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Original Article

Effect of velvet Timor deer (*Rusa timorensis*) supplementation to fertility status of male Wistar rats (*Rattus norvegicus*)

Nur Hafidzah Devi Kartika[1], Daud Samsudewa[1,*], and Nor Basid Adiwibawa Prasetya[2]

[1] Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, 50275, Indonesia
[2] Department of Chemistry, Faculty of Science and Mathematic, Universitas Diponegoro, Semarang, 50275, Indonesia
*Correspondence: daudreproduksi@gmail.com

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Abstract

Objective: To determine the increase in calcium (Ca) and zinc (Zn) content in Timor deer blood before and after velvet powder supplementation. This study also investigates the effect of velvet powder supplementation on male rats' fertility status.
Methods: This study employed twenty males Wistar rats weighing 200 – 250 g and aged 90 days. The sample was divided into four groups: the control group (T0), the25 mg kg^{-1} WB^{-1} velvet powder (T1), the 50 mg kg^{-1} WB^{-1} velvet powder (T2) and the 100 mg kg^{-1} WB^{-1} velvet powder (T3). Ca and Zn concentrations in rat blood were determined using an atomic absorption spectrophotometer (AAS) in the Feed Nutrition Laboratory at Universitas Diponegoro. The fertility status (sperm motility, mortality and abnormality) of male rat sperm was studied. In this study, statistical analysis was performed using analysis of variance (ANOVA).
Results: The results revealed a significant difference $P<0.05$ ANOVA which was shown in blood Ca and Zn parameters before and after treatment, motility, sperm death and sperm abnormality in the rats.
Conslusions: In conclusion, supplementation with 100 mg kg^{-1} WB^{-1} Timor deer velvet powder will improve the fertility status of male rats.

Keywords: Calcium; Fertility; Timor deer; Velvet powder; Zinc

INTRODUCTION

Timor deer (Rusa timorensis) is an endemic resource animal with economic, ecological and aesthetic value. The primary product of Timor deer is a low fat, high protein meat. Velvet, the growing stage of buck antler, is a product derived from Timor deer[1]. Deer life span is between 3- and 20-years old [2] and velvet regeneration occurs annually [3].

Fertility refers to an individual's ability to fertilize gamete cells which produce new individuals. Physics and psychology have an impact on fertility rates [4]. Sperm analysis can be used to calculate fertility rates [5]. Sperm quality has become a requirement and specification of the fundamental aspects of species reproductive biology [6].

Velvet is a raw material that is frequently used in traditional Chinese and Korean for antioxidants, immunomodulation and libido stimulation. This material contains minerals, fatty acids, amino acids, polysaccharides, protein and phospholipids in which the combination forms bioactive substances with pharmaceutical properties [7]. Calcium [Ca], phosphor [P], magnesium [Mg], natrium [Na], ferric [Fe], kalium [K], zinc [Zn], mangan [Mn] and cuprum [Cu] are all found in Sika and Red deer velvet [3]. Among them, Ca and Zn are two elements that have been linked to improved male reproductive health and sperm quality [8]. Nothing scientific report of Timor deer velvet that has been commercialized, but no scientific report. The aim of this study is to compare the levels of Ca and Zn in blood before and after supplementation with velvet

powder. This study also looked at the effect of velvet powder on sperm quality (motility, abnormality and mortality) in male rats. Therefore, the potential utilization value of Timor deer can be optimized.

MATERIAL AND METHODS

Ethic Statement

Protocol of experiment and animal were approved by the Animal Research Ethics Committee of Universitas Diponegoro Number. 58-06/A-6/KEP-FPP.2022.

Material

Twenty male Wistar rats weighing of 200–250 g and aged 90 days old were used in this study. Velvet powder used for rats treatments in classification the control group (T0), 25 mg kg^{-1} WB^{-1} velvet powder (T1), 50 mg kg^{-1} WB^{-1} velvet powder (T2), and 100 mg kg^{-1} WB^{-1} velvet powder (T3). Surgical tools used for rats surgery, microscope for observed the sperm cells, dilution tools for dilute the semen of the rats, mineral analysis tools for observed Ca and Zn of velvet powder, and semen quality tools (motility, abnormality, and mortality percentage rats semen) for observed the rats sperm.

Methods

Treatments

This study used five steps, 1) Ca and Zn analysis of velvet powder, 2) analysis of the increased levels of calcium and zinc in rat blood, 3) rearing of Wistar rats, 4) surgery of Wistar rats, 5) sperm quality analyze of Wistar rats.

Ca and Zn analysis of velvet powder

Calcium and zinc content in blood rats were examined using an atomic absorption spectrophotometer (AAS).

Analysis of the increased levels of calcium and zinc in rat blood

The analysis of the increased levels of Ca and Zn were done twice, first was blood analyze before the rearing of Wistar rats and the last was blood analyze after the rearing of Wistar rats. The analyze were using an atomic absorption spectrophotometer (AAS).

