of semen was measured in terms of DPPH and ABTS radical scavenging ability according to El-Seadawy *et al.* [33]. Briefly, 0.1 mM DPPH and 2.45 mM ABTS solutions were prepared. Subsequently, freshly collected semen was homogenized, sonicated at 20 KHz for 30 s, and centrifuged at 800 ×g for 5 min followed by the collection of supernatants. For the DPPH assay, 100 µl semen supernatant plus 100 µl of DPPH solution was distributed to a 96-well plate following incubation at 25°C for 30 min and measured at the absorbance of 517 nm in a microplate reader (SPECTROstar® Nano, BMG LABTECH, Ortenberg, Germany). For ABTS assay, 10 µl of semen supernatant plus 190 µl ABTS+ solution was transferred to a 96-well plate and kept at room temperature for 30 minutes. After that, the sample was measured at the absorbance of 735 nm in a microplate reader (SPECTROstar® Nano, BMG LABTECH, Ortenberg, Germany). In both cases, the control sample was prepared by replacing semen supernatant with PBS. The only supernatant absorbance value was measured as blank. In both cases, the radical scavenging activity was calculated as a percentage (%) using the following formula – Radical Scavenging activity by ABTS or

$$\text{DPPH (\%)} = 1 - \frac{\text{Sample} - \text{Blank}}{\text{Control}} \times 100$$

In vitro fertility of sperm

The fertilizing ability of sperm was assessed through *in vitro* fertilization according to our previously developed protocol [34]. Briefly, the oocytes were collected from local slaughterhouse ewe ovaries and *in vitro* matured in 5% BSA supplemented TCM-199 medium at 37°C with 70% relative humidity and 5% CO_2 for 24 h in a CO_2 incubator. Meanwhile, fresh semen was collected from treated or untreated ram and capacitated in Bracket and Oliphant (BO) medium upon seminal plasma removal. Finally, the matured oocytes were fertilized *in vitro* with capacitated sperm and the rate of fertilization was calculated accordingly.

Slaughtering and testicular morphometry

The rams were slaughtered following the halal method. Upon slaughter and complete bleeding, both testes were collected from the rams belonging to three distinct treatment groups. Subsequently, the length (cm) and breadth (cm) of the testes were measured using a measuring tape, while the weights (gm) were measured using a digital balance, according to our previous study [31].

Histological study

The testes collected from slaughtered rams were trimmed and fixed overnight at 4°C in a 4% (w/v) paraformaldehyde solution in PBS. Subsequently, the testis samples underwent dehydration in ethanol, clearing in xylene, and embedding in paraffin to prepare the block. Then, 5 µm thick sections were cut from the paraffin block, mounted on slides, deparaffinized, and rehydrated using ethanol. The sections were then incubated in xylene and stained with hematoxylin-eosin, following morphological examination, including the biometry of the tunica albuginea and seminiferous tubules, under a phase contrast microscope (Zeiss, Germany), according to Karimi *et al.* [35].

Statistical analysis

The data recorded in each group, whether treated or untreated controls, were subjected to one-way analysis of variance (ANOVA) followed by LSD post-hoc test using Statistical Package for the Social Sciences (SPSS) for Windows, Version 20.0 (IBM, 2011).

RESULTS

Effect of seaweed on growth performance, scrotal circumference, and testicular morphology of rams

The growth performance, scrotal circumference, and testicular morphology of rams recorded are compiled in Table 2. The initial body weight of the ram ranged from 10.52 kg to 10.64 kg, showing no significant ($p>0.05$) difference among all treated and untreated groups. However, the final body weight, total weight gain, and daily weight gain in 28 days (the experimental period) were significantly ($p<0.05$) increased in the seaweed-treated group compared to those of the control (Table 2). The initial scrotal circumferences were similar ($p>0.05$) among all treated or untreated groups (17.96, 18.03, and 17.92, respectively); however, the final scrotal circumferences in both the seaweed-treated groups increased significantly ($p<0.05$) compared to the control. Similarly, the average testicular weight, length, and breadth were significantly ($p<0.05$)

higher in the seaweed-treated group than in the control group (Table 2). However, no significant ($p>0.05$) difference was observed in all parameters between SW 7g/d and SW 14g/d treatment groups.

Table 2. Effect of seaweed on growth performance, scrotal circumference, and testicular morphology of rams.

