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Semen quality of Bali bulls fed supplement of Mungbean sprouts and mineral mix

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Abstract The results showed that MBS and MBM supplementation significantly ($P<0.05$) increased semen volume, concentration, motility, viability, and integrity of membrane plasma and had no significant effect ($P>0.05$) on semen abnormality. MBS and MBM supplementation at P2 (basal diet + 1 kg MBS + 0.25 kg MBM) was more effective in improving the semen quality of Bali bulls.

Keywords: Bali bull, Mung bean sprouts, Mineral mix, Semen quality

Introduction

Breeding is the primary key to success in achieving the desired cattle population. Cattle breeding needs to be supported by the application of technology so that cattle can optimize their reproductive potential. One technology that has been applied massively is artificial insemination. The application of artificial insemination allows optimal use of superior bulls, increases the efficiency of cattle breeding at every business scale, accelerates efforts to improve the genetic quality of cattle, and suppresses the spread of certain diseases in cattle. The Central Government, through the Ministry of Agriculture of the Republic of Indonesia, has evaluated the implementation of artificial insemination, and the result is that the implementation of artificial insemination has not been optimal. Several obstacles cause the implementation of artificial insemination to be not optimal, and technical problems are also found. All of this must be resolved jointly by the central and local governments.

In practice, the success of artificial insemination is determined by many factors: semen, livestock, breeders and inseminators, and livestock management. The sementing factor in semen quality (almost all frozen semen packaged in

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straws) depends on the production, distribution, storage, and treatment processes. The main factor influencing the success of artificial insemination is the quality of frozen semen. Therefore, to ensure the quality of frozen cattle semen in circulation, it is necessary to set standards for frozen cattle semen. The quality of frozen semen that meets the standards must be supported by excellent and correct handling so that the quality of frozen semen can be maintained until it is ready for insemination. Implementation in the field, due to several reasons such as long distances, poor handling, and lack of liquid N2 during trips to breeders, so that the quality of frozen semen that meets these standards may decrease; this is feared as one of the reasons for the failure of artificial insemination (Susilawati, 2011).

The success of the application of reproductive biotechnology in the form of artificial insemination with frozen semen is influenced by several biological and technical factors. Semen quality is one factor that plays a role in the success of artificial insemination technology and will affect the fertility rate. The excellent quality of semen produced by healthy males is closely related to giving appropriate feed with good nutritional quality (Soeparna *et al*., 2013). Nutritional deficiency or insufficiency directly affects reproduction efficiency, low performance, and productivity (Salem *et al*., 2006). A good ration has a balance of nutrients, especially energy and protein content, that can meet the needs of livestock for production activities. To obtain a balanced nutritional ration, it is necessary to use alternative sources of feed containing carbohydrates, proteins, fats, vitamins, and minerals (Soeparna *et al.,* 2013; Hafid *et al*., 2019).

Providing all the nutrients needed, especially micro-elements such as vitamins and minerals, is often challenging in feeding formulation. Therefore, the strategy of feeding supplements with certain micro-element content is needed, especially for the reproductive process. Microelements act directly or indirectly on sperm metabolism; if there is a deficiency, it can lead to poor sperm quality through defective spermatogenesis and by intense the body's oxidative stress (De Delis *et al*., 1996; Agarwal and Prabakaran, 2005; Garratt *et al*., 2013; Lipovac *et al*., 2016). Oxidative alterations provoke sperm dysfunctions, such as loss of motility and viability and impairment of sperm-oocyte fusion. Thus, antioxidants can play a crucial role in protecting male germ cells against oxidative damage, preventing the loss of motility and the decreased capacity for sperm–oocyte fusion (Aitken *et al*., 1993; Fraga *et al*., 1991; Agarwal *et al*., 2003). Feed supplements as a source of vitamins and mineral functions as antioxidants could improve the quality of semen (Soeparna *et al*., 2013). Spermatozoa are especially susceptible to peroxidative damage because of their high polyunsaturated fatty acids. As they can produce highly reactive oxygen species (ROS) that can negatively affect fertility, prevention of oxidative damage is considered crucial for normal fertility (Angrimani *et al*., 2017).

A previous study found that mung bean sprouts contain vitamins E, C, riboflavin, niacin, folate, and minerals such as selenium, manganese, zinc, and iron (Soeparna *et al*., 2013; Diartha *et al*., 2016). Vitamin E in the mung bean sprouts is an antioxidant that protects different organs against oxidative stress and stabilizes the sperm membranes by complex formation (Raederstorff *et al*., 2015). Some studies have reported that vitamin E protects the testis from oxidative damage and improves sperm quality in goats, sheep, and rabbits (Yousef *et al*., 2003; Aydilek *et al*., 2004; Yue *et al*., 2010; Hong *et al*., 2010). The study aimed to investigate the effect of supplementation of mung bean sprouts and mineral mix on the semen quality of Bali bull.

