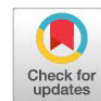


Research Article



Differences in Microminerals Zinc (Zn) and Iron (Fe) in Gembrong and Boerka Goats and its Correlation with Spermatozoa Quality

TEUKU ARMANSYAH^{1*}, AMALIA SUTRIANA¹, JULI MELIA², TASYA FAKHIRA HARAHAP³, TONGKU NIZWAN SIREGAR², HUSNURRIZAL HUSNURRIZAL², HAFIZUDDIN HAFIZUDDIN², DWINNA ALIZA⁴, BUDIANTO PANJAITAN⁵, ARMAN SAYUTI⁵

¹Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia;

²Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia;

³Study Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; ⁴Laboratory of Pathology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; ⁵Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Abstract | Low semen volume and sperm concentration, indicators of poor semen quality in Gembrong goats, may be associated with nutritional factors, particularly the microminerals zinc (Zn) and iron (Fe). These microminerals are established biomarkers for semen quality in cattle, where higher concentrations in the blood are typically associated with better semen quality. This study aimed to determine the differences in Zn and Fe concentrations between Gembrong and Boerka goats and to assess the correlation of these minerals with semen quality parameters, including semen volume and sperm concentration. In this study, blood serum samples were collected from six male goats, three Gembrong and three Boerka, each aged 3 years, with an average body condition score of 3.83 ± 0.29 , and maintained under identical feeding and management conditions. Zinc and iron concentrations were measured using the Atomic Absorption Spectrophotometry (AAS) method. Differences between breeds were analyzed using the independent t-test, and correlations between mineral concentrations and semen quality parameters were assessed using Pearson's correlation. The average Zn concentrations in Gembrong and Boerka goats were 0.009 ± 0.013 mg/dL and 0.075 ± 0.002 mg/dL, respectively, with a statistically significant difference ($P < 0.05$). The Fe concentrations were 2.683 ± 0.500 mg/dL for Gembrong goats and 2.780 ± 0.599 mg/dL for Boerka goats, showing no significant difference ($P > 0.05$). A significant positive correlation was observed between Zn concentration and both semen volume ($r = 0.88$, $P < 0.05$) and sperm concentration ($r = 0.94$, $P < 0.05$). In contrast, Fe concentration was not significantly correlated with semen volume ($r = 0.10$, $P > 0.05$) or sperm concentration ($r = 0.07$, $P > 0.05$). In conclusion, the breed of goat influenced Zn concentrations but not Fe concentrations. Furthermore, Zn concentration was positively correlated with semen volume and sperm concentration, whereas Fe concentration showed no correlation with these semen quality parameters.

Keywords | Boerka goats, Fe, Gembrong goats, Sperm quality, Zn

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***Correspondence** | Teuku Armansyah, Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; **Email:** t_armansyah@usk.ac.id

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Gembrong goats are one of Indonesia's indigenous genetic resources, originating from Karangasem Regency, Bali Province. These goats are characterized by their distinctive long, shiny white fur that covers most parts of the body, including the neck and face. However, their population has declined drastically, with only 26 individuals recorded in 2014 (Hariyono and Endrawati, 2023). Based on a report by the Board on Agriculture National Research Council (1993), the Gembrong goat has been classified as an endangered species.

In light of these findings, efforts to enhance reproductive performance in goats through the use of artificial insemination (AI) technology are challenged by limitations in semen quality. Previous studies have shown that the spermatozoa quality of Gembrong goats is relatively lower than that of Boerka goats. Among the reproductive performance parameters evaluated, semen volume and spermatozoa concentration showed significant differences ($P < 0.05$) between the two breeds. Specifically, Gembrong goats had lower semen volume and sperm concentration, with values of 0.50 ± 0.00 mL and $1,557 \pm 712.0 \times 10^6$ cells/mL, respectively, compared to Boerka goats, which recorded 1.00 ± 0.20 mL and $4,500 \pm 317.0 \times 10^6$ cells/mL (Husnurrisal *et al.*, 2023). When compared with other goat breeds, it can be seen that the semen volume of Gembrong goats is relatively lower. The semen volume of Saburai goats was reported to be 0.78 ± 0.40 mL (Saputra *et al.*, 2019), Kejobong goats was 0.60 ± 0.20 mL (Syamyono *et al.*, 2014) and Boer goats were 1.54 ± 0.78 mL cited by Saputra *et al.* (2019), 1.05 ± 0.15 mL (Rosmaidar *et al.*, 2013), 1.00 ± 0.24 mL (Lestari *et al.*, 2014), 1.00 ± 0.20 mL (Pahriadi *et al.*, 2022).