Parameters	C	SW 7g/d	SW 14g/d	Level of significance (p)
Initial body weight (kg)	10.58±0.71^a	10.64±0.89^a	10.52±0.55^a	0.194
Final body weight (kg)	11.74±0.85^b	12.27±0.46^a	12.20±0.74^a	0.041
Total gain (kg)	1.15±0.09^b	1.62±0.13^a	1.69±0.17^a	0.032
Average daily gain (g)	41.33±3.04^b	57.83±4.66^a	60.37±6.21^a	0.004
Initial scrotal circumference (cm)	17.96±0.86^a	18.03±1.27^a	17.92±1.02^a	0.212
Final scrotal circumference (cm)	20.57±0.48^b	21.69±0.28^a	21.70±0.32^a	0.018
Testicular weight (g) at slaughter	58.43±2.47^b	63.80±3.26^a	63.41±2.04^a	0.022
Testicular length (cm) at slaughter	6.10±0.13^b	7.20±0.31^a	7.85±0.22^a	0.013
Testicular breadth (cm) at slaughter	4.18±0.24^b	4.73±0.31^a	4.85±0.12^a	0.021

Values are Mean ± SD of five replicates. Different lowercase letters superscripts (a or b) in the same parameter (row) differ significantly ($p<0.05$) among the treatments. C – Control; SW 7g/d – Seaweed treated group (7g/d/head); SW 14g/d – Seaweed treated group (14g/d/head).

Effect of seaweed on semen volume and sperm quality parameters of rams

The semen volume and sperm quality parameters, including sperm concentration, proportion of live sperm, abnormal sperm, sperm mass motility, and progressive motility of rams treated with or without seaweed, are compiled in Figure 1. It was observed that the semen volume of all rams was increased with the advancement of age; however, the seaweed-treated groups showed significantly ($p<0.05$) higher measurements over the control, throughout the treatment period (Figure 1A). Moreover, the sperm concentration, percentage of live sperm, sperm mass motility, and progressive motility were significantly ($p<0.05$) increased (Figure 1B, D-F) while decreasing ($p<0.05$) the percentage of abnormal sperm (Figure 1C) compared to those of control. However, no significant ($p>0.05$) difference was observed between the treatment groups SW 7g/d and SW 14g/d (Figure 1).

Effect of seaweed on sperm antioxidant activity and *in vitro* fertilization of oocytes

The antioxidant activity assessed by DPPH and ABTS radical scavenging ability of sperm as well as the rate of *in vitro* fertilization of oocytes using the sperm obtained from ram treated or untreated with seaweed are shown in Figure 2. It was observed that the DPPH scavenging ability was significantly ($p<0.05$) increased in the SW7g/d treated group (62.48%), which was further significantly ($p<0.05$) increased by SW14g/d treatment (73.41%) compared to those of control (53.98%). Similarly, the ABTS scavenging ability was significantly ($p<0.05$) increased by seaweed treatment compared to the control (39.07%); however, no significant ($p>0.05$) difference was observed between the treatment groups SW 7g/d and SW 14g/d (56.37% and 58.09%, respectively). The rate of *in vitro* fertilization was observed to be similar ($p>0.05$) between the two treatment groups (67.14% and 71.71%, respectively); however, the rates observed in the control groups (45.43%) were significantly ($p<0.05$) lower compared to the seaweed treatment groups.

