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The effect of providing *Moringa oleifera* leaf meal in the diet on the semen quality of cross-bred Landrace boars

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Abstract

The research aims to examine the impact of providing *Moringa oleifera* leaf meal (MLM) in the diet on the quality of Landrace-cross boar semen, as well as to determine the optimal shelf life of semen from Landrace-cross boar. The design used was a completely random design with a split time pattern. The treatments consisted of: Landrace-cross-bred boar fed without *Moringa* leaf meal (P0) and Landrace-cross-bred boar fed with *Moringa* leaf meal (P1). Semen observations were carried out every 12 hours, starting from the 0th hour to the 72nd hour for both treatments and repetitions were carried out three times for each observation. The results of this study indicate that the provision of *Moringa* leaf flour in the diet for Landrace-cross boar has a positive impact on the quality and quantity of semen, especially in increasing sperm volume, motility, viability and concentration. It was concluded that giving 1% *Moringa* oleifera leaf meal in feed had a positive effect in maintaining sperm quality over a certain period of time, especially when it was fresh or at the beginning of storage.

Keywords: Boar; Semen; Landrace-cross; *Moringa oleifera*

1. Introduction

One of the factors causing the low birth rate of livestock in Indonesia is the quality of the boar used. Low quality males cause failed fertilization and lengthen birth intervals, which will cause losses and threaten the sustainability of the livestock business[1].

The success of an artificial insemination (AI) program is influenced by several factors and one of them is the quality of the semen used [2]. Fresh semen does not last long in *in vitro* storage due to the rapid death of spermatozoa cells. This death occurs due to free radical attacks as a result of electron transport in mitochondria to the plasma membrane of sperm cells.

Semen quality is influenced by feed, maturity, season [3], and breed [4]. The nutrients contained in feed influence the production of spermatozoa, secretion of gonadotropin hormones, and the development of sexual behavior. The capacity of the testes to produce spermatozoa cells and the hormone testosterone is influenced by the ability of the seminiferous tubules and leidig cells or interstitial cells to be stimulated by the hormones Follicle Stimulating Hormone and Leteinizing Hormone [5].

Herbal plants contain phytochemical compounds which are responsible for sexual activity and spermatogenesis. These elements include: saponins, alkaloids, avonoids, ferulic acid, and chlorogenic acid [6]. As reported by [7] that giving 1% mung bean sprouts can improve the quality and quantity of semen in Yokshire-cross boars, and can maintain the quality

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for up to 72 hours of storage. Similar research results were also reported by [8] who stated that giving green bean sprout waste as a substitute for grass was able to increase fresh semen productivity in ruminant livestock.

One plant that contains all these elements is *Moringa oleifera* leaves. *Moringa* leaves contain vitamin A, vitamin C, and vitamin E. *Moringa* leaves also contain vitamin K, vitamin B, and Zn minerals [9]. The process of forming spermatozoa cells which produces high quality semen, vitamins A, E, and C, as well as the minerals Zn and Se [10]. The Zn mineral content in *Moringa* leaves is 31.03 mg/kg [11] which functions to stimulate Leydig cells in the testes to produce the hormone testosterone.

Moringa leaves are classified as a tree plant, found in many tropical countries, and have the potential to be used as animal feed. *Moringa* leaves are a good alternative animal feed, especially during the dry season where animal feed is very limited [11]. *Moringa* leaf dry matter production reaches 4.2-8.2 tonnes/ha and is an alternative that can substitute for commercial livestock rations [12] and is good for use as supplement feed [13].

Several previous studies related to the use of *Moringa* leaves as animal feed and their effect on the quality of livestock sperm. Abu et al. [14] reported that *Moringa* leaf flour up to 15% did not have a negative effect on the quality of spermatozoa in the epididymis of rabbits. Raji and Njidda [15] stated that supplementation with 50% *Moringa* leaf feed in goats can increase sperm count. Syarifuddin et al.[16] showed that giving dried *Moringa* leaves as much as 15% of the concentrate can increase testosterone hormone concentration, libido and cow spermatozoa motility. The aim of this study was to examine the effect of *Moringa oleifera* leaf flour supplementation on the quality and quantity of Landrace-cross boar spermatozoa.

