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Supplementation of Moringa (*Moringa oleifera*) leaf meal block on the quality of Bali bull semen

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Abstract. The low calving rate of Bali cattle followed by a decrease in productivity is related to the low quality of semen produced by a male as a genetic carrier for their offspring. This study aimed to determine the effect of Moringa (*Moringa oleifera*) leaf meal block on the semen quality of Bali bull. This study was using 10 Bali bulls aged from 1.5 to 2 years which were divided into two treatment groups. Each group consisting of 5 bulls that were supplemented with Moringa leaf block flour in the diet (P1) and the other 5 bulls as control, P2 (without treatment). The parameters measured in this study were the percentage of microscopic evaluation (mass motility, individual motility, progressive motility). Each parameter in each treatment was compared using the T-test (independent sample t-test). The results of this study indicate that the macroscopic evaluation of semen in P1 was a slightly thick consistency, a slightly milky white color, a distinctive odor, while in P2 it is moderate consistency, slightly translucent color, and specific odor. The volume and pH at which the mean P1 was 1.23 ± 0.57 , 6.86 ± 0.33 , at P2 was 1.44 ± 0.56 , 7.05 ± 0.39 , respectively ($P > 0.05$). The results of this study showed that microscopic semen evaluation of bulls supplemented with Moringa leaf block flour in the diet (P1) had significantly ($P < 0.05$) higher than without supplementation (P2). Microscopic evaluation of semen on P1 showed that the mean values of individual motility, concentration, and abnormality were 80.65 ± 5.26 , $1.156 \times 10^6/\text{ml}$, and 17.31 ± 3.60 , respectively, and P2 were 73.23 ± 7.36 , $724 \times 10^6/\text{ml}$, and 24.10 ± 6.34 , respectively. The mean values of mass motility, progressive motility and viability of P1 were (++) 80%, 67.20 ± 6.32 , 91.58 ± 7.93 , respectively and P2 were (++) 70%, 60.19 ± 7.49 , 88.08 ± 11.34 , respectively. It can be concluded that supplementation of Moringa leaf block in the diet of Bali bulls can improve the quality of semen.

1. Introduction

The low calving rate in the population of cattle is usually affected by multi factors that influence reproductive performance. One of the factors is bull fertility in which has a vital role in reproductive outcomes. Every individual bull has their ability to fertilize the oocyte which describes their potential to have a lot of offspring during their life. A high potential of a bull is affected by many factors including feeding and/or supplementation of such ingredient in the feeding. Better feeds in the diet as well as those feeding meet the requirement needed by the bull will subsequently allow the reproductive organs of the bull to produce high-quality semen. On the other hand, bulls are fed low quality of feeding, even they are having a good genetic to produce high-quality semen.



Low-quality semen produced by a bull will decrease productivity and finally reduces the possibility to achieve an optimum population in a certain area. The potential of the bull can be assessed based on the quality of the semen produced. The quality of semen can be assessed by evaluating the macroscopically and microscopically using special equipment. In order to have high-quality semen during the assessment, the bull should have a good condition when semen is collected. High-quality semen can be achieved if it is fed normally according to the requirements. However, supplementation of feed in the feeding might increase the quality of semen. This due to that high-quality semen determine the success rate of the reproductive process.

The success of the reproductive process is determined by the condition of the bull. The most important male assessment is the quality of semen produced by the male to fertilize ovum from female cattle [1]. The low quality of semen will have a negative impact on reducing the conception rate, thereby reducing the calving rate [2]. Therefore, to achieve high-quality semen, it is hypothesized that supplementation of feed especially Moringa (*Moringa oleifera*) leaf meal block in the feeding increase the quality of bull semen. This study aimed to determine the effect of Moringa leaf meal block on the semen quality of Bali bull.

2. Materials and methods

2.1. Materials

This study took place at the Samata Integrated Farming System (SIFS), Gowa Regency and the Semen Processing Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The tools used in the study consisted of an artificial vagina, microscope with Computer-Assisted Sperm analysis (CASA) software, and spectrophotometer. The main material used was semen that were collected from Bali bulls aged 1.5 to 2 years old and blocks of Moringa flour. Supporting materials included in this study were moringa flour, salt, minerals, molasses, urea, warm water at 40°C, Vaseline, 70% alcohol, litmus paper, andromed, aquabidest, object-glass, and cover glass.