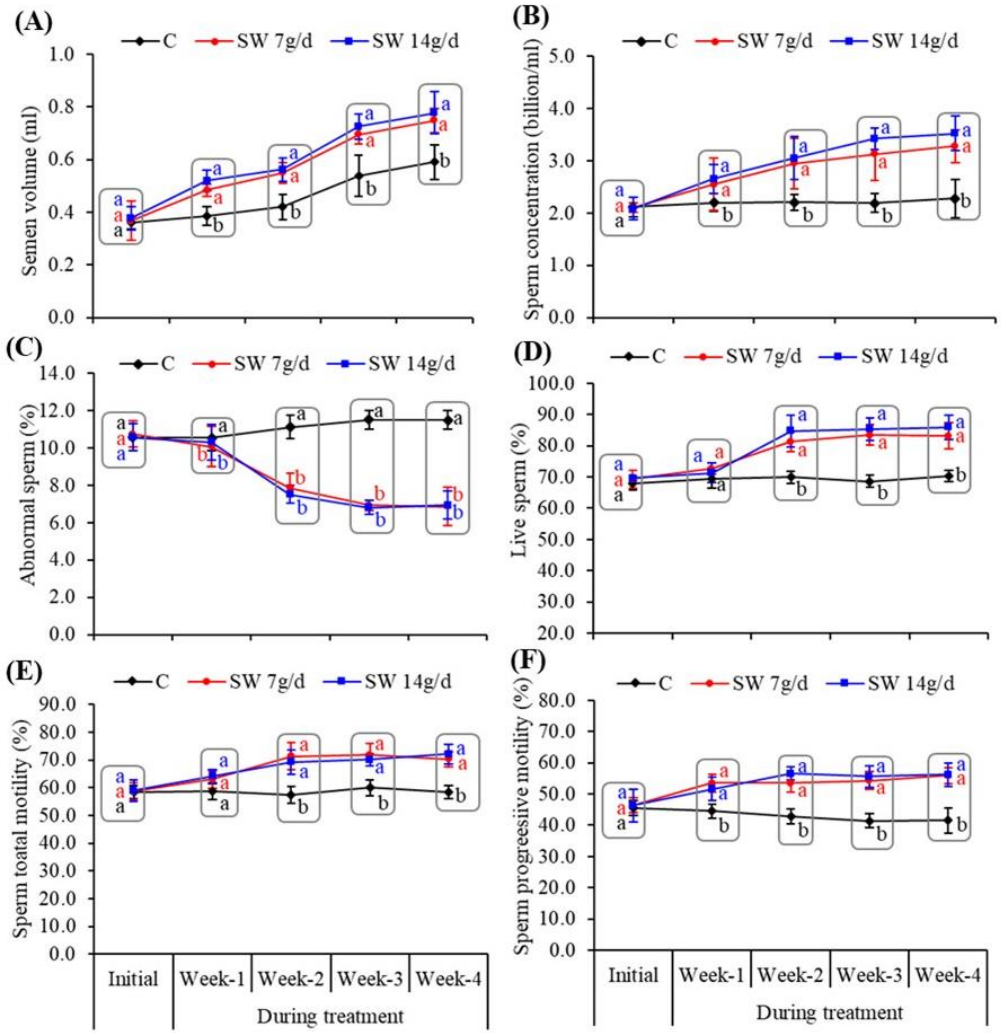

Figure 1. The semen volume and sperm quality parameters of ram treated with or without seaweed. Where (A) indicates Semen volume, (B) Sperm concentration, (C) Percentage of abnormal sperm, (D) Percentage of live sperm, (E) Sperm total motility and (F) Sperm progressive motility. Values are Mean ± SD of five replicates. Different lowercase letters superscripts (a or b) in the same week or duration (rectangular boxes) indicates the significant ($p<0.05$) differences among the treatments. C – Control; SW 7g/d – Seaweed treated group (7g/d/head); SW 14g/d – Seaweed treated group (14g/d/head).

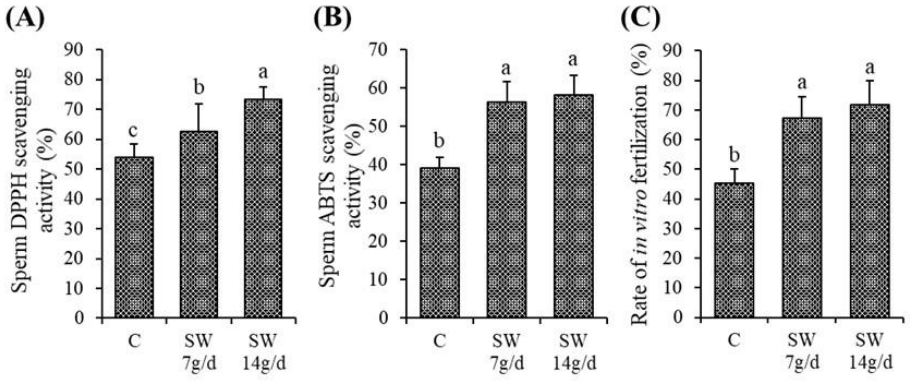

Figure 2. The radical scavenging activity and fertilizing ability of sperm. Where (A) indicates sperm DPPH scavenging activity, (B) ABTS scavenging activity, and (C) Rate of *In vitro* fertilization. Values are Mean ± SD of five replicates. Different lowercase letters on the bar (a or b) indicate the significant ($p<0.05$) difference among the treatments. C – Control; SW 7g/d – Seaweed treated group (7g/d/head); SW 14g/d – Seaweed treated group (14g/d/head).

Effect of seaweed on testicular histomorphology, thickness of tunica albuginea, and biometry of the seminiferous tubules

The testicular histological section of the testis of the rams treated or untreated with seaweed is shown in Figure 3A and the thickness of tunica albuginea in Figure 3B. It was observed that the average thickness of tunica albuginea of the testis of control group rams was (316.60 μm), which was significantly ($p<0.05$) higher than those of seaweed-treated rams (241.25 μm and 207.88 μm, respectively); however, no significant ($p>0.05$) difference was observed between two treatment groups (Figure 1B).

The biometry of seminiferous tubules in terms of length and width and the number of spermatogenic cells per seminiferous tubules are shown in Figure 4. The average length of seminiferous tubules of the testis of the rams without seaweed treatment was 473.20 μm, which was significantly ($p<0.05$) increased to 548.61 μm in SW 7g/d group and 572.72 μm in SW 14g/d; however, no significant ($p>0.05$) differences observed between two treatment groups (Figure 4 A, B). The sectional breadth of the seminiferous tubules in seaweed treated group SW 14g/d (221.40 μm) was significantly ($p<0.05$) increased followed by SW 7g/d group (199.67 μm) compared to those of control (152.36 μm) as shown in Figure 4 A and C. Similarly, the average number of spermatogenic cells per seminiferous tubules was significantly ($p<0.05$) increased in seaweed treated group SW 14g/d (557.43) and SW 7g/d group (545.24) compared to those of control (411.62) (Figure 4D). However, no significant ($p>0.05$) difference was observed between the two treatment groups.

