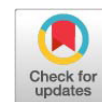


Research Article



Dietary L-Arginine Improves Semen Quality of Kacang Goats Under Dry Climate Conditions

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Abstract | This study evaluated the effect of L-arginine added to feed on semen quality of Kacang goats under dry climate conditions. Twelve male Kacang goats aged 1.5–2 years were randomly assigned to four treatments: T0 (0%), T1 (0.5%), T2 (0.75%) and T3 (1.0%) L-arginine supplementation. The basal diet, formulated on a dry matter basis, consisted of 30% concentrate, 40% *Leucaena leucocephala* leaf meal and 30% natural grass. Semen quality was evaluated on the basis of macroscopic parameters (volume, colour, odour, consistency and pH) and microscopic parameters (motility, viability, abnormality and sperm concentration). Semen colour, odour and consistency remained within the normal physiological range for Kacang goats, while pH did not differ significantly among treatments ($P = 0.205$). Semen volume was significantly affected by treatment ($P = 0.002$), with T2 (0.80 mL) significantly higher than the other groups. Among the microscopic parameters, only individual sperm motility was significantly affected by L-arginine supplementation ($P = 0.025$), increasing from 80.00% in T0 to 93.33% in T2. Sperm concentration ($P = 0.340$), abnormality ($P = 0.086$) and viability ($P = 0.644$) showed numerical improvements with increasing L-arginine level but were not statistically significant. Based on these results, dietary supplementation of 0.75% L-arginine (T2) was identified as the optimal level, as it produced the highest semen volume and the only statistically significant improvement in sperm motility among the tested treatments. This study highlights the potential of L-arginine supplementation as a nutritional strategy to enhance the semen quality of Kacang goats under dry climate conditions.

Keywords | L-arginine, Semen quality, Kacang goat, Sperm motility, Dry climate

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INTRODUCTION

The population of Kacang goats in East Nusa Tenggara (NTT) increased by 4.96% in 2022, accompanied by a 5.22% increase in meat production (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2022). Goat meat consumption in Indonesia has also shown a gradual increasing trend, driven by population growth, cultural and religious demand, and increasing consumer preference for small-ruminant meat. Consequently, continuous improvement in goat productivity remains necessary to

support future meat demand, including in major goat-producing regions such as East Nusa Tenggara (Christie, 2007; Sujarwanta *et al.*, 2024).

Kacang goat farming systems in NTT are generally small-scale and are not yet oriented toward integrated and sustainable livestock production systems. Goats are commonly kept as a form of non-cash savings rather than as a primary source of income. The number of animals owned by farmers is relatively low, ranging from 5 to 13 goats, with only 1-2 males per farmer (Tulle *et al.*, 2021).

The limited number of breeding males per herd, combined with restricted genetic exchange among smallholder farms, may reduce reproductive efficiency and slow genetic improvement.

Feeding management is still conventional and highly dependent on seasonal availability. During the rainy season, feed resources are abundant and of good quality, whereas during the dry season feed availability becomes limited and of poor nutritional quality. This condition leads to nutritional imbalance, which may adversely affect reproductive performance, particularly semen quality (Bindari *et al.*, 2013).

Semen quality is a key factor determining male reproductive performance, whether achieved through natural mating or artificial insemination (AI). Although natural mating remains the predominant breeding practice in smallholder goat production systems in Indonesia and the application of artificial insemination is still limited (Tambing *et al.*, 2001), semen quality remains physiologically relevant because it directly reflects buck fertility potential and influences the likelihood of successful conception regardless of the breeding method employed (Rodríguez-Martínez, 2003; Morrell, 2006). Adequate nutrient intake, especially of protein and energy, plays an essential role in spermatogenesis and sperm maturation. Therefore, improving feed quality and nutritional balance is a critical strategy to enhance reproductive performance in goats.