2. Material and methods

2.1. Animal treatments

This research was carried out at the Technical Implementation Unit (UPT) of the Regional Artificial Insemination Center, in Baturiti District, Tabanan Regency, Bali, Indonesia. This research used Landrace-cross boars aged 8 years with healthy conditions and good semen quality, namely a spermatozoa concentration of more than $150x10^6$ cells/ml and spermatozoa motility of more than 60%. Cement collection was carried out twice a week using the massage method. The feed given in the study consisted of: 40% yellow corn, 35% pollard, and 25% protein concentrate, as well as additional *Moringa* leaf flour according to the treatment, namely (P0) ration without giving *Moringa* leaf flour and (P1) ration with giving *Moringa* leaf flour as much as 1% in the ration. The drinking water provided comes from well water which is provided *ad libitum*.

2.2. Experimental design

The design used was a completely random design with a split time pattern. The treatments consisted of: Landrace-cross-bred boar fed without *Moringa oleifera* leaf meal (P0) and Landrace-cross-bred boar fed with *Moringa oleifera* leaf meal (P1). Semen observations were carried out every 12 hours, starting from the 0th hour to the 72nd hour for both treatments and repetitions were carried out three times for each observation.

2.3. Equipment

Equipment used in this research included: dummy-saw, tamping tool, measuring cup, water bath, elemeyer tube, dropper pipette, thermometer, binocular microscope, glass object, preparation box, pH meter, BTS diluent, 2% eosin dye, and other tools.

Characteristics of fresh semen include: volume, color, consistency, pH, mass movement of spermatozoa, concentration of spermatozoa, percentage of spermatozoa motility, and viability of spermatozoa [17]. The percentage of motility was assessed subjectively by comparing motile spermatozoa moving forward (progressive) with those that were not progressive (linear). Ratings were given from 0% (not motile) to 100% (all motile). Spermatozoa viability and percentage viability were calculated using 2% eosin dye and examination was carried out under a microscope.

2.4. Data analysis

The data obtained were analyzed using variance, if the treatment average had a significant effect ($P<0.05$), then the analysis continued with Duncan's multiple range test.

3. Results and discussion

The results of the evaluation of fresh cement are the initial stage which is used as a basis for determining the suitability of cement for further processing. Evaluation of semen characteristics in the Landrace cross boar group fed without Moringa (P0) and those given 1% *Moringa* leaf meal in the diet is presented in Table 1.

Table 1 Semen characteristics of Landrace crossbred boars

Cement characteristics	Landrace crossbred boar group		Standard
	P0	P1	
Volume (ml)	323±24.97[a]	432±34.58[b]	150-200[18]
Color	clear	clear	Milky-white[19]
Consistency	runny	runny	Runny[19]
pH	6.34±0.58[a]	6.57±0.28[b]	7.3-7.8[18]
Mass movement of spermatozoa	+++ (very good)	+++ (very good)	(+++)[19] (very good)
Concentration (10^6 sel/ml)	226.41±11.75[a]	277.71±13.97[b]	200-300[18]
Spermatozoa viability (%)	58.21±9.01[a]	67.37±4.56[b]	70–90[18]
Spermatozoa motility (%)	0.42±0.01[a]	0.55±0.01[b]	50-80[18]

Note: (ab) Different letters on the same line indicate significantly different (P<0.05).

Semen volume is an important indicator for assessing reproductive capacity and fertilization potential in pigs. The observation results showed that the P1 boar group had an average semen volume of 432 ml, which was higher than the control (P0 boar group) which only had an average volume of 323 ml. The results obtained from this research show a higher value compared to the results of research conducted by [18], namely the volume of boar cement ranges between 240-250 ml and ranges between 200-250 ml [20].