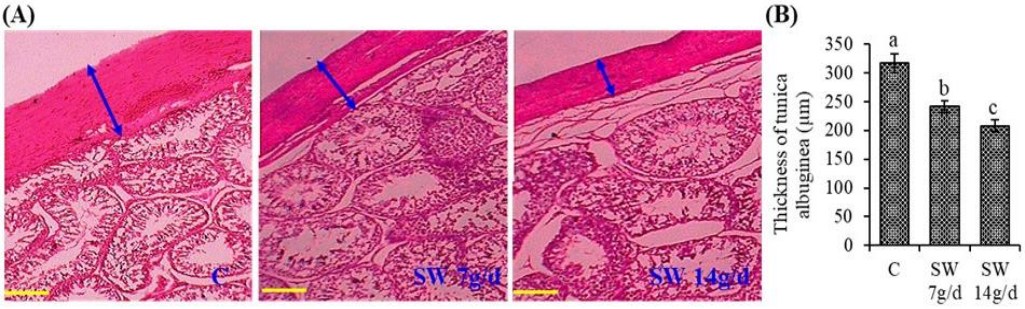

Figure 3. The histological sections and the thickness of tunica albuginea of testis. Where (A) indicates testicular morphology showing the tunica albuginea, (B) the thickness of tunica albuginea. Values are Mean ± SD of five replicates of the average of both right and left testis. Different lowercase letters on the bar (a or b) indicate the significant ($p<0.05$) difference among the treatments. C – Control; SW 7g/d – Seaweed treated group (7g/d/head); SW 14g/d – Seaweed treated group (14g/d/head). Scale bar is 200 μm.