One potential approach to improving reproductive performance is dietary supplementation with L-arginine. L-arginine is a functional amino acid involved in various metabolic pathways, and its metabolism produces bioactive compounds such as nitric oxide (NO), polyamines, agmatine and creatine (Nieves and Langkamp-Henken, 2002; Li *et al.*, 2007). These compounds play important roles in cellular metabolism, including energy production in the form of adenosine triphosphate (ATP) and physiological regulation. In reproductive systems, nitric oxide has been reported to contribute to sperm motility and function by enhancing mitochondrial activity and ATP production, as well as maintaining membrane integrity and protecting sperm cells from oxidative damage (Rosselli *et al.*, 1998; Wu *et al.*, 2009).

Nitric oxide may also contribute to improved blood circulation to the reproductive organs, thereby potentially supporting spermatogenesis and sperm quality (Moncada and Higgs, 1993; Wu *et al.*, 2009). Previous studies have reported that L-arginine supplementation improves reproductive performance in various livestock species. In poultry, L-arginine supplementation has been shown to enhance semen quality and fertility (Ahangar *et al.*, 2017), while in pigs it improves sperm quality under heat stress

conditions (Chen *et al.*, 2018; Fahlevi *et al.*, 2022).

Evidence in small ruminants is more recent and still limited. Oral supplementation with rumen-protected L-arginine increased ejaculate volume, individual sperm motility, sperm concentration and viability in rams, and simultaneously reduced sperm abnormalities while raising serum testosterone and glutathione peroxidase activity (Hussein *et al.*, 2022). Comparable responses have been reported for L-citrulline, the immediate metabolic precursor of arginine, which increased seminal plasma pyruvate and amino acid concentrations and improved semen quality and cryotolerance in rams (Zhao *et al.*, 2023; Fan *et al.*, 2024). The response is not uniform across species, however: in boars, dietary arginine at up to 1.77% modified the abundance of several seminal plasma proteins associated with reproduction without measurably altering sperm motility or morphology (Oladejo *et al.*, 2025).

Information on dietary L-arginine supplementation in Kacang goats raised under dry climate conditions nevertheless remains scarce. Most previous studies have focused on poultry, pigs and laboratory animals, whereas studies evaluating semen quality responses in local goat breeds under nutritional stress conditions are still few. L-arginine plays important roles in ruminant nutrition and metabolism, particularly in physiological processes associated with reproduction and cellular metabolism (Wu *et al.*, 2022). Dry climate regions such as East Nusa Tenggara are characterised by prolonged drought periods, fluctuating temperatures and low-quality feed resources, which may negatively affect reproductive performance and semen quality (Armansyah *et al.*, 2018). Elevated ambient temperature reduces sperm production and motility and increases the proportion of morphologically abnormal spermatozoa in small ruminants (Arero and Ozmen, 2025), and seasonal heat load has been shown to compromise testicular thermoregulation and semen quality in rams maintained in desert environments (Barragán *et al.*, 2023). Research on Kacang goat semen in Indonesia has so far concentrated on preservation and extender technology rather than on nutritional intervention in the live animal (Susilowati *et al.*, 2021, 2022; Mustofa *et al.*, 2023).

Therefore, the present study was conducted to evaluate the effect of graded levels of dietary L-arginine supplementation on the macroscopic and microscopic semen quality of Kacang goats raised under dry climate conditions.

MATERIALS AND METHODS

TIME AND LOCATION

This study was conducted in Sasi Village, Kefamenanu District, North Central Timor Regency, East Nusa Tenggara Province, Indonesia, from August to November

2024. According to climatological records of the Meteorological, Climatological and Geophysical Agency (BMKG) of the East Nusa Tenggara Region, ambient temperature during the dry season generally ranged from 28–34 °C and reached up to 38.4 °C during peak drought periods (BMKG, 2024).

EXPERIMENTAL ANIMALS AND MANAGEMENT

The experimental animals consisted of 12 clinically healthy male Kacang goats aged 1.5–2 years with an initial body weight of 13.67 ± 2.54 kg (mean $\pm$ SD). Two adult female goats exhibiting natural oestrus were used as teaser animals during semen collection. Each animal was housed individually in a pen measuring 0.7 m $\times$ 1.5 m equipped with feeding and drinking facilities. The animals were maintained under standard management conditions. A 14-day adaptation period was provided to allow the animals to adjust to the experimental diets, housing conditions, handling and semen collection procedures before commencement of the experiment.