2.2. Methods

A total of 10 Bali bulls aged 1.5-2 years old, as semen producers were divided into two treatment groups. Group 1 consisted of 5 bulls and was treated with supplementation of Moringa leaf flour blocks in the diet (P1) and the other 5 bulls as control (without treatment; P2). The semen collection was carried out twice a week using an artificial vagina in the morning time at about 07.00 a.m. After obtaining semen from each bull in both treatments, the semen was then evaluating macroscopically (volume, pH, color, consistency, odor), and microscopically (mass motility, individual motility, progressive motility) in the laboratory.

2.3. Parameters and data analysis

The parameters measured in this study were evaluation of semen macroscopically (volume, pH, color, consistency, odor), and microscopically (mass motility, individual motility, progressive motility). The data obtained in this study were tabulated in an Excel program including data on mass motility, individual motility, and viability. Two treatment groups (P1 and P2) were compared using a comparative t-test (t-test) with an independent sample design.

3. Results and discussion

3.1. Macroscopic evaluation of Bali bull fresh semen at different treatments

Macroscopic evaluation of Bali bulls' semen with and without supplemented with Moringa leaf meal block are shown in table 1. Based on table 1, the consistency, color, and smell of semen in the group of bulls without supplemented with Moringa leaf powder block had a slightly clear consistency and distinctive odor, while in the treated group, the leaf flour block was cream color thick consistency and specific odor. The results of the t-independent test showed that the average volume of semen without Moringa leaf powder block was 1.44 ± 0.56 mL and pH of 7.05 ± 0.39 , while the volume of semen treated

with Moringa leaf powder block was 1.22 ± 0.56 mL and pH 6.88 ± 0.33 . These results did not showing any significant difference ($P > 0.05$) between the two groups.

Table 1. Macroscopic evaluation of Bali bulls fresh semen at different treatments.

Parameter	Treatment group	
	P1	P2
Volume (mL)	1.23 ± 0.57	1.44 ± 0.56
pH	6.86 ± 0.33	7.05 ± 0.39
Color	Cream	Slightly clear
Consistency	Slightly thick	Medium
Odor	Specific	Specific

In a study [3], supplementation of Moringa leaves in the feed increase libido, scrotal circumference, and semen quality of Bali bulls. The volume before supplementation of Moringa leaves in feed was 2.49 mL, pH was 6.99, medium consistency, and milky white color. After supplementing with Moringa leaf in the feed, the volume of semen increase to 4.24 mL with a pH of 6.99, slightly thick consistency and has a creamy color. In general, the volume and color of the semen obtained in this study differ from the previous study [3], this may due to that the age of bulls used in the present study was relatively younger. This in line with a study [4], that the volume of semen will increase with age increased. However, the pH and consistency of the semen did not differ significantly.

The pH of semen obtained in this study, both P1 and P2 groups were still at normal levels. If the pH of the semen is too acidic, even if it is wet, it cannot be further processed. This has been well described [5] that the pH of semen ranged from 6.58-7.00 for further processed. A study [6] added that the pH of the bull semen is in the range of 6.4 - 7.8. The difference in the pH value of each bull semen is influenced by several factors, including if the high semen pH is caused by a lot of dead spermatozoa while the pH of the semen tends to be alkaline, this is due to the more fluids produced by the accessories gland [7]. In this study, this was not found because in general, the pH of each bull semen was normal, both the treated and untreated groups.

The semen consistency of the Bali bulls treated with Moringa leaf flour blocks was classified as good, with a slightly thick consistency when compared to the semen consistency of the untreated group with moderate consistency. According to a study [1], one of the factors affecting the viscosity of semen is the quality of the feeding. The better quality of the feeding, it will produce thick semen and vice versa. Supplementation of the block of Moringa leaf flour has been shown to improve sperm consistency in comparison to the untreated one.

The color of semen in the group of bulls treated with Moringa leaf flour block was milky white, and it can be classified as very good when compared to the color of the semen in the control group which was a bit clear. The color is milky or creamy, whitish, and cloudy, whereas if the cow's semen is not normal with a yellowish-green color, it indicates the presence of *Pseudomonas aeruginosa* germs in the semen.

