http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2025.169.177

Research Article
Microalgae *Spirulina platensis* Fortification Enhance Mice Spermatozoa Quality in Combination with *Anadara granosa* Blood Shell Powder

[1]Eddyman Waliman Ferial, [1]Andi Ilham Latunra, [1]Muhtadin Asnady Salam, [1]Asadi Abdullah, [2]A. Muh Anshar, [3]Fika Yuliza Nur, [4]Fatmawati Nur, [5]Saiful Anuar Bin Karsani and [1]Mustika Tuwo

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[3]Veterinary Study Program, Faculty of Veterinary Medicine, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia
[4]Biology Study Program, Faculty of Science and Technology, Alauddin Islamic State University, Gowa 92113, South Sulawesi, Indonesia
[5]Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia

Abstract

Background and Objective: Infertility, a condition in reproductive health, refers to the inability to achieve conception after a year of regular unprotected intercourse. This study builds on previous research involving a patented food supplement containing *Anadara granosa* L. bloodshell powder. The goal is to enhance the quality of blood shell capsules from AnadaraMAN by fortifying them with *Spirulina platensis*, a highly nutritious microalga. **Materials and Methods:** Thirty male mice, weighing 20-30 g and aged 8-11 weeks, were divided into 6 treatment groups using the CRD method. The fortification process involved dissolving *Anadara granosa* L. and *Spirulina platensis* in a 0.5% Na-CMC solvent according to specific doses, administered orally to the mice twice daily for 21 days. Spermatozoa samples were collected from the cauda epididymis of euthanized mice. Sperm morphology was observed using 1% eosin dye under a 400x microscope, while motility was assessed in a 0.9% physiological NaCl suspension. Quantitative data was then analyzed using the Analysis of Variance (ANOVA) test for normally distributed data and continued with the Least Significant Difference (LSD) test. **Results:** Findings revealed a safe daily dose for mice is 4.16 mg/20 g of *Anadara granosa* L. blood shell and 2.6 mg/20 g of *Spirulina platensis* microalgae. Fortifying significantly increased viscosity in treatment groups Q (50% *Anadara granosa* L. with 50% *Spirulina platensis*) and T (100% *Anadara granosa* L.), as shown by a Kruskal Wallis test with a p-value of 0.002 which is less than the threshold of 0.05. Furthermore, mice spermatozoa displayed normal coloration during examination, suggesting no adverse effects from the supplementation. **Conclusion:** The study suggests the potential benefits of combining *Anadara granosa* L. and *Spirulina platensis* to enhance reproductive health, highlighting the need for further research.

Key words: Male fertility, microalgae, mineral-rich shellfish, reproductive health, normal coloration

Citation: Ferial, E.W., A.I. Latunra, M.A. Salam, A. Abdullah and A.M. Anshar *et al.*, 2025. Microalgae *Spirulina platensis* fortification enhance mice spermatozoa quality in combination with *Anadara granosa* blood shell powder. Pak. J. Biol. Sci., 28: 169-177.

Corresponding Author: Mustika Tuwo, Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University, Makassar 90245, South Sulawesi, Indonesia Tel: +62 852-9985-2835

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mice are creatures known for their fertility, characterized by relatively short life cycles, high birth rates, varied traits, ease of handling and well-characterized anatomical and physiological features[1]. They typically live between 1-3 years, though this can vary among strains, primarily based on environmental sensitivity and diseases. Mice exhibit exceptionally high fertility rates, capable of producing approximately one million offspring in about a year. Their sexual productivity spans 7-8 months, with an average litter size ranging from 6-10 offspring per birth. Various methods are being developed to enhance reproductive organ fertility, particularly in males, focusing on internal factors such as improving sperm quality and hormonal regulation, primarily applicable to humans. Adequate intake of both macro and micronutrients is crucial for maintaining the functions of reproductive cells, tissues and organs[2]. Infertility can arise due to insufficient nutritional intake that supports the male reproductive organs fertility, leading to abnormalities in semen volume, sperm quality and quantity[3-5]. Macro-nutrients (carbohydrates, proteins, and fats) along with micro-nutrients (vitamins and minerals) play pivotal roles in determining sperm quality. Parameters used to determine and evaluate sperm quality and quantity include volume, cell count, morphology, motility, aroma, viscosity and color of spermatozoa[6,7].

Shellfish, notably abundant in the waters of Eastern Indonesia, serve as a rich nutritional resource. Their delectable flavor contributes to their popularity among seafood enthusiasts. Beyond their taste, shellfish offer substantial nutritional value and vitamins[8,9]. Shells, both in meat and shell, contain zinc (Zn), a micromineral known for its capacity to elevate testosterone levels in humans and other mammals[10,11]. *Anadara granosa* L., a type of shellfish known as blood shell, is notable for its potential to improve male reproductive health due to its rich mineral composition, including iron (Fe), phosphorus (P), fluorine (F), iodine (I), calcium (Ca), potassium (K), zinc (Zn) and selenium (Se). Moreover, shellfish are an excellent source of high-quality animal protein, classified as complete protein, with 85-95% essential amino acids that are easily absorbed. They also contain fat-soluble vitamins and B-complex nutrients, further enhancing their nutritional value[12,13].