FEED COMPOSITION AND TREATMENTS

The basal diet consisted of 30% concentrate, 40% *Leucaena leucocephala* leaf meal and 30% natural grass on a dry matter basis. The ingredient composition and proximate chemical composition of the experimental diets are presented in Table 1. The concentrate consisted of ground maize, fish meal and L-arginine at the respective supplementation levels (0, 0.5, 0.75 and 1.0%), whereas the proportions of *Leucaena*

leucocephala leaf meal (40%) and natural grass (30%) were kept constant across all treatments. Proximate analysis showed only slight differences in nutrient composition among treatments, mainly due to the increasing inclusion levels of L-arginine while the same basal diet formulation was maintained.

L-arginine was supplemented at four dietary levels, namely T0 (0%), T1 (0.5%), T2 (0.75%) and T3 (1.0%). Crystalline L-arginine in free (non-rumen-protected) form was used. L-arginine was thoroughly mixed with the concentrate immediately before feeding to ensure homogeneous distribution. The concentrate was offered individually once daily between 07:00 and 08:00 h, while *Leucaena leucocephala* leaf meal and natural grass were provided only after the concentrate had been consumed. This feeding sequence was applied to maximise consumption of the supplemented concentrate before forage feeding. Drinking water was available *ad libitum* throughout the experimental period. The concentrate for each treatment was formulated on a dry matter basis and prepared in two to three batches over the experimental period, with sufficient stock maintained at all times, so that feed quality and availability remained consistent. Daily feed refusal was monitored visually, and no rejection of the L-arginine-supplemented concentrate was observed at any inclusion level, including 1.0%. The actual L-arginine concentration in the prepared diets was not verified analytically; inclusion levels are therefore reported as formulated proportions.

Table 1: Ingredient composition and analyzed chemical composition of the experimental diets.

A. Ingredient composition (% of diet)		Treatments		
Ingredient	T0	T1	T2	T3
Ground maize	23.00	22.50	22.25	22.00
Fish meal	7.00	7.00	7.00	7.00
L-Arginine	0.00	0.50	0.75	1.00
<i>Leucaena leucocephala</i> leaf meal	40.00	40.00	40.00	40.00
Natural grass	30.00	30.00	30.00	30.00
Total	100	100	100	100
B. Selected chemical composition of the experimental diets				
Chemical composition	T0	T1	T2	T3
Dry matter (%)	91.72	91.09	91.00	89.48
Ash (% DM)	6.97	6.87	6.80	6.75
Organic matter (% DM)	93.03	93.13	93.20	93.25
Crude protein (% DM)	14.08	14.55	15.26	15.97
Ether extract (% DM)	5.13	5.11	5.02	5.10
Crude fiber (% DM)	9.03	8.88	8.69	8.63
Carbohydrate (% DM)	73.20	72.99	72.92	72.19
Nitrogen-free extract (% DM)	64.17	64.12	64.23	63.56
Gross energy (MJ/kg DM)	17.98	18.01	18.03	18.10
Metabolizable energy (kcal/kg DM)	3361.70	3369.95	3374.78	3381.74

Notes: T₀ = basal diet without L-arginine; T₁ = basal diet + 0.5% L-arginine; T₂ = basal diet + 0.75% L-arginine; T₃ = basal diet + 1.0% L-arginine. Chemical analyses were conducted at the Feed Chemistry Laboratory, Faculty of Animal Science, Universitas Nusa Cendana, Indonesia (2024).

Table 2: Effect of dietary L-arginine supplementation on macroscopic and microscopic semen characteristics of Kacang goats.