Material and methods

Animal and diets

The study was conducted in the Technical Service Unit of the Livestock Breeding Center, Department of Food Crops, Horticulture, and Livestock of Jambi Province for three months. Twelve Bali bulls aged 4-5 years were used, with an average body weight of 397.50 ± 38.61 kg. Each bull was reared in an individual stall 1.5 x 2.5 m. The bull was fed basal diets with 70% grass and 30% commercial concentrate (called P-135). The chemical composition of grass, called P-135, is presented in Table 1.

Table 1. Nutrients composition of *P.purpureum*, calfeed P-135 and basal diets

Nutrients	*P. purpureum*	Calfeed P-135	Basal Diet
Dry matter, %	87.25	85.52	86.73
Crude protein, %	12.75	13.16	12.87
Crude fiber, %	22.00	11.78	18.93
Crude fat,%	1.5	5.58	2.72
Nitrogen free extract	48.57	58.92	51.68
Ash, %	15.18	10.56	13.79
TDN			

In an early study, the bull was given the basal diet for 14 days for an adaptation period. The diet treatments of Mung bean Sprouts (MBS) and Milton Block Minerals® (MBM) supplementation were P0: Basal diet + 0% MBS + 0% MBM (control), P1: Basal diet + 0.5 kg MBS + 0.25 kg MBM, P2: Basal diet + 1.0 kg MBS + 0.25 MBM, P3: Basal diet + 1.5 kg MBS + 0.25 kg MBM. The diet was given twice daily at 07.00 am and 2.00 pm for 30 days. The composition of Milton Block Minerals® is presented in Table 2.

Table 2. Composition of milton block minerals

Minerals	Composition per kg
Calcium	5%
Phosphor	2%
Natrium	70%
Cobalt	0.065 g
Copper	0.3 g
Ferrous	2.4 g
Magnesium	2.7 g
Manganese	0.75 g
Potassium	0.3 g
Zinc	0.5 g

Semen collection and evaluation procedures

The semen samples had collected after 30 days of the applied experimental rations, starting at 06.00 am. The semen collection had done once a week before the bulls fed. The semen was collected using an artificial vagina (AV) and evaluated macro-and microscopically according to the procedures (Arifiantini, 2012). The macroscopic evaluation included volume, color, pH, and consistency. Semen volume was determined by the graded collection tube as soon after collection. Meanwhile, color and viscosity are determined visually. pH was measured using a pH meter, and the concentration was measured using a spectrophotometer SpermaCue.

The microscopic evaluation includes mass activity, sperm progressive motility, sperm concentration, sperm viability, sperm morphology, and sperm plasma membrane integrity. The sperm's progressive motility (movement) had evaluated by transferring a drop of semen diluted with four drops of physiological NaCl on a warm slide (37°C), then covered by a cover glass and observed under a microscope at a magnification of 10x40 with 5-10 fields of view. The assessment can use a comparison with a total value of 10. If the active and inactive spermatozoa ratio is 5:5 or 7:3, the motility value is 50% or 70%.

The procedure for assessing viability and morphology was using eosin staining with the same observed, where one drop of semen and fours drop of 2% eosin were then homogenous, smeared, and dried above a heating plate for 10-15 seconds. At least the sperms have counted in 10 fields of the slides viewed randomly with a minimum number of cells > 200 cells. The sperm had classified as life (colorless) and dead (red color of head sperm). Sperm viability (%) = (total sperm life/total sperm) X 100 %. The sperm abnormality had prepared the same with viability observation, where diluting a drop of sperm with 8-10 drops of eosin without drying was then determined visually in 10 fields under a microscope at a magnification of 10 x 40. Sperm abnormality (%) = (abnormal sperm/total sperm) X 100%. Sperm membrane integrity assessment used hypoosmotic solution (HOS) prepared by mixing 0.9 g fructose and 0.49 g sodium citrate in 100 ml distillate water. A total of 10 micro milliliters of semen has added with 1 ml of HOS solution and incubated for 45 minutes at 37°C. Take 15 ml of a good sample and place it on a warm slide (37°C), covered with cover glass, and observe under a microscope at a magnification of 10x40. Sperm had been classified by seeing the presence or absence of coiled tails. Membrane plasma integrity (%) = (total sperm with coiled tails/total sperm) X 100%.

Statistical analysis

The effect of mung bean sprouts and mineral mix supplementation on semen volume, concentration, motility, viability, abnormalities, and plasma membrane integrity was analyzed using analysis of variance (ANOVA) in a Completely randomized Design (CRD). Comparisons among treatments were performed by applying Duncan Multiple Range Test (DMRT) with significant differences at $P<0.05$. The data were analyzed using the statistical software SPSS version 20.