Rearing of Wistar rats

Wistar rats reared for 49 days in a row. In the rearing process the rats were administered orally treatments on every day, in accordance with rat spermatogenesis [9]. Twenty rats were randomly assigned to one of four group treatments. Tables 1 and 2 show the ingredients of rats feed, as well as the calcium and zinc content.

Table 1. Ingredients of rats ration

Ingredients	Quantity
Water content	14%
Crude protein	19%
Crude lipid	3%
Starch	8%
Ash	8%
Calcium	1.2%
Phospor	1%

Table 2. Ingredients of Ca and Zn in Velvet of Timor Deer

Ingredients	Quantity
Calcium (Ca)	15.7308 %
Zinc (Zn)	83.9822 ppm

Surgery of Wistar rats

Surgical was aimed to take the cauda epididymis of Wistar rats, because the mature sperm stored in cauda epididymis.

Sperm quality analyze of Wistar rats

After 49 days of treatment, semen samples were taken to analyze the quality. The sperm quality analysis namely, motility, abnormality, and mortality parameters were counted in the Genetic Breeding and Reproduction laboratory of Universitas Diponegoro using the method of [10].

Data analysis

In this study, ANOVA was used for statistical analysis. When the ANOVA revealed significant differences, DMRT was used. The statistical analysis was supported by SPSS 25.

RESULTS

The increase of calcium and zinc in rat blood before and after administration of velvet powder supplementation

The administration of Timor deer velvet powder supplementation for 49 days had a significant difference in the increase in blood Ca in rats as shown in Table 3. The results showed that the effect of giving Timor deer velvet powder at different doses had a significant effect on the rate of change in blood Ca $P<0.05$.

The administration of Timor deer velvet powder supplementation for 49 days had a significant difference on changes in Zn in the blood of rats as shown in Table 3. The results showed that the effect of giving Timor deer velvet powder in different doses had no significant effect on the rate of change in blood Zn $P<0.05$.

Table 3. Rate changes of ca and zn in blood rats

Variable	Ca[1)]	Zn[1)]
	---%---	--ppm--
Con*	6.4178[a]	0.2393[a]
T1	6.9628[a]	0.2578[ab]
T2	7.4962[a]	0.3138[bc]
T3*	13.6716[b]	0.6288[c]
Average	8.6371	0.3599

Con, control; T1, treatment dose 25 mg/KgBB; T2, treatment dose 50 mg/KgBB; T3, treatment dose 100 mg/KgBB.
[1]) Comparisons between Con and Trt groups on a multivariate analysis of variance (ANOVA).
* $p<0.05$

Rat Semen Analysis

The fertility capacity to the fertilization process was demonstrated by the motility of sperm rats. The results of the motility parameter are shown in Table 4. The results showed that different doses of Timor deer velvet powder had effect on motility $P<0.05$. Furthermore, T3 had higher motility than T0, T1, and T2. In addition, the results of sperm mortality measurement showed that the treatment had a significant difference ($P<0.05$) on spermatozoa mortality. The mortality rate in the T3 treatment group was significantly lower ($P<0.05$) than in the T0, T1, and T2 groups. Similarly, analysis of abnormalities revealed that all treatments were significantly different ($P<0.05$) for sperm abnormalities. The $P<0.05$ abnormality was significantly lower in the T3 treatment group compared to the T0, T1, and T2 groups. Figure 1 shows normal sperm images, while Figure 2 shows abnormal sperm images.

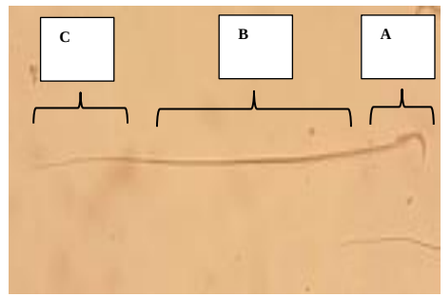

Figure 1. Shape of Normal Sperm. A = Head, B = Body, C = Tail

DISCUSSION

The significant increase in calcium (Ca) content in the blood may be attributed to the use of Timor deer velvet powder. This could be due to the presence of calcium in the velvet powder itself. Furthermore, the calcium content of the feed has been linked to an increase in blood calcium levels. This finding is consistent with the findings of [11], who discovered that calcium intake through feed can affect calcium levels in the blood.

The significant increase in zinc (Zn) content in the blood can be attributed to the amount of Zn determined by the Zn content in the feed. According to [12], Zn consumption through feed is determined not only by the Zn content of the feed, but also by the availability and digestibility of Zn in the intestine.

