
Semen evaluation of Boer breeder bucks as influenced by lysine supplementation

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Abstract Artificial insemination (AI) is widely used biotechnology tool applied to improve reproduction and genetics in livestock. The viability of the sperm cells is the most important factor to ensure successful insemination which can be achieved through proper nutrition management. The effect of amino acid supplementation, particularly additional of lysine in the diet of breeder bucks is studied. The production performance and quality of sperm cells produced by semen donors without supplementation (T₁) is differentiated with donors receiving 1.75% lysine or low rate (T₂), 2.45% or moderate rate (T₃), and 3.15% or high rate of supplementation (T₄) within two periods: Period 1 (Days 1-21) and Period 2 (Days 22 and onwards). The results showed that with lysine supplementation regardless of rate, animals are heavier than the animals that were not supplemented ($p < 0.05$). Lysine supplementation positively affected on animal's libido in terms of period consumed and the number of mountings before a successful ejaculation ($p < 0.05$). The volume of semen increased ($p < 0.001$); while sperm cell concentration, motility, fast progressive, slow progressive, circle and local motility rates were not significantly affected; however, positive effect on rate of immotile ($p < 0.01$) and velocity ($p < 0.05$) was observed. Considering all parameters, Treatment 2 lysine supplementation resulted to improve animal production and higher viability of sperm cells in both fresh and frozen-thawed state. Thus, 1.75% of lysine is recommended for inclusion to daily dietary concentrate ration.

Keywords: Goat, Spermatogenesis, Amino acid, Lysine

Introduction

Artificial insemination (AI) was the first assisted reproductive biotechnology to be applied commercially for genetic improvement of animals since 1900. At present, AI remains the most widely used biotechnology tool applied to improve reproduction and genetics in livestock (Foote, 2002). Among the advantages of AI include disease control, faster distribution of frozen semen and availability of breeding records. AI has become the foundation for expanded breeding schemes such as estrus synchronization programs (synchronized breeding, including timed AI), embryo transfer (ET), in-vitro embryo production (IVP), the use of sexed semen, cloning, and transgenics. Progress in AI and the

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associated technologies including semen collection, dilution and cryopreservation of germ cells have enabled elite male animals to be used in insemination program. In the Philippines, AI was introduced since 1900s in large ruminant and swine breeding programs to produce genetically superior animals and to improve herd quality in lesser time than traditional breeding method (Hernandez and Gifford, 2013). However, efforts to utilize the technology in small ruminant at community-level commenced in 2009 (Nayga *et al.*, 2011).

To maintain good quality sperm cells for AI, proper nutrition management program is recommended as this will influence sex libido and fertility of the donors (Benatta *et al.*, 2020). Amino acids or AA are building blocks of protein and it is important for proper cell synthesis (Wu, 2009). AAs that cannot be synthesized in the cells of any animals are considered as nutritionally essential and must be included in the diet to maintain physiological functions of cells, tissues and the body. Methionine and lysine are known as top limiting AA for ruminant (Greenwood and Titgemeyer, 2000). In human sperm cells, lysine is associated with sperm functions, including motility, capacitation, acrosome reaction and sperm-egg interaction (Sun *et al.*, 2014). In animals, several studies show positive effect of lysine in production and reproduction including improved milk production and health of perinatal dairy cows, muscle growth of monogastric animals such as pigs (Liao *et al.*, 2015; Dong *et al.*, 2016), and sperm capacitation in mouse (Ritagliati, 2018). However, for goats there are limited studies conducted to assess the effect of lysine in male fertility.

This study was conducted to evaluate the effect of different levels of dietary lysine supplementation at two different periods to the production and reproduction performances of Boer breeder bucks.