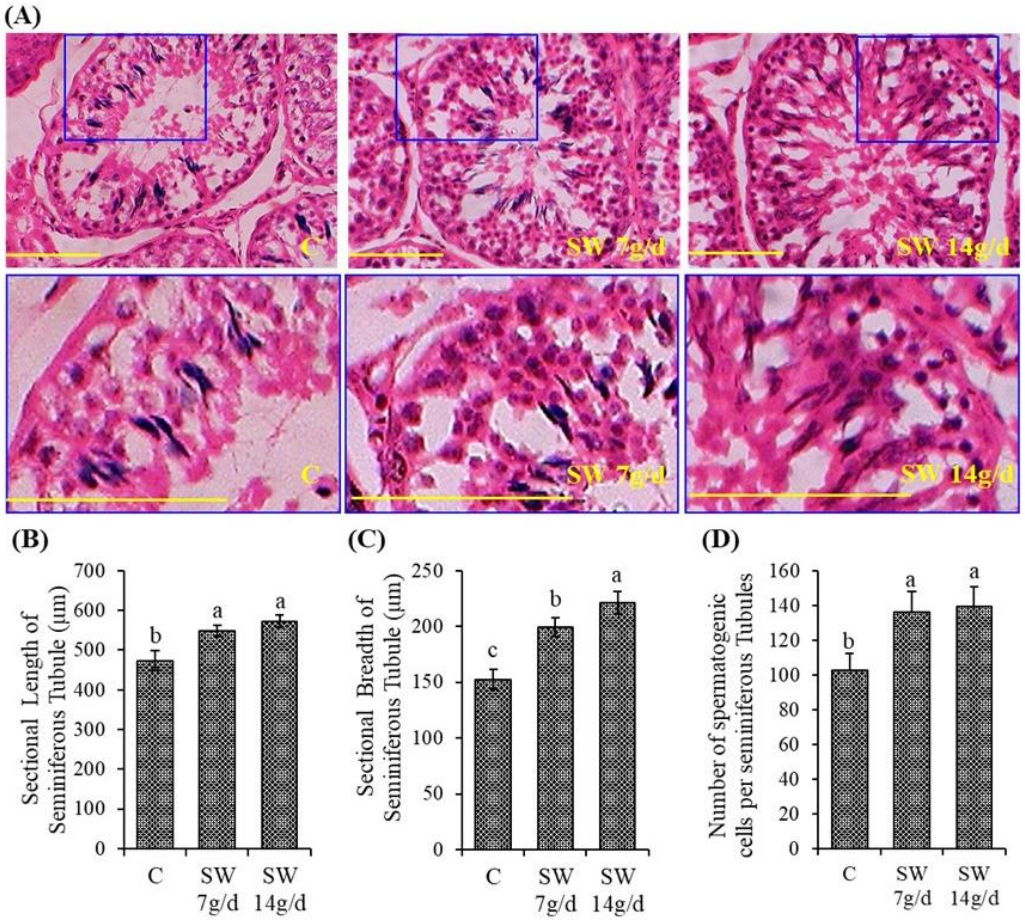

Figure 4. The histological sections show the seminiferous tubules of testis. Where (A) indicates morphology of seminiferous tubules, (B) the length of seminiferous tubules, (C) the breadth of seminiferous tubules and (D) the number of spermatogenic cells in the seminiferous tubules. Values are Mean ± SD of five replicates of the average of both right and left testis. Different lowercase letters on the bar (a or b) indicate the significant ($p<0.05$) difference among the treatments. C – Control; SW 7g/d – Seaweed treated group (7g/d/head); SW 14g/d – Seaweed treated group (14g/d/head). The scale bar is 100 µm.

DISCUSSION

The current study evaluated the effects of dietary seaweed supplementation on various physiological and reproductive parameters in rams including growth, testicular development, semen quality, antioxidant potential, and testicular histology. Initially, the body weight, scrotal circumferences and semen quality of rams were similar ($p>0.05$) among all treated and untreated rams, indicating comparable starting conditions for all groups. However, over the treatment period, seaweed supplemented rams demonstrated a notable increase ($p<0.05$) in final body weight, total and daily weight compared to the control group. Moreover, the scrotal circumference, and testicular weight, length, and breadth, showed a significant ($p<0.05$) improvement in the seaweed-treated group. These findings suggest that seaweed supplementation may enhance both physical growth and testicular development in rams, potentially improving their productive and reproductive capacity. Several studies have reported that seaweeds and their active compounds have various beneficial effects on livestock, which include increasing feed intake, enhanced growth performance, boosting average daily body weight gain [36], and improved digestive function, gut health, and reducing diarrhea in ruminants and pigs [37]. In our previous study, dietary supplementation of

G. parvispora in diets significantly improved CP digestibility, average daily gain, and immunity, while reducing the pathogenic microbial load in lambs [38].

Moreover, seaweed has been found to possess significant antioxidants, which can directly affect gut mucous membranes, gut-associated lymph nodes, and lymphoid tissues, thereby enhancing gut integrity and stimulating immune function within the digestive tract [39]. Furthermore, seaweed has been shown to influence cytokine output, exerting an immunomodulatory effect on the digestive system [40]. Other research has indicated that diets containing seaweed extracts can downregulate the expression of pro-inflammatory chemokines and cytokines in intestinal and hepatic cells [41]. This down-regulation of pro-inflammatory cytokines may allow animals to better absorb nutrients and consequently experience improved growth rates, in addition to strengthening the immune system [42]. The enhanced growth observed in our study may be attributed to these intracellular events facilitated by seaweed supplementation in the diet. Moreover, dietary supplementation of seaweed has been shown to enhance testicular development, semen quality, hyaluronidase enzyme activity, and blood testosterone levels in experimental rats [43]. Similarly, seaweed antioxidants can neutralize the excessive reactive oxygen species (ROS) and improve sperm motility without affecting normal sperm morphology [44]. In our study, analysis of semen quality parameters indicates that dietary seaweed supplementation positively influenced semen volume, sperm concentration, viability, and motility, while reducing abnormal sperm counts. Seaweeds are rich sources of nutrients and bioactive compounds [22], which may support spermatogenesis, leading to increased sperm concentration, viability, and motility. Consequently, rams receiving seaweed supplementation exhibited higher sperm quality compared to the control groups in this study. Moreover, several studies have demonstrated the positive effects of seaweed in reducing oxygen-free radicals and oxidative stress intensity, as well as improving sperm motility, in humans [45], rabbits [46], goats [47], bulls [48], and pigs [49].

In our study, sperm obtained from seaweed-treated rams demonstrated enhanced DPPH and ABTS radical scavenging abilities. Moreover, the rate of *in vitro* fertilization was noticeably higher in seaweed-treated rams compared to the control group. These findings suggest that seaweed supplementation not only improves semen quality but also has a beneficial impact on sperm antioxidant capacity and fertilization potential. The motility of sperm is the primary factor enabling spermatozoa movement within the female reproductive tract to the site of fertilization. Seaweeds are rich sources of valuable nutrients, particularly chelated micro-minerals, and their inclusion in the diet has shown a positive impact on sperm motility and fertility in ruminants [26,50]. In another study, dietary supplementation of red seaweed resulted in an increased level of testosterone and sperm motility while decreasing DPPH and MDA levels in rats [51]. The improved sperm quality in terms of motility, viability, and *in vitro* fertilization rate in our study may be attributed to the micronutrient and antioxidant content of seaweed, which protects spermatogenic cells from oxidative damage.