Results

Macroscopic evaluation

The Macroscopic evaluation of semen is to know the early condition of semen quality. The visible semen evaluation result is shown in Table 3. Observation of semen consistency and color showed differences in semen consistency and color as influenced by MBS and MBM supplementation of Bali bull cows fed treatment P0, P1, and P2, P3.

Bali bulls fed the P0 and P1 treatments showed semen with liquid consistency and milky white color compared to P2 and P3 with thick and creamy white color. The pH of the semen obtained in this study varied from 6.70-6.73 and was not significantly ($P>0.05$) affected by the level of supplementation of MBS and MBM in the diets. However, the pH obtained is still in normal pH conditions. The volume of Bali bull semen was affected ($P<0.05$) by the increased supplementation of MBS and MBM in the diets. The semen volume of the Bali bull-fed diet of P2 and P3 was higher ($P<0.05$) than P0 and P1.

Table 3. Macroscopic Evaluation of Bali Bull Semen

Semen Characteristics	Treatments				Sig.
	P0	P1	P2	P3	
Consistency	Liquid	Liquid	Viscous	Viscous	
Color	White Milky	White Milky	Cream	Cream	
pH	6.70 ± 0.00	6.73 ± 0.06	6.70 ± 0.00	6.70 ± 0.00	0.441
Volume (ml)	4.87 ± 0.42^{b}	5.03 ± 0.06^{b}	6.57 ± 0.40^{a}	6.6 ± 0.53^{a}	0.001

(a-b) the letters differ in each row, indicating a significant difference at level P< 0.05.

Microscopic evaluation

The results of microscopic observation of the semen quality of Bali bulls is shown in Table 4. Microscopic observation of the semen quality of male Bali cows showed that supplementation of GBM and MBM significantly (P<0.05) increased semen concentration, motility, viability, and sperm membrane integrity.

Table 4. Semen Microscopic characteristic of Bali Bulls

Microscopic Characteristics	Treatments				Sig.
	P0	P1	P2	P3	
Concentration, 10^{6} ml^{-1}	871.33±130.01^{a}	907.33±31.64^{ab}	1056.67±56.36^{bc}	1110.00±110.53^{c}	0.034
Motility,%	50.00±00.00^{a}	60.00±11.30^{ab}	76.67±11.55^{c}	70.00±00.00^{bc}	0.009
Viability, %	71.49±3.18^{a}	73.74±2.09^{ab}	79.82±4.66^{b}	79.66±2.81^{b}	0.03
Abnormality, %	0.92±0.22	1.09±0.59	0.91±0.56	1.17±0.91	0.943
Plasma membrane integrity,%	66.95±4.20^{a}	69.82±1.00^{ab}	75.76±3.98^{c}	74.97±1.14^{bc}	0.020

(a-c) the letters differ in each row, indicating a significant difference at level P< 0.05.

Semen concentration, motility, viability, and sperm membrane integrity of Bali bulls fed P3 (basal diet + 1.5 kg GBM + 0.25 kg MBM) showed higher compared with P0, P1, but not different (P>0.05) with P2. Furthermore, GBM and MBM supplementation did not significantly (P>0.05) affect semen abnormality.

Discussion

Macroscopic evaluation

The semen color results are still classified as usual. Arifiantini (2012) and Prastiya *et al*. (2021) stated that the average standard color of cow and goat semen is generally milky white, cloudy white, creamy, yellowish, and grayish white to meet further examination criteria. Semen color in cattle is influenced by vesicular glands, which are accessory glands in the reproductive organs. MBS and MBM supplementation in the ration affects the color of semen produced in male Bali cows. The semen color of Bali bulls fed P2, and P3 was slightly creamier than P0 and P1. In addition, semen color can also be influenced by the quality of feed in the ration, especially the riboflavin content in MBS, where every 100 g of MBS contains 7% RDA of riboflavin. Thus, increasing MBS supplementation in the diet tends to cause the semen color to be creamier. Putri *et al*. (2020) found that riboflavin can affect semen color. A creamier semen color also affects the number of spermatozoa in the semen. According to Waluyo (2014), semen color can describe the interpretation of viscosity and concentration. Generally, the creamier the color of the semen, the thicker the consistency and the higher the concentration. Sperm motility is an essential factor in determining semen quality and fertilization ability. The chemical environment of semen can have a profound effect on sperm quality, such as the seminal plasma pH, and may contribute to male fertility (Zhou *et al*., 2015). The pH obtained in this study ranged from 6.7-6.73, which is still in normal conditions and has no effect on semen quality, especially sperm motility and concentration, which can reduce fertility Hafez (Arifiantini, 2012; Zhou *et al*., 2015; Hafez, 1993). Furthermore, Zhou *et al*. (2015) found that spermatozoa can be directly affected by semen pH. Acidic conditions have more effect on reducing sperm viability than alkaline. Bali cattle sperm have an optimum pH of 5.9-7.3 (Nirwana and Suparman, 2017). Meanwhile, sperm cells have an optimum pH to live well. Contri *et al*. (2013) found that sperm at low pH conditions between 6.0-6.5 had motility of 53.3%-60.7%. At high pH between 8.0-8.5, sperm have low motility of 33.9%-23.1%. Sperm showed good motility at pH optimum at pH 7.0-7.5 with motility of 66.8%-71.1%.