Spirulina platensis is a microalgae that has been widely used as food, feed and medicine. *Spirulina platensis* is a food ingredient that is very rich in vitamin B12[14,15]. *Spirulina platensis* also contains several vital elements such as zinc (Zn), magnesium (Mg), manganese (Mn), calcium (Ca), selenium (Se) and also vitamins such as vitamin C and vitamin E[16]. The vitamins and minerals contained in the microalga *Spirulina platensis* can increase and improve sperm quality[17]. In the dry state, it contains 55-75% protein consisting of amino acids such as methionine, cysteine and lysine, compared to protein from eggs and milk[18,19]. According to WHO, food fortification is the addition of specific nutrients to food to improve the quality of the food, which can be beneficial for health. In the food fortification process, what is usually added are micronutrients, for example, vitamins and minerals such as calcium, sodium, iodine and iron, as well as vitamin A and vitamin D. Adding micronutrients to a food can make the product have added value in terms of nutrition. Therefore, by fortifying *Anadara granosa* L. blood shell powder with *Spirulina platensis* microalgae, it is hoped that it can improve the spermatozoa quality of mice *Mus musculus*[20]. This study aimed to improve the quality of mice spermatozoa by administering blood shell capsules from AnadaraMAN enriched with *Spirulina platensis*, a nutrient-rich microalgae. This study evaluated the safety and effectiveness of the combination of sperm characteristics, including morphology and motility using a rat model.

MATERIALS AND METHODS

Study area: This research was conducted from January to June, 2023. Sample fortification was carried out at the Zoology and Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences and Biopharmaceutical Laboratory, Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Indonesia. Thirty male mice weighing 20-30 g and aged 8-11 weeks were placed and treated in the Animal Cage, Faculty of Pharmacy, Hasanuddin University, Indonesia. Spermatozoa observations were conducted at the Veterinary Education Clinic, Department of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Extraction process of *Anadara granosa* L.: Samples of 1 kg blood shells are washed clean and then boiled until the shells open. Separate the contents of the clam from the shell, then dry in the oven (Memmert Universal Oven (Model UF, German) at a temperature of 50-60°C for 18-20 hrs. The dried shells are ground with a blender (Philips Blender HR2094, China) to form blood shellfish powder.

***Spirulina* extraction process:** A total of 1 g of *Spirulina platensis* is added with $CaCO_3$ as a neutralizing agent and sodium ascorbate as a neutralizing antioxidant (tip of a small spatula), then ground until smooth. The samples were

extracted with 10 mL of polar organic solvents, namely acetone, acetonitrile, Dimethyl Sulfoxide (DMSO), ethanol and methanol, for 20 min (2 extraction times) at a speed of 250 rpm (MR Hei-Standard, Heidolph)[21]. The extract obtained was then partitioned with diethyl ether and ethyl acetate (1:1; v/v) and added to a saturated salt solution and tap water until separation occurred. The top layer containing the extract was collected and concentrated using a rotary evaporator (Laborta 4010 digital, Heidolph) and dried with argon gas (UHP).

Fortification of *Anadara granosa* L. powder with *Spirulina platensis* microalgae: The mixing process is a process required in making medicinal preparations. Mixing is necessary to produce the distribution of two or more materials which will later undergo a joint metabolic process in the body. The doses that have been determined from the two natural ingredients for mice with a body weight between 20-30 g will then be diluted respectively using CMC-Na solvent to form a suspension. Furthermore, the dosage for the treatment that will be given to mice is taken from the second dose of natural ingredients which have been converted from human doses to mice. The process of combining the two natural ingredients was carried out using a 1 mL syringe by aspirating the *Anadara granosa* L. blood shell suspension solution according to the treatment requirements, then using the same syringe, the *Spirulina platensis* suspension solution was aspirated according to the treatment requirements. Mixing is carried out to produce the distribution of two or more materials which will later undergo metabolic processes simultaneously in the body.

Preparation of test animals: In this study, 30 male mice were used with a body weight of 20-30 g and an age of around 8-11 weeks. Mice were divided into 6 treatment groups with different cages for each group and each group contained 5 mice. Before the research began, mice were acclimatized for 7 days in laboratory conditions. Every day the mice were given standard food in AD-2 and drinking water. Acclimatization is carried out so that the mice can adapt to the environmental conditions they will occupy during the research.

