



## Movement Patterns of Bali Bull (*Bos sondaicus*) Sperms After Supplementation of Micronutrients Zn, Selenium, and Vitamin E

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### ABSTRACT

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In ruminant livestock, it is very important to pay attention to the adequacy of micro minerals and vitamins, considering that minerals and vitamins are absolute and must be present in the feed so that the health and productivity of the livestock is not disturbed. To produce quality semen, vitamin E, selenium and zinc are needed. These micronutrients play a role in the spermatogenesis process which will have an impact on the quality of spermatozoa produced by male livestock. The aim of this study was to determine the effect of supplementation of micronutrient on sperm movement pattern of Bali bull. This study was used two Bali bull aged 4-5 years with a body weight of 300-350 kg. The study was divided into two periods of treatments and each period was carried out for three weeks. In the first period (control), experimental Bali bull was fed natural grasses and concentrate (1% of body weight). Furthermore, in the second period (treatment period), the feed was similar as the first period with the addition of a mixture of minerals (Zn and Se) and vitamin E. The volume, color, pH, viscosity and movement pattern of the bull's sperm were recorded. Likewise, sperm concentration, motility, viability and abnormality were measured. Fresh semen was diluted with Andromed® accordingly before subsequent processing. Data were analyzed using paired student t-test. The results of the study showed that the macroscopic quality of fresh semen of Bali bull improved after supplementation of micronutrient. The movement pattern of spermatozoa after administration of micronutrient supplements did not have a significant effect ( $P > 0.05$ ). It can be concluded that micronutrient supplementation in Bali bulls can improve macroscopic evaluation, abnormality, viability, concentration and maintained spermatozoa movement patterns.

**Keywords:** Bali bull, spermatozoa, micronutrient, semen quality, and movement pattern sperm.

## INTRODUCTION

One of the causes of low calving rates in cattle in tropical areas, including Indonesia, is the low quality of the bulls used. Low male quality causes failed fertilization and lengthened calving intervals. This problem will ultimately cause farmers losses, which will threaten the sustainability of the livestock business, and is of concern [1].

Several factors influence the age at which male puberty occurs, including social interactions, genetics, environment, nutrition, hormones, age, and experience, [2]. This puberty is the beginning of semen production in cattle. Semen quality itself is influenced by feed administered to the animal [3]. The nutritional content of feed influences spermatozoa production, gonadotropin hormone secretion, and the development of sexual behavior. The capacity of the testicles to produce spermatozoa and the hormone testosterone is influenced by the ability of the seminiferous tubules and Leydig cells or interstitial cells or stimulation and Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) [4].

The spermatogenesis process that produces high-quality semen requires the amino acids methionine, cysteine [5], and arginine [6], a fatty acid,  $\alpha$ -linoleic acid, vitamins A, C, and E, Zn, and Se [7]. Zn is important in producing several hormones, including testosterone and GnRH. An optimal feed and nutritional management program will impact reproductive health and gonadotropin secretion. The levels of energy, protein, minerals, and vitamins at each stage of livestock growth and reproduction are very important to maximize the fertility potential of the bull [8]. The quality of spermatozoa or spermatogenesis is improved through energy or protein balance, and mineral supplementation in bulls during the prepubertal period. Determining nutrition in male cattle requires information including the condition and body weight of the bull, type, and composition of feed, for example, dry matter, TDN, protein, and mineral sources [9].

Artificial Insemination (AI) program is a mating method that is more efficient in using male semen than natural mating. Furthermore, the success of AI is largely determined by the quality of the male's semen; in this case, macroscopic and microscopic. Apart from that, it is also determined by the movement pattern of spermatozoa because the movement pattern of spermatozoa itself greatly determines male fertility. This is very important for the capacitation process in the female reproductive tract. The movement pattern and distance traveled by spermatozoa in the female reproductive organ canal, to support high fertility, must be able to reach the target fertilization site and have the ability to fertilize egg cells. The importance of movement patterns in spermatozoa can also be seen in abnormalities in the normal shape of sperm, such as progressive, non-progressive, and immotile (not moving) movements [10].