Materials and methods

Mature Boer bucks of age 3 to 4 years old raised at the Semen Processing Laboratory of the Cagayan Valley Small Ruminants Research Center, Isabela State University, Echague, Isabela served as experimental animals. The animals were managed under confinement production system. In this approach, the animals were stall-fed and provided with diet composed of grasses and legumes. Each experimental animal received fresh forages at a rate of 30% of its body weight. Roughage was further separated into a 60:40 (grass: legume) ratio. The ration was divided into four equal portions and distributed to the animals at 8 and 11 a.m., and 2 and 4 p.m.

Every morning, the experimental animals received a formulated feed concentrate for breeders (Orden *et al.*, 2018) shown in Table 1. Lysine (AjiPro-L, manufactured by Ajinomoto BioPharma, Japan) was supplemented in the

concentrate based on the 18% available crude protein (CP), as recommended by EFSA Journal (2015).

Table 1. Feed Formulation for Breeder Bucks at 18% Crude Protein, 100 kg

Ingredients, parts	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Corn grits	54.50	58.75	59.75	60.35
Rice bran	11.00	11.00	11.00	11.00
Soybean meal	25.50	20.00	18.30	17.00
Fish meal	4.00	4.00	4.00	4.00
Molasses	4.00	4.00	4.00	4.00
Salt	1.00	0.50	0.50	0.50
Lysine	0.00	1.75	2.45	3.15

There were four dietary lysine supplementations evaluated in the study to include without supplementation (T₁), with 1.75% lysine or low rate (T₂), 2.45% or moderate rate (T₃), and 3.15% or high rate of supplementation (T₄). The supplementation was carried-out in two periods: Period 1 (Days 1-21) and Period 2 (Days 22 and onwards). The study was laid-out in Completely Randomized Design (CRD) and the data were subjected to Analysis of Variance (ANOVA).

Assessment on production performance

The experimental animals' production performance was assessed to establish the influence of supplementation on overall animal productivity. The initial and final weights were obtained and recorded for evaluation.

Assessment on semen production and fresh sperm cell viability

The experimental animals were subjected to semen collection three times a week on alternate days. Collection took place at 6:00 a.m. using an artificial vagina (AV). During collection, the sexual behavior or libido of the experimental animals was observed, as evidenced by the time spent (sec) and the number of mountings performed by the semen donors in every successful ejaculation.

The semen collected was immediately submitted to the laboratory for analysis. The materials utilized in the evaluation were pre-warmed at 37°C, the normal temperature for semen evaluation. Similarly, the collected sample was kept inside a water bath at 37°C during the evaluation time.

Semen evaluation

Physical characterization

The volume of the collected semen was measured to assess whether the sample is suitable for further processing. For goats, a minimum of 0.4 mL of semen can be processed.

Microscopic evaluation of sperm cells

Prior to the evaluation, the sample was diluted by adding 0.3 mL of prepared semen extender to 0.1uL of semen sample. After dilution, 0.1uL of mixture was placed in pre-warm slide and was viewed at 40x magnification under the video microscope Computer Aided Sperm Analyzer or CASA Machine installed with AndroVision™. The viability of the sperm cells was evaluated using parameters such as concentration, motility and velocity.

Results

Animal productivity

The effect of the lysine supplementation on animal productivity to include initial and final weight, and gain in weight is presented in Table 2.

Table 2. Initial, final and gain in weight of experimental animals supplemented with lysine

Treatments	Production Performance		
	Initial Weight, kg	Final Weight, kg	Gain in Weight, kg
Treatment 1	53.50	56.00	2.50 ^b
Treatment 2	62.00	68.00	6.50 ^a
Treatment 3	57.00	63.50	6.50 ^a
Treatment 4	51.00	57.00	6.00 ^a
CV %	23.03%	20.47%	11.39%

Note: Means with the same letter are not significantly different with each other.

ns- not significant

*- Significant at 5% level

There was no significant difference between the treatments' initial weights, which ranged from 51.00 kg to 62.00 kg. The results imply that the experimental animals are uniform or homogeneous. Furthermore, same statistical result was obtained in final weight between the treatments, which vary from 56.00 kg to 63.50 kg. However, weight gain varies significantly between treatments. Animals that received lysine supplementation obtained an average of 6 to 6.50

kgs, whereas animals that were not supplemented gained an average of 2.50 kgs over the study period.

