Short Note [Nota Corta]

SEMEN QUALITY OF HORNED AND POLLED BALI BULLS TREATED WITH BEAN SPROUT (*Phaseolus radiatus L.*) SUPPLEMENTED FEEDING †

CALIDAD DEL SEMEN DE TOROS BALI DE CUERNO Y SIN CUERNO SUPLEMENTADOS CON GERMINADO DE FRIJOL (*Phaseolus radiatus L.*)

Sri Gustina[1], Hasbi Hasbi[1*], Zulkharnaim Zulkharnaim[1], Herry Sonjaya[1], Sudirman Baco[1], Siti Nuraisya Hamsir[1], Mutmainna Mutmainna[1], Husnul Qhatimah[1] and Siti Farida[2]

[1]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, 90245, Indonesia
[2]Service of Livestock and Animal Health, Province of South Sulawesi, Jl. Veteran Selatan No. 234, Makassar, Indonesia 90131. Email: hasbi_fapetunhas@yahoo.com
**Corresponding author*

SUMMARY

Background. Horned and Polled Bali cattle are big meat producing livestock and have various other advantages, so they need to be maintained regarding their existence by improving semen quality. Providing bean sprouts (*Phaseolus radiatus L.*) in feeding is an effort to improve semen quality because of its antioxidant content that can scavenge free radicals. **Objective.** To determine the quality of semen from Bali Polled bull and Bali Horned bull fed with bean sprout feed supplements. **Methodology.** Four Horned and four Polled Bali bulls aged 5-7 years were fed bean sprouts-supplemented twice a day in the amount of 1 kg/bull for two months. Parameters observed in fresh semen include color, smell, pH, volume, concentration, and followed by motility, viability, abnormality, membrane integrity, and acrosome integrity for both fresh and frozen semen. **Results.** The results of the macroscopic evaluation of fresh semen showed that the color, smell, pH, and volume were not different (P>0.05) in Polled compared to Horned. Meanwhile, the microscopic evaluation showed that motility, viability, abnormality, membrane integrity, and acrosome integrity were not different (P>0.05) between Horned and Polled, but the sperm concentration of Horned was higher (P<0.05) than Polled ($1.160 \pm 1.16 \times 10^9$ vs $0.840 \pm 0.92 \times 10^9$). The quality of frozen semen showed that the motility and membrane integrity of Polled is higher (P<0.05) than Horned (48.57 ± 3.77% vs. 35.38 ± 12.15% and 76.60 ± 4.30% vs. 70.00 ± 5.79%), whereas recovery rate was higher (P<0.01) of Polled than Horned (69.39 ± 3.88% vs. 50.54 ± 9.02%), but in the parameters of viability, abnormality, and acrosome integrity were not different (P>0.05). **Implications**. This information from this study can be used to improve semen the quality of Horned and Polled Bali bulls fed bean sprouts. **Conclusion.** In conclusion, after bean sprouts (*Phaseolus radiatus L.)* supplemented feeding, the semen concentration of Horned increases compared to the Polled one. While the color, smell, pH, volume, motility, viability, abnormality of membrane integrity, and acrosome integrity of fresh semen were not affected. The motility, recovery rate, and membrane integrity of frozen semen are increased in Polled compared to the Horned one, while viability, abnormalities, and acrosome integrity remained similar.

Key words: Bean Sprout; Horned Bali Bulls; Polled Bali Bulls; Fresh Semen Quality; Frozen Semen Quality.

RESUMEN

Antecedentes. El ganado Bali con cuernos y sin cuernos es un ganado productor de carne y tiene otras ventajas, por lo que es necesario mantener su existencia mejorando la calidad del semen. Proporcionar brotes de frijol (*Phaseolus radiatus* L.) en la alimentación es un esfuerzo por mejorar la calidad del semen debido a su contenido de antioxidantes que pueden eliminar los radicales libres. **Objetivo**. Determinar la calidad del semen de ganado Bali sin cuernos y ganado Bali con cuernos alimentado con suplementos alimenticios de brotes de frijol. **Metodología.** Se emplearon cuatro toros Bali con cuernos y cuatro toros sin cuernos de 5 a 7 años de edad que fueron alimentados con brotes de frijol suplementados dos veces al día en una cantidad de 1 kg por toro durante dos meses. Los parámetros observados en el semen fresco incluyen color, olor, pH, volumen, concentración y seguidos de motilidad, viabilidad, anormalidad, integridad de la membrana e integridad del acrosoma tanto para el semen fresco como para el congelado. **Resultados.**

