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# The Effects of Mineral Mix Supplementation on Reproductive Performance of Bali Bulls

**Alfian A. M.[1], M. Yusuf[2*], A. Ako[2], A. L. Toleng[2], A. M. Diansyah[2], M. F. Amrullah[2] R. Rahmat[2] and H. Hasrin[3]**

[1] *Post-graduated of Animal Science and Technology, Faculty of Animal Husbandry, Hasanuddin University, Jl. Perintis Kemerdekaan 10 Tamalanrea Makassar, South Sulawesi, Indonesia*

[2] *Faculty of Animal Husbandry, Hasanuddin University, Jl. Perintis Kemeredekaan Km. 10 Tamalanrea Makassar, South Sulawesi, Indonesia. 90245*

[3] *Faculty of Vocation, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea Makassar, South Sulawesi, Indonesia. 90245*

**Abstract**

THIS study aims to evaluate the effectiveness of mineral mix supplementation on the reproductive performance of Bali bulls. This study used 10 Bali bulls. The Urea Molasses Multi-Nutrient Block is manufactured using two raw material formulas (T1 = non-supplementation of mineral mix and T2 = with supplementation of mineral mix). The assessment in this study was regarding testosterone hormone profiles, scrotal circumference, and libido. The evaluation of sperm quality was analysed by macroscopic (volume, pH, colour, odour, and consistency) and microscopic (motility, concentration, abnormality, and live sperm). The result showed the testosterone profile of Bali bulls that the morning testosterone profile in T1 was significantly ($p < 0.05$) lower than in T2. However, the daytime testosterone profile in the T1 did not differ significantly ($p > 0.05$) from T2. The scrotum circumference of Bali in T1 did not differ significantly ($p > 0.05$) from T2. However, the libido of Bali bulls T1 was significantly ($p < 0.05$) lower than in T2. The quality of sperms of Bali bulls, in terms of color, odor, and consistency, showed the same results. The volume of Bali bulls in T1 did not differ significantly ($p > 0.05$) from T2. However, pH in T1 was significantly ($p < 0.05$) higher than in T2. The motility in T1 was significantly ($p < 0.05$) lower than in T2. Likewise, live sperm, concentration, and abnormality did differ significantly between the two treatments ($p < 0.05$). Based on the results of the study, mineral mix supplementation can improve the reproductive performance of Bali bulls. Furthermore, the increase is due to the content of mineral mix supplemented in UMMB. Therefore, it is necessary to improve the reproductive ability and reproductive performance of Bali bulls.

**Keywords**: Bali Bulls, Supplementation, Mineral Mix, UMMB, Reproductive Performance, Sperm.

## Introduction

The productivity of beef cattle is one of the main factors in determining the business's success in the livestock industry [1] and the bull is an important aspect. In Indonesia, Bali cattle is one of the cattle breeds that plays a pivotal role in increasing cattle population in Indonesia, representing one of the most promising local Indonesian cattle breeds [2]. Bali bulls often face challenges in maintaining optimal reproductive performance [3]. A Breeding Soundness Examination (BSE) is conducted to guarantee the quality of bulls. This is a standard procedure utilized to evaluate the reproductive suitability of bulls. BSE includes various aspects such as physical evaluation, examination of reproductive organs, sperm quality, and general health status [4]. However, BSE results are influenced by various factors, including the nutritional status of the bulls, which is often less than optimal. Nutrition is critical in maintaining and enhancing the reproductive performance of bulls [5].

Deficiencies in essential minerals have been linked to adverse effects on reproductive health, including a decline in sperm quality and libido [6]. Several minerals, including copper, zinc, and manganese, have been demonstrated to influence spermatogenesis, hormone synthesis, and the functioning of reproductive organs [7]. Therefore, ensuring adequate mineral intake is very important to

*Corresponding authors: Muhammad Yusuf, E-mail: myusuf@unhas.ac.id Tel.: +628124727802
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maintain the reproductive performance of Bali bulls. Another method of treating mineral deficiencies is through the administration of mineral supplementations [8]. This supplementation is to rectify mineral deficiencies that should be addressed through daily feed. Supplying mineral mix will enhance, sperm quality, elevate libido, and augment BSE results [9].