Micronutrients play a role in spermatogenesis, impacting the quality of spermatozoa produced by male livestock. Vitamin supplementation acts as an antioxidant to break the chain of free radical capture in cell membranes. Micro minerals such as Zn and Se also play a role in livestock reproduction, especially bulls, and as antioxidants that protect spermatozoa from free radicals and external disturbances, thereby reducing damage and abnormal spermatozoa. Zn supplementation can significantly increase the motility of fresh or frozen spermatozoa, spermatozoa concentration, and the percentage of acrosome integrity [11].

This study aimed to determine the effect of supplementing micronutrients, Zinc, Selenium, and Vitamin E, on the movement patterns of Bali bulls.

## MATERIALS AND METHODS

This study was conducted from November 2023 to January 2024 at the Samata Integrated Farming System (SIFS), Samata, and the Laboratory of Animal Reproduction, Hasanuddin University, Makassar. This study was used two Bali bulls, aged 4-5 years old, with a body weight of 300 - 350 kg, and they were kept in an individual barn. The study was divided into two periods of experiments, and each period was conducted for three weeks. In the first period, the experimental bulls were fed with natural grasses and rice straw ad lib. They were also fed with concentrates (1% of body weight) (control).

Furthermore, in the second period, they were fed a diet similar to that used in the first period with an additional micronutrient mix (Zinc, Selenium, and Vitamin E). Before semen collection, the false mounting was conducted twice. Semen was collected by using an artificial vagina. The collected semen was observed macroscopically and microscopically. According to Septiani et al. [12], macroscopic evaluation consists of volume, color, pH, and viscosity. Microscopic evaluation consists of motility, viability, abnormality, and concentration [13]. Evaluation of movement patterns includes Distance Average Path (DAP), Distance Curvilinear (DCL), Distance Straight Line (DSL), Velocity Average Path (VAP), Velocity Curvilinear (VCL), Velocity Straight Line (VSL), Linearity (LIN), Wobble (WOB), Amplitude of Lateral Head Displacement (ALH), and Beat Cross Frequency (BCF) [14]. Evaluation of semen quality at each collection was conducted soon after collection. Sperm concentration was evaluated using the Photometer SDM 6. Computer Assisted Semen Analyzer (CASA) with Sperm Vision™ Version 3.7.5 software was used to determine the motility and movement patterns of sperm. At the same time, viability and abnormality were assessed in a trinocular microscope. Andromed® was used as an extender to dilute the fresh semen.

### Statistical Analysis

The data obtained were analyzed statistically using the T-test. Differences in sperm concentration, motility, viability, abnormality, and movement patterns of Bali bull sperm were analyzed using the paired Student's t-test with SPSS Version 29 software.

## RESULTS AND DISCUSSIONS

### Fresh Semen Quality

Table 1 shows the macroscopic and microscopic evaluation of Bali bull fresh semen before and after micronutrient supplementation. Volume, pH, smell, and color of the bull semen were not significantly different ( $P > 0.05$ ) between the two treatments. Mean volume per ejaculation, pH, and semen color in both groups were 3.2-4.8 ml, 6.3-6.4, and yellowish. The viscosity of the semen was medium-high.

These results were similar to those reviewed by Roy et al. [15]. They reported that the Zn mineral supplementation increases the ejaculate volume of several mammal species, such as bulls, goats, rabbits, and buffalo. The volume of semen treated with micronutrient supplementation can prevent a decrease in the number of reservoirs, in other words, micronutrient supplementation can prevent a decrease in livestock libido, because the amount