The low fertility of Gembrong goats, characterized by lower semen quality compared to other goats, is most likely caused by breed differences that affect their nutrition. Several reports have showed the interactions between nutrition and reproduction in sheep and goats (Vázquez-Armiño *et al.*, 2011; Chao *et al.*, 2023). In the breeding of these species, one of the nutrients that are of concern is microminerals (trace elements). Based on their needs, minerals are divided into macro and micro minerals, and this classification is carried out based on their dietary requirements. A mineral required in quantities greater than 100 ppm needed in food is classified as macrominerals, including calcium, phosphorus, magnesium, potassium, sulfur, sodium, and chloride. While, a mineral required in quantities less than 100 ppm needed in food is classified as micro-mineral, including cobalt, copper, iodine, iron, manganese, selenium, and zinc. Microminerals play an important role in many physiological activities such as energy production, enzyme activity, hormone production, collagen formation,

vitamin and tissue synthesis, oxygen transport, and other physiological processes related to growth and reproduction (Ibrahim *et al.*, 2013).

Among several microminerals, zinc (Zn) and iron (Fe) play a great role in determining semen quality. Widhyari *et al.* (2015) reported that dietary zinc (Zn) supplementation can enhance spermatozoa concentration and motility in Friesian Holstein cattle. Hindrawati *et al.* (2020) added that Zn supplementation tends to increase spermatozoa motility, semen volume, and reduce spermatozoa abnormalities. In sheep, it was reported that administration of Zn could increase spermatozoa motility and viability (Mousavi-Esfokhi *et al.*, 2023). The quality of Samosir goat semen was also improved following the administration of selenium and zinc (Zn) (Siswoyo *et al.*, 2018). Similarly, in cashmere goats, Zn supplementation enhanced semen quality by increasing the antioxidant capacity of spermatozoa (Liu *et al.*, 2020).

The Zn has many important functions in spermatozoa physiology, including influencing lipid flexibility, stabilizing membrane, capacitation, and spermatozoa acrosome reactions (Fallah *et al.*, 2018). However, Fe minerals play an important role in spermatogenesis and semen quality because Sertoli and Leydig cells are an important source of Fe protein storage, ferritin (Knazicka *et al.*, 2021). To date, information regarding the Zn and Fe content in various goat breeds which is related to semen quality has never been reported. Pipan *et al.* (2021) reported that semen suitable for cryopreservation is associated with high levels of Zn and Fe in blood serum, suggesting that these mineral concentrations can serve as biomarkers for sperm freezing potential. This finding forms the basis for selecting Zn and Fe as the focus of mineral measurements in the present study. Iron also plays a crucial role in spermatogenesis and spermatozoa metabolism, as it is a key component of cytochrome enzymes involved in the electron transport chain, which supports sperm motility (Pal *et al.*, 2017).

The effect of Zn and Fe on male animal fertility is related to the maintenance of oxidative balance to protect sperm (Zečević *et al.*, 2025). High levels of semen ROS can cause sperm dysfunction, sperm DNA damage and reduced male reproductive potential. Oxidative stress occurs commonly in spermatozoa through lipid peroxidation because the sperm membrane contains high amounts of polyunsaturated fatty acids (Donnelly *et al.*, 1999). Antioxidants protect sperm from ROS production, DNA damage, plasma and mitochondrial membrane damage, decreased motility and increase sperm motility, viability and fertility (El-Speiy *et al.*, 2023).