In this context, testosterone is of significant importance regarding male reproductive function. Testosterone is involved in the development of secondary sexual characteristics, sperm production, and increased libido [10]. Low testosterone levels are often associated with decreased reproductive quality, so managing this hormone level is one of the focuses of livestock reproduction programs. Several studies have shown that mineral mix supplementation can positively increase testosterone levels in the blood. Minerals such as zinc play a role in synthesizing and regulating the testosterone hormone, which can improve overall reproductive performance [11]. Thus, mineral mix supplementation not only has a direct impact on sperm quality but also through increasing testosterone levels. In Bali bulls, which have adapted to tropical environments but often lack mineral intake through natural feed, mineral mix supplementation becomes increasingly important [12]. This mineral deficiency can directly impact reproductive health, so appropriate mineral supplementation is expected to improve the reproductive performance of bulls, including increasing BSE results and insemination success rates [13].

Various studies [14] have evaluated the effectiveness of mineral mix supplementation on the reproductive performance of bulls, including Bali bulls. In general, these studies showed an increase in sperm quality and libido, results after supplementation [15]. This supports the assumption that mineral mix supplementation is an effective solution for increasing the productivity of bulls [16]. However, implementing mineral mix supplementation in the field is challenging. Factors such as the proper dosage, the type of minerals used, and interactions with other nutrients must be carefully considered. In addition, regular monitoring of the nutritional status and reproductive health of bulls is needed to ensure the success rate of this supplementation program [17]. Therefore, the study is to assess the efficacy of mineral mix supplementation on the reproductive performance of Bali bulls.

## Materials and Methods

This study was carried out from January to July 2024 at the Samata Integrated Farming System in Samata Village, Sumbaopu District, Gowa Regency (collection of semen), Laboratory of Animal Reproduction and Semen Processing Unit, Faculty of Animal Science, Hasanuddin University, Makassar (processing and analysis of semen) and the Laboratory of Physiology, Faculty of Veterinary Medicine, Syiah Kuala University, Aceh (testosterone assay). This study used10 Bali bulls aged between 3 and 5 years, with an average weight ranging from 150 to 300 kg. The bulls were housed in individual stalls and fed a diet consisting of 80% forage and 20% concentrate. The Animal Ethics Committee of Hasanuddin University, Makassar, Indonesia approved all procedures conducted in the study.

*Treatment and Supplementation of Mineral-mix*

A ten bulls used in the present study were arranged and divided into two groups; five bulls for each group; T1 = without supplementation of mineral mix and T2 = supplementation of mineral mix. The mineral mix was made in the form of Urea Molasses Multi-Nutrient Block (UMMB) after modification with the addition of minerals, which is given for 60 days the following procedure of [18]. The materials of UMMB in two treatment groups are shown in Table 1. Furthermore, the contents of minerals (*Trypi*, Indonesia) are presented in Table 2. The bulls were fed (licking) UMMB block approximately 500g per day.

*Testosterone hormone profiles*

Testosterone hormone profiles were assessed according to the methodology outlined by [19]. Blood samples, up to 5 ml each, were collected in the morning and afternoon via the jugular vein. Subsequently, the plasma was adjustedto centrifugation at 1005,4 xgfor 10 minutes, after which plasma was collected and stored at -20°C until the testosterone hormone test was conducted. The enzyme-linked immunosorbent assay (ELISA) method was employed to profilethe testosterone hormone.

*The assessment of scrotal circumference*

The assessment of scrotal circumference followed the procedure of [20], carried out during the day using a measuring tape to measure the widest part of scrotum.