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ORCID = H. Hasbi: http://orcid.org/0000-0002-6069-2171

Los resultados de la evaluación macroscópica del semen fresco mostraron que el color, olor, pH y volumen no fueron diferentes (P>0.05) en sin cuernos respecto a con cuernos. Mientras tanto, la evaluación microscópica mostró que la motilidad, la viabilidad, la anormalidad, la integridad de la membrana y la integridad del acrosoma no fueron diferentes (P>0.05) entre los cuernos y los sin cuernos, pero la concentración de esperma de los con cuernos fue significativamente mayor (P <0.05) que los de los sin cuernos (1.160 ± 1.16 x 10^9 frente a 0.840 ± 0.92 x 10^9). La calidad del semen congelado mostró que la motilidad e integridad de la membrana de los toros sin cuerno es mayor (P<0.05) que de los toros con cuerno (48.57 ± 3.77% vs. 35.38 ± 12.15% y 76.60 ± 4.30% vs. 70.00 ± 5.79%), mientras que la recuperación la tasa fue mayor (P<0.01) en los sin cuernos que en los con cuernos (69.39 ± 3.88 % frente a 50.54 ± 9.02 %), pero en los parámetros de viabilidad, anormalidad e integridad del acrosoma no fueron diferentes (P>0.05). **Implicaciones**. Esta información se puede utilizar para mejorar la calidad del semen de toros Bali con cuernos y sin cuernos alimentados con brotes de frijol. **Conclusión.** La alimentación suplementada con brotes de frijol (*Phaseolus radiatus* L.), aumenta la concentración de semen de los toros con cuerno en comparación con los sin cuernos. Mientras que el color, olor, pH, volumen, motilidad, viabilidad, anormalidad de la integridad de la membrana y la integridad del acrosoma del semen fresco no se vieron afectados. La motilidad, la tasa de recuperación y la integridad de la membrana del semen congelado aumentan en el semen sin cuernos en comparación con el con cuernos, mientras que la viabilidad, las anomalías y la integridad del acrosoma se mantienen similares.
Palabras clave: Brote de frijol; Toros de Bali; Calidad del Semen Fresco; Calidad del Semen Congelado.

INTRODUCTION

Bali cattle, one of the beef cattle can produce quite a large amount of meat and have high production performance and reproductive capacity. Therefore, it needs to maintain its existence and use it sustainably (Hikmawaty *et al*., 2014). Meanwhile, there are Polled Bali cattle which are now being developed in Indonesia, especially in the South Sulawesi area. According to Baco *et al*. (2020) in the 1980s, hornless Bali cattle were found in Sidenreng Rappang and are currently termed Polled Bali cattle. However, the reproductive ability of Polled Bali cattle, especially males, has not been studied much. Hasbi *et al*. (2021) reported that Polled Bali bull took longer from approaching the teaser to ejaculating compared to Horned. This condition is thought to be closely related to the quality of the semen produced.

Semen is a total of spermatozoa and plasma produced by accessory glands. The collected semen is evaluated to determine the quality of the semen produced so that it meets standards and is suitable for use (Fazrien *et al*., 2020). Semen quality can be influenced by several factors, including genetics, environmental factors, and the feed provided (Suyadi *et al*., 2016). Hindrawati *et al*. (2020) stated that the quality of semen is determined by the quality of the feed consumed. Nutritious feed for males will determine the quantity and quality of semen produced. Bean sprouts (*Phaseolus radiatus L*.) are one feed supplement that is thought to be able to improve and maintain semen quality.