However, the testicular parenchyma comprises seminiferous tubules containing spermatogenic cells responsible for spermatozoa production [52], alongside sertoli cells that are responsible for spermatocyte maturation and germ cell differentiation [53]. The intracellular antioxidants are effective against the harmful impact of free radicals that occurred during the metabolic process required for spermatogenesis, thereby improving testis functions [21]. The histological examination of testicular tissue in our study revealed a thinner tunica albuginea with expanded seminiferous tubules containing a higher number of spermatogenic cells in seaweed-treated ram testis compared to the control group. These findings suggest that seaweed supplementation

exerts positive impacts on testicular architecture and spermatogenic cell proliferation. Several studies have also reported that dietary seaweed supplementation improves spermatogenesis and enhances testicular architecture while protecting seminiferous tubule morphology in rats [54] and mice [55]. Moreover, various antioxidants from seaweed have proven helpful in treating male infertility [56].

Nonetheless, seaweed meal and extracts have been supplemented in ruminant diets for decades to enhance the immunity and antioxidative status of cattle, sheep, and goats [26]. In lambs and kids, seaweed meal has also been found to boost immunity, enhance overall health, and protect against oxidative stress [57]. In recent years, the popularity of seaweed has surged in both Western and developing nations due to its abundant nutritional benefits and pharmacological properties [25,58]. Therefore, *G. parvispora* could be used for enhancing male fertility not only in sheep but also in other livestock species. However, further research is recommended to understand the specific mechanisms driving these effects and to refine seaweed supplementation protocols for practical implementation in animal husbandry.

CONCLUSIONS

In this study, dietary supplementation with seaweed *G. parvispora* exerted a beneficial impact on various aspects of growth and reproductive physiology of ram. Moreover, it improved testicular morphology and spermatogenesis in rams, thereby enhancing the antioxidant activity and sperm quality, leading to improve *in vitro* fertilization capacity. These findings suggest that seaweed could serve as a valuable and effective dietary supplement for enhancing male reproductive performance, thus potentially improving breeding programs within the livestock industry.

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AUTHOR CONTRIBUTIONS

SAMH and ASMS were involved in conception and design of the experiments. SAMH, MMI, and MRI contributed to perform the experiments. SAMH and IJM analyzed data. SAMH and ASMS contributed to drafting the article. MMR contributed to revising it critically for important intellectual content. SAMH, ASMS, and MMR made the final approval of the version to be published.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Ben Moula A, Moussafir Z, et al. Heat stress and ram semen production and preservation: Exploring impacts and effective strategies. Journal of Thermal Biology. 2024;119:103794.
[2] DLS. Livestock Economy at a Glance: Livestock population of Bangladesh. 2022.