*The assessment of libido*

The assessment of libido followed the procedure of [21], which was carried out before semen collection in the morning. The bulls are approached by the teaser and libido behaviour is observed. The libido scores are presented in Table 3.

*Collection and evaluation semen*

Semen collection was according to the procedure of [22], which was carried out in the morning using an artificial vagina twice a week. Semen quality assessment was evaluated macroscopically (odor, color, volume, pH, and consistency) and

microscopically (, abnormality, concentration, motility, and live sperm rate).

*Macroscopic Evaluation*

Volume was measured by reading the scale on the collection tube, while colour was assessed visually, ranging from milky white to creamy. Odor assessment is the inhalation of the characteristic odor of semen [23]. Semen consistency was evaluated by tilting the collection tube, returning it to an upright position and timing how long it took for the semen to settle back at the bottom. The consistency was categorized into three types: dilute (semen quickly settles), medium (semen slowly settles with some residue on the tube wall), and thick (semen takes a long time to settle and leaves significant residue on the tube wall). The pH was measured using pH indicator paper, ranging from of 6.0 to 8.0 [24].

*Microscopic Evaluation*

*Sperms Motility*

Spermatozoa motility was assessed by placing ten microliters of the sperm suspension onto a glass slide. The motility was analysed using the computer-assisted semen analysis (CASA) system with Vision Version™ 3.7.5 software (Minitub, Germany) [25].

*Sperms Concentration*

The The concentration of spermatozoa was measured using a photometer (SDM 6, Minitub, Germany). First, a cuvette filled with 3 ml of physiological NaCl solution was placed into the device with the marked line facing forward, and the zero button was pressed to calibrate it. The cuvette was then removed and replaced with another cuvette containing the same physiological NaCl solution but with the addition of 30 µl of fresh semen. After pressing the result button, the spermatozoa concentration was displayed as the number of spermatozoa per ml [26].

*Live and Abnormality Sperm Rate*

The assessment of live and abnormalities sperms were conducted using a specific method: ten microliters of semen were mixed with ten microliters of 2% Eosin on a glass slide. The mixture was then examined under a trinocular microscope (Primo Star, Zeiss, Germany) at 400x magnification, with the analysis performed using Indomicro View 3.7 software [27]. Spermatozoa were categorized as either dead or alive based on their colouration dead spermatozoa appeared red, while live ones remained colourless. Abnormalities in spermatozoa, such as severed tails, broken tails, or irregular head shapes, were also noted. Each sample included observations of at least 200 sperm cells [28].

*Statistical analysis*

The data in this study were expressed as mean values with corresponding standard deviations. The statistical significance of differences in scrotal circumference, libido, semen quality, and testosterone levels, was determined using a t-test. A p-value of less than 0.05 was considered to indicate statistical significance. All analyses were performed using IBM SPSS Statistics software (version 25).

## Results

### Testosterone Hormone Profile

Figure 1 shows the testosterone profile of Bali bulls at different feed supplementations. Statistical analysis showed that the morning testosterone profile in T1 was significantly ($p < 0.05$) lower than in T2 (3.15 ng/ml vs. 6.75 ng/ml). However, the daytime testosterone profile in T1 did not differ significantly ($p > 0.05$) from T2 (0.93 ng/ml vs. 2.76 ng/ml). Testosterone hormone profile of Bali bulls with different supplemented Feeds can be seen in Figures1.

*Scrotal Circumference and Libido*

Fig. 2 shows the scrotum circumference of Bali bulls at different feed supplementations. Statistical analysis showed that scrotal circumference in T1 did not differ significantly ($p > 0.05$) from T2 (20.5 cm vs. 21.78 cm). However, figure 3 shows the libido of Bali bulls at different feed supplementations. Statistical analysis showed that libido in T1 was significantly ($p < 0.05$) lower than in T2 (2.4 vs. 3.8). Scrotal circumference and libido of Bali bulls with different supplemented feeds can be seen in Figures2 and 3.