Treatments: The treatment used in this research was a Completely Randomized Design (CRD) with 6 treatments with 5 replications. This treatment will be given twice a day, namely in the morning and evening for 21 days, carried out sonde (orally). The treatment is as follows:

K = Control
P = Fortification 70% *A. granosa* L.: 30% *S. platensis*
Q = Fortification 50% *A. granosa* L.: 50% *S. platensis*
R = Fortification 30% *A. granosa* L.: 70% *S. platensis*
S = *Anadara granosa* L. 100%
T = *Streptomyces platensis* 100%

The CRD method begins with collecting 30 male mice in the same container according to the required criteria. Next, 6 cages for mice were provided, marked 1 to 6. After that, the mice that were still in the container were then taken randomly or (randomly) moved into cages that had been numbered 1 to 6 until each cage contained 5 mice tails. Determination of treatment (K, P, Q, R, S and T) in each cages containing 5 mice was carried out using the lot method or drawing. Repetition determinations were carried out 5 times, marked by giving different colors to the body parts of each individual in one cage and the same thing was done in every other cage.

Mice spermatozoa extraction: Removal of mice spermatozoa is carried out surgically. Before surgery, anarchosis was performed on the mice with chloroform, the abdomen was dissected and both testicles were taken, then spermatozoa were obtained from the cauda epididymis. The cauda epididymis is isolated by cutting the proximal end of the corpus epididymis and the distal end of the vas deferens. It is then placed in a watch glass containing 1 mL of 0.9% NaCl solution. A small incision is made at the proximal end of the cauda using scissors and gentle pressure is applied to release the epididymal fluid, which is subsequently suspended in the 0.9% NaCl solution.

Spermatozoa viscosity analysis: The analysis of the viscosity of mice spermatozoa is carried out subjectively by touching the surface of the sperm with a pipette or stirring rod and then pulling it will form a thread. Viscosity analysis is also carried out by looking at the concentration (density) of spermatozoa under a microscope. The consistency of semen depends on the concentration of spermatozoa and seminal plasma, semen that has a thick consistency contains more spermatozoa than semen that has a thin consistency[22].

Spermatozoa color analysis: Sperm that has been collected in a watch glass is observed using a white background using sufficient lighting. Check the color of the sperm and check for turbidity. Normal sperm is usually cloudy white like starch, sometimes slightly grayish. The normal color of spermatozoids is like glue or starch or grayish white. If the abstinence is

long, it will have a yellowish color. If the spermatozoids are clear/translucent, it is usually interpreted as watery semen. If red blood cells are found, the sperm will be brownish due to hemoglobin[23].

Statistical analysis: Observation data consists of qualitative and quantitative data. Quantitative data were then analyzed using the Analysis of Variance (ANOVA) test for normally distributed data, with results considered significant at a $p<0.05$. If significant differences were found, the analysis was followed by the Least Significant Difference (LSD) test to determine pairwise differences. A non-parametric test was conducted using Kruskal-Walli's test, with significance determined at $p<0.05$ for data that were not normally distributed. If significant differences were detected, further pairwise comparisons were carried out using the Mann-Whitney test to identify specific groups with significant differences[24,25].

RESULTS AND DISCUSSION

Spermatozoa viscosity: The results of observations of the viscosity of mice spermatozoa were carried out after treatment for 21 days on 30 male mice test animals by performing surgery where the sperm was taken through the epididymis.

Figure 1 shows that the average value of sperm viscosity or thickness for mice in the K treatment group (control) is 3.2. Treatment group P was 3.75. Treatment group Q was 4.8. Treatment group R was 3.4. Treatment group S was 4.8 and for treatment group T was 3.4. After obtaining data on sperm viscosity in mice, the data was then processed statistically using a normality test which aims to determine whether the data is normally distributed or not. The data obtained from the averaging results were then tested for normality using the Shapiro-Wilk test and obtained a p-value = 0.000 so it could be concluded that the normality test on viscosity was not evenly distributed (normal). The viscosity data was then analyzed using the Kruskal Wallis test because the data obtained was not evenly distributed. Data from the Kruskal Wallis test results can be seen in Table1.

Table 1 shows the results of the sperm viscosity analysis of mice in the treatment group which had the highest mean, namely groups Q and S, each of which was 4.80. Based on the results of non-parametric tests using the Kruskal Wallis test, a value of $p = 0.002$ was obtained, so it can be concluded that there is a significant influence because the significant level value obtained was less than 0.05 ($p<0.05$). Next, further tests were carried out using the Mann Whitney *post-hoc* test to see whether there was a significant effect in each treatment group which can be seen in Table 2. Table 2 shows that the K treatment group has a significant difference from the Q and S groups with a p-value of 0.007 or $p<0.05$, respectively. Group P has a significant difference from groups Q and S with a p-value of 0.014 or $p<0.05$, respectively. The Q treatment group had a significant difference from the R and T groups with a p-value of 0.011 or $p<0.05$, respectively. The R treatment group had a significant difference from the T group with a p-value of 0.01 or $p<0.05$ and the S treatment group had a significant difference from the T group with a p-value of 0.01 or $p<0.05$. Overall, comparisons between groups with a $p<0.05$ show that there is a significant or real effect. Collecting spermatozoa from mice is done by dissecting