Bean sprouts contain several vitamins, including vitamins A, C, and E, as well as several minerals, such as sodium, magnesium, zinc, potassium, and iron, which can increase male fertility. The antioxidants are also contained in bean sprouts which are thought to be able to protect spermatozoa from damage caused by free radicals, so that they can improve and maintain the quality of spermatozoa (Soeparna *et al*., 2013; Sumarmin *et al*., 2018). The use of bean sprouts as a feed supplement has been previously reported by Nurcholis *et al*. (2015) and can increase semen volume, motility, viability and spermatozoa concentration. Furthermore, Winarso *et al*. (2004) reported that giving bean sprouts was able to increase the motility and viability of spermatozoa. Therefore, this research was conducted to determine the quality of semen from Bali Polled bull and Bali Horned bull fed with bean sprout feed supplements.

MATERIALS AND METHODS

Ethical approval

The experimental research has been approved by The Animal Ethics Commission of Hasanuddin University with number 302/UN4.6.4.5.31/PP36/2021.

Animals Treatment

This research used four male Horned and four male Polled Bali bulls aged 5-7 years with a body weight of 350-450 kg, The basal feed used was elephant grass (*Pennisetum purpureum*) at 10% of body weight and additional feed (NuFeed®) at 1% of body weight with a concentration of crude protein of 16%. They were fed bean sprouts-supplemented twice a day of 1 kg/bull for 60 days with crude protein 38.54%.

Semen Collection

Semen collection was carried out using an artificial vagina filled with 42 – 45°C temperature of water (Priyanto *et al.* 2015), and it was then examined macroscopically and microscopically. The evaluation of the microscopic was carried out by looking at the mass movement of spermatozoa. Semen that can be

further processed with sperm motility of 70% (Baracaldo *et al*., 2007).

Concentration of Sperm

The spermatozoa concentration was calculated by mixing 0.035 mL of semen with 3.5 mL of 0.9% NaCl solution into a cuvette. Next, it was calculated using an SDM 6 photometer (Minitube, Germany) (Prastowo *et al*., 2018).

Sperm Motility

Spermatozoa motility was measured by placing 10 µL of fresh semen samples on an object glass covered with a cover glass, then observed using a microscope (Olympus CX33, Japan) equipped camera (Olympus EP50, Japan) with a magnification of 400 times (Adiputra *et al*. 2022). Spermatozoa motility is assessed subjectively by looking at the number of spermatozoa moving forward/progressively. The motility assessment standard uses a scale between 0-100% (Gebreyasus *et al*. 2021).

Viability and Abnormality of Sperm

Spermatozoa viability was assessed by dripping 10 µL of diluted fresh semen and 20 µL of eosin-nigrosin dye and then homogenized. The preparations were observed using microscope (Olympus CX33, Japan) equipped camera (Olympus EP50, Japan) with a magnification of 400 times. The principle of assessing spermatozoa viability is based on live spermatozoa do not absorb color or are colorless, while dead ones absorb color (red) (Handayani et al., 2021), while abnormalities are by looking at abnormalities in the head, neck, and tail. Evaluation of spermatozoa viability and abnormalities was carried out by observing a minimum of 200 cells. The percentage of spermatozoa viability and abnormality was calculated using the following formula (Blegur *et al*., 2020):

$$\text{Viability Rate} = \frac{\sum \text{Live Sperm}}{\sum \text{Total of Spermatozoa}} \times 100\%$$

$$\text{Abnormality Rate} = \frac{\sum \text{Abnormality Sperm}}{\sum \text{Total of Spermatozoa}} \times 100\%$$

Membrane Integrity

The intact plasma membrane of spermatozoa was evaluated using the hypoosmotic swelling (HOS) test by incubating 10 µL semen sample in 1 mL of HOS test solution at a temperature of 37°C for 30-45 minutes (Adiputra *et al*., 2022). Curved or swollen tails characterize spermatozoa with an intact plasma membrane, whereas straight indicate that the spermatozoa are deformed or dead (Paddrik *et al*., 2012). The preparations were observed using a microscope (Olympus CX33, Japan) equipped camera (Olympus EP50, Japan) with a minimum magnification of 400 times on 200 spermatozoa cells. The percentage of membrane integrity was calculated using the following formula (Alhuur *et al*., 2020):

$$\text{Membrane Integrity} = \frac{\sum \text{Intact Membrane of Sperm}}{\sum \text{Total of Spematozoa}} \text{ x } 100\%$$