*Semen quality*

Table 4 shows the quality of sperms of Bali bulls with different supplemented feeds. The colour, odour, and consistency showed the same results. Statistical analysis showed that volume in the T1 did not differ significantly ($p > 0.05$) from T2 (2.47ml vs. 3.17ml). However, pH in T1 was significantly ($p < 0.05$) higher than in T2 (6.64 vs. 6.4). The motility in T1 was significantly ($p < 0.05$) lower than in T2 (68% vs. 89.76%). Likewise, viability (63.33% vs 87.39%), concentration (0.160×109/ml vs 1.127×109/ml), and abnormality (17% vs 12.45%) did differ significantly between the two treatments ($p < 0.05$). The quality of Bali bull semen with different supplemented feed can be seen in Table 4.

## Discussion

In Mineral mix supplementation in cattle, especially Bali bulls, has become the research focus to improve reproductive performance. This supplement is expected to recover Bull soundness examination results by various aspects of reproductive health, such as libido, testosterone hormone, and semen quality. Several studies have shown that mineral mix supplementation can significantly affect several of these parameters, although not on all physiological aspects of Bali

bulls [29]. Breeding Soundness examination (BSE) is a comprehensive evaluation of their reproductive health, including assessment of physical condition, agility, and reproductive organ function. Providing mineral mix supplements has improved the results of BSE. This is because essential minerals such as zinc and magnesium in the supplement can improve muscle and nerve function and the quality of sperm produced [30].

Testosterone is the chief hormone that regulates the development of secondary sexual characteristics in bulls and plays vital role in sperm production and libido. Mineral mix supplementation has been shown to increase testosterone levels in bulls (Figure1), which in turn contributes to increased libido and semen quality. In addition, adequate mineral intake influences libido or sexual drive in bulls [31]. Research showed the intake of mineral mix supplementation has higher libido that non-supplements (Figure 2). The mineral content, such as zinc, plays a role in the composition of hormones related to libido, including testosterone. Semen quality is an indicator that evaluates bulls' reproductive ability [9]. Providing mineral mix supplements to Bali bulls has improved semen quality, especially in concentration, motility, viability, abnormalities, and pH (Table 4). Minerals such as zinc play a role in maintaining the integrity of the sperm membrane and protecting sperm from oxidative damage, thereby increasing the chances of fertilization [32]. Copper and manganese are important for a bull's productivity, affecting sperm quality and reproductive health. Copper supports hemoglobin synthesis and reproductive organ function, while manganese plays a role in testosterone production and sperm quality. Lack or excess of either can reduce a bull's fertility [14].

Supplementation of mineral mix has many benefits, but research shows that not all physiological aspects of bulls are affected [33]. One aspect that did not show significant changes ($p>0.05$) was scrotal circumference (Figure 3). Scrotal circumference is a physical indicator related to sperm production capacity, but mineral supplementation did not significantly affect this parameter. This may be due to genetic factors being more dominant in determining scrotal circumference than supplementation's influence. Scrotal circumference in Bali bulls may have reached an optimal size according to their genetics, so adding mineral supplements does not provide significant additional effects [34]. Overall, mineral mix supplementation in Bali bulls can improve several aspects of reproductive health, such as testosterone hormone profile, libido, and semen quality. However, its effect on scrotal circumference is limited, indicating that this supplementation is more effective on other aspects than physical effects such as scrotal size [35]. However, this study has several limitations, including the duration of supplementation, which may not be long enough to observe long-term effects, and the absence of isolation of the effects of each mineral mix. In addition, individual variation in response to supplementation and the potential long-term side effects of mineral accumulation have not been evaluated.

## Conclusion

Based on the results of the study, mineral mix supplementation can improve the reproductive performance of Bali bulls. Furthermore, the increase is due to the content of mineral mix supplemented in UMMB. Therefore, it is necessary to improve the reproductive ability and reproductive performance of Bali bulls.