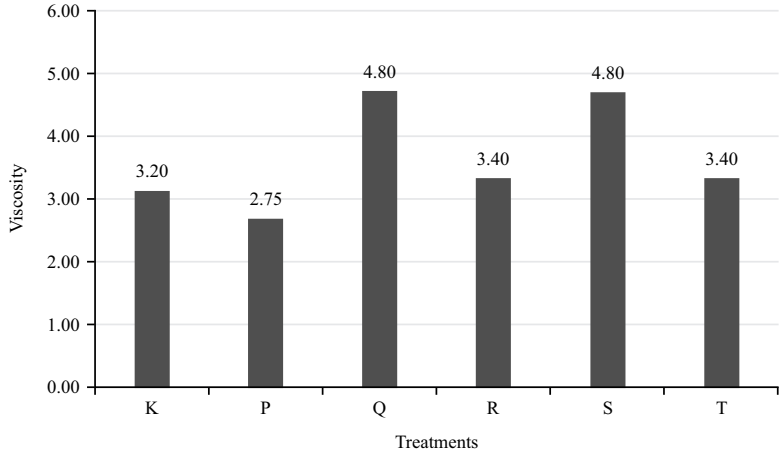

Fig. 1: Histogram of observations of the average viscosity of mice

K: Control, P: Fortification 70% *A. granosa* L.: 30% *S. platensis,* Q: Fortification 50% *A. granosa* L.: 50% *S. platensis,* R: Fortification 30% *A. granosa* L.: 70% *S. platensis,* S: *A. granosa L.* 100% and T: *S. platensis* 100%

Table 1: Results of sperm viscosity analysis

Groups		Viscosity
K	Mean	3.20
	SD	0.45
P	Mean	3.60
	SD	0.55
Q	Mean	4.80
	SD	0.45
R	Mean	3.40
	SD	0.55
S	Mean	4.80
	SD	0.45
T	Mean	3.40
	SD	0.55
p-value		0.002

Table 2: Results of sperm analysis

Comparison between groups		Viscosity mean difference (I-J)	p-value
K	P	-0.40	0.221
	Q	-1.60	0.007
	R	-0.20	0.513
	S	-1.60	0.007
	T	-0.20	0.513
P	Q	-1.20	0.014
	R	0.20	0.549
	S	-1.20	0.014
	T	0.20	0.549
Q	R	1.40	0.011
	S	0.00	1.000
	T	1.40	0.011
R	S	-1.40	0.011
	T	0.00	1.000
S	T	1.40	0.011

the stomach and taking both testicles, then spermatozoa are obtained from the cauda epididymis. Sperm produced from the cauda epididymis of mice is not considered seminal plasma because it does not go through accessory glands that produce seminal plasma and does not go through the ejaculation process in the penis[26]. Semen is the male's genital secretions which are normally ejaculated into the female's genital tract during copulation. Semen consists of spermatozoa and plasma, spermatozoa are male sex cells produced by the testes while semen plasma is a mixture of secretions produced by the vesicular and prostate glands[27,28]. The reproductive system of male mice consists of the testes stored in the scrotum, epididymis and vasa deferentia and the former embryonic excretory system which functions as a spermatozoa duct, accessory glands, urethra and penis[29]. The function of the male sexual accessory glands is to secrete seminal plasma which functions as a medium for spermatozoa during their journey from the reproductive tract of male animals to the reproductive tract of female animals[30]. The seminal plasma produced from this gland contains, among other things, cations, buffers, bicarbonate ions, free amino acids, fatty acids, vitamins, enzymes and carbohydrate complexes[31]. The accessory glands of the male reproductive organs do not contain and carry germ cells, but assist in the function they are supposed to perform, namely as a means of transportation[32]. The accessory glands consist of the seminal vesicles, three pairs of prostate glands (coagulant glands), ampullary glands, bulbourethral glands and prepuce glands[33]. Determination of the viscosity of mice spermatozoa is determined by looking at the number of spermatozoa contained therein. The number of spermatozoa is seen from under a microscope so that the level of consistency or viscosity contained in mice sperm can be determined[34]. According to Iskandar *et al.*[35] semen depends on the concentration of spermatozoa and seminal plasma, semen that has a thick consistency contains more spermatozoa than semen that has a thin consistency. Giving treatment to mice for 21 days had an effect on the viscosity of mice sperm. The average value of sperm viscosity or viscosity of mice in the K treatment group (control) was 3.2. Treatment group P was 3.75. Treatment group Q was 4.8. Treatment group R was 3.4. Treatment group S was 4.8 and for treatment group T was 3.4. The data obtained from the averaging results were then tested for normality using the Shapiro-Wilk test and obtained a $p = 0.000$, so it could be concluded that the normality test on viscosity was not evenly distributed (normal). The viscosity data was then analyzed using the Kruskal Wallis test because the data obtained was not evenly distributed. Based on the results of non-parametric tests using the Kruskal Wallis test, a value of $p = 0.002$ was obtained, so it can be concluded that there is a significant influence because the significant level value obtained was less than 0.05 ($p<0.05$). Treatment group K has a significant difference to groups Q and S. Group P has a significant difference to groups Q and S. Treatment group Q has a significant difference to groups R and T. Treatment group R has a significant difference to group T and group treatment S had a significant difference to group T. Overall the comparison between groups with a $p<0.05$ showed that there was a significant or real effect. The data obtained showed that there was an effect of treatment from *Anadara granosa* L. and *Spirulina platensis* which increased the average level of viscosity or viscosity of mice spermatozoa. The very high Zn content in the blood shells *Anadara granosa* L. plays a role in the spermatogenesis process and can improve sperm quality[36]. Based on the average percentage of viscosity level and comparative analysis between groups, it can be seen that treatments R and T have the lowest average percentage of