[3] Hoque SM, Islam MM, et al. Estimation of total methane emission from enteric fermentation of ruminant livestock in Bangladesh. Asian Journal of Medical and Biological Research. 2017;3:245–53.
[4] BSS. Sheep farming becomes blessing for ethnic minority female students | Special Stories 2022.
[5] Khanal P, Dhakal R, et al. Sustainable Livestock Production in Nepal: A Focus on Animal Nutrition Strategies. Agriculture 2022;12.
[6] Morris ST. Overview of sheep production systems. Advances in Sheep Welfare, Elsevier; 2017, 19–35.
[7] Islam SS, Hasan MS, et al. Prospects and problems of indigenous sheep production in south-western coastal regions of Bangladesh. Journal of Agricultural Sciences - Sri Lanka. 2021;16:54–66.
[8] Rakib MRH, Desha NH, et al. Environmental adaptability, morphometric features with reproductive and productive potentialities of indigenous sheep in Bangladesh. Journal of Advanced Veterinary and Animal Research. 2022;9:649–62.
[9] Haile A, Mirkena T, et al. Community based sheep breeding programs: Tapping into indigenous knowledge. Livestock Research for Rural Development. 2013;25:219.
[10] Gutu Z, Haile A, Rischkowsky BA, Mulema AA, Kinati W, Tesfahun G. Evaluation of community-based sheep breeding programs in Ethiopia 2015.
[11] Alvarez M, Anel-Lopez L, et al. Current challenges in sheep artificial insemination: A particular insight. Reproduction in Domestic Animals. 2019;54:32–40.
[12] Islam M, Apu A, et al. Comparative study on the libido, semen quality and fertility of Brahman cross, Holstein Friesian cross and Red Chittagong breeding bulls. Bangladesh Journal of Animal Science. 2018;47:61–67.
[13] Alvarez M, Anel-Lopez L, et al. Current challenges in sheep artificial insemination: A particular insight. Reproduction in Domestic Animals. 2019;54:32–40.
[14] Miguel-Cruz EE, Mejía-Villanueva O, et al. Induction of fertile estrus without the use of steroid hormones in seasonally anestrous Suffolk ewes. Asian-Australasian Journal of Animal Science. 2019;32:1673–85.
[15] Sathe SR. Laparoscopic artificial insemination technique in small ruminants-A procedure review. Frontiers in Veterinary Science. 2018;5:1–9.
[16] Sharma A, Minhas S, et al. Male infertility due to testicular disorders. Journal of Clinical Endocrinology and Metabolism. 2021;106:e442–59.
[17] Mendoza N, Casao A, et al. Influence of Non-conventional Sperm Quality Parameters on Field Fertility in Ovine. Frontiers in Veterinary Science. 2021;8:1–10.
[18] Guan Y, Malecki IA, et al. Under-nutrition reduces spermatogenic efficiency and sperm velocity, and increases sperm DNA damage in sexually mature male sheep. Animal Reproduction Science. 2014;149:163–172.
[19] Zhang W, Min L, et al. Beneficial Effect of Proline Supplementation on Goat Spermatozoa Quality during Cryopreservation. Animals. 2022;12.
[20] Rey-Crespo F, López-Alonso M, et al. The use of seaweed from the Galician coast as a mineral supplement in organic dairy cattle. Animals. 2014;8:580–6.
[21] Vizzari F, Massányi M, et al. Effects of dietary plant polyphenols and seaweed extract mixture on male-rabbit semen: Quality traits and antioxidant markers. Saudi Journal of Biological Sciences. 2021;28:1017–25.
[22] Ghosh S, Sarkar T, et al. Novel Bioactive Compounds From Marine Sources as a Tool for Functional Food Development. Frontiers in Marine Science. 2022;9.
[23] Mišurcová L. Chemical composition of seaweeds. In: Kim S-K, (editor). Handbook of Marine Macroalgae. Biotechnology and Applied Phycology. 2011; 171–192.
[24] Usov AI. Polysaccharides of the red algae. Advances in Carbohydrate Chemistry and Biochemistry. 2011;65:115–217.
[25] Morais T, Inácio A, et al. Seaweed potential in the animal feed: A review. Journal of Marine Science and Engineering. 2020;8:1–24.
[26] Evans FD, Critchley AT. Seaweeds for animal production use. Journal of Applied Phycology. 2014;26:891–9.
[27] Wang PT, Sudirman S, et al. Oral supplementation of fucoxanthin-rich brown algae extract ameliorates cisplatin-induced testicular damage in hamsters. Biomedicine and Pharmacotherapy. 2020;125:109992.
[28] Matshogo T Ben, Mnisi CM, et al. Effect of Pre-Treating Dietary Green Seaweed with Proteolytic and Fibrolytic Enzymes on Physiological and Meat Quality Parameters of Broiler Chickens. Foods. 2021;10.
[29] Blanz M, Mainland I, et al. Identifying seaweed consumption by sheep using isotope analysis of their bones and teeth: Modern reference δ13C and δ15N values and their archaeological implications. Journal of Archaeological Science. 2020;118.
[30] Selim A, Islam M, et al. Nutritional Value of Seaweed Species for Ruminants: Impact of Dietary Supplementation of Gracilaria Parvispora on Digestibility, Growth Performance, Blood Parameters, and Immune Response of Lambs. SSRN Electron J 2023.
[31] Kabiraj SK, Hoque SAM, et al. Testicular biometry and its relationship with body weight and semen output of black Bengal bucks in Bangladesh. Journal of Cell and Animal Biology. 2011;5:27–32.
[32] Khan MKI, Hossain MI, et al. Impact of protein supplementation on semen quality, fertility, and BMP1R gene expression in sheep of Bangladesh. Translational Animal Science. 2022;6:1–8.
[33] El-Seadawy IE, Kotp MS, et al. The impact of varying doses of moringa leaf methanolic extract supplementation in the cryopreservation media on sperm quality, oxidants, and antioxidant capacity of frozen-thawed ram sperm. Tropical Animal Health Production. 2022;54:344.
[34] Karmaker S, Apu A, et al. Effect of supplementation of BSA on in vitro maturation and fertilization of Black Bengal goat oocytes. Fundamental and Applied Agriculture. 2020;5:1.
[35] Karimi H, Ranjbar SM, et al. Influence of laterality on testis anatomy and histology in Ghezel rams. Veterinary Medicine and Science. 2019;5:151–6.
[36] Nhlane LT, Mnisi CM, et al. Nutrient digestibility, growth performance, and blood indices of Boschveld chickens fed seaweed-containing diets. Animals. 2020;10:1296.