Previous studies have reported that mineral concentrations in animals can vary depending on species and breed. Sogutlu *et al.* (2022) examined mineral concentrations in

seven dog breeds maintained under identical feeding and management conditions. Their findings showed that the highest magnesium (Mg) concentration was observed in the Pointer breed, while the highest copper (Cu) concentration was found in the Turkish Tazi breed. In pigs, breed differences are believed to influence feed intake behavior, which in turn affects mineral concentrations in the blood. Aritonang and Silalahi (2001), found that purebred Landrace pigs consumed more feed than purebred Yorkshire pigs, even when both were kept under the same management and feeding conditions. In the present study, despite both Gembrong and Boerka goats being maintained under identical conditions and receiving the same diet, differences in breed are believed to have influenced blood mineral concentrations, particularly Zn and Fe levels. The aim of this study was to compare the Zn and Fe concentrations between these two goat breeds and assess how these minerals correlate with semen quality parameters, such as semen volume and sperm concentration. Improving semen quality through dietary supplementation with Zn and Fe is crucial for preserving the reproductive health of both Gembrong and Boerka goats, as high-quality semen is essential for producing viable frozen semen used in artificial insemination programs.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study had obtained an ethical approval certificate from the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala (No. 169/KEPH/IX/2022).

STUDY PERIOD AND LOCATION

Semen quality evaluation was conducted at BSIP Small Ruminants, Sei Putih, North Sumatra. The examination of Zn and Fe levels was carried out at the Chemical Engineering Laboratory, Faculty of Engineering, Universitas Syiah Kuala, Banda Aceh.

RESEARCH DESIGN

In this study, six blood plasma samples were used from male Gembrong goats (G, n=3) and male Boerka goats (B, n=3). All goats used were 3 years old, with the same body condition score of 3.83 ± 0.29 , and were kept at BSIP Small Ruminants, Sei Putih, North Sumatra, with the same care and feeding management. The small sample size in this study was due to the limited availability of samples. In Sumatra, Gembrong goats were only available at BSIP Small Ruminants, Sei Putih, North Sumatra. There were only 6 male Gembrong goats available. Among these 6 goats, only 3 Gembrong goats were suitable for semen collection.

SEMEN COLLECTION AND EVALUATION

Semen collection was carried out using the artificial vagina

method, and the quality of the collected semen was immediately evaluated macroscopically (semen volume) and microscopically (spermatozoa concentration). The selection of these two variables was based on previous reports that the semen quality variables were significantly different between Gembrong and Boerka goats were semen volume and spermatozoa concentration (Husnurrizal *et al.*, 2023). Semen volume was immediately measured in the collection tube after the collection process (Arifiantini, 2012).

The sperm concentration was calculated using a hemocytometer. Semen was withdrawn to the 0.5 mark on the scale, then 3% NaCl solution was added up to the 101 mark. The mixture was gently homogenized for 2-3 minutes. A few drops of solution were discarded. Further, the Neubauer counting chamber was covered with a cover glass, and the sample was put into the Neubauer counting chamber. The spermatozoa counting was carried out in five large squares, with a magnification of 40×10 . The spermatozoa concentration was calculated using the equation of $Y \times 5 \times 10^6$ (Y= number of spermatozoa in 5 squares) (Arifiantini, 2012).

COLLECTION AND PREPARATION OF BLOOD PLASMA SAMPLES

Blood samples were collected via the jugular vein and transferred into a vacutainer tube containing EDTA, homogenized, and put in a cool box in cold conditions. The blood samples were taken to the laboratory and centrifuged at 3000 rpm for 15 minutes. The blood plasma formed was put into a microtube and stored in a freezer at -20°C before Zn and Fe concentration analysis. Examination of Zn and Fe concentrations in the blood plasma of Gembrong and Boerka goats was carried out using the Atomic Absorption Spectrophotometer (AAS, Shimadzu 700) method. The limit of detection (LOD) and measurement accuracy for Zn and Fe using this method were $0.5 \mu\text{g/L}$ and 2%, respectively.

DATA ANALYSIS

Data on Zn and Fe concentration in male Gembrong and Boerka goats were analyzed using the T-test, while the correlation between Zn or Fe concentration on semen volume and spermatozoa concentration was analyzed using Pearson correlation analysis.

RESULTS AND DISCUSSION

The differences in Zn or Fe minerals in the blood plasma of Gembrong and Boerka goats are presented in Table 1. The present results showed that the Zn concentration in Gembrong goats was below normal range while in Boerka goats were above the normal range for goat blood.

