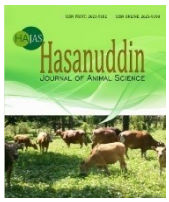

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Correlation Between Blood Metabolite Profile and Spermatozoa Quality in Bali Semen by Micronutrient Addition

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ABSTRACT

The hematological profile and blood metabolite status are indicators of the physiological condition of livestock. This study aimed to determine the correlation between blood metabolite profiles (blood glucose and cholesterol) and sperm quality (motility and abnormality) in fresh Bali bull semen. Blood collection and semen sampling were conducted twice a week for 5 weeks. The collected blood and fresh semen were evaluated from two treatments: before and after the addition of micronutrients (including Zn, Se, and Vitamin E) in the feed. The parameters observed in this study were blood metabolite profiles (blood glucose and cholesterol levels) and sperm quality (percentage (%) motility and % abnormality of spermatozoa) before and after the addition of micronutrients. The data obtained were analyzed using simple linear regression and correlation tests. The results of this study showed that both treatments had a correlation between blood metabolite profiles and sperm quality in fresh Bali bull semen. Blood glucose levels correlated with sperm motility in fresh Bali bull semen, and good sperm motility values were obtained in all treatments before and after micronutrient administration in the feed. Cholesterol levels correlated with sperm abnormalities in fresh Bali bull semen, and all treatments before and after micronutrient addition in the feed showed abnormality values above the standard value. The blood metabolite profile (glucose and cholesterol) correlates with spermatozoa quality (motility and abnormalities) in fresh semen from Bali bull, where the addition of micronutrients in feed can increase spermatozoa motility but is not effective in suppressing the decrease in spermatozoa abnormalities.

Keywords: Blood metabolite, blood sugar, cholesterol, spermatozoa quality

INTRODUCTION

The livestock cattle business in Indonesia requires special attention to maintain and support population growth, where appropriate technology in the fields of reproduction and feed is applied easily and efficiently. The use of artificial insemination (AI) technology in optimizing efforts to increase reproductive efficiency involves striving for each breeding cow to produce offspring every year with the desired sex [1]. The success of AI is determined by the quality of frozen semen from males, which is influenced by the characteristics of their fresh semen, which can be evaluated both macroscopically and microscopically [2]. The semen quality of a male is closely related to fertility [3] and has a high economic significance in livestock production programs [4].

One of the factors that causes the low pregnancy rate is the poor quality of semen used [5]. The quality of spermatozoa in semen is determined by the number, motility, and morphology of spermatozoa [6]. Sperm count, motility, and the morphology of spermatozoa are the parameters used to estimate the fertility of males [7]. Motility of spermatozoa is one of the factors that play an important role in the determination of normal spermatozoa [8].

In semen, white blood cells (leukocytes) play a role in the immune system and phagocytosis of abnormal spermatozoa. The discovery of leukocytes that increase in number in semen is an indication of inflammation or infection in the reproductive tract [9]. Leukocytes are colorless blood cells capable of moving in an amoeboid manner [10]. Red blood cells (erythrocytes) have a role in binding oxygen and circulate to all tissues of the animal farm body [11]. Erythrocytes are made in the spinal cord, which, under normal circumstances, are biconcave in shape, nucleusless, and inside contain hemoglobin. Bone strength is closely related to the mineral content in bones, especially calcium [12]. Bone strength is influenced by several factors, including age, genetics, physical activity, nutrition, and hormones such as estrogen, growth hormone, thyroid hormone, insulin, and testosterone [13]. Androgens are synthesized and secreted into the bloodstream and mostly form testosterone. After entering its target cells, testosterone is also metabolized by aromatase to form estradiol in the hypothalamus, where determination of sexual behavior occurs [14]. Hematological features or profiles and blood metabolite status are one of the determining indicators of the physiological condition of an animal farm [15]. Hematological profiles and blood metabolite profiles have been widely used to monitor health status, assess nutrient metabolism, identify dietary causes of low productivity, and evaluate heat stress levels and animal welfare [16]. According to a study, insulin levels in the blood affect the plasma membrane and acrosome of sperm. As a result, spermatogenesis is hindered in individuals with diabetes with insulin resistance or insufficiency [17]. Diabetes can negatively impact erectile and ejaculatory function, as well as decrease sperm volume, sperm counts, sperm motility, and result in abnormal sperm morphology [18]. Cholesterol also affects mitochondrial function and tyrosine phosphorylation during capacitation, playing a role in maintaining sperm structure, function, and high fertilization ability [19].

MATERIALS AND METHODS

Time and Place of Research

This research was conducted from November 2023 to January 2024 at the Samata Integrated Farming System in Gowa Regency and the Laboratory of Animal Reproduction, Faculty of Animal Husbandry, Hasanuddin University, Makassar.