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*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

*Ethical of approval*

The Animal Ethics Committee of Hasanuddin University, Makassar, Indonesia approved all procedures conducted in the study.

**TABLE 1. The Formulation of Urea Molasses Multi-Nutrient Block (UMMB)**

| Material | Treatment | |
|---|---|---|
| | T1 | T2 |
| **Rice bran (%)** | 43 | 40 |
| **Molasses (%)** | 35 | 32 |
| **Urea (%)** | 7 | 7 |
| **Salt (%)** | 7 | 7 |
| **Cement (%)** | 8 | 8 |
| **Mineral Mix (*Trypi*) (%)** | 0 | 6 |
| **Total** | 100 | 100 |

**TABLE 2. Composition of mineral mix (*Trypi,* Indonesia)**

| Nutrients | Content |
|---|---|
| **Carbohydrate (%)** | 0.38 |
| **Protein (%)** | 0.27 |
| **Fat (%)** | 0.27 |
| **Ca (%)** | 0.09 |
| **Cu (%)** | 0.002 |
| **P (%)** | 0.10 |
| **Fe (%)** | 0.008 |
| **Mg (%)** | 0.002 |
| **K (%)** | 0.048 |
| **Zn (%)** | 0.0003 |
| **Na (%)** | 17.06 |
| ***Miristat* (%)** | 0.55 |
| ***Arachidonat* (%)** | 0.33 |
| ***Palmitat* (%)** | 3.30 |
| ***Behenat* (%)** | 1.42 |
| ***Streat* (%)** | 1.21 |
| ***Ernsat* (%)** | 0.74 |
| ***Oleat* (%)** | 0.45 |
| ***Lighnoserat* (%)** | 0.38 |

**TABEL 3. Score of libido**

| Score | Description |
|---|---|
| **1+** | Bull show no libido response. |
| **2+** | Bull show approach, lick, Flehmen, and Erection but do not mount Teaser |
| **3+** | Bull show approach, lick, Flehmen, Erection, and Mounting but do not ejaculate |
| **4+** | Bull show approach, lick, Flehmen, Erection, Mounting, and ejaculation but do not show a sexual response after |
| **5+** | Bull show approach, lick, Flehmen, Erection, Mounting, and ejaculation and again show a sexual response after ejaculation |

**TABEL 4. Bali bulls semen quality with different supplementation feed**

| | Parameter | T1 | T2 |
|---|---|---|---|
| **Macroscopic** | Colour | Creamy | Creamy |
| | Odor | Typical | Typical |
| | Consistency | Medium | Medium |
| | Volume(ml) | 2.47±0.5 | 3.17±1.44 |
| | pH | $6.64±0.12^{a}$ | $6.4±0.12^{b}$ |
| **Microscopic** | Motility (%) | $68±2.65^{a}$ | $89.76±2.30^{b}$ |
| | Viability (%) | $63.33±5.77^{a}$ | $87.39±4.22^{b}$ |
| | Concentration($\times 10^{9}$/ml) | $0.160±0.02^{a}$ | $1.127±0.31^{b}$ |
| | Abnormality (%) | $17.00±1.00^{a}$ | $12.45±3.54^{b}$ |

$^{a,b}$The mean ± SD with different superscript letters differs significantly from each other ($p < 0.05$)

