Bantara Journal of Animal Science **p-ISSN: 2656-9701**
Vol. 7, No. 2, October 2025 **e-ISSN: 2657-1587**
DOI: https://dx.doi.org/10.32585/bjas.v7i2.7226 Journal homepage: https://journal.univetbantara.ac.id/index.php/bjas/index

The Effect of Young Timor Deer (*Rusa timorensis*) Hornbill Flour Supplementation on the Testicular Size of Male Wistar Rats (*Rattus norvegicus*)

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* *Received for review September 16, 2025 Accepted for publication October 31, 2025*

Abstract: The processing of young deer antlers is an example of using deer as wildlife, with the principle of continuity and without harming them. The purpose of this study was to determine the effect of young Timor deer antler meal and to determine the impact of its supplementation on the size of the testes of male Wistar rats. Twenty male Wistar rats with a body weight of 200–250 g and an age of 90 days were used. The samples were divided into four groups: a control group and three treatment groups of young Timor deer antler meal at doses of 25 mg kg^{-1} BW^{-1}, 50 mg kg^{-1} BW^{-1}, and 100 mg kg^{-1} BW^{-1}. Ca and Zn measurements were carried out using an atomic absorption spectrophotometer at the Feed Nutrition Laboratory of Diponegoro University. The size of the left and right testes (length, width, diameter, and weight) was analyzed using Analysis of Variance (ANOVA) as a statistical test, and Duncan's Multiple Range Test was used for testis weight. Data on Ca and Zn content were analyzed descriptively. The results showed a significant difference ($P<0.05$) in testicular weight parameters, while testicular length, width, and diameter were not significantly different ($P\geq0.05$). Therefore, it can be concluded that supplementation with young Timor deer antler flour affected testicular weight, but not testicular length, width, and diameter.

Keywords: Timor deer; Young deer antlers; Mice; Supplementation; Reproductive organs.

Introduction

Deer have economic, aesthetic, educational, and medical value. Young Timor deer antlers are a byproduct of deer, which regenerate annually (Wu et al., 2013). Deer can live up to 20 years (Mahre et al., 2016). According to Takadjandji et al. (2019), young deer antlers contain chemical compounds, antioxidants, and minerals such as calcium, phosphorus, magnesium, sodium, and zinc. Antioxidants protect sperm from Reactive Oxygen Species (ROS) during the spermatogenesis phase. Minerals play an essential role in male reproductive health and sperm quality (Minamniha et al., 2019). In China and Korea, young deer antlers are believed to increase fertility due to their antioxidant and mineral content (Ismanto, 2016). Fertility levels are also reflected in testicular size, which is related to the number of seminiferous tubules for spermatogenesis. The high calcium (Ca) and zinc (Zn) content of young deer antlers has the potential to improve reproductive performance in animals, specifically male Wistar rats. Male Wistar rats are commonly used as research subjects before being used directly in livestock or humans due to their genetic and biological similarities to humans, ease of care and maintenance, and relatively short life cycle.

The development of biotechnology utilizing wildlife products is increasingly oriented towards sustainability principles, with young Timor deer antlers an ideal example, as they naturally regenerate each year without endangering deer populations (Orassay et al., 2024). A recent metabolomics study by Tansathien et al. (2019) revealed that young antlers contain over 200 bioactive compounds, including type I collagen, chondroitin sulfate, and essential amino acids, all of which play a role in tissue regeneration. These compounds are not only beneficial for reproductive health but also show potential as immunomodulators and anti-aging agents (Zang et al., 2016). These unique characteristics make this deer byproduct a high-value commodity in the pharmaceutical and nutraceutical industries.