The smell of semen obtained in the study, both groups of bulls was the distinctive odor. This is consistent in a study [9], that normal semen, in general, has a distinctive fishy odor accompanied by an odor from the animal itself. A foul odor usually occurs when the semen contains pus which is caused by an infection of the organs or reproductive tract of male animals. Generally, the smell of semen is categorized as a specific odor.

3.2. Microscopic evaluation of Bali bull fresh semen at different treatments

Microscopic evaluation of Bali bulls' semen with and without supplemented with Moringa leaf meal block including mass motility, individual motility, progressive motility, and concentration.

3.2.1. *Mass motility.* The mass motility of Bali bulls' sperms with and without supplemented with Moringa leaf meal block are shown in table 2.

Table 2. Mass motility evaluation of Bali bulls fresh semen at different treatments.

Mass Motility	Treatment group	
	P1 (%)	P2 (%)
+	10	30
++	80	70
+++	10	0
Total	100	100

In table 2, it can be seen that the average mass motility of the sperms in the treated group was (++) equivalent to 80%, while in the control group was (++) equivalent to 70%. Based on these data, showed no significant difference among the groups. The mass motility in this study is suitable for further processing because it was equivalent to (++) . This is in accordance with a previous study [7], that the minimum mass movement that can be processed is the category (++) . Thus the semen from the two groups of this study met the category.

Observation of mass movement in semen is only to determine the feasibility of processing, the factors that influence sperms motility are sperm age, sperm maturation, energy storage of ATP (Adenosine Triphosphate), active agents, biophysical and physiological, fluid suspension, and inhibition stimulation [10].

3.2.2. *Individual motility.* The individual motility of spermatozoa of Bali bulls' semen with and without supplemented with Moringa leaf meal block are shown in figure 1.

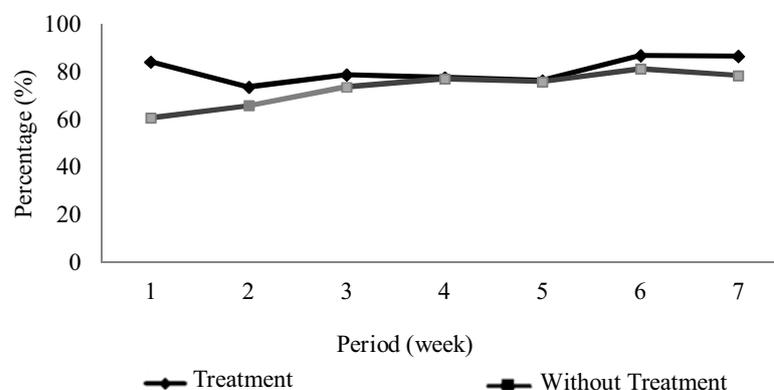


Figure 1. Individual motility of spermatozoa of Bali bulls' semen with and without supplemented with Moringa leaf meal block.

Independent t-test showed that the mean individual spermatozoa motility of the P1 and P2 groups differed significantly ($P < 0.05$). The mean individual motility of the P1 group was 80.65 ± 5.26 %, while in the P2 group was 73.23 ± 7.36 %. The individual motility obtained in the P1 group in this study similar to a study [11], that the average motility of all breeds of bulls is above 80%. One of the factors that caused the difference in the mean value of individual motility in the group of bulls is the supplementation of the block of moringa leaf powder in the treated group.

Figure 1 shows that there is a difference in the increase in the quality of individual spermatozoa motility between the two groups. P1 group of bulls from week 1-3 the movement of the graph is still

fluctuating (unstable), entering week 4-5 the graph movement begins to stabilize and enters week 6 of the movement. The graph has increased by 10.52% from week 5, the movement of the graph remains stable until week 7, while in the group of P2 from week 1-4 continues to increase, entering week 5 the movement of the graph has decreased by 1.29%, entering the 6th week there was an increase of 5.35% and on the 7th week the movement of the chart decreased by 2.65%. Based on the movement of the graph in the P1 group, it can be seen that the increase in individual motility of spermatozoa begins at week 6. This is in accordance with the opinion in a previous study [12], that giving or replacing quality supplements and forage should be done 30-60 days before the mating period to show good reproductive performance. An increase in the motility of individual spermatozoa proves that the nutritional content of Moringa leaf powder blocks is good for sperm motility.

3.2.3. *Progressive motility.* Progressive motility of spermatozoa of Bali bulls' semen with and without supplemented with Moringa leaf meal block are shown in figure 2.