T1= UMMB non-supplementedmineral mix

T2= UMMB supplemented mineral mix

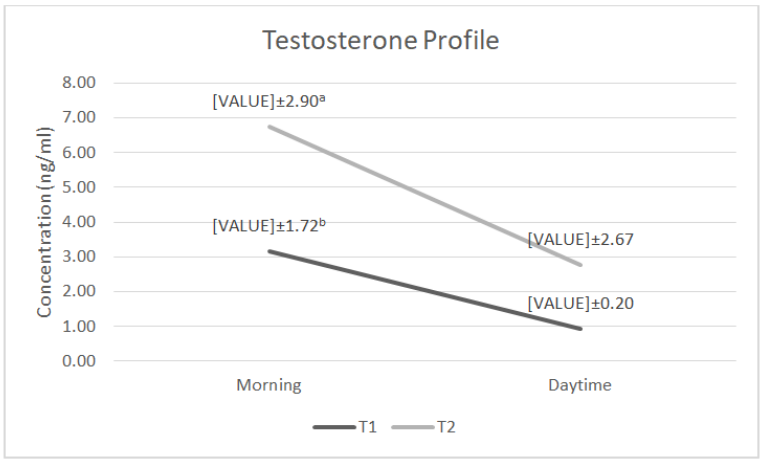


**Fig. 1. Testosterone hormone profile of Bali bulls with different supplemented**

[a,b]The mean ± SD with different superscript letters differs significantly from each other ($p < 0.05$).
T1= UMMB non supplemented mineral mix
T2= UMMB supplemented mineral mix

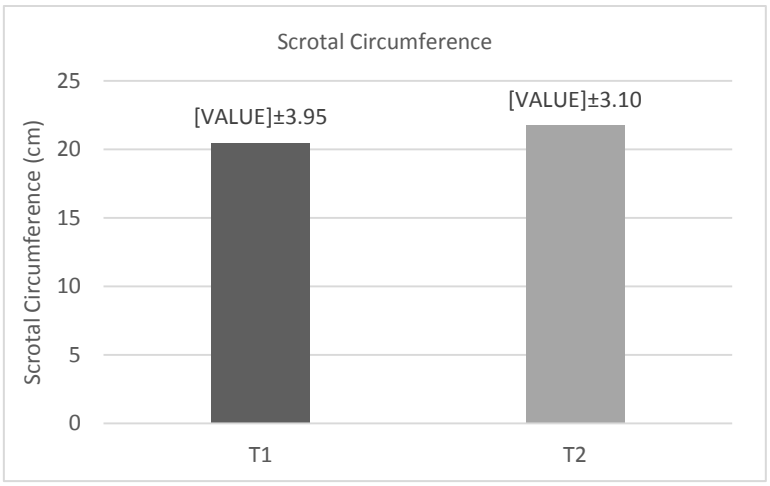


**Fig. 2. Scrotal circumference of Bali Bulls with different supplemented feeds**

T1= UMMB non supplemented mineral mix
T2= UMMB Supplemented mineral mix

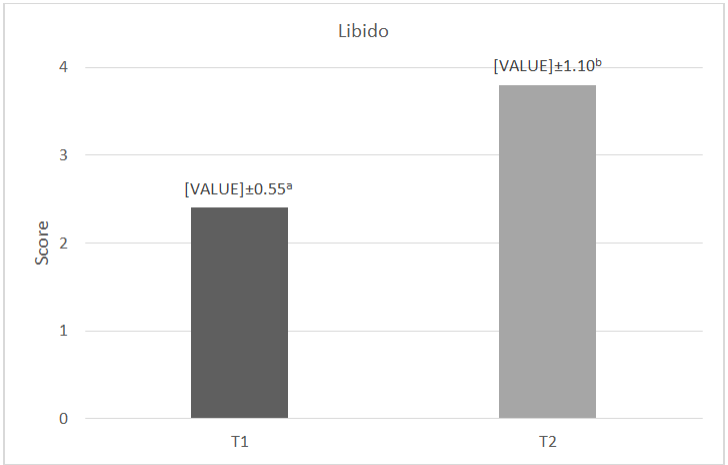


**Fig. 3. Libido of Bali Bulls with different feeds**

[a,b] The mean ± SD with different superscript letters differs significantly from each other ($p < 0.05$)
T1= UMMB non Supplemented mineral mix
T2= UMMB Supplemented mineral mix

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