Semen production

Semen volume. In the first period of lysine supplementation, a significant difference was noted between treatments. Treatment 2 and 3 had the highest average of 0.78 mL and 0.76 mL, respectively; Treatment 1 had an average of 0.49 mL, and Treatment 4 had the lowest average of 0.43 mL.

Furthermore, a significant difference was further observed in Period 2 of lysine supplementation. Treatment 2 had the highest average of 1.11 mL, followed by Treatment 3 at 0.90 mL. Treatments 1 and 4 produced the least amount, with averages of 0.73 mL and 0.68 mL, respectively, which are statistically comparable.

Semen concentration. During the first period of lysine supplementation, there was a significant difference in semen concentration ($\times 10^9$) between treatments. Treatment 2 produced the most concentrated sample (1.17×10^9 sperm cells/mL), whereas Treatment 4 generated the least (0.74×10^9 sperm cells/mL).

However, there was no significant difference in Period 2 of lysine supplementation. The concentration of sample ranges from 0.95×10^9 to 1.67×10^9 cells/mL.

Sperm cell viability

Motility. There was no significant difference in motility (%) throughout the first period of lysine supplementation. The treatment mean varies from 77.03% to 89.83%. Similarly, in Period 2, no significant difference was found across treatments. The average result ranged from 69.32% to 92.31%.

Fast progressive motility. Significant differences among the mean value obtained from different treatments was observed in Period 1. The motility observed in Treatment 2 at 79.51% and Treatment 3 with a mean value of 76.78% were the fastest among treatments but are statistically equal. The slowest was observed in Treatment 4 with an average mean value of 62.50%.

However, in Period 2 of lysine supplementation, no significant difference among the treatment means was observed, which ranges from 44.64% to 85.15%.

Slow progressive motility. In Period 1, no significant difference was noted between the treatments' means. The computed treatment means ranged between 13.98% and 19.69%. Similarly, no significant differences were found in Period 2 of lysine supplementation. The means varied from 11.96% to 24.71%.

Immotile. There was no significant difference between treatments in Period 1 of lysine supplementation. The treatments produced mean values of immotile sperm cells range from 9.63% to 24.21%.

However, there was a significant difference between treatments during Period 2 of lysine supplementation. Treatment 4 had the highest rate of immotile sperm cells at 26.86%, whereas Treatment 2 had the lowest rate at 7.33%. The results of the study show the positive response of low rate of supplementation on the motility sperm cells.

Velocity. A significant difference in the velocity (um/sec) of sperm cells collected from different treatments was noted during Period 1 of lysine supplementation. The fastest rate was found in Treatment 1, with a mean value of 96.17 um/sec, while the slowest was observed in Treatment 3, with an average of 77.83 um/sec.

Furthermore, a significant difference was also noted in Period 2 of supplementation. The fastest rate was recorded in Treatment 2 with an average velocity of 128.36 um/sec, while the slowest was observed in Treatment 4 with an average of 35.71 um/sec.

Table 3. Semen Production and Sperm Cell Quality from Period 1 of Lysine Supplementation

Treatments	Semen Production		Sperm Cell Viability				
	Volume, mL	Concentration, x10 ⁹	Motility, %	Fast progressive motility, %	Slow progressive motility, %	Immotile, %	VCL, um/sec
Treatment 1	0.49 ^b	0.92 ^b	83.40	69.91 ^b	19.69	24.21	96.17 ^c
Treatment 2	0.78 ^a	1.17 ^a	89.83	79.51 ^a	13.98	9.63	91.04 ^b
Treatment 3	0.76 ^a	0.89 ^{bc}	89.07	76.78 ^a	18.52	17.36	77.83 ^b
Treatment 4	0.43 ^b	0.74 ^c	77.03	62.50 ^c	18.47	22.23	44.30 ^d
<i>ANOVA</i>	*	*	ns	*	ns	ns	*
<i>LSD</i>	0.12	0.15	-	6.82	-	-	4.22
<i>CV</i> %	11.05 %	9.45%	7.26%	5.22	26.92%	21.34%	8.01%