Parameter	Treatment				P-value
Macroscopic parameters	T0 (0%)	T1 (0.5%)	T2 (0.75%)	T3 (1.0%)	
Volume (mL)	0.40 ± 0.17 ^a	0.40 ± 0.11 ^a	0.80 ± 0.04 ^b	0.27 ± 0.06 ^a	0.002
Colour	Creamy	Creamy	Creamy	Creamy	NA
Odour	Typical	Typical	Typical	Typical	NA
Consistency	Thick	Thick	Thick	Thick	NA
pH	6.49 ± 0.03 ^a	6.57 ± 0.09 ^a	6.47± 0.11 ^a	6.42 ± 0.05 ^a	0.205
Microscopic parameters					
Individual motility (%)	80.00 ± 4.08 ^a	86.67 ± 2.89 ^{ab}	93.33 ± 2.36 ^b	85± 4.08 ^a	0.025
Viability (%)	88.07 ± 3.10 ^a	89.94 ± 3.55 ^a	86.68 ± 2.91 ^a	89.88 ± 4.45 ^a	0.644
Abnormality (%)	11.67 ± 1.45	9.42 ± 1.89	7.64 ± 2.23	6.57 ± 2.86	0.086
Concentration (×10 ⁹ spermatozoa/mL)	2.08 ± 0.51 ^a	2.64 ± 0.82 ^a	2.74 ± 0.04 ^a	2.02 ± 0.61 ^a	0.340

Notes: Values are presented as mean ± standard deviation (SD). Means within the same row bearing different superscript letters (^{a-b}) differ significantly according to Duncan's Multiple Range Test ($P < 0.05$). Means sharing the same superscript are not significantly different. NA = not applicable.

Feed samples of the experimental concentrate, *Leucaena leucocephala* leaf meal and natural grass were subjected to proximate analysis to determine dry matter (DM), ash, organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), carbohydrate (CHO), nitrogen-free extract (NFE), gross energy (GE) and metabolisable energy (ME). The analyses were conducted at the Feed Chemistry Laboratory, Faculty of Animal Science, Universitas Nusa Cendana, using standard AOAC methods. The results of the proximate analysis are presented in Table 1.

EXPERIMENTAL DESIGN

The experiment was arranged in a completely randomised design (CRD) consisting of four dietary treatments (T0 = 0%, T1 = 0.5%, T2 = 0.75% and T3 = 1.0% L-arginine supplementation) with three replications. Each treatment consisted of three male Kacang goats randomly assigned to the dietary treatments.

SEMEN COLLECTION AND EVALUATION

Semen was collected using an artificial vagina maintained at 42–45 °C with the assistance of teaser female goats. Semen collection was conducted twice weekly (Monday and Thursday) at 09:00 h throughout the experimental period. Macroscopic evaluation included semen volume, colour, odour, consistency and pH. Microscopic semen evaluation included sperm motility, viability, abnormality and concentration. Sperm motility was evaluated under a light microscope at 400× magnification by examining five microscopic fields and assessing at least 200 spermatozoa per sample. Sperm viability and abnormality were evaluated using eosin-nigrosin staining by counting at least 200 spermatozoa per sample. Sperm concentration was determined using a Neubauer haemocytometer (Marienfeld, Germany).

STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation (SD). Prior to analysis, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Data were analysed by one-way analysis of variance (ANOVA) based on a completely randomised design. When significant differences among treatments were detected, Duncan's multiple range test was used for post-hoc comparisons. Statistical significance was declared at $P < 0.05$. All analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

The effects of dietary L-arginine supplementation on the macroscopic and microscopic semen quality of Kacang goats are presented in Table 2. Among the parameters evaluated, only semen volume ($P = 0.002$) and individual sperm motility ($P = 0.025$) were significantly affected by treatment. Semen pH ($P = 0.205$), sperm viability ($P = 0.644$), sperm abnormality ($P = 0.086$) and sperm concentration ($P = 0.340$) did not differ significantly among treatments, although numerical trends were observed. Semen colour, odour and consistency were assessed qualitatively and were uniform across all treatments. Throughout this section, changes in the non-significant parameters are therefore described strictly as numerical trends and are not interpreted as treatment effects.