Cement color is closely related to concentration and consistency (viscosity). The higher the concentration of spermatozoa can cause the increase in the consistency and color of the semen. Semen in normal boars has a thin consistency and is milky white in color, because there is riboflavin from the secretions of the vesicular glands. The average color of the cement obtained in this study was clear with a thin consistency, and very good mass movement (+++). This is in accordance with the opinion of [20] who states that the color and consistency of pig semen depends on the fraction being collected, namely the pra-spermatozoa fraction is watery with a white-grey color, and the spermatozoa-rich fraction is milky (nonviscous) with a creamy white color.

The data from this research shows that the pH of the cement in the treatment (pH 6.57) was slightly higher than the control (pH 6.34). The results of this study were lower than research conducted by [21], namely pH 7.3-7.8 and pH 7.2-7.5 [22]. If there is a decrease in pH, the metabolism and motility of spermatozoa will also decrease [23]. Johnson et al.[22] added that pH below 7.2 will reduce spermatozoa motility.

The spermatozoa concentration in this study showed that administration of *Moringa* leaf flour had a higher semen concentration (277.71x106 cells/ml) compared to the control (226.41x106 cells/ml). This result is not much different from the research results of [18] and [21] which ranged from 200-300x106 cells/ml.

Increased motility and viability of spermatozoa also reflects an increase in overall semen quality. The increased motility from 0.42 (P0 boar group) to 0.55 (P1 boar group) indicates an increase in the movement ability of spermatozoa, which is very important in the fertilization process. This is supported by the opinion of [24], who said that the mineral Zn contained in *Moringa* leaves can stimulate Leydig cells in the testes to produce testosterone, while in the process of spermatogenesis the mineral Zn plays a role in ribonuclease activity, maturation of spermatozoa and increasing sperm motility as well as maintaining the germinative epithelium and seminiferous tubules. Providing *Moringa* leaf flour was able to increase the nutritional value of the ration, thereby enabling more optimal spermatozoa metabolism. Likewise, the increase in spermatozoa viability from 58.21% (P0) to 67.37% (P1) indicated that sperm had a better ability to

survive and function properly. both in the reproductive process. The viability of spermatozoa is observed by looking at the color of the head of the spermatozoa.

The increase in motility and viability of spermatozoa in the P1 boar group shows that the provision of *Moringa* leaf meal in the diet has had a positive impact on the quality and quantity of semen of Landrace cross boars. The good ability of spermatozoa to move and survive can increase the occurrence of successful fertilization, thereby increasing reproductive productivity in the boar population that is given this treatment. These results are in accordance with the results of previous research which highlighted the potential of *Moringa* leaf flour as a feed supplement to improve sperm quality in livestock [25]. Previous studies by [26] showed that administering *Moringa* leaf flour can increase sperm viability in chickens, which confirms the positive effect of this feed ingredient on animal reproduction.

The protein and mineral Zn in *Moringa* leaves play a role in the maturation of spermatozoa during the spermatogenesis process and stimulate Leydig cells in the testes to produce testosterone [24]. High spermatozoa viability can increase the success of fertilization and pregnancy in female animals, thereby increasing productivity and reproductive efficiency on livestock [27]. Research by [28] confirmed that increased spermatozoa motility is positively correlated with the success rate of fertilization in farm animals, indicating the importance of this parameter in evaluating sperm quality. In this study, significant changes in spermatozoa motility between control and treatment showed that the provision of *Moringa* leaf flour in the diet had a positive impact on the motility of spermatozoa. A better ability of sperm to move actively can increase the possibility of egg fertilization, thereby increasing reproductive success in female animals [17].

4. Conclusion

Providing 1% *Moringa oleifera* leaf meal in the feed can improve the quality and quantity of boar semen from Landrace crosses. The optimal shelf life of cement quality is at the beginning of storage, up to 12 hours.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experimental animals in this research have been approved by the Animal Ethics Commission from the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

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