Previous finding showed that the Zn concentration in

goat blood serum was 273.0-1,090.0 µg/L with an average of 587.4±236.0 µg/L (Schweinzer *et al.*, 2017). This data suggested that species differences may influence mineral concentrations in the blood. These results were supported by the report of Soch *et al.* (2010), that mineral concentrations in the blood are influenced by species differences. Mahusoon *et al.* (2004) stated that differences in mineral metabolism can result in differences in mineral concentrations between sheep breeds.

Table 1: Concentrations of Zn and Fe in the blood plasma of gembrong and boerka goats.

Types of Goats	Sample	Microminerals (mg/dL)	
		Zinc (Zn)	Iron (Fe)
Gembrong	G1	0.024	3.23
	G2	0.001	2.57
	G3	0.001	2.25
	Average (±SD)	0.009±0.013 ^a	2.683±0.500 ^a
Boerka	B1	0.076	3.43
	B2	0.076	2.25
	B3	0.076	2.66
	Average (±SD)	0.075±0.002 ^b	2.780±0.599 ^a

^a ^bDifferent superscripts in the same column indicate significant differences (P<0.05); **G**: Gembrong; **B**: Boerka.

The findings of this study proved that differences in goat breeds affect the concentration of Zn minerals. In contrast, Sogutlu *et al.* (2022) reported no breed-related influence on Zn concentrations in dogs. Zn concentration in Labrador dog breeds, German Shepherd, Instruction, Turkish Tazi, Setters, Malinois, and Golden Retriever were measured at 44.19±4.64; 43.72±3.98; 47.18±4.08; 41.76±3.65; 42.68±3.99; 40.92±4.06; and 46.78±4.14 mmol/L, respectively. These variations in results may be attributed to species differences. Dhami *et al.* (2001) reported that breed differences in cattle did not significantly affect blood plasma zinc (Zn) concentrations. For instance, the Zn concentrations in the blood plasma of different cattle breeds were as follows: Gir – 1.36 ± 0.05 ppm, Jersey – 0.98 ± 0.09 ppm, Holstein-Friesian – 1.25 ± 0.12 ppm, Crossbred – 1.42 ± 0.10 ppm, and Jafarabadi – 1.31 ± 0.09 ppm. However, the findings of the present study align with those of Kalita *et al.* (1999), who reported that Zebu cattle tend to have lower mineral concentrations compared to other breeds.

The average Fe concentration was not significantly different, but the average Fe concentration of Gembrong goats tended to be lower than the Fe concentration of Boerka goats. In this study, Fe concentration in both breeds was higher than the Fe concentration in goats reported by Schweinzer *et al.* (2017) with concentration of 1,280-1,410 µg/L (average of 1,886.5±416.3 µg/L). Similar results were reported by Sogutlu *et al.* (2022), that Fe con-

centration in dogs was not influenced by breed. The iron (Fe) concentrations in the blood of various dog breeds were reported as follows: Labrador – 6.63 ± 1.21 mmol/L, German Shepherd – 6.88 ± 1.32 mmol/L, Instruction – 7.04 ± 1.34 mmol/L, Turkish Tazi – 6.91 ± 1.04 mmol/L, Setters – 6.42 ± 1.03 mmol/L, Malinois – 6.76 ± 1.12 mmol/L, and Golden Retriever – 7.10 ± 1.12 mmol/L. In contrast, Dhami *et al.* (2001) reported that differences in cattle breeds affect blood plasma Fe concentrations. The highest Fe concentration was found in the Jafarabadi and crossbred breeds, while the lowest was found in the Jersey breeds. The iron (Fe) concentrations in the blood plasma of different cattle breeds were reported as follows: Gir – 5.56 ± 0.15 ppm, Jersey – 5.31 ± 0.49 ppm, Holstein-Friesian (HF) – 5.92 ± 0.50 ppm, Crossbred – 6.13 ± 0.62 ppm, and Jafarabadi – 6.89 ± 0.46 ppm.