Acrosome Integrity

Evaluation of the intact acrosomal cap of spermatozoa was carried out by placing 10 µL semen sample into 30 µL of formol saline solution and then homogenizing. Next, a 10 µL semen sample was placed on an object glass and covered using a cover glass, then was observed using a microscope (Olympus CX33, Japan) equipped camera (Olympus EP50, Japan) with a magnification of 400 times a minimum of 200 spermatozoa cells. Spermatozoa that have an intact acrosome cap are characterized by 1/2 to 2/3 of the head being dark colored (Priyanto *et al*., 2015). The percentage of acrosomal integrity can be calculated using the following formula (Fatah *et al*., 2018):

$$\text{Acrosome Integrity} = \frac{\sum \text{Intact Acrosome of Sperm}}{\sum \text{ Total of Spermatozoa}} \text{ x } 100\%$$

Recovery rate

The preparation of frozen semen is carried out following the procedure developed by Said *et al*. (2015). The collected semen was diluted using an Andromed® extender to a concentration of 25 million and then put into a 0.25 mL polyvinyl straw. The packed semen in straws was equilibrated at a temperature of 4°C for 2 hours, then placed on a horizontal rack and exposed to liquid nitrogen at a height of 5 cm for 10 minutes. It is then put into a container at a temperature of -196°C.

The recovery rate of spermatozoa was evaluated by calculating spermatozoa motility after thawing with a minimum value of 50% (BSN, 2017). The Recovery rate was calculated using the following formula:

$$\text{Recovery Rate} = \frac{\text{Post Thawing Motility}}{\text{Fresh Semen Motility}} \text{ x } 100\%$$

Statistical Analysis

The data obtained was analyzed using IBM SPSS® statistics for windows version 26 (Inc. USA). Evaluation of the normality data using the Shapiro Wilk Test. The data were abnormally distributed, so the test continued by using a non-parametric test (Mann-Whitney U Test) to compare the sample of Horned and Polled Bali bulls treated with supplementation of bean sprout (*Phaseolus radiatus L.*) in feeding.

RESULTS AND DISCUSSION

Quality of Fresh Semen

Evaluation of the quality of fresh semen must be done to find out whether the collected semen is suitable for further processing into frozen semen. The parameters observed in this study include macroscopic (color, smell, pH, and volume) and microscopic (concentration, motility, viability, abnormality, membrane integrity, and acrosome integrity) evaluations. The quality of fresh semen of Horned and Polled Bali bulls is presented in Table 1.

Macroscopic evaluation of fresh semen from Horned Bali and Polled Bali bulls showed that the color, smell, pH, and volume were not significantly different (P>0.05). The results of this study indicated that the giving of bean sprouts (*Phaseolus radiatus L.*) in the feed does not affect the color, smell, pH, and volume of the semen of both Horned and Polled Bali bulls. The color, smell, and pH of the semen in this study did not differ from previous research reported by Hasbi *et al*. (2023) that Bali Polled and Horned have creamy color, distinctive smell, and pH 6.40±0.00. Meanwhile, the volume is lower than previously reported by Gustina *et al*. (2024), was 4.25 ± 1.28 mL in the Bali Polled, but higher than that reported by Hasbi *et al*. (2023), was 6.02 ± 2.22 mL in Bali Polled and 5.29 ± 1.79 mL in Horned.