Table 3: Results of sperm color

Treatment	Spermatozoa color
K1	Yellowish white
K2	Yellowish white
K3	White
K4	Yellowish white
K5	White
P1	Yellowish white
P2	Yellowish white
P3	White
P4	Yellowish white
P5	Yellowish white
Q1	Yellowish white
Q2	Yellowish white
Q3	Yellowish white
Q4	White
Q5	White
R1	Yellowish white
R2	White
R3	Yellowish white
R4	Yellowish white
R5	White
S1	Yellowish white
S2	White
S3	White
S4	White
S5	Yellowish white
T1	Yellowish white
T2	Yellowish white
T3	Yellowish white
T4	Yellowish white
T5	Yellowish white

viscosity level of all treatment groups that received intervention (groups P, Q, S and T) and have a difference in average very low (small) average compared to the control treatment group (K). This is thought to be due to the presence of active compounds that have antifertility properties in *Spirulina platensis* as mentioned by Yuniati *et al.*[37] and Bortolini *et al.*[38] that *Spirulina platensis* biomass contains flavonoids, steroids, phenols and saponins, so that in the R treatment group with the intervention of 30% *Anadara granosa* blood shells and 70% *Spirulina platensis* microalgae and the T treatment group with the intervention of 100% *Spirulina platensis* had similar results. different from the control group. Meanwhile, between the control group and the treatment group given the intervention of 50% *Anadara granosa* L. blood shells fortified with 50% *Spirulina platensis* microalgae and 100% *Anadara granosa* L., there was a fairly high difference. Malpani *et al.*[39] stated that steroids, alkaloids, flavonoids and tannins are compounds that have antifertility properties, especially in males, where these compounds are also contained in the microalgae *Spirulina platensis*. Flavonoids have a structure similar to the hormone estrogen and are antiandrogens which can inhibit the work of the aromatase enzyme so that the testosterone hormone decreases[40]. Domíngues-López *et al.*[41] reported that flavonoids are able to stimulate and increase estrogen levels, so that they will provide negative feedback, namely by not releasing LH and FSH. High levels of estrogen caused by the active substances alkaloids, flavonoids and steroids cause inhibition of FSH secretion which results in the immediate cessation of spermatogenesis and sterilization if it continues[42]. Disruption of FSH release will cause FSH levels to fall and affect sertoli cells in producing nutrients for the formation and maturation of spermatozoa[43].

Spermatozoa color: The results of observing the color of spermatozoa in mice can be seen in Table 3 which shows that the color of the sperm of mice in each treatment group was white and also yellowish white. The difference in the color of the mice's sperm is not that significant and is almost the same. Sperm color is considered normal if it is in the range of white to yellowish white. If the spermatozoids are clear/translucent, it is usually interpreted as watery semen[44]. If red blood cells are found, the sperm will be brownish, due to the presence of hemoglobin. The blood shell *Anadara granosa* L. contains various important components such as macro nutrients, namely protein, total fat and carbohydrates. Meanwhile, for micronutrients such as minerals, namely Ca, Fe, Mg, P, Zn, Cu, Mn and Se, essential amino acids and vitamins include: vitamins A, E, B complex and C[45]. The reported chemical composition of the *Anadara granosa* L. blood shell is 9-3% protein, 0.2% fat, 1-7% glycogen and has a calorific value in 100 g of fresh meat. It is also known that shellfish are a type of food that is believed to be an aphrodisiac. Providing nutrition with the blood shell *Anadara granosa* L. affects the spermatozoa of mice. This is due to the presence of a substance that plays an important role in the process of spermatozoa formation contained in the blood shell *Anadara granosa* L., namely the mineral zinc (Zn). Blood shells which have a very high Zn content play a role in the spermatogenesis process and can improve sperm quality. The high nutritional content of *Spirulina platensis* is known to be widely used as a health food ingredient[46]. The main nutrients contained in *Spirulina* are carbohydrates, proteins, fats (gamma linoleic, omega 3, 6 and 9), vitamins (B-complex, E), minerals (Fe, Ca, K) and natural pigments (beta carotene, chlorophyll, xanthophyll, phycocyanin). *Spirulina platensis* also produces compounds that can function as antioxidants (prevent cancer and free radicals), increase the body's immune system (resistance to environmental fluctuations and disease attacks), and lower

