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Research Article
Effects of Nutritional Status on Semen Characteristics of Kacang Goats

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Abstract

Background and Objective: Nutritional status of livestock has impact on growth performance and development of reproductive organs. Testicular mass, semen characteristics, spermatogenesis and reproductive hormones can be influenced by feeding levels. This study was conducted to evaluate semen characteristics of Kacang goats given various feeding levels for 24 weeks. **Methodology:** Nine adult male Kacang goats were divided into 3 feeding groups (G1, G2 and G3). After a 2 week adaptation period, all three groups of goats were fed fully for 8 weeks (full feeding). Then the goats were fed an amount that was 60% (G1), 50% (G2) or 40% (G3) of full feeding levels for the next 8 weeks (restricted feeding). Finally, the goats were refed fully for the last 8 weeks (re-alimentation). Semen samples were collected using an artificial vagina at the end of each feeding period. **Results:** Full feeding followed by restricted feeding and resumption of full feeding had no significant effects on the ejaculate volume, sperm cell concentration, sperm cell motility and proportion of live sperm values in Kacang goats. Semen parameters were maintained in the physiological range that was normal for Kacang goats. **Conclusion:** Kacang goats have a good capacity to preserve semen quality at various levels of feeding.

Key words: Semen, feed restriction, re-alimentation, Kacang goat

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The goat population in Indonesia is approximately 19.6 million, which represents 35% of all ruminants in the country[1]. The Kacang breed of goat is indigenous and found in almost all regions of Indonesia. Kacang goats are widely bred as a source of meat, organic fertilizer and to fulfill various other economic needs, especially those of small farms[2-4]. Kacang goats are well-adapted to the tropical environment of Indonesia and retain good reproductive performance under limited environmental conditions as evidenced by frequent multiple births [single birth (1.41 head doe^{-1}/year), twins (2.98 head doe^{-1}/year), triplets (4.58 head doe^{-1}/year 2.6%)][2,3]. Nonetheless, Kacang goat production in tropical areas of Indonesia often faces fundamental nutritional challenges such as unbalanced feed availability during the rainy and dry seasons[4,5].

Nutrition plays a key role in the growth and development of reproductive systems in livestock. For rams in particular, nutritional availability has a significant impact on physiological responses and seasonal reproductive performance[6-8]. Nutrition greatly affects testicular mass and in turn seminiferous tissue amounts and spermatogenic capacity in sheep and goats[9]. Changes in body mass loss are also closely related to the amount of sperm produced in goats[9,10]. Meanwhile, several studies showed a strong and direct relation between nutrition, testicular mass and amount of sperm[11,12]. Decreased semen quality is associated with feed intake shortages (undernutrition) that are longer than the spermatogenesis cycle, which in goats was shown to last nearly for 7 weeks[9,13,14]. However, Ebtihal *et al.*[15] reported a significant decrease in semen quality in rams fed one-third the amount of basal diet for 21 days. In contrast, Widiyono *et al.*[16] reported that feed restriction for 4 weeks did not change semen characteristics of Indonesian indigenous goats. Another study showed rams with restricted feeding for 20 weeks regained normal size and function of reproductive organs (testicles) after resumption of feeding that fulfilled nutritional requirements[17]. Currently there is limited information about how feed restriction followed by refeeding lasted about 7 weeks affects reproductive characteristics of male goats in Indonesia. This research investigated the impacts of various levels of restricted feeding and refeeding (re-alimentation) on semen characteristics of Kacang goats.

MATERIALS AND METHODS

All methods in this study were approved by the Commission of Ethics, Integrated Research and Testing Laboratory, Gadjah Mada University (Project No. 202/KEC-LPPT/XI/2014).

Animals and research procedures: Nine male Kacang goats aged 1.5 years with good libido were used in this study. Each animal was kept in an individual pen and fed a ration that consisted of 60% green fodder (leafy part of peanut plants) and 40% concentrate (PT Japfa Comfeed Indonesia) wherein the dry matter amount was 3.5% of the body weight (full feeding). Drinking water was provided *ad libitum*. After a 2 week adaptation period on this diet, the animals were divided into 3 groups (G1, G2 and G3) that underwent a series of feed treatments for 24 weeks. The groups of goats were fed fully for 8 weeks (full feeding). Then the goats were fed an amount that was 60% (G1), 50% (G2) and 40% (G3) of full feeding levels for the next 8 weeks (restricted feeding). Finally, the goats were refed fully for the last 8 weeks (re-alimentation).