Research Methodology

The materials used in this study included blood and semen samples collected from a single adult Bali bull (*Bos sondaicus*). Supporting materials comprised 70% alcohol and aquadest (distilled water). Equipment used included Easy Touch® GCHb device (for glucose, cholesterol and hemoglobin analysis), BD Vacutainer® Flashback Blood Collection Needle 22GA × 1" (0.7 mm × 25 mm), vacutainer holder, Vaculab® EDTA tubes, semen reservoir (artificial vagina), K-Y® personal lubricant jelly, sperm tubes, gloves, aluminum foil, label paper, tissue paper, microscope slides and cover slips, autoclave, water bath, photometer and Computer Assisted Semen Analysis (CASA).

Semen was collected using an artificial vagina (AV), which is a critical tool in artificial insemination. The AV consists of an internal and external rubber casing filled with hot water (up to 60 °C), ensuring the inner lining maintains a temperature of 40–45°C [20]. The AV is a cylindrical device with a deep sleeve, a funnel, and a collection tube. To facilitate insertion and promote penile erection, the interior is adequately lubricated and securely positioned around the male cattle's genitalia [21]. At the time of shelter, temperature conditions, and others, the inside of the artificial vagina can be adjusted like the vagina of a cow in lust. Bring the cattle to the cage to be met with the angler female to stimulate the libido of the males, who will accommodate the semen. The prepared artificial vagina is given Pelican (K-Y® personal lubricant jelly). If there are signs that the male will climb the male or female angler, then an artificial vagina is installed on the penis to collect semen. Blood sampling is done after semen collection using a tool that has been provided. Take the bull to the tongs to prepare. Then take a bull blood sample of about 5 ml or enough through the jugular vein using a venoject BD Vacutainer® flashback blood collection needle 22GA x 1'' (0.7 mm x 25 mm) and a Vaculab® EDTA tube that has been paired. Jugular venipuncture using a Vacutainer needle and tubes (<100 ml): using the halter, position the animal's head so that it is slightly elevated and drawn to the side to expose the jugular vein. Screw the 18G x 1.5" or 20G x 1.5" vacutainer needle onto the vacutainer holder. Insert the vacutainer tube into the other end of the vacutainer holder. Do not puncture the stopper. Hold these assembled materials in one hand. Disinfect the venipuncture site with alcohol. Disinfect the venipuncture site with alcohol. Occlude the jugular vein by applying pressure in the jugular groove located in the lower neck. Position the needle bevel-up and insert it into the distended jugular vein at a 45° angle cranial to the jugular groove. Once the needle is positioned in the vein, insert a Vacutainer tube into the needle to collect the blood. When the desired volume has been collected (a minimum of 5 ml is suggested), remove the occluding pressure from the vein. If collecting more than one sample (from different tube types), leave the needle and vacutainer holder in place and insert the next blood tube. Once the sample has been collected, detach the

tube from the needle and withdraw the needle from the jugular vein [22]. The collar can be removed if it impedes access to the sampling site. Then, directly analyze blood samples for sugar and cholesterol levels using the Easy Touch GCHb (Glucose and Cholesterol) device.

Assessment process of bull blood samples using Easy Touch® GCHb to see blood sugar and cholesterol levels. The process of assessing fresh semen of Bali bull is macroscopically by looking at the volume of fresh semen and microscopically (motility and abnormality of spermatozoa).

The parameters observed in this study were blood metabolite profile (blood sugar and cholesterol levels) and spermatozoa quality (percentage (%) motility and % spermatozoa abnormalities) before and after the addition of micronutrients in the feed (Zn, Se, and Vitamin E). Two feeding periods: a control period, during which cattle received only concentrate and forage; and a treatment period, during which cattle received micronutrient supplementation in their feed. The micronutrients included Zinc (40,000 mg), Selenium (100 mg), and Vitamin E (10,000 IU), which were mixed into the concentrate.

Data obtained in this study were tabulated in an Excel program, namely data on blood metabolite profiles (including: blood sugar and cholesterol levels) and spermatozoa quality (including: percentage (%) of individual motility and percentage of spermatozoa abnormalities) collected. The relationship between blood metabolites and the quality of spermatozoa in collected semen was tested using a simple linear regression analysis. The test will be followed by a correlation test to determine the relationship between blood metabolite profile values and spermatozoa quality.

RESULTS AND DISCUSSIONS

Fresh Semen Volume of Bali Bull at Five Collection Times

Based on the volume of fresh semen of Bali bull at five times of collection (before and after the addition of micronutrients in the feed (Zn, Se, Vitamin E), the following results were obtained, then assessed by paying attention to Table 1.