Feed supplementation is the daily administration of feed in specific amounts and at specific intervals, in addition to the main feed, for particular purposes (Sudrajad and Utomo, 2021). The mechanism of action of young antlers in increasing male fertility primarily occurs through three main pathways: (1) activation of the endogenous antioxidant system by increasing the expression of SOD and catalase enzymes (Orassay et al., 2024), (2) stimulation of gonadotropin hormone secretion through natural steroid compounds (Wang et al., 2023), and (3) improvement of blood microcirculation in the testes through L-arginine, which plays a role in nitric oxide (NO) synthesis. The use of male Wistar rats in this study is supported by the findings of De et al. (2007), which demonstrated similarities in spermatogenesis patterns between Wistar rats and humans, particularly in sensitivity to nutritional stimuli and hormonal regulation. This animal model also allows for the observation of supplementation effects over a relatively short period (4-8 weeks), given that the spermatogenesis cycle in mice lasts only 52 days (compared to 74 days in humans), making it highly efficient for preliminary studies. Feed supplementation is defined as providing feed at specific levels and intervals daily in addition to the main feed for a particular purpose (Sudrajad and Utomo, 2021). Therefore, this study was conducted to increase the potential utilization of Timor deer.

Biogas production from anaerobic digestion of livestock waste offers a practical, eco-friendly solution. With a high methane content of 4,800 to 6,700 kcal/m³, cow manure is an efficient source of renewable energy. Biogas not only reduces environmental pollution but also provides alternative energy for rural households, contributing to economic savings and environmental conservation. Sruni Village has been developing a Biogas-Based Energy Self-Sufficiency Village (Desa Mandiri Energi, DME) program since 2010, collaborating with governmental and private institutions to provide training and technical support. However, the program faces challenges, including limited technical skills among farmers, insufficient financial resources, and infrastructure inefficiencies, which hinder the maximization of biogas utilization.

Given these conditions, this study aims to analyze the strengths, weaknesses, opportunities, and threats (SWOT) associated with biogas utilization in Sruni Village. Furthermore, it seeks to formulate strategic empowerment initiatives to optimize biogas production and utilization, supporting the realization of a sustainable Energy Self-Sufficiency Village (DME) model that can enhance rural livelihoods and contribute to Indonesia's renewable energy transition.

Materials and Methods

The research protocol was approved by the Animal Research Ethics Committee of Diponegoro University (No. 58-06/KEP-FPP.2022). The research was conducted in the experimental animal laboratory of the Breeding and Reproduction Genetics Laboratory in 2022. The material used was twenty male Wistar rats weighing 200–250 g and aged 90 days. Young Timor deer Ranggah flour was dissolved in distilled water and sodium carboxymethylcellulose (Na-CMC), and then given orally using the force-feeding method after eating once a day, and the feed was given twice a day (morning and evening) for 49 consecutive days, according to the rat spermatogenesis cycle (Toeliehere, 1993). The research design used was ANOVA with four treatments and five replications, detailed as follows:

T0: Basal feed
T1: Basal feed + supplementation with young deer antler meal 25 mg/kg/body weight
T2: Basal feed + supplementation with young deer antler meal 50 mg/kg/body weight
T3: Basal feed + supplementation with young deer antler meal 100 mg/kg/body weight

The rat ration content is presented in Table 1.

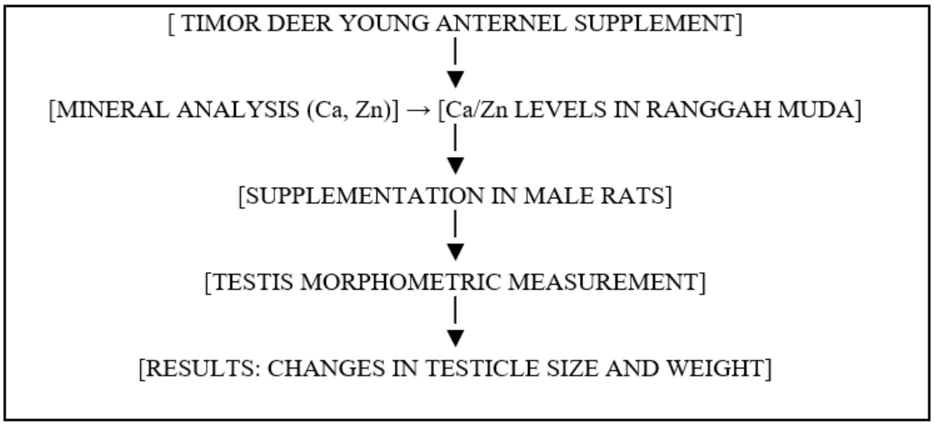
Table 1. Rat Feed Ration Composition

Composition	Quantity
Water Content	14%
Crude Protein	19%
Crude Fat	3%
Carbohydrate	8%
Ash	8%
Calcium	1.2%
Phosphorus	1%

The calcium (Ca) and zinc (Zn) content of young Timor deer antlers was analyzed using atomic absorption spectrophotometry (AAS) to obtain precise mineral composition data. This analysis refers to the method developed by Amin et al. (2023), which validates AAS for the measurement of minerals in biological materials. The nutritional potential of young deer antlers as a supplement is also supported by the findings of Takadjandji et al. (2019), who reported high mineral content in young antlers of other deer species.