The feed was divided equally into 2 portions and offered to the animals at 8:00 a.m. and 4:00 p.m. The amount of feed provided for each animal was adjusted according to the results of the weekly animal weight check.

During the study, general health care management was given to prevent diseases, including infestations by endo- and ectoparasites. Every week semen samples were collected from the goats by ejaculation into artificial vaginas.

Collection and evaluation of semen: At the end of the full feeding, restricted feeding and re-alimentation periods, semen samples were collected using an artificial vagina. Semen characteristics were evaluated using methods described by Ghorbankhani *et al.*[18]. Immediately after semen collection, the ejaculate volume (EV) was measured. The semen sample was microscopically analyzed for sperm cell concentration (SCC), sperm cell motility (SCM) and the proportion of live sperm (PLS). The sperm cell concentration was measured with a Neubauer hemocytometer after a semen aliquot was mixed with a 0.05% solution of formalin or normal salt solution (1:400). To evaluate SCM, the semen was diluted with a 0.1 M sodium citrate solution, placed on a glass slide, covered with a coverslip and examined at a magnification of 400X with a light microscope. The SCM was assessed microscopically using a scale of 0-100%. Semen smears were made and stained with eosin-nigrosin stain for PLS measurement that involved counting unstained sperm.

Statistical analysis: Data for semen parameters (EV, SCC, SCM, PLS) at the end of the feeding periods are presented as the mean and standard error of the mean. The effects of feeding

period and feed restriction treatment and interactions were analyzed using General Linear Model procedures. Duncan's multiple range test was used to determine significant differences between the means. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The mean ejaculate volume (EV) of the Kacang goats in all feeding groups ranged from 0.41-0.86 mL, which was consistent with the range observed in, Peshawar goats (0.3-0.8 mL)[19], Zaraibi goats (0.35-0.98 mL)[20], Damascus goats (0.72-1.31 mL)[21] and Merkhoz goats older than 20 months (0.6-1.2 mL)[22,23]. However, this range was lower than that for Gorno-Altai goats (1.55-2.00 mL), South African indigenous goats (1.32-1.43 mL)[24] and Saanen goats (1.15 mL)[25]. The difference from previously observed values could be due to younger age of goats which were used in the present study, younger goats are known to have lower EV than adult goats[21], as well as the method used to collect semen (artificial vagina vs. electro ejaculator). Pineda *et al.*[26] reported that semen collection methods involving electro ejaculators yielded higher semen volumes due to high levels of accessory gland secretions.

The 8 week feed restriction did not change the EV in groups G2 and G3 but the EV of group G1 decreased from 0.76±0.15-0.43±0.05 mL at the end of the full feeding and restricted feeding periods, respectively (Table 1). Nonetheless, even this reduced EV (0.43 mL) was comparable to EV of group G3 during the full feeding (*ad libitum*) period (0.41±0.10 mL) and the EV range reported by Barkawi *et al.*[20] and Qureshi *et al.*[19]. In contrast, after the 8 week refeeding (re-alimentation) period, the EV tended to increase with respect to the restricted feeding period (Table 1). This finding is consistent with a previous study of sheep conducted by Guan *et al.*[27], who reported that animals fed lower amounts than the recommended amount had lower EV, whereas animals with high feed intake had correspondingly higher EV. Parker and Thwaites[14] also reported that feed restriction upto 50 or 70% of normal amounts for 11-15 weeks significantly reduced both EV and sperm concentration. Corresponding changes in feed intake status and EV were also described for various breeds of goats in Malaysia[28]. The increased EV in the re-alimentation period likely reflects the improvement of reproductive organ function. Rekwot *et al.*[29] reported that bulls with feed restriction from 25-45% of full feeding for 90 days regained normal reproductive function after refeeding, whereas studies on steers, heifers and sheep showed increases in growth hormone during the refeeding period after feed restriction[30-32]. This increase in growth hormone may affect semen quality, as suggested by Storer[33], who reported that administration of growth hormone to horses increased the volume of gel-free semen. Furthermore, the results of research on rams also showed that nutrition status significantly affected levels of both growth hormone and EV. Meanwhile, Ghorbankhani *et al.*[18] investigated the effects of nutrition status on semen characteristics in sheep and discovered that improved nutrition status was associated with increased testosterone levels and EV. A study on Zaraibi goats also showed that changes in EV correspond to changes in blood testosterone levels[20].