Note: Means with the same letter are not significantly different with each other.

ns- not significant

*- Significant at 5% level

Table 4. Semen Production and Sperm Cell Quality from Period 2 of Lysine Supplementation

Treatments	Semen Production			Sperm Cell Viability			
	Volume, mL	Concentration, x10 ⁹	Motility, %	Fast progressive motility, %	Slow progressive motility, %	Immotile, %	VCL, um/sec
Treatment 1	0.73 ^c	0.98	81.49	72.07	18.93	18.96 ^b	85.11 ^b
Treatment 2	1.11 ^a	1.67	92.31	85.14	11.96	7.33 ^c	128.36 ^a
Treatment 3	0.90 ^b	0.98	84.06	68.96	24.71	14.69 ^b	86.79 ^b
Treatment 4	0.68 ^c	0.95	69.36	44.64	17.31	26.86 ^a	35.71 ^c
CV %	9.28%	19.97%	14.35%	19.13%	27.46%	15.14%	25.09

Note: Means with the same letter are not significantly different with each other.

ns- not significant

*- Significant at 5% level

** - Significant at 1% level

Discussion

The major function of lysine in animals is to serve as one of the building blocks for synthesis of body proteins and peptides. Without lysine-involved protein and peptide syntheses, living cells or living animals could not exist. Lysine can affect animal metabolism of other nutrients, hormone production, and immunity (Wu, 2009). Lysine supplementation is important for growth and body weight improvement of ruminants (Hsin-Tai *et al.*, 2021; Klemesrud *et al.*, 2000); which is exactly observed among monogastric animal particularly pigs (Liao *et al.*, 2015).

In mature animals, poor nutrition can reduce production of ova and spermatozoa (Guan *et al.*, 2014). Martin *et al.* (2010) on their study with buck fertility concluded that the daily rate of sperm production is reduced by underfeeding. Moreover, the quality of the semen produced can be improved by providing all required nutrients to support reproduction function of the animal, and can induce sperm production due to the increase of testosterone, an androgen which is required for initiating and maintaining spermatogenesis. Some essential amino acids that are likely involved in the sperm motility are arginine, methionine, leucine and lysine (Rurangwa *et al.*, 2004). Moreover, amino acid supplementation can also be attributed to improve membrane integrity (Santiago-

Moreno *et al.*, 2019) which is important to maintain sperm cell viability. Lysine in particular, helps the body absorb calcium, which is important in many physiological processes including spermatogenesis, sperm motility and fertilization.

The results of the study showed that the volume of semen and its concentration increase as the lysine supplementation progresses (Dong *et al.*, 2016; Ren *et al.*, 2015). The effect is in time on sperm maturation cycle which happens every 7 weeks (Staub and Johnson, 2018; Srivastava *et al.*, 1993). The positive response of Boer breeder bucks to the intervention is manifested in sperm cell viability parameters to include faster motility and velocity and lower rates of sperm cells that move in circle and local patterns, as well as rate of immotile cells (Rurangwa *et al.*, 2004). In relation to male physiologically, the supplementation of lysine is also important as it serves as a regulatory mechanism of sperm function including volume of semen, concentration of sperm cells, and its motility (Sun *et al.*, 2014).

Based from the results presented, it is therefore concluded that lysine supplementation can improve animal productivity in terms of weight gain, can affect sexual behavior of the Boer breeder bucks as indicated by shorter time consumed and fewer mounting or attempts recorded, and can improve semen volume and concentration. Low to moderate rate or 1.75g- 2.45g lysine supplementation can improve the sperm cell viability in terms of concentration, motility and velocity. Therefore, inclusion of lysine with a rate of 1.75% to 2.45% is recommended for breeder buck aged 3-4 years old.

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