The color, smell, and pH of the semen in this study were classified as normal, as previously reported by Bria *et al*. (2022), who stated that the normal color of fresh semen from Bali bull is creamy. Furthermore, Lestari *et al*. (2014) stated that the creamy color of semen is caused by the presence of riboflavin from the secretions of the vesicular glands and the large number of spermatozoa in the semen, which will result in the semen having a deeper color. The quality of semen affects the color of the semen, where normal semen is creamy, while yellowish green semen means it is contaminated by *Pseudomonas aeruginosa* bacteria, and if the semen is red means it is contaminated with blood (Damayanti *et al*., 2022). Yendraliza *et al*. (2019) reported that bull semen has a distinctive smell, which indicates that the semen is not contaminated or normal, whereas if the semen smells bad it means the semen has been mixed with pus (Lestari *et al*., 2014). Mila *et al*. (2021) reported that the normal pH of bull semen ranges from 6.2 to 6.8 and is one of the parameters in evaluating the quality of fresh semen. Furthermore, Fitriani *et al*. (2022) stated that the pH value of semen is influenced by seminal plasma fluid in the semen, which causes the semen to become more alkaline. Changes in pH values depend on the production capacity of the accessory glands and the frequency of ejaculation (Tambing *et al*., 2003). Semen pH high or low can affect semen quality, and can even cause spermatozoa to die more quickly (Widhyari *et al*., 2015). The volume of semen in this study was classified as normal, as previously reported by Garner and Hafez (2008) that the normal volume of bull semen ranged from 5-8 ml per collection, but slightly lower than previously by Hasbi *et al*. (2023) that the average semen volume of Bali Polled bull is 6.02 ± 2.22 ml. Semen volume can be influenced by various factors such as breed, age, temperature, feed, body, and testicular size, frequency of semen collection, and the condition of the bull (Bria *et al*., 2022).

Table 1. The quality of fresh semen from Horned and Polled Bali bulls with the supplementation of bean sprouts (*Phaseolus radiatus L.*) in feeding

Parameters	Horned Bali bull (Mean±SD)	Polled Bali bull (Mean±SD)
Macroscopic		
Color	Creamy	Creamy
Smell	Distinctive	Distinctive
pH	6.40±0.00	6.40±0.00
Volume (mL)	5.25±2.80	5.77±1.61
Microscopic		
Concentration ($x10^9$)	$1.160±1.16^a$	$0.840±0.92^b$
Motility (%)	70.00±0.00	70.00±0.00
Viability (%)	90.11±4.80	90.71±3.87
Abnormality (%)	11.00±3.93	11.28±2.07
Membrane Integrity (%)	78.74±4.37	81.40±3.27
Acrosome Integrity (%)	88.37±3.62	91.49±3.85

^{a,b}Values with different superscripts in the same row differ significantly (P<0.05).

Microscopic evaluation showed that motility, viability, abnormality, membrane integrity, and acrosome integrity were not significantly different (P>0.05) between Horned and Polled Bali bulls, but the spermatozoa concentration of Horned was significantly higher (P<0.05) than Polled one. The results of this study indicated that giving bean sprouts (*Phaseolus radiatus L.*) in the feed does not affect the motility, viability, abnormality, membrane integrity, and acrosome integrity of spermatozoa of both Horned and Polled Bali bull, but can increase the sperm concentration in Horned one. The motility of spermatozoa in this study was classified as normal and had good quality, and it met the standards for further processing into frozen semen in accordance with the Indonesian National Standard (INS) of bull frozen semen no. 4869.1-2017. Savitri *et al*. (2014) reported that fresh semen must have individual motility of sperm of at least 70% to be processed and made into frozen semen. Sperm Motility in this study was lower than previous research reported by Hasbi *et al*. (2023), where fresh semen motility in Horned bulls was 77.78 ± 2.64% and in Polled bull it was 73.33 ± 2.50%. Spermatozoa motility can be influenced by several factors, such as feed, age, maintenance management, frequency of semen collection, and membrane integrity (Arvioges *et al*., 2020). The viability of the spermatozoa obtained was within the normal range and can be processed further. This was in accordance with what was reported by Blegur *et al*. (2020) that fresh bull semen must have a minimum viability of 60-75% so that it can be processed into frozen semen. The results of this study were lower than previous research reported by Hasbi *et al*. (2023) was 91.92 ± 1.51% in Horned, and 92.73 ± 1.83% in Polled. Furthermore, Blegur *et al*. (2020) and Ardhani *et al*. (2020) reported that factors that can influence spermatozoa viability are pH, feed, diluent used, and membrane integrity.