Table 1: Mean Ejaculate Volume (EV, mL) at the end of full feeding, restricted feeding (60% (G1), 50% (G2) and 40% (G3) of full feeding) and re-alimentation periods in Kacang goats

	Ejaculate volume (EV, mL)		
Groups	Full feeding	Restricted feeding	Re-alimentation
G1	0.76 ± 0.15^{ab}	0.43 ± 0.05^{b}	0.86 ± 0.23^{a}
G2	0.50±0.20	0.50±0.10	0.60±0.17
G3	0.41±0.10	0.45±0.21	0.50±0.26

Values are Mean±SD (n = 3), values in the same row with different superscripted letters indicating significant differences ($p<0.05$)

The mean Sperm Cell Concentration (SCC) in Kacang goats at the end of the full feeding period ranged from 366.67×10^6-633.33×10^6 mL^{-1} (Table 2), which was similar to that observed in South African indigenous goats in a previous study ($663.6\pm33.3\times10^6$ mL^{-1})[34]. Compared to other goat breeds, the SCC level in Kacang goats was higher than that of Gorno Altai goats ($161.3\pm83.6\times10^6$ mL^{-1})[24] but lower than Saanen goats (3.69×10^9 mL^{-1})[25], Jermasia goats (3.97×10^9 mL^{-1}), Boer goats ($4.20\pm0.62\times10^9$ mL^{-1}), Malaysian Kacang goats ($5.35\pm0.98\times10^9$ mL^{-1})[28] and Peshawar dairy goats (1.027×10^9 mL^{-1})[19]. The different levels of SCC could be influenced by breed and environmental factors such as age, temperature and ejaculation frequency, as suggested by a study by Ax *et al.*[35].

The 8 week feed restriction period did not significantly affect the SCC of Kacang goats in this study (Table 2, $p>0.05$). This result could be due to the fact that feed restriction for 8 weeks did not cause any significant changes in spermatogenesis, which is consistent with an earlier study on pigs which showed only feed restriction periods that were longer than 8 weeks reduced spermatogenesis[36]. Another study involving different goat breeds in Malaysia showed that reducing feed intake to 50% of maintenance amounts for 4 months did not change SCC significantly[28]. The tendency toward increased SCC in Kacang goats in group G1 and the significant increased SSC in group G3 at the end of the

re-alimentation period ($p<0.05$) suggested that reproductive function increased after the refeeding. This outcome is similar to a study of bulls by Rekwot *et al.*[29] and findings by Kheradmand *et al.*[37], who showed that refeeding for several weeks significantly increased SCC in sheep. Meanwhile, Ghorbankhani *et al.*[18] suggested that improved nutritional status in sheep increased testosterone levels and SCC. Another previous study also reported that sheep subjected to high levels of feeding showed increased SCC, total sperm in the reproductive tract, scrotal circumference, testicular mass and plasma LH than the sheep given lower amounts of feed[17].