MACROSCOPIC SEMEN QUALITY

The macroscopic characteristics of semen volume, colour, odour, consistency and pH are important initial indicators of reproductive health and semen quality. With the exception of semen volume, these parameters were uniform across treatments and remained within the ranges reported

as normal for Kacang goat semen (Widiyono *et al.*, 2017), indicating that dietary L-arginine supplementation up to 1.0% did not adversely affect the basic physical characteristics of semen.

SEMEN VOLUME

Semen volume was the only macroscopic parameter significantly affected by dietary L-arginine supplementation ($P = 0.002$). Semen volume increased from 0.40 ± 0.17 mL in T0 to 0.80 ± 0.04 mL in T2 and then decreased to 0.27 ± 0.05 mL in T3 (to agree with Table 2), indicating a non-monotonic response. Because orthogonal polynomial contrasts were not performed, this pattern is described as non-monotonic rather than as a statistically confirmed dose-dependent response.

The greater semen volume observed in T2 may be associated with enhanced secretory activity of the accessory sex glands, which contribute most of the seminal plasma volume (Hafez and Hafez, 2000; Salamon and Maxwell, 2000). A comparable increase in ejaculate volume has been reported in rams supplemented with rumen-protected L-arginine, in that case accompanied by ultrasonographically confirmed enlargement of the seminal vesicles and bulbourethral glands, which the authors attributed to nitric-oxide-mediated increases in blood flow to the reproductive tract (Hussein *et al.*, 2022). However, accessory gland function and seminal plasma composition were not evaluated in the present study; therefore, this explanation remains speculative.

The reduction in semen volume observed at the highest supplementation level (T3) should be interpreted with caution. Feed intake was not quantified; however, no visible feed refusal was observed in any treatment during the experimental period. Consequently, it cannot be concluded whether the lower semen volume was associated with reduced nutrient intake or with other physiological responses to the higher level of L-arginine supplementation. Additional studies including measurements of feed intake and metabolic indicators, such as blood urea nitrogen and plasma ammonia, are needed to clarify the mechanism underlying this response.

SEMEN COLOUR, ODOUR AND CONSISTENCY

Semen from all treatments was creamy in colour, exhibited the characteristic odour of goat semen and was thick in consistency. A creamy colour and thick consistency are generally associated with high sperm concentration, whereas a watery or translucent appearance indicates lower sperm concentration (Hafez and Hafez, 2000; Roca *et al.*, 2005). The absence of abnormal odour indicates the absence of obvious contamination or clinical abnormalities of the reproductive tract (Salamon and Maxwell, 2000).

Because semen colour, odour and consistency are qualitative (categorical) characteristics and were identical across all treatments, no statistical analysis was performed. Dietary L-arginine supplementation therefore had no observable effect on these macroscopic semen characteristics under the conditions of the present study.

SEMEN pH

Semen pH ranged from 6.42 ± 0.10 to 6.57 ± 0.06 (to agree with Table 2) and did not differ significantly among treatments ($P = 0.205$). These values are within the normal physiological range reported for goat semen (Salamon and Maxwell, 2000). Semen pH is an important indicator of the biochemical environment of the ejaculate and contributes to the maintenance of sperm viability and motility, whereas marked deviations from the physiological range may impair sperm function. The absence of significant differences among treatments indicates that dietary L-arginine supplementation did not measurably alter the seminal biochemical environment under the conditions of this study.

MICROSCOPIC SEMEN QUALITY

Of the four microscopic parameters evaluated, only individual sperm motility was significantly affected by L-arginine supplementation ($P = 0.025$). Sperm viability ($P = 0.644$), abnormality ($P = 0.086$) and concentration ($P = 0.340$) did not differ significantly among treatments. The changes described below for these three parameters are numerical trends and must be interpreted with caution, particularly in view of the limited number of experimental units ($n = 3$ per treatment), which restricts the statistical power available to detect moderate treatment effects.

SPERM MOTILITY

Individual sperm motility increased significantly from 80.00% in T0 to 93.33% in T2 ($P = 0.025$) and declined thereafter in T3 (Table 2). Motility is among the most informative indicators of semen quality, as it reflects the capacity of spermatozoa to traverse the female reproductive tract and reach the oocyte (Roca *et al.*, 2005; Brito, 2010).