The length and width of the left and right testicles were measured using vernier calipers. This procedure followed the standard protocol. Supriyadi et al. (2018) stated the importance of repeating measurements (three times) to minimize errors. The accuracy of the measuring instruments was verified in a study by Pratiwi et al. (2020), which found that vernier calipers and digital scales were reliable instruments for biomedical research.

Testicular diameter was measured by wrapping a thread around the broadest part, while testicular weight was measured using a high-accuracy digital scale. These morphometric data were then linked to the effects of supplementing young Timor deer antlers. The research chart is shown in Illustration 1.



Calcium and Zinc Content

Table 2 shows that young Timor deer antlers in this study showed higher Ca than Zn content. This mineral content is higher than that reported by Takadjanji et al. (2019) in Dramaga, Bogor. According to Zhang et al. (2005), differences are influenced by the age of the deer, with Ca, Zn, P, K, and Al

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increasing with age.

Table 2. Feed Ration Composition

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

Results and Discussion

The study findings showed no significant difference (P≥0.05) in testicular length between the young antler supplementation treatment groups (Table 3). These results are consistent with Berger's (2019) study, which found that after reaching puberty, dimensional growth in ruminant testicles tends to plateau because Sertoli cell division has reached saturation. Similarly, Chen et al.'s (2015) study reported that growth factor supplementation from deer antlers had a significant effect only on prepubertal mice, not on adult mice.

Table 3. Testicular Length

Treatment	Quantity (cm)	
	Left	Right
T0	17.378^{a}	17.148^{a}
T1	16.5693^{a}	17.166^{a}
T2	17.810^{a}	18.170^{a}
T3	18.0027^{a}	17.908^{a}

^{abc} Different superscripts in one column indicate significant differences (p<0.05). T0 (Control parameter); T1 (Dose 25 mg kg^{-1} BW$^{-1)}$; T2 (Dose 50 mg kg^{-1} BW^{-1}); T3 (Dose 100 mg mg kg^{-1} BW^{-1}).

Nevertheless, the pattern of testicular length increase in groups T2 and T3 (especially the right testis) is noteworthy. This finding aligns with the report by Zang et al. (2016), which identified IGF-1 and TGF-β in young Cervus nippon antlers as capable of stimulating Leydig cell proliferation. However, as noted by Gao et al. (2021), the morphometric response of testicles to supplementation is highly dependent on the sexual developmental status of the test animals. Therefore, further research using a prepubertal animal model is recommended to optimize the anabolic potential of young Timor hornbills.

Similar results were observed for testicular width (Table 4), which is also influenced by puberty [10]. The testicular width measurements in Table 4 show variation between treatments (T0–T3), but no statistically significant differences were found (P≥0.05). This finding is consistent with Berger (2019), who reported that after puberty, dimensional growth in mammalian testicles tends to stabilize because Sertoli cell division and spermatogenesis have reached their peak. These results are also supported by a study by Chen et al. (2015), which found that supplementation with active ingredients from young hornbills had no significant effect on testicular morphometric parameters in adult animals.

Table 4. Testicular Width

Treatment	Quantity (cm)	
	Left	Right
T0	9.198^{a}	9.197^{a}
T1	8.865^{a}	8.742^{a}
T2	9.633^{a}	9.584^{a}
T3	9.704^{a}	9.384^{a}

^{abc} Different superscripts in one column indicate significant differences (p<0.05). T0 (Control parameter); T1 (Dose 25 mg kg^{-1} BW$^{-1)}$; T2 (Dose 50 mg kg^{-1} BW^{-1}); T3 (Dose 100 mg mg kg^{-1} BW^{-1}).