The spermatozoa abnormalities obtained in this study were higher compared to previous studies reported by Hasbi *et al*. (2023) that 5.52 ± 0.92% in Horned, and Polled had 4.01 ± 0.94%, but it is still within the normal range and can be processed further. This was in accordance with what was previously reported by Yendraliza *et al*. (2019) that spermatozoa abnormalities must be less than 20% so that they can be processed further into frozen semen. Furthermore, Zakaria *et al*. (2020) reported that spermatozoa abnormalities can be influenced by various factors, such as differences in osmotic pressure when diluting. Membrane integrity in this study was higher than previous research reported by Hasbi *et al*. (2023), that 72.43 ± 7.32% for Horned, and 76.58 ± 6.53% for Polled, but it can still be processed further into frozen semen. As reported by Syafi'i and Rosadi (2022), fresh semen must have membrane integrity with a percentage of more than 60% in order to be used in AI programs. Membrane integrity can be influenced by various factors such as feed, age, living environment, and handling of semen evaluation (Anwar *et al*., 2015). The acrosome integrity obtained in this study was 88.37±3.62% in Horned bull and 91.49±3.85% in Polled bull was higher than previous research by Anwar *et al*. (2015), namely 68.25 ± 3.20% in Bali Horned bull, and lower than the research reported by Diansyah *et al*. (2022), namely 96.36 ± 1.48% in Bali Polled bull.

Quality of Frozen Semen

Evaluation of frozen semen quality needs to be done to determine whether it is suitable for use in Artificial Insemination (AI) activities in accordance with the Indonesian National Standard (INS) of bull frozen semen no. 4869.1-2017. The quality of frozen semen from Horned and Polled Bali bull is presented in Table 2.

The motility and membrane integrity were significantly higher (P<0.05) and the recovery rate was very significantly higher (P<0.01) of frozen semen from Polled Bali bulls than Horned ones, but in the parameters of viability, abnormality, and acrosome integrity were not significantly different (P>0.05). The motility, membrane integrity, and recovery rate of frozen semen in Polled Bali bull was higher than that of Horned one so this indicates that the frozen semen of Polled Bali bull has better resistance during the freezing process. This is thought to be because spermatozoa are able to utilize the nutrients contained in bean sprouts (*Phaseolus radiatus L.*) better.

Table 2. The quality of frozen semen of Horned and Polled Bali bulls with bean sprout (*Phaseolus radiatus L.*) supplemented feeding.

Parameters	Horned Bali bull (Mean±SD)	Polled Bali bull (Mean±SD)
Motility (%)	35.38±12.15^{a}	48.57±3.77^{b}
Recovery Rate (%)	50.54±9.02^{A}	69.39±3.88^{B}
Viability (%)	80.76±9.73	85.28±5.86
Abnormality (%)	17.57±3.03	18.72±3.64
Membrane Integrity (%)	70.00±5.79^{a}	76.60±4.30^{b}
Acrosome Integrity (%)	81.71±5.34	86.55±4.28

^{a,b}Values with different superscripts in the same row differ significantly (P<0.05).
^{A,B} Values with different superscripts in the same row very differ significantly (P<0.01).

The motility of frozen semen in the Polled Bali bulls in this study was higher than that previously reported by Gustina *et al*. (2022), 45.41 ± 0.83%, and in the Horned bulls, it is lower, 49.58 ± 0.83%. Meanwhile, membrane integrity was higher than previously reported by Hasbi *et al*. (2023), 54.79 ± 3.73% in Polled and 53.08 ± 7.23% in Horned bulls. Whereas, the recovery rate in Horned Bali bull and Polled Bali bulls is lower than previously reported by Hasbi *et al*. (2023), 61.62 ± 10.46% in Horned and 71.72 ± 9.83% in Polled ones. It is thought that the nutrients contained in bean sprouts (*Phaseolus radiatus L.*) can be used to increase the motility, membrane integrity, and recovery rate of frozen semen in Polled Bali bull include antioxidants that are able to prevent free radical attacks and can minimize the occurrence of cool shock experienced by spermatozoa during storage process. This is in accordance with what was previously reported by Suharyati and Hartono (2013) that antioxidants were able to maintain semen motility in Boer goats. There are several factors that can influence frozen semen motility, including, as reported by Savitri *et al*. (2014), that spermatozoa motility can be influenced by the equilibration and prefreezing processes. The recovery rate obtained in Horned Bali bull in this study was lower than that reported by Sukmawati *et al*. (2014) in Limousine (59.70 ± 3.23%), Simmental (58.46 ± 1.06%), and Friesien Holstein (57.53 ± 1.74%), while in Bali Polled was higher.