The improvement in motility observed in T2 is consistent with the reported role of L-arginine as the substrate for nitric oxide (NO) synthesis. In other species, NO has been associated with mitochondrial function and adenosine triphosphate (ATP) generation, both of which are required for flagellar movement (Rosselli *et al.*, 1998; Wu *et al.*, 2009). The magnitude of the response reported here is comparable with that observed in small ruminants supplemented with the same amino acid or its precursor: individual motility rose from 76.9% to 85.2% in rams given rumen-protected L-arginine (Hussein *et al.*, 2022), and L-citrulline supplementation improved

semen quality in rams in association with higher seminal plasma pyruvate and amino acid concentrations (Zhao *et al.*, 2023). Direct evidence linking this pathway to the amino acid in a ruminant is available from rams, in which L-arginine administration altered seminal plasma nitric oxide concentrations and arginase activity alongside changes in spermatological parameters (Ozer Kaya *et al.*, 2020). However, nitric oxide, ATP and oxidative stress biomarkers were not measured in the present study. This mechanism should therefore be regarded as a biologically plausible explanation rather than as direct evidence derived from the present experiment.

The crude protein content of the experimental diets increased slightly from 14.08% to 15.97% DM because crystalline L-arginine contributed additional nitrogen to the concentrate. This modest increase in dietary crude protein may also have contributed to the observed improvement in sperm motility through enhanced nutrient availability. Nevertheless, the response was not proportional to dietary crude protein concentration, as both sperm motility and semen volume reached their highest values in T2 despite the highest crude protein content occurring in T3. This pattern suggests that crude protein alone is unlikely to explain the observed response. Because the experimental diets were not formulated to be isonitrogenous, the independent effects of L-arginine and of dietary crude protein cannot be distinguished in the present study.

The decline in sperm motility observed at the highest supplementation level (T3) suggests that increasing dietary L-arginine beyond the optimal level provided no additional biological benefit. Similar findings have been reported in studies evaluating excessive amino acid supplementation, in which higher inclusion levels did not further improve reproductive performance (Chen *et al.*, 2018). Although oxidative stress has been implicated as a potential contributor to reduced sperm motility through lipid peroxidation of the sperm membrane and impairment of mitochondrial function (Aitken and Baker, 2006; Agarwal *et al.*, 2014; Wang *et al.*, 2025), oxidative stress biomarkers were not evaluated in the present study. Consequently, the physiological mechanism underlying the decline in motility in T3 remains unresolved.

SPERM VIABILITY

Sperm viability remained high across all treatments, ranging from 86.68% to 89.94%, and was not significantly affected by dietary L-arginine supplementation ($P=0.644$). High viability indicates that most spermatozoa retained intact plasma membranes and maintained normal physiological function (Hafez and Hafez, 2000; Roca *et al.*, 2005). The consistently high viability observed in all treatments suggests that the bucks were in good reproductive

condition and that dietary L-arginine supplementation neither improved nor impaired sperm membrane integrity under the conditions of this study.

Although L-arginine has been reported to support reproductive function through its role as a precursor of nitric oxide, which contributes to blood circulation, nutrient delivery and cellular homeostasis (Rosselli *et al.*, 1998; Wu *et al.*, 2009), these mechanisms were not evaluated in the present study. Likewise, adequate dietary protein and energy are known to support membrane integrity during spermatogenesis (Beconi *et al.*, 1993; Roca *et al.*, 2005). It is also possible that dietary arginine acts primarily on seminal plasma composition rather than on routine sperm quality endpoints, as reported in boars in which supplementation altered the abundance of twelve seminal plasma proteins associated with reproduction while leaving sperm motility and morphology unchanged (Oladejo *et al.*, 2025). The consistently high sperm viability observed in this study therefore most likely reflects the good reproductive status of the experimental bucks rather than a measurable effect of dietary L-arginine supplementation.