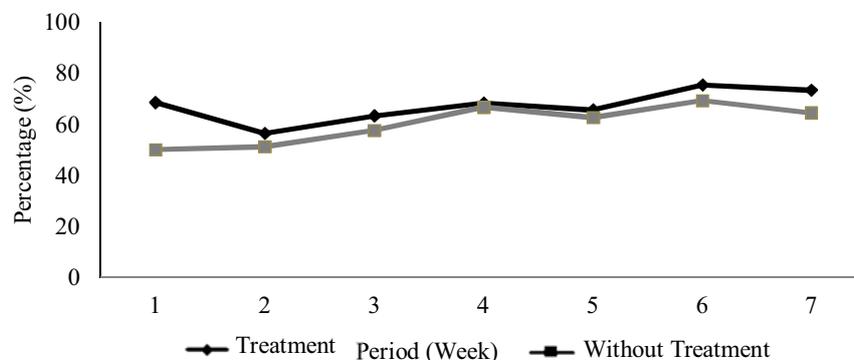


Figure 2. Progressive motility of spermatozoa of Bali bulls' semen with and without supplemented with Moringa leaf meal block.

Progressive motility plays an important role in successful fertilization. According to a study [8], fertile males have progressive motile sperms of 50-80%. The results of this study indicate that the two groups, both treated and untreated block were fertile bulls, although the progressive motility of untreated bulls had lower than treated bulls.

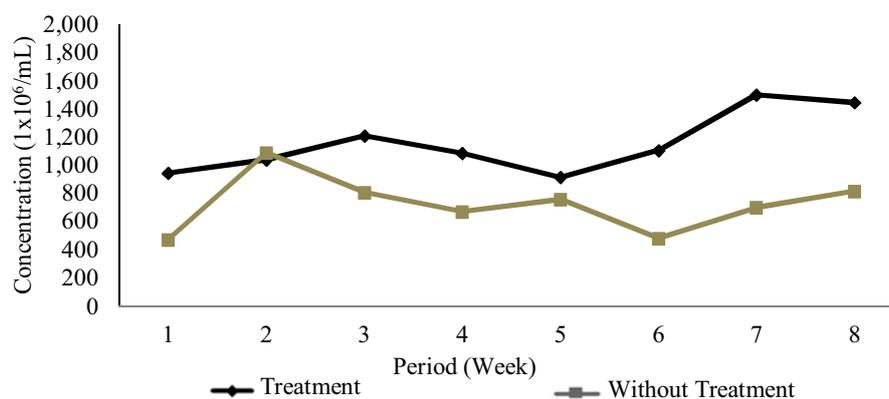


Figure 3. Concentration of spermatozoa of Bali bulls' semen with and without supplemented with Moringa (*Moringa oleifera*) leaf meal block.

3.2.4. *Concentration of spermatozoa.* The spermatozoa concentration of spermatozoa of Bali bulls' semen with and without supplemented with Moringa leaf meal block are shown in figure 3. In this study, the mean spermatozoa concentration in P1 group was $1.156 \times 10^6/\text{mL}$, while in the P2 group the concentration was $724 \times 10^6/\text{mL}$. Statistical analysis showed that the sperms concentration in P1 group had significantly ($P < 0.05$) higher than in P1 group. Figure 3 shows that the sperms concentration of the Bali bulls' semen treated with Moringa leaf powder block increase until the end of the period. The sperms concentration of the treated bulls group is considered as a normal category. This in line with the previous study [6] stated that the normal bull sperm concentration is 800-2,000 million/mL, whereas, in the other study [13], the normal concentration of semen is 800-1,200 million/mL.

Sperms concentration is influenced by several factors, including male sexual maturity, ejaculate volume, the interval of collection, reproductive health, testicular size, age, season, geographical differences, and feed quality [14]. This is in line with a study [4], which stated that in general, the concentration of spermatozoa in semen is in line with the sexual development and maturity of the bulls, according to the quality of feeding. In this study, it showed that supplementation of Moringa leaf powder block to the treated group of bulls had a large effect on male reproduction and had a positive correlation with the increase in sperms concentration.

4. Conclusion

This study concluded that supplementation of Moringa leaf meal block to Bali bulls could improve the quality of semen (consistency, color of individual motility, and sperms concentration).

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