Viability, abnormality, and acrosome integrity obtained in this study showed that giving bean sprouts (*Phaseolus radiatus L.*) in feed gave the same response to Horned Bali bull and Polled Bali bull. The viability of the frozen semen obtained is very good and suitable for use in AI programs, as reported by Malinda *et al*. (2021), the viability of spermatozoa is at least 50% so that it can be used in AI programs. The viability of spermatozoa in this study was higher than that of previous research by Hasbi *et al*. (2023), with 54.58% in Bali Horned and 56.05% in Polled one. Spermatozoa viability can be influenced by several factors including pH, diluent used, freezing process, thawing, and membrane integrity (Malik *et al*., 2017 and Tethool *et al*., 2022). The spermatozoa abnormalities obtained were low and still suitable for use, as previously reported by Ardhani *et al*. (2020) that abnormalities of spermatozoa must be ≤20% to be used. However, it is higher than that reported by Hasbi *et al*. (2023), that the abnormality of frozen semen in Bali Horned was 6.32% and in Polled was 8.85%. Malik *et al*. (2017) stated that factors that can influence spermatozoa abnormalities are abnormalities that occur during the process of spermatogenesis in the testicles (primary abnormalities), abnormalities that occur in the reproductive organ tract (secondary abnormalities), and abnormalities that occur after ejaculation to semen evaluation. Ardhani *et al*. (2020) added that high abnormalities can also be caused by repeated container transfers during the distribution process and the lack of liquid nitrogen in the containers.

Membrane integrity in this study was higher because it was influenced by adequate nutrition for spermatozoa, an unsuitable environment, the dilution and freezing process, the number of abnormal spermatozoa, and the occurrence of cell damage (Ardhani *et al*., 2020). The acrosome integrity values obtained showed that frozen semen is suitable for use in AI program, as reported by Syafi'i and Rosadi (2022) that the minimum percentage of spermatozoa acrosome integrity for AI program is 30%. The results of this study were lower than previous research by Hasbi *et al*. (2023), 89.49% of Horned Bali bull and 92.98% of Polled one. Ardhani *et al*. (2020) stated that the percentage of acrosome integrity can decrease with increasing storage time, damage due to handling processes, and semen freezing.

CONCLUSION

Bean sprouts contain several vitamins, including vitamin A, C, E, as well as several minerals such as sodium, magnesium, zinc, potassium and iron which can increase male fertility. After bean sprouts (*Phaseolus radiatus L.)* supplemented feeding, the semen concentration of Horned Bali bull increases compared to the Polled one. While the color, smell, pH, volume, motility, viability, abnormality of membrane integrity, and acrosome integrity of fresh semen were not affected. The motility, recovery rate, and membrane integrity of frozen semen are increased in Polled Bali bull compared to the Horned one, while viability, abnormalities, and acrosome integrity remained similar. In the future, research needs to be carried out to test its fertility capabilities both in vivo and in vitro.

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Conflict of interest statement. The authors declare there is no conflict of interest.

Compliance with ethical standards. This study was ethically approved by The Animal Ethics Commission of Hasanuddin University approved this study (approval number: 302/UN4.6.4.5.31/PP36/2021).

Data availability. Data are available from the corresponding author upon reasonable request.

Author contribution statement (CRediT): S. Gustina - Conceptualization original draft., **H. Hasbi -** Designing the study and project administration., **S.N.**

Hamsir – Formatted the manuscript, **M. Mutmainna –** Data analysis., **H. Qhatimah -** Edited the manuscript., **S. Farida** - Collected the sample and data., **Z. Zulkharnaim –** Validated of data., **H. Sonjaya –** Review and data curation., **S. Baco** – Writing and investigation.

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