Semen volume was affected by factors such as; feeding quality, age of the bull, body weight, frequency, and months of semen collection (Suyadi *et al*., 2020). The semen volume per ejaculate varied from 4-10 ml, averaging 6 ml (Waluyo, 2014). The increasing semen volume of Bali Bull is affected by the supplementation of antioxidants and other nutrients supplied in the diet from mung bean sprouts and mineral mixes such as vitamin E, vitamin C, amino acids, Zn, and Cu (Soeparna *et al*., 2013). Geary *et al*. (2021) reported that Zn was affecting an increase in lactate dehydrogenase activity as the enzyme in the mitochondria sheath of the sperm for ATP production and effective in increasing the sperm volume and percentage.

Microscopic evaluation of Bali bull semen

The total concentration of sperm in the diet of P3 is strongly related to antioxidants and minerals contained in the mung bean sprouts and mineral mix, which are required for hormone reproduction, namely testosterone which has the function of forming and maturation spermatozoa in tubules seminiferous (Hafid *et al*., 2021). Meanwhile, Mulyani (2018) found that mung bean sprouts have a role as natural antioxidants because it contains vitamins E, C, and zinc. Furthermore, Martinelli *et al*. (2020) state that antioxidants can increase the concentration of semen. Supplementation, zinc, and other minerals contained in supplements also increase semen concentration, such as helping to regulate testosterone levels in the body. Testicular Zn is crucial for normal spermatogenesis and sperm physiology; it maintains genomic integrity in the sperm and fixes the attachment of sperm head to tail (Roy *et al*., 2013).

It had shown a positive effect of adding mung bean sprouts and mineral mix in the feeding of Bali bull on sperm motility. Mung bean sprouts and mineral mix have vitamin E and Se content and play a role in maintaining sperm motility and viability. Ratnani *et al*. (2020) classified vitamins E and Se as antioxidants that protect sperm membranes from damage caused by free radicals. The result of this study is consistent with the previous studies that indicate a positive effect of vitamin E, and Se increased sperm motility and viability (Ali *et al*., 2021; Ghafarizadeh *et al*., 2020). In supplementation, the mineral content of Zn in mung bean sprouts and the mineral mix has a role in increasing sperm quality, germination, and fertility (Fallah *et al*., 2018). Zn acts as an anti-inflammatory and is involved in the sperm's oxidative metabolism, and has many functions in sperm physiology, including effects on lipid flexibility and sperm membrane stabilization (Chia *et al*., 2000). The treatments of P2 and P3 were better than P0 and P1 in improving the quality of individual sperm motility. The minimum percentage of individual sperm motility is 70% to could be processed into frozen semen. So, when viewed from the average sperm motility, the treatments of P2 and P3 meet these requirements. Meanwhile, the sperm viability obtained in this study was still in the normal range of 60-80% (Hafez, 1993).

The sperm abnormality value obtained is still below 20% and is still included in the criteria for sperm to be used in the production of frozen semen (Waluyo, 2014). Sperm abnormalities could affect the total of sperm infertile, so it is necessary to use it as an indicator in the production of frozen semen for artificial insemination. Abnormal sperm count is one of the main factors that affect sperm quality because abnormal sperm are the cause of failure in fertilization. Abnormalities in the head shape make it difficult to penetrate the egg cell wall, while abnormalities of sperm in the tail will be delicate to reach the egg or ovum (Mulyani, 2018).

It shows a positive role of mung bean sprouts and mineral mix in the diets on plasma membrane integrity, one of which is the role of vitamin E and minerals that act as antioxidants. Siswandoko (2017), antioxidants can prevent and reduce lipid oxidation and prevent free radicals that can damage the sperm plasma membrane. In supplementation, the mineral mix containing sodium and potassium can maintain the functional integrity of the plasma membrane (Reis *et al*., 2014).

According to the findings, the study concluded that the treatments of P2 (basal diet + 1 kg mung bean sprouts + 0.25 kg mineral mix) effectively increased the sperm quality of Bali bulls cows. Therefore, mung beans combined with a mineral mix in the diets as a feed supplement is necessary to improve the quality of sperm.

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