of storage is a reflection of the level of libido of the animal, but is not able to prevent a decrease in the volume of semen produced. The pH of Bali bull semen used in the study is normal. These results were similar to those reviewed by Mila et al. [16]. They reported that the pH greatly influences the survival of bull spermatozoa. Meanwhile, according to reviews by Roy et al. [15], Zn supplementation does not affect pH. The colour of Bali bull semen is normal. Results were similar to those reviewed by Isnaini and Fazrien [17]. They reported that the semen color of Bali cattle was normal, white, and yellowish. The smell is normal, typical of livestock sperm. This is the opinion of Eni [18], who states that normal bull semen generally has a distinctive smell accompanied by the smell of the livestock itself. This smell indicates that the semen is in a normal condition and there is no contamination [19]. The higher the Semen, the higher the concentration of the Semen produced. Results were similar to those reviewed by Nubatonis et al. [20], which showed that a higher semen viscosity has a higher spermatozoa concentration value than a medium semen viscosity.

Table 1. Macroscopic and microscopic evaluation of Bali bull fresh semen before and after micronutrient supplementation

Parameter	Micronutrien Supplementation	
	Before	After
Macroscopic Evaluation		
Volume (ml)	3.2 ± 2.28	4.8 ± 1.20
pH	6.3 ± 0.17	6.4 ± 0.08
Color	Yellowish	Yellowish
Viscosity	Medium	High
Microscopic Evaluation		
Motility (%)	85.74 ± 5.17	84.95 ± 1.30
Viability (%)	55.20 ± 17.16	63.20 ± 50.33
Abnormality (%)	72.80 ± 22.21	45.80 ± 20.09
Concentration (10 <sup>6</sup> /ml)	1.251 ± 0.307	1.543 ± 0.560

Table 1 shows that motility, viability, and abnormality of Bali bulls before and after the supplementation of micronutrients were similar ( $P > 0.05$ ). After micronutrient supplementation, the percentage of motility was increased in small amounts. This is due to the short period of micronutrient supplements, which is only three weeks. These results were similar to those reviewed by Sabhapati et al. [21]. They reported that the organic Zn supplementation increases semen volume and sperm concentration and improves the integrity of the bull spermatozoa membrane. Zn supplementation (80 ppm) in the bulls for 90 days increased all parameters (volume, motility activity, individual motility, number of live sperm, acrosome integrity, and Hypo Osmotic Swelling Test (HOST) for determining sperm quality by evaluating the membrane integrity of spermatozoa).

Viability is the percentage of spermatozoa cells that are alive [22]. According to Khairi et al. [23], supplementation can also be done by adding micro minerals to feed, which can increase feed consumption and semen quality. Micro minerals that can influence the reproduction of bulls are the mineral Zinc (Zn) and the mineral Selenium (Se). Observation of spermatozoa viability can be used as an indicator of the structural integrity of spermatozoa membranes [24].

Evaluating sperm abnormality in bulls is important for semen analysis because it greatly affects semen quality. The spermatozoa abnormality in this study were included in the normal category. Opinion of Blegur et al. [25] bull semen generally contains between 5% and 35% abnormal spermatozoa.

Evaluation of spermatozoa concentration is very important because this factor describes the properties of sperm, which are used as one of the criteria for determining semen quality. Concentration values are included in the normal category. The opinion of Aisah et al. [26], semen concentration varies from 1,000 to 1,800 × 10<sup>6</sup>/ml.

### Movement Patterns Bali bull

Table 2 evaluates the movement patterns of Bali bulls before and after micronutrient supplementation. The Distance Curve (DCL), Distance Average Path (DAP), and Distance Straight Line (DSL) of the bull semen were not significantly different ( $P > 0.05$ ) between the two treatments.