Table 1. Fresh semen volume of Bali bull at five collection times (before and after micronutrients supplementation on feed)

Collection	Value (ml)	
	Before Micronutrients Supplementation	After Micronutrients Supplementation
I	5	3
II	2	4.5
III	0.8	5.5
IV	2	2
V	2	5
Average	2.36	4
Standart	4-8 (Toelihere, 1981)	

The average semen volume value of Bali bull in five times collection before the addition of micronutrients was 2.36 ml. This can be caused by various factors, including the insufficient nutritional value of the feed given. Inadequate dietary intake and imbalanced nutrient adequacy

in livestock can delay the onset of puberty and increase the age at first calving, prolong the gestation period, extend the calving interval, and disrupt the hormonal regulatory system, including the process of spermatogenesis in males [23]. These conditions can significantly impact reproductive efficiency in Bali bulls.

The average semen volume value of Bali bull in five times collection before the addition of micronutrients was 4 ml. The semen volume of Bali bull ranges between 2–15 ml with an average of 4–8 ml [24]. Climate, which encompasses temperature, moisture, rainfall, and seasons, can impact livestock productivity [25, 26]. Each semen volume collected after micronutrient supplementation showed an increase during the second, third, and fifth collections. This indicates that the addition of micronutrients to the feed can help prevent a decline in semen volume in bulls. The results of research by Khairi et al. [27] found that supplementation with a combination of zinc (Zn), selenium (Se), and vitamin E in Bali bulls can reduce the decline in semen production, sperm motility, and spermatozoa concentration during periods of high rainfall and humidity.

Correlation between Blood Sugar Level and Spermatozoa Motility

The correlation of blood sugar levels to spermatozoa motility (before and after the addition of micronutrients in the feed (Zn, Se, Vitamin E), the following results were obtained, then assessed by paying attention to Tables 2 and 3.

Table 2. Correlation between blood sugar level and motility of fresh semen spermatozoa Bali bull on five collection times (before micronutrient supplementation)

Collection	Blood Sugar Levels (mg/dL)	Motility (%)
I	62	94.76
II	100	84.49
III	187	81.49
IV	56	88.83
V	78	84.13
Average	96.6±53.32	86.47±5.19
Sig. (2-tailed)	0.17>0.05	
Pearson Correlation	-0.71 (Negatively correlated strong category)	

Table 2 showed that the value of blood sugar levels had no effect (P > 0.05) on the motility of spermatozoa of Bali bulls before the addition of micronutrients to the feed. The results of research by Assumpção et al. [28] found that protein levels, sugar, and amino acids in semen plasma have no relation to fertility or fertility of male Nelore bull (*Bos taurus indicus*).

It can be seen that in collections I and IV, the blood sugar level of Bali bull is below the standard (62 and 56 mg/dL), then in collections II, III, and IV, blood sugar levels were above the standard (100, 187, and 78 mg/dL). Normal blood glucose levels in male Bali bull range between: 68.96–72.81 mg/dL [29]. Glucose metabolism that does not function properly can damage the body's organs. High glucose levels can cause hyperglycemia [30]. However, if the energy intake is too low, it can actually lead to hypoglycemia [31].

The motility value of fresh semen spermatozoa of Bali bull in collections I to V was above the standard (94.76, 84.49, 81.49, 88.83, and 84.13%). Subclinical hypoglycemia events will cause reproductive disorders. The average low blood glucose level was found in dairy cows with reproductive disorders of 48.58 mg/dL [32].

Table 3. Correlation between blood sugar level and motility of fresh semen spermatozoa Bali bull on five collection times (after micronutrients supplementation)

Collection	Blood Sugar Levels (mg/dL)	Motility (%)
I	89	90.06
II	89	83.76
III	71	82.66
IV	58	84.45
V	66	83.85
Average	74.6±13.93	84.95±2.92
Sig. (2-tailed)	0.385>0.005	
Pearson Correlation	0.505 (positively correlated medium category)	

Table 3 shows that blood sugar levels had no effect (P > 0.05) on the motility of Bali bull spermatozoa after the addition of micronutrients to the feed. Nutritional deficiencies increase oxidative stress and further decrease sperm motility in farm animals [33]. Nutritional factors are related to the diversity or type of feed, which determines energy content and affects cattle's blood glucose levels [34]. The feed consumed undergoes carbohydrate hydrolysis in the presence of enzymes, breaking carbohydrates into glucose. Additionally, glucose is a nutrient that the body quickly uses as its primary energy source [35].