SPERM ABNORMALITY

The proportion of morphologically abnormal spermatozoa decreased numerically from 11.67% in T0 to 6.57% in T3; however, the differences among treatments were not statistically significant ($P=0.086$). This reduction should therefore be interpreted as a numerical trend rather than as evidence of improved sperm morphology.

Abnormal sperm morphology is commonly associated with impaired spermatogenesis and oxidative damage during sperm development (Aitken and Baker, 2006; Agarwal *et al.*, 2014; Wang *et al.*, 2025). L-arginine has been reported to support reproductive function through its involvement in nitric oxide metabolism and improved nutrient delivery to reproductive tissues (Wu *et al.*, 2009; Khalil *et al.*, 2018). However, oxidative stress biomarkers, nitric oxide and reproductive physiological parameters were not evaluated in the present study. Consequently, the possible role of L-arginine in reducing sperm abnormalities cannot be confirmed and remains a biologically plausible explanation.

Although the supplementation period covered approximately two spermatogenic cycles in goats, the present study was not designed to evaluate cumulative protective effects over successive cycles. Further studies incorporating sequential semen evaluation and oxidative stress measurements are required to clarify this response.

SPERM CONCENTRATION

Sperm concentration increased numerically from 2.08×10^9 spermatozoa/mL in T0 to 2.74×10^9 spermatozoa/mL in T2 before declining in T3. However, the differences

among treatments were not statistically significant ($P=0.340$). The values obtained fall within the normal range reported for sexually mature goat bucks (Hafez and Hafez, 2000; Roca *et al.*, 2005) and are consistent with baseline values reported for Kacang bucks in Indonesia (Susilowati *et al.*, 2021; Mustofa *et al.*, 2023).

Although the numerical increase of approximately 32% between T0 and T2 appears biologically relevant, the lack of statistical significance indicates that this difference could not be distinguished from normal variation among animals, particularly given the limited number of experimental units. Spermatogenesis depends on an adequate supply of protein and energy for germ cell proliferation and differentiation (Roca *et al.*, 2005; Gholami *et al.*, 2010). However, because the experimental diets were not isonitrogenous, the independent effects of dietary crude protein and of supplemental L-arginine could not be distinguished. Therefore, no causal relationship between dietary L-arginine supplementation and sperm concentration can be established.

Nitric oxide has been reported to regulate testicular blood flow and to support spermatogenic activity in several mammalian species (Wu *et al.*, 2009). Nevertheless, neither nitric oxide nor reproductive hormones were measured in the present study. Accordingly, the physiological mechanism underlying the numerical increase in sperm concentration remains speculative.

INTEGRATED INTERPRETATION AND SELECTION OF THE OPTIMAL SUPPLEMENTATION LEVEL

Taken together, the results indicate that dietary L-arginine supplementation produced the most favourable semen quality response at 0.75% (T2). Semen volume and individual sperm motility, the only two parameters showing statistically significant treatment effects, both reached their highest values at this supplementation level. Sperm concentration followed the same numerical pattern, sperm viability remained consistently high across treatments, and sperm abnormality decreased numerically but did not differ significantly among treatments.

The recommendation of 0.75% dietary L-arginine supplementation was based exclusively on parameters that showed statistically significant treatment effects. Although sperm abnormality continued to decrease numerically in T3, this trend was not statistically significant and therefore cannot justify recommending the higher supplementation level. Considering the significant decline in semen volume and sperm motility in T3, supplementation above 0.75% provided no additional measurable benefit under the conditions of the present study.

It should be emphasised that the present study evaluated

laboratory indicators of semen quality rather than fertility. No artificial insemination or natural mating trial was conducted; therefore, the reproductive consequences of the improved sperm motility observed at 0.75% L-arginine remain unknown. Likewise, no economic evaluation of dietary L-arginine supplementation was performed.