Mortality in T3 is significantly different from other groups, which may be influenced

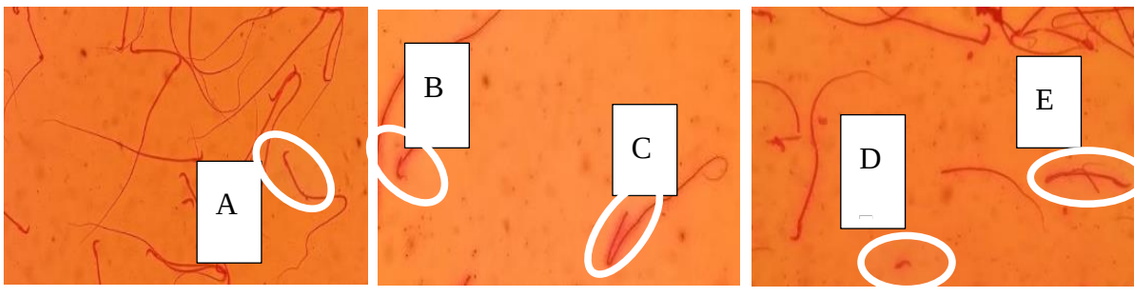

Figure 2. Picture of Abnormal Sperm. A = Crooked body of sperm, B = Crooked head of sperm, C = Sperm without head, D = Sperm without head and tail, E = Crooked tail of sperm

Table 4. Results of Sperm Test After Treatments

Variable	Motility[1)]	Mortality[1)]	Abnormality[1)]
	----------%----------		
Con	*61,3332 ± 4,22 [a]	*18,3654 ± 4.22[bc]	16,6961 ± 4,22[b]
T1	59,6665 ± 2,27 [a]	22,6773 ± 4.49[c]	18,3701 ± 4,49[b]
T2	65,3333 ± 4.98 [ab]	14,0443 ± 3.94[b]	15,1724 ± 2,65[b]
T3	*68,7333 ± 6.66 [b]	* 8,0110 ± 2.02[a]	8,3535 ± 1,94 [a]

T0, control; T1, treatment dose 25 mg/KgBB; T2, treatment dose 50 mg/KgBB; T3, treatment dose 100 mg/KgBB.
1) Comparisons between Con and Trt groups on a multivariate analysis of variance (ANOVA).
* $p<0.05$

by the use of Timor deer velvet powder, which contains zinc. Zinc is a cofactor of the Superoxide Dismutase (SOD) enzyme. Zinc is a cofactor of the SOD enzyme, which protects the sperm from reactive oxygen species (ROS), resulting in optimal sperm viability [17]. It could also be caused by the Ca content in Timor deer velvet. Ca's ability to stimulate and release testosterone is well-known. According to [18], calcium is involved in the production of steroid hormones. The steroid hormone testosterone is used in male reproduction. [19] stated that testosterone hormones play an important role in spermatogenesis until sperm release.

The lowest sperm mortality score revealed by the third treatment (velvet supplementation in 100 mg/kgBB) is 8.01±2.02%. Because of the low mortality rate, sperm was highly viable. Reported that the membrane plasm plays an important role in protecting sperm from extracellular injury [20]. Plasma membranes' function is to protect sperm from disruption. This is similar to [21], who stated that plasma membranes become sperm protectors, with a role in maintaining the capacitation process, acrosome reaction, and spermatozoa metabolisms. Because of sperm mortality, mortal sperm turns red while live sperm does not. The red color of mortal sperm caused by eosin passes through the membrane cell of mortal sperm. According to Malinda et al. Mortal sperm turns red due to increased permeability and absorbs eosin, whereas non-mortal sperm does not absorb color from eosin [22]. The red color of mortal sperm is caused by damaged plasma membranes, so the red color of eosin is absorbed [23].

The abnormality in T3 is significantly different from other groups, which may be influenced by Timor deer velvet powder supplementation. It could be caused by the Zn content of Timor deer velvet powder. According to [24], the role of zinc in the testis is to stimulate the maturation of germinal cells into spermatozoa, which can reduce the causes of sperm morphology abnormalities. Sperm with a low rate of abnormality is considered to be of high quality. The abnormality rate of fertile sperm is less than 20% [25]. Figure 1 depicts a typical sperm with a hook-like head and a long, straight tail. This is consistent with that normal rats sperm has a falciform head [26]. Lin et al. [27] found that rat sperm has a falciform-shaped head. However, sperm abnormalities can manifest themselves in a variety of ways, including crooked head shapes, sperm without a tail, and tails without a head. [26] classified sperm abnormalities into four types: double heads, round and reduced heads, round and enlarged heads, and deformed acrosomes with separate heads. [29] found that abnormal sperm with irregularly shaped heads, excessive bending, and non-straight tails. Figure 2 shows an abnormal shape of sperm. There are two types of sperm abnormalities: primary abnormality and secondary abnormality. A primary abnormality is an abnormal form of sperm caused by a failure of spermatogenesis. The primary abnormality of sperm is caused by an imperfect process of sperm formation or by damage to sperm cells from within the testicles [28]. [30] found that secondary abnormalities in sperm were caused by human error during staining in the sample of smear preparation and the storage process.

CONCLUSIONS

The ability of Timor deer velvet powder to improve the reproductive quality of male Wistar rats has been demonstrated, making it an alternative ingredient for fertility boosters. A dose of 100 mg kg^{-1} WB^{-1} supplementation has the greatest ability to improve reproductive quality.

CONFLICT OF INTEREST

This research did not have to another conflict of interest.

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