cholesterol[16]. Administration of *Spirulina platensis* has the potential to act as a protective agent in testicular tissue because it contains the main antioxidant substances, namely phycocyanin and phycocyanobilin, which are able to protect against oxidative damage to the P450 system in Leydig cells[47]. The results of observations of the color of mice spermatozoa showed that the color was still considered normal because in each treatment group, the mice sperm was white and also yellowish white. The difference in the color of the mice's sperm is not that significant and is almost the same. Standards sperm color is considered normal if it is in the range of white to yellowish white[44]. In research Gao *et al*.[45] also showed that spermatozoids before and after consuming blood shell nutrition were normal, namely grayish white. If the spermatozoids are clear/translucent, then they can be said to be watery semen. If red blood cells are found, the sperm will be brownish, this is due to the presence of hemoglobin. The color of the mice spermatozoa was said to be normal because no difference was found between the color of the spermatozoa of mice in group K (control) which were not given intervention by the blood shell *Anadara granosa* and the microalgae *Spirulina platensis* and the color of the spermatozoa of mice in groups P, Q, R, S and T which were given intervention of blood shell *Anadara granosa* and microalgae *Spirulina platensis*.

CONCLUSION

The safe dose of blood shell *Anadara granosa* L. for mice is 4.16 mg/20 g of mice body weight per day and the safe dose of the microalga *Spirulina platensis* for mice is 2.6 mg/20 g of mice body weight p er day. By administering the intervention of *Anadara granosa* L. blood shell powder which was fortified with *Spirulina platensis* microalgae, the highest average percentage of viscosity was obtained in the Q and T treatment group. When observing the color of mice spermatozoa, normal results were obtained. Future studies should focus on optimizing the dosage, understanding the mechanisms and evaluating the long-term safety and efficacy of the fortified supplement in both animal models and humans. Additionally, efforts should be made to develop standardized, sustainable formulations for broader applications in reproductive health.

SIGNIFICANCE STATEMENT

This study explores an innovative approach to addressing infertility by enhancing the quality of a patented *Anadara granosa* L. blood shell supplement through fortification with *Spirulina platensis* microalgae. The findings indicate that this fortified supplement significantly improves sperm quality parameters, including viscosity, without causing adverse effects, as shown by the normal coloration of spermatozoa. These results suggest that combining *Anadara granosa* L. and *Spirulina platensis* has potential benefits for reproductive health, providing a promising direction for the development of supplements aimed at improving male fertility.

ACKNOWLEDGMENTS

We would like to thank the Hasanuddin University, Indonesia for providing financial support through Collaborative Fundamental Research Scheme, grant numbers: 00323/UN4.22/PT.01.03/2023, Zoology and Botany Laboratory, Faculty of Mathematics and Natural Sciences, Biopharmaceutical Laboratory, Faculty of Pharmacy and Veterinary Education Clinic, Faculty of Medicine, Hasanuddin University for the facilitations.

REFERENCES

1. Perlman, R.L., 2016. Mouse models of human disease: An evolutionary perspective. Evol. Med. Public Health, 2016: 170-176.
2. Bayne, K., 2018. Environmental enrichment and mouse models: Current perspectives. Anim. Models Exp. Med., 1: 82-90.
3. Skoracka, K., P. Eder, L. Łykowska-Szuber, A. Dobrowolska and I. Krela-Kaźmierczak, 2020. Diet and nutritional factors in male (in)fertility-underestimated factors. J. Clin. Med., Vol. 9. 10.3390/jcm9051400.
4. Leslie, S.W., T.L. Soon-Sutton and M.A.B. Khan, 2023. Male Infertility. StatPearls Publishing, Treasure Island.
5. Chao, H.H., Y. Zhang, P.Y. Dong, S. Gurunathan and X.F. Zhang, 2023. Comprehensive review on the positive and negative effects of various important regulators on male spermatogenesis and fertility. Front. Nutr., Vol. 9. 10.3389/fnut.2022.1063510.
6. Ferramosca, A. and V. Zara, 2022. Diet and male fertility: The impact of nutrients and antioxidants on sperm energetic metabolism. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms23052542.
7. Langyan, S., P. Yadava, F.N. Khan, Z.A. Dar, R. Singh and A. Kumar, 2021. Sustaining protein nutrition through plant-based foods. Front. Nutr., Vol. 8. 10.3389/fnut.2021.772573.
8. Jayasekara, C., E. Mendis and S.K. Kim, 2020. Seafood in the Human Diet for Better Nutrition and Health. In: Encyclopedia of Marine Biotechnology, Kim, S.K. (Ed.), John Wiley & Sons Ltd., Hoboken, New Jersey, ISBN: 9781119143802, pp: 2939-2959.