[37] Ruiz ÁR, Gadicke P, et al. Supplementing nursery pig feed with seaweed extracts increases final body weight of pigs. Austral Journal of Veterinary Sciences. 2018;50:83–7.
[38] Selim ASM, Islam R, et al. Nutritional Value of Seaweed Species for Ruminants: Impact of Dietary Supplementation of Gracilaria Parvispora on Digestibility, Growth Performance, Blood Parameters, and Immune Response of Lambs. SSRN Electron J 2023.
[39] Rajauria G, Ravindran R, et al. Molecular characteristics and antioxidant activity of laminarin extracted from the seaweed species Laminaria hyperborea, using hydrothermal-assisted extraction and a multi-step purification procedure. Food Hydrocolloids. 2021;112:106332.
[40] Vazirzadeh A, Marhamati A, et al. Immunomodulation, antioxidant enhancement and immune genes up-regulation in rainbow trout (Oncorhynchus mykiss) fed on seaweeds included diets. Fish and Shell fish Immunology. 2020;106:852–8.
[41] Sweeney T, Meredith H, et al. Extracts of laminarin and laminarin/fucoidan from the marine macroalgal species Laminaria digitata improved growth rate and intestinal structure in young chicks, but does not influence Campylobacter jejuni colonisation. Animal Feed Science and Technology. 2017;232:71–9.
[42] Walsh AM, Sweeney T, et al. Effect of dietary laminarin and fucoidan on selected microbiota, intestinal morphology and immune status of the newly weaned pig. British Journal of Nutrition. 2013;110:1630–8.
[43] Ghareeb DA, Abd-Elgwad A, et al. Ulva lactuca methanolic extract improves oxidative stress-related male infertility induced in experimental animals. Archives of Physiology and Biochemistry. 2021;127:397–405.
[44] Sobhani A, Eftekhaari TE, et al. Antioxidant effects of brown algae sargassum on sperm parameters:CONSORT-compliant article. Medicine (Baltimore) 2015;94:1–6.
[45] Aleksandrov A, Mollova D, et al. Influence of bioactive substances from Ascophyllum nodosum, Lycium barbarum and Tribulus terrestris on the quality characteristics of human spermatozoa. Journal of Bioscience and Biotechnology. 2020;9:11–6.
[46] Okab AB, Samara EM, et al. Effects of dietary seaweed (Ulva lactuca) supplementation on the reproductive performance of buck and doe rabbits. Journal of Applied Animal Research. 2013;41:347–55.
[47] Ren F, Fang Q, et al. Lycium barbarum and Laminaria japonica polysaccharides improve Cashmere goat sperm quality and fertility rate after cryopreservation. Theriogenology. 2019;129:29–36.
[48] Saberivand A, Sarvarzadeh F, et al. The effect of Caulerpa sertularioides extract on bull sperm freezablity and subsequent embryo development. Theriogenology. 2022;189:167–76.
[49] Han H, Zhou Y, et al. Alginate oligosaccharides increase boar semen quality by affecting gut microbiota and metabolites in blood and sperm. Frontiers in Microbiology. 2022;13:982152.
[50] Yates DT, Salisbury MW, et al. Effects of Tasco-14 supplementation on growth and fertility traits in young male Boer goats experiencing heat stress. Texas Journal of Agriculture and Natural Resources. 2010;23:12–8.
[51] Ibrahim NM, Ibrahim SR, et al. The effect of Red Seaweed (Chondrus crispus) on the fertility of male albino rats. Saudi Journal of Biological Sciences. 2021;28:3864–3869.
[52] Ilacqua A, Francomano D, et al. The physiology of the testis. In: Belfiore A, LeRoith D, editors. Principles of Endocrinology and Hormone Action, Endocrinology. Switzerland. Springer International Publication. AG, 2016;1–38.
[53] Wistuba J, Neuhaus N, et al. Physiology of Testicular Function. Androl. Male Reprod. Heal. Dysfunct. Springer; 2023;15–54.
[54] Kong ZL, Sudirman S, et al. Fucoxanthin-rich brown algae extract improves male reproductive function on streptozotocin- nicotinamide-induced diabetic rat model. International Journal of Molecular Sciences. 2019;20.
[55] Khairuddin K, Sudirman S, et al. Caulerpa lentillifera polysaccharides-rich extract reduces oxidative stress and proinflammatory cytokines levels associated with male reproductive functions in diabetic mice. Applied Sciences. 2020;10:1–14.
[56] Sinclair S. Male infertility: nutritional and environmental considerations. Alternative Medicine Review: a Journal of Clinical Therapeutic. 2000;5:28–38.
[57] Kannan G, Saker KE, et al. Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. Small Ruminant Research. 2007;73:221–7.
[58] Ruocco N, Costantini S, et al. Polysaccharides from the marine environment with pharmacological, cosmeceutical and nutraceutical potential. Molecules. 2016;21:551.