The mean of sperm cell motility (SCM) in Kacang goats at the end of the full feeding period ranged from 60.0-83.3% (Table 3). These values were similar to other goat breeds such as Boer (73%)[38], Jermasia (82.50%)[28], Peshawar (52.17-74.30%)[19] and Iranian Merkhoz (69.1-83.9%)[22,23] but was higher than that seen for Indian indigenous goats (44.17-57.5%)[39]. No significant effect of feeding period or feed treatment was observed on SCM ($p>0.05$) because the SCM for the feed restriction and re-alimentation period was similar. These findings support results of previous studies in pigs showing that SCM was not significantly influenced by the level of feeding. Moreover, there were no differences between SCM levels of pigs fed *ad libitum* for 7 weeks followed by restricted feeding for 8 weeks[36]. It is possible that the restricted feeding-refeeding in this study did not significantly affect the biochemical composition of semen (Ca, Mg, P, total protein, citric acid and fructose levels), which, as Aguiar[40] noted, is correlated with sperm motility and vigor. Fructose is especially rich in semen and provides nutrient energy for the spermatozoa[41]. Studies on nutrition in rams have demonstrated that reduced energy or feed intake results in a decrease in the fructose content of ejaculate[42] and in the sperm motility[14]. Our earlier study of Kacang goats showed that feeding at 50% of full feeding for 4 weeks did not significantly decrease the total protein and glucose concentrations in the blood plasma and that the blood chemistry parameter values remained within the range of physiological reference value for these goats[16]. Therefore, further examination of the effects of feed restriction and refeeding for 8 weeks on the biochemical composition of semen in Kacang goats is needed.

The mean of the proportion of live sperm (PLS) in Proportion of Live Sperm (PLS) of Kacang goats ranged between 53.67 and 92.67%, which was similar to that seen for Iranian Merghoz goats (79.4-90.2%)[23], Damascus goats (56.35-81.08%)[21] and Iranian Markhoz goats (80.2-90.7%)[22] but higher than Iranian Rayini goats (60.2-77.6 %)[43]. No significant effects of feed restriction followed by re-alimentation on PLS of Kacang goats was observed ($p>0.05$, Table 4). These results reinforce the results of a study by Guan *et al.*[27] who reported similar PLS values for sheep fed lower than the adequate levels of feed (undernutrition) and those fed higher than adequate levels for 9 weeks.

Table 2: Mean of sperm cell concentration (SCC, 10^6 mL^{-1}) at the end of full feeding, restricted feeding [60% (G1), 50% (G2) and 40% (G3) of full feeding] and re-alimentation periods in Kacang goats

	Sperm cell concentration (10^6 mL^{-1})		
Groups	Full feeding	Restricted feeding	Re-alimentation
G1	416.68±160.73	691.67±62.91	941.67±853.79
G2	366.67±14.43	750.00±216.51	741.67±670.97
G3	633.33±57.75[b]	750.00±35.35[b]	1,591.67±590.73[a]

Values are Mean±SD (n = 3), values in the same row with different superscripted letters indicating significant differences ($p<0.05$)

Table 3: Mean of sperm cell motility (SCM) at the end of full feeding, restricted feeding [60% (G1), 50% (G2) and 40% (G3) of full feeding] and re-alimentation periods in Kacang goats

	Sperm Cell Motility (%)		
Groups	Full feeding	Restricted feeding	Re-alimentation
G1	66.66±15.27	70.00±10.00	30.00±26.45
G2	60.00±26.45	66.66±15.27	53.33±37.86
G3	83.33±05.77	70.00±14.14	70.00±10.00

Values are Mean±SD (n = 3)

Table 4: Mean of proportion of live sperm (PLS) at the end of full feeding, restricted feeding [60% (G1), 50% (G2) and 40% (G3) of full feeding] and re-alimentation periods in Kacang goats

	Proportion of Live Sperm (%)		
Groups	Full feeding	Restricted feeding	Re-alimentation
G1	74.33±06.35	87.00±05.19	53.67±44.29
G2	71.60±21.38	89.00±03.46	68.00±43.31
G3	90.67±06.11	91.50±02.12	92.67±03.05

Values are Mean±SD (n = 3)

In general, in Kacang goats with feed restrictions of up to 40% of full feeding amounts for 8 weeks followed by an 8 week re-alimentation period did not show significant changes in semen characteristics or fertility. Thus, these feeding patterns can be applied to the breeding management of Kacang goats to reduce the amount of feed given and in turn increase feed efficiency and reduce environmental impacts of livestock.

CONCLUSION

The results of this study support the hypothesis that Indonesian Kacang goats can maintain semen quality after feed restriction. Kacang goats that had feed restriction regained reproductive function upon resumption of full feeding (refeeding). These results suggest that patterns of feed restriction and refeeding in Kacang goats can be applied for breeding management of goats in Indonesia.

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