9. Bavinck, M., M. Ahern, H.M. Hapke, D.S. Johnson and M. Kjellevold *et al*., 2023. Small Fish For Food Security and Nutrition. Food & Agriculture Org., Rome, Italy, ISBN: 9789251379103, Pages: 170.
10. Astuti, P., C.M. Airin, S. Sarmin, A. Nururrozi and S. Harimurti, 2019. Effect of shell as natural testosterone boosters in Sprague Dawley rats. Vet. World, 12: 1677-1681.
11. Yuneldi, R.F., C.M. Airin, H.T.S. Saragih, S. Sarmin, P. Astuti and A.R. Alimon, 2023. Growth, pectoralis muscle performance, and testis of pelung cockerels (*Gallus gallus gallus* [Linnaeus, 1758]) supplemented with blood clam shell powder (*Anadara granosa* [Linnaeus, 1758]). Vet. World, 16: 474-482.
12. Solang, M., 2017. Blood cockle (*Anadara granosa*) supplementation to increase serum calcium level and femur growth of low-protein diet rat. Nusantara Biosci., 9: 62-67.
13. Dalólio, F.S., J.P. Oliveira, H.S. Rostagno, L.F.T. Albino, C.C. Silva and C. Lozano, 2023. Effect of increasing levels of fat-soluble and water-soluble vitamins on improving performance and plasma vitamin concentration in modern hybrids pig's growth and finishing phase. Arquivo Bras. Med. Vet. Zootecnia, 75: 303-312.
14. Rey, A.I., A. de-Cara, A. Rebolé and I. Arija, 2021. Short-term spirulina (*Spirulina platensis*) supplementation and laying hen strain effects on eggs' lipid profile and stability. Animals, Vol. 11. 10.3390/ani11071944.
15. Ahda, M., Suhendra and A. Permadi, 2024. *Spirulina platensis* microalgae as high protein-based products for diabetes treatment. Food Rev. Int., 40: 1796-1804.
16. AlFadhly, N.K.Z., N. Alhelfi, A.B. Altemimi, D.K. Verma, F. Cacciola and A. Narayanankutty, 2022. Trends and technological advancements in the possible food applications of spirulina and their health benefits: A review. Molecules, Vol. 27. 10.3390/molecules27175584.
17. Putri, M.W.D., Prayogo and D.S. Budi, 2020. Effect of *Spirulina platensis* supplementation in the diet to sperm performance of silver rasbora (*Rasbora argyrotaenia*). IOP Conf. Ser.: Earth Environ. Sci., Vol. 441. 10.1088/1755-1315/441/1/012041.
18. Prandi, B., F. Boukid, S. van de Walle, S. Cutroneo and J. Comaposada *et al*., 2023. Protein quality and protein digestibility of vegetable creams reformulated with microalgae inclusion. Foods, Vol. 12. 10.3390/foods12122395.
19. Panaite, T.D., G.M. Cornescu, N.C. Predescu, A. Cismileanu, R.P. Turcu, M. Saracila and C. Soica, 2023. Microalgae (*Chlorella vulgaris* and *Spirulina platensis*) as a protein alternative and their effects on productive performances, blood parameters, protein digestibility, and nutritional value of laying hens' egg. Appl. Sci., Vol. 13. 10.3390/app131810451.
20. Thakur, S., A. Singh, B. Insa and S. Sharma, 2023. Food fortification in India as malnutrition concern: A global approach. Sustainable Food Technol., 1: 681-695.
21. Chaiklahan, R., N. Chirasuwan, V. Loha, S. Tia and B. Bunnag, 2018. Stepwise extraction of high-value chemicals from *Arthrospira* (*Spirulina*) and an economic feasibility study. Biotechnol. Rep., Vol. 20. 10.1016/j.btre.2018.e00280.
22. Otčenášková, T., E. Macíčková, J. Vondráková, M. Frolíková, K. Komrskova, R. Stopková and P. Stopka, 2023. Proteomic analysis of the mouse sperm acrosome-towards an understanding of an organelle with diverse functionality. Eur. J. Cell Biol., Vol. 102. 10.1016/j.ejcb.2023.151296.
23. Abdollahi, M.B., S.F. Dehghan, F.A. Balochkhaneh, M.A. Moghadam and H. Mohammadi, 2021. Comparison of mice' sperm parameters exposed to some hazardous physical agents. Environ. Anal. Health Toxicol., Vol. 36. 10.5620/eaht.2021013.
24. Mishra, P., U. Singh, C.M. Pandey, P. Mishra and G. Pandey, 2019. Application of student's *t*-test, analysis of variance, and covariance. Ann. Card. Anaesth., 22: 407-411.
25. Sharma, L.R. and S. Jha, 2023. Applying major parametric tests using SPSS in research. Int. Res. J. MMC, 4: 85-97.
26. James, E.R., D.T. Carrell, K.I. Aston, T.G. Jenkins, M. Yeste and A. Salas-Huetos, 2020. The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21155377.
27. Druart, X., J.P. Rickard, G. Tsikis and S.P. de Graaf, 2019. Seminal plasma proteins as markers of sperm fertility. Theriogenology, 137: 30-35.
28. Wang, F., W. Yang, S. Ouyang and S. Yuan, 2020. The vehicle determines the destination: The significance of seminal plasma factors for male fertility. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21228499.
29. Wu, E.H. and F.L. de Cicco, 2022. Anatomy, Abdomen and Pelvis, Male Genitourinary Tract. StatPearls Publishing, Treasure Island.
30. Vitku, J., L. Kolatorova and R. Hampl, 2017. Occurrence and reproductive roles of hormones in seminal plasma. Basic Clin. Androl., Vol. 27. 10.1186/s12610-017-0062-y.
31. Ugur, M.R., T. Dinh, M. Hitit, A. Kaya, E. Topper, B. Didion and E. Memili, 2019. Amino acids of seminal plasma associated with freezability of bull sperm. Front. Cell Dev. Biol., Vol. 7. 10.3389/fcell.2019.00347.
32. Ješeta, M., A. Pospíšilová, L. Mekiňová, K. Franzová and P. Ventruba *et al*., 2023. Non-invasive diagnostics of male spermatogenesis from seminal plasma: Seminal proteins. Diagnostics, Vol. 13. 10.3390/diagnostics13152468.
33. Wong, A.D., K.A. Keller, A.J. Cerreta, K. Cook and J.K. Webb *et al*., 2024. Visualization of radiographic mineral opacities in guinea pigs in the location of the bulbourethral glands may be underdiagnosed. J. Exot. Pet Med., 48: 25-30.
34. Tomaiuolo, G., F. Fellico, V. Preziosi and S. Guido, 2022. Semen rheology and its relation to male infertility. Interface Focus, Vol. 12. 10.1098/rsfs.2022.0048.