LIMITATIONS OF THE STUDY

This study has several limitations. First, the relatively small number of experimental animals (three bucks per treatment) may have reduced the statistical power to detect moderate treatment effects. Second, physiological parameters related to nitric oxide metabolism, oxidative stress biomarkers, ATP production and reproductive hormones were not directly measured, so the biological mechanisms underlying the observed responses could not be confirmed. Third, because the experimental diets were not formulated to be isonitrogenous, the independent effects of dietary crude protein and of supplemental L-arginine could not be distinguished.

A fourth limitation concerns the form in which the amino acid was supplied. Free amino acids are extensively degraded by ruminal microorganisms before reaching the small intestine, and the crystalline L-arginine used here was not rumen-protected; the proportion of supplemental L-arginine actually absorbed is therefore unknown. Studies in small ruminants have addressed this problem either by using rumen-protected preparations (Hussein *et al.*, 2022) or by supplying L-citrulline, which is not taken up by ruminal microbes and is subsequently converted to arginine in the kidney (Zhao *et al.*, 2023; Fan *et al.*, 2024). The responses reported here may consequently underestimate the effect achievable with a protected preparation, and direct comparison of protected and unprotected forms in Kacang goats is warranted.

A fifth limitation is that much of the mechanistic literature cited for the L-arginine–nitric oxide pathway derives from human and rodent studies; ruminant-specific evidence remains comparatively scarce, being largely confined to rams (Ozer Kaya *et al.*, 2020; Hussein *et al.*, 2022), and no goat-specific measurement of nitric oxide in blood or reproductive tissue was available for comparison. In addition, the L-arginine content of the prepared diets was not confirmed by analytical assay, so the delivered doses are nominal rather than measured. Finally, although the experiment was conducted under dry climatic conditions, direct physiological indicators of heat load such as rectal temperature, respiration rate, scrotal surface temperature or the temperature-humidity index were not recorded, so the thermal status of the animals cannot be characterised (Barragán *et al.*, 2023; Arero and Ozmen, 2025). Future studies should include larger sample sizes, isonitrogenous diets, rumen-protected supplementation, measurements of

oxidative stress biomarkers, nitric oxide metabolism and reproductive hormones, explicit heat load indicators, and fertility performance, in order to clarify the physiological mechanisms and practical benefits of dietary L-arginine supplementation in Kacang goats.

CONCLUSION AND RECOMMENDATIONS

Dietary L-arginine supplementation significantly affected semen volume ($P = 0.002$) and individual sperm motility ($P = 0.025$) in Kacang goats reared under dry climate conditions, with both parameters reaching their highest values at the 0.75% supplementation level (T2). Semen colour, odour, consistency and pH did not differ among treatments and remained within the normal physiological ranges reported for Kacang goats, indicating that supplementation up to 1.0% did not adversely affect the basic physical characteristics of semen. Sperm concentration ($P = 0.340$), sperm abnormality ($P = 0.086$) and sperm viability ($P = 0.644$) did not differ significantly among treatments. Based exclusively on the parameters that showed statistically significant treatment effects, dietary supplementation with 0.75% L-arginine is considered the most favourable level among those evaluated for improving the semen quality of Kacang goats under dry climatic conditions. Because the present study assessed laboratory indicators of semen quality rather than fertility, further studies incorporating larger sample sizes, isonitrogenous diets, physiological measurements and fertility trials are required before practical recommendations can be made for goat production systems in East Nusa Tenggara.

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NOVELTY STATEMENT

This is the first study to evaluate graded levels of dietary L-arginine supplementation on both the macroscopic and the microscopic semen quality of Kacang goats maintained

under the dryland feeding systems of East Nusa Tenggara, Indonesia. The study identifies 0.75% dietary L-arginine as the level associated with the highest semen volume and sperm motility, and thereby provides a nutritional entry point for improving buck reproductive performance in smallholder goat production systems in semi-arid regions.

AUTHORS' CONTRIBUTION

AN conceived and designed the study, supervised the field experiment and drafted the manuscript. YK performed semen collection and laboratory evaluation and contributed to data analysis. AAD prepared the experimental diets, managed the animals and contributed to interpretation of the results. All authors read, revised and approved the final manuscript.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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