The correlation test revealed that zinc (Zn) was significantly associated (P < 0.05) with both semen volume and sperm concentration. The correlation value of Zn with semen volume and spermatozoa concentration was 0.88 and 0.94 respectively. This is in line with the report by Osadchuk *et al.* (2021) that Zn concentration was positively correlated with semen volume, spermatozoa concentration, motility, and normal spermatozoa morphology. Zn supplementation has been justified could significantly increase semen volume, spermatozoa motility, and the percentage of normal spermatozoa morphology (Zhao *et al.*, 2016; Rahman *et al.*, 2014) The increase in semen volume and spermatozoa concentration may be related to the role of Zn in nucleic acid and protein metabolism (Mousavi-Esfokhi *et al.*, 2023). In addition, low Zn concentrations has been associated with low testosterone concentrations (Dhami *et al.*, 2001). Although the correlation between Zn with semen volume and spermatozoa concentration was relatively strong, these results have limitations to show the true relationship between variables, particularly in large populations given the limited number of available samples that was used in this study.

The mineral Zn plays an important role in the spermatogenesis process and plays a major role in regulating spermatogonia reproduction and germ cell meiosis. It is necessary to ensure normal function of the hypothalamic-pituitary-gonadal axis. The mineral Zn has a major role in the 5α reductase enzyme required for the transformation of testosterone into the biologically active form, 5α dihydrotestosterone, which is very important for the production and secretion of testosterone from Leydig cells (Fallah *et al.*, 2018). The increase in spermatozoa volume and concentration due to the influence of Zn is possibly related to an increase in the testosterone hormone through antioxidant action and steroidogenic enzyme interactions in Leydig cells (Santos and Teixeira, 2021).

Zinc (Zn) supplementation has been shown to enhance fertility in both Samosir goats and rams (Siswoyo *et al.*, 2018; Mousavi-Esfikhahi *et al.*, 2023). In Samosir goats, dietary Zn supplementation generally improved semen quality (Siswoyo *et al.*, 2018). Similarly, in rams, Zn administration led to improvements in sperm quality, membrane integrity, and spermatozoa viability (Mousavi-Esfikhahi *et al.*, 2023). A deficiency of Zn in the diet can impair the antioxidant defense system, potentially affecting reproductive performance (Agarwal *et al.*, 2012).

The correlation test showed that iron (Fe) was not significantly associated with semen volume or sperm concentration ($P > 0.05$). The correlation between Fe and semen volume was negative, with a coefficient of 0.10, while the correlation with sperm concentration was weakly positive at 0.07. These results were in line with the report by Nenkova *et al.* (2017), which reported that there was no significant relationship between Fe concentration and spermatozoa concentration, motility, viability, and morphology. Although not significant, Fe tended to show a negative influence on semen volume. Hashemi *et al.* (2018) in their study reported that Fe can reduce spermatozoa quality and motility through the induction of lipid peroxidation and the formation of hydroxyl radicals. Different results were reported by Pipan *et al.* (2021) and Liu *et al.* (2020) that Fe was positively correlated with spermatozoa concentration, total spermatozoa count, and spermatozoa motility. The absence of a correlation between Fe and semen volume and spermatozoa concentration in the study should be carefully examined due to the limited number of samples available in the study for the forementioned reasons. When evaluate the data in detail, the results of this study indicated that the tendency of increasing Fe concentration resulted in increasing semen volume and concentration as stated by Pipan *et al.* (2021) and Liu *et al.* (2020).

Fe plays an important role in the synthesis of nucleic acids and proteins, electron transport, cellular respiration, proliferation, and differentiation, all of which are related to spermatogenesis and spermatozoa metabolism. Sertoli cells and Leydig cells are important ferritin reservoirs. Fe mineral is also an important component of catalase, the main antioxidant enzyme to prevent fluctuations in free radical production and protect cellular structure and function from oxidative damage (Pal *et al.*, 2017). However, Fe can be toxic at high levels in which high Fe concentrations produce fewer spermatozoa, and when concentration increases, daily spermatozoa production decreases (Tvrdá *et al.*, 2012).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, breed differences between Gembrong and Boerka goats influenced zinc (Zn) concentrations but had

no effect on iron (Fe) concentrations. Zinc levels were significantly correlated with both semen volume and sperm concentration, whereas iron levels showed no significant correlation with either parameter.

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NOVELTY STATEMENTS

This research is the first research to examine the relationship between Zn and Fe minerals in Gembrong goats and compare them with Boerka goats.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the manuscript.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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