It can be seen that in collections IV and V, the blood glucose levels of Bali cattle are below the standard (58 and 66 mg/dL). In shelters I and III, the sugar content values were above the standard (89 and 71 mg/dL). This may be due to blood sampling being performed after the animals were fed, which causes glucose levels to vary or be inconsistent, ranging between 58 and 89 mg/dL. Normal blood glucose levels in male Bali cattle range from 68.96 to 72.81 mg/dL [36]. Glucose is a nutrient in the blood whose concentration can change easily over time. Therefore, glucose levels mainly depend on the time of blood collection [37].

Correlation Of Cholesterol Levels to Spermatozoa Abnormalities

Based on the results of the correlation of cholesterol levels to spermatozoa abnormality (before and after the addition of micronutrients in the feed (Zn, Se, Vitamin E), the following results were obtained, then assessed by paying attention to Tables 4 and 5. Table 4: The value of cholesterol levels had no significant effect (P > 0.05) on the abnormality of spermatozoa in Bali bulls before the addition of micronutrients to the feed. The study by Martin et al. [38] found that the diet composition of high-cholesterol feed significantly reduced the percentage of spermatozoa with normal morphology and increased the percentage of spermatozoa with abnormal morphology in New Zealand White rabbits.

Table 4. Correlation of cholesterol levels to abnormalities of fresh semen of Bali bull in five time collections (before micronutrients supplementation)

Collection	Cholesterol (mg/dL)	Abnormality (%)
I	285	48
II	210	34
III	73	46
IV	224	21
V	215	34
Average	201.40±77.85	36.60±10.89
Sig. (2-tailed)	0.752 > 0.005	
Pearson Correlation	0.196 (Negatively correlated with strong categories)	

The blood glucose levels in collections I, II, IV, and V for the Bali bull exceed the standard values (285, 210, 224, and 215). Conversely, the cholesterol level in the third collection falls below the standard (73 mg/dL). The normal range for blood cholesterol levels in dairy cows is 130–200 mg/dl [39]. Cholesterol biosynthesis primarily occurs in liver tissue, with 33% derived from dietary sources and 67% synthesized within the body [40].

The abnormal value of fresh semen spermatozoa from a Bali bull in five collections, labeled I to V, is above the minimum standard value. Semen suitable for AI has spermatozoa abnormalities that should not exceed 20%, and if abnormalities surpass 20%, fertility will decrease [41].

Table 5. Correlation of cholesterol levels to abnormalities of fresh semen of Bali bull in five time collections (after micronutrients supplementation)

Collection	Cholesterol (mg/dL)	Abnormality (%)
I	214	23
II	200	38
III	222	46
IV	198	21
V	201	27
Average	207.00±10.48	31.00±10.65
Sig. (2-tailed)	0.365 > 0.005	
Pearson Correlation	0.524 (Strongly positively correlated)	

Table 5 shows that the value of cholesterol levels had no effect (P > 0.05) on the abnormality of spermatozoa in Bali bulls after the addition of micronutrients to the feed. Abnormal morphological rates between 8–10% do not significantly affect fertility, but if abnormalities are more than 25% of one ejaculate, then a decrease in fertility cannot be anticipated [42].

It is evident that in collections I to V, the blood cholesterol levels of Bali bulls exceed the standard normal value. The study results of Turk et al. [43] showed that normal cholesterol levels range from 130 to 252 mg/dl. Cholesterol levels are influenced by the fat content of the feed.

Feed fats, especially saturated fatty acids like laurate, myristate, and palmitate, can raise total cholesterol levels in the blood.

The abnormal values of fresh semen spermatozoa from Bali bulls in five collections, numbered I to V, are above the minimum standard. Tambing et al. [44] explained that the abnormal percentage should not exceed 15%, while Garner and Hafez [45] state that spermatozoa abnormalities should not exceed 20%.

CONCLUSIONS

Based on the research results and discussion, it can be concluded that blood glucose levels were negatively correlated with sperm motility in fresh Bali bull semen before micronutrient supplementation; however, a positive correlation was observed after micronutrient administration. Cholesterol levels were negatively correlated with spermatozoa abnormalities in fresh Bali cattle semen before micronutrient supplementation; however, a positive correlation was observed following micronutrient administration. Micronutrient supplementation in Bali bulls can improve blood metabolite profiles, which in turn enhances sperm quality in terms of motility and abnormalities in fresh semen.

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AUTHORS' CONTRIBUTIONS

Conceptualized the study and drafted and revised the manuscript. All Author: Conducted experiments and analyzed data. All authors have read and approved the final manuscript.

COMPETING INTERESTS

The authors have to declare that they have no competing interests.

ETHICAL CLEARANCE

Authors must have ethical clearance to use animals as research objects, either in the form of direct contact with animals or in the use of their products in research.

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