However, the pattern of increase in testicular width in groups T2 and T3 (especially the left testicle) indicated a positive, albeit insignificant, response. This may be explained by the growth factor content in young hornbills, as reported by Zang et al. (2016), such as IGF-1 and TGF-β, which have the potential to stimulate Leydig cell proliferation. However, as Guan and Martin (2017) noted, the testicular morphometric response to supplementation is highly dependent on the sexual developmental status of the test animals. Therefore, further research using prepubertal animal models is needed to examine further the potential anabolic effects of young Timor antlers on male reproductive organ development.

The testicular diameter measurements in Table 5 show variation between treatments (T0-T3), but no statistically significant differences (P≥0.05). This finding aligns with previous research on testicular length and width and reinforces the conclusion that young antler supplementation does not significantly affect testicular morphometric parameters in adult mice. These results are consistent with the report by Mirsky et al. (2016), who found that testicular diameter is a relatively stable parameter in sexually mature animals.

Table 5**.** Testicular diameter

Treatment	Quantity (cm)	
	Left	Right
T0	3.133^{a}	3.526^{a}
T1	3.067^{a}	3.04^{a}
T2	3.540^{a}	3.526^{a}
T3	3.473^{a}	3.38^{a}

^{abc} Different superscripts in one column indicate significant differences (p<0.05). T0 (Control parameter); T1 (Dose 25 mg kg^{-1} BW$^{-1)}$; T2 (Dose 50 mg kg^{-1} BW^{-1}); T3 (Dose 100 mg mg kg^{-1} BW^{-1}).

However, the pattern of testicular diameter increase in groups T2 and T3 shows an interesting trend. According to a study by Xu et al. (2022), young deer antlers have the potential to influence testicular interstitial cell development. However, as revealed in Berger's (2019) study, the morphological response of testicles to external stimulation in adult animals tends to be limited. This finding further underscores the importance of conducting additional research using prepubertal animal models to explore the potential anabolic effects of young antlers comprehensively.

Testicular weight differed significantly (P<0.05) between treatments (Table 6). Supplementation with young Timor deer antlers, rich in antioxidants, can inhibit reactive oxygen species (ROS). This can enhance antioxidant activity, thereby increasing the number of spermatogenic cells and thickening the seminiferous tubules, thereby increasing testicular weight (Lea et al., 2014). Testicular weight is also influenced by the development of germinal epithelial cells that produce sperm (Atang and Palupi, 2020). Significant differences in testicular weight are also caused by germinal epithelial cell development. Junqueira et al. (2007) stated that the testes are composed of seminiferous tubules composed of epithelial cells.

Table. 6 Testicular weight

Treatment	Quantity (cm)	
	Left	Right
T0	1.595^{a}	1.601 ^{a}
T1	1.610^{a}	1.654 ^{a}
T2	1.748^{ab}	1.732 ^{ab}
T3	1.995^{b}	2.038 ^{b}

^{abc} Different superscripts in one column indicate significant differences (p<0.05). T0 (Control parameter); T1 (Dose 25 mg kg^{-1} BW$^{-1)}$; T2 (Dose 50 mg kg^{-1} BW^{-1}); T3 (Dose 100 mg mg kg^{-1} BW^{-1}).

The results showed a significant difference (P<0.05) in testicular weight between treatment groups (Table 6). Group T3, supplemented with a dose of 100 mg/kg body weight, showed the highest increase in testicular weight (1.995-2.038 g) compared to the control group (T0). This finding aligns with research by Zang et al. (2016), who reported that young antler extract can increase testicular weight through mechanisms such as increased spermatogenic cell proliferation and thickening of the seminiferous tubules. Fitria et al. (2015) stated that testicular weight is related to the number of spermatozoa filling the lumen.

Two main mechanisms can explain this increase in testicular weight. First, the antioxidant content in young antlers, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), as reported by Qamar et al. (2023), can suppress the production of reactive oxygen species (ROS), thereby protecting germ cells from oxidative stress. Second, research by Liu et al. (2023) shows that growth factors in young antlers, such as IGF-1 and TGF-β, can stimulate the development of the germinal epithelium and improve testicular germinal histology, thereby contributing to increased testicular weight.

Conclusion

Supplementation of young Timor deer antlers in male Wistar rats affected testicular weight, but not testicular length, width, and diameter.

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