Table 2. Movement patterns of the Bali bull before and after micronutrient supplementation

Parameter	Micronutrien Supplementation	
	Before	After
DCL (µm)	46.26 ± 8.32	40.37 ± 14.88
DAP (µm)	23.55 ± 3.47	22.62 ± 1.62
DSL (µm)	14.95 ± 1.58	14.59 ± 1.08
VCL (µm/det)	112.89 ± 21.54	110.62 ± 10.87
VAP (µm/det)	57.83 ± 9.08	55.77 ± 3.88
VSL (µm/det)	37.00 ± 4.17	36.25 ± 2.55
STR (VSL/VAP)	0.64 ± 0.03	0.64 ± 0.00
LIN (VSL/VCL)	0.33 ± 0.02	0.33 ± 0.01
WOB (VAP/VCL)	0.51 ± 0.02	0.50 ± 0.02
ALH (µm)	6.26 ± 0.37	6.21 ± 0.29
BCF (Hz)	21.08 ± 1.31	20.84 ± 0.52

This study examines several factors, such as genetics, age, body weight, feed, environmental conditions, and the frequency of short-term administration of micronutrients. This study was supported by Feradis [27], Herry [28], Bindari, et al. [29]. Motility (movement) occurs due to a mitochondrial sheath in the middle of the spermatozoa's tail, which acts as a site for energy synthesis for movement. Movement of spermatozoa tails depends on ATP production by mitochondria, with the metabolic energy source coming from fructose contained in the diluent. The mineral Zn can provide movement energy for sperm so that sperm are more active. The mineral Zn can help the maturation of spermatozoa, increase androgen levels in blood plasma, and is associated with normal spermatogenesis activity, resulting in increased motility. Supplementation of organic Zn also affects the energy synthesis process for spermatozoa

motility. Zn supplementation significantly increases the motility of fresh or frozen sperm. Organic Zn supplementation increases semen volume and sperm concentration and improves the integrity of the male spermatozoa membrane. Zn supplementation (80 ppm) in bulls for 90 days increased all parameters (volume, motility activity, individual motility, number of live sperm, acrosome integrity, and Hypo Osmotic Swelling Test (HOST) for determining sperm quality by evaluating the membrane integrity of spermatozoa).

Velocity Curvilinear (VCL), Velocity Average Path (VAP), and Velocity Average Path (VAP) of the bull semen were not significantly different ( $P>0.05$ ) between the two treatments. The motility pattern of this study was in the transition group between hyperactive and non-hyperactive. This study was supported by Susilawati [30], there are three patterns of spermatozoa motility, namely hyperactivation with a VCL value of  $\geq 100 \mu\text{m}/\text{second}$ ,  $\text{LIN} < 60\%$ , and  $\text{ALH} \geq 5 \mu\text{m}/\text{second}$ , and non-hyperactivation if it has a VSL value of  $\geq 40 \mu\text{m}/\text{sec}$ ,  $\text{LIN} \geq 60\%$ ,  $\text{ALH} < 5 \mu\text{m}/\text{sec}$ , and both transition groups. The results of testing hyperactivation motility patterns using CASA can be a good effort to predict the fertilization ability of spermatozoa. Hyperactivation of spermatozoa is required immediately before the acrosomal reaction in vitro and movement in the oviduct during fertilization. The motility of hyperactivated sperm is positively correlated with the ability to penetrate the zona Pellucida. The fertility rate of the hyperactivation group was higher than that of the non-hyperactivation group. This can strengthen the suspicion that the mineral Zn plays a role in increasing the sperm motility characteristics of Bali bulls, so that they experience hyperactivity, which is likely to produce more fertile sperm.

Percentages of Linearity (LIN), Straightness (STR), and Wobble (WOB). There was no significant difference before and after micronutrient supplementation. The lateral distance of sperm head movement in each average path (ALH) and sperm movement frequency (BCF) were not significantly different ( $P>0.05$ ) before and after micronutrient supplementation.

## **CONCLUSIONS**

It can be concluded that supplementation with micronutrients such as Selenium (Se), Zinc (Zn), and Vitamin E for three weeks in Bali bull, can improve macroscopic evaluation, abnormality, viability, concentration and maintained spermatozoa movement patterns.

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## **AUTHORS' CONTRIBUTIONS**

All authors have contributed in conceptualized the study, drafted and revised the manuscript, conducted experiments, analysed data, and read and approved the final manuscript.

## **CONFLICT OF INTERESTS**

The authors declare that there is no conflicts of interest in doing the study as well as writing this article.

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