35. Iskandar, H., H. Sonjaya, R.I. Arifiantini and H. Hasbi, 2022. The quality of fresh and frozen semen and its correlation with molecular weight of seminal plasma protein in Bali cattle. Trop. Anim. Sci. J., 45: 405-412.
36. Fallah, A., A. Mohammad-Hasani and A.H. Colagar, 2018. Zinc is an essential element for male fertility: A review of Zn roles in men's health, germination, sperm quality, and fertilization. J. Reprod. Infertility, 19: 69-81.
37. Yuniati, R., M. Zainuri and H. Kusumaningrum, 2020. Qualitative tests of secondary metabolite compounds in ethanol extract of *Spirulina platensis* from Karimun Jawa Sea, Indonesia. Biosaintifika: J. Biol. Biol. Educ., 12: 343-349.
38. Bortolini, D.G., G.M. Maciel, I. de Andrade Arruda Fernandes, A.C. Pedro, F.T.V. Rubio, I.G. Branco and C.W.I. Haminiuk, 2022. Functional properties of bioactive compounds from *Spirulina* spp.: Current status and future trends. Food Chem.: Mol. Sci., Vol. 5. 10.1016/j.fochms.2022.100134.
39. Malpani, A., N. Mahurkar and U. Aswar, 2020. Phytochemical analysis and antifertility potential of *Cynodon dactylon* in female Wistar rats: A herbal approach towards contraception. Chin. Herb. Med., 12: 281-288.
40. Kiyama, R., 2023. Estrogenic flavonoids and their molecular mechanisms of action. J. Nutr. Biochem., Vol. 114. 10.1016/j.jnutbio.2022.109250.
41. Domínguez-López, I., M. Yago-Aragón, A. Salas-Huetos, A. Tresserra-Rimbau and S. Hurtado-Barroso, 2020. Effects of dietary phytoestrogens on hormones throughout a human lifespan: A review. Nutrients, Vol. 12. 10.3390/nu12082456.
42. Bhatt, N. and M. Deshpande, 2021. A critical review and scientific prospective on contraceptive therapeutics from ayurveda and allied ancient knowledge. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.629591.
43. Santi, D., P. Crépieux, E. Reiter, G. Spaggiari and G. Brigante *et al*., 2020. Follicle-stimulating hormone (FSH) action on spermatogenesis: A focus on physiological and therapeutic roles. J. Clin. Med., Vol. 9. 10.3390/jcm9041014.
44. Vasan, S.S., 2011. Semen analysis and sperm function tests: How much to test? Indian J. Urol., 27: 41-48.
45. Gao, L., X. Xiong, C. Chen, P. Luo and J. Li *et al*., 2023. The male reproductive toxicity after nanoplastics and microplastics exposure: Sperm quality and changes of different cells in testis. Ecotoxicol. Environ. Saf., Vol. 267. 10.1016/j.ecoenv.2023.115618.
46. Ramírez-Rodrigues, M.M., C. Estrada-Beristain, J. Metri-Ojeda, A. Pérez-Alva and D.K. Baigts-Allende, 2021. *Spirulina platensis* protein as sustainable ingredient for nutritional food products development. Sustainability, Vol. 13. 10.3390/su13126849.
47. Barkallah, M ., A. Ben Slima, F. Elleuch, I. Fendri, C. Pichon, S. Abdelkafi and P. Baril, 2020. Protective role of *Spirulina platensis* against bifenthrin-induced reprotoxicity in adult male mice by reversing expression of altered histological, biochemical, and molecular markers including MicroRNAs. Biomolecules, Vol. 10. 10.3390/biom10050753.