

Inclusion of ginger (*Zingiber officinale* Roscoe) in the diet of rams and its effect on sperm quality

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ABSTRACT

Objective: To compare changes in sperm quality in adult rams when ginger pellets were included in the diet during the breeding season.

Design/methodology/approach: The study was conducted for 16 weeks with 31 adult rams (eight Pelibuey, eight East Friesian, eight Damara and seven Dorper) at the Sheep and Goat Reproduction Laboratory (LaROCa) of the Colegio de Postgraduados, Campus Montecillo, located at 19° LN (north latitude) during short days (September-December). During the first 8 weeks, the rams received a diet based on commercial concentrate, minerals, lucerne, oats and water ad libitum; during the following 8 weeks (from 9 to 16), 2 g of dehydrated ginger pellets per day per animal were added to the diet. Weekly measurements were made of scrotal circumference, body weight and semen variables (volume, sperm concentration, mass motility, individual progressive motility, percentage of live sperm and normality).

Results: An increase in body weight and scrotal circumference was observed in all four breeds during the experiment, with increases of 4.94 kg and 2.41 cm, 12.85 kg and 7.48 cm, 10.88 kg and 5.9 cm and 6.48 kg and 3.77 cm in Pelibuey, East Friesian, Damara and Dorper breeds respectively. A treatment effect ($p < 0.05$) was found on sperm volume, live sperm percentage, sperm normality and sperm concentration. Differences ($p < 0.05$) were found in the breed effect for the variables time to ejaculate, volume, mass motility, percentage of live sperm, normality and sperm concentration and in the breed by treatment interaction for the variables volume, mass motility, individual motility, percentage of live sperm, normality and sperm concentration.

Limitations on study/implications: The study was carried out in only one season of the year (breeding season), it would be of great interest to carry out evaluations throughout the year (breeding vs. non-breeding season).

Findings/conclusions: Including ginger in the diet of sheep during the breeding season is a viable management option to improve sperm quality.

Keywords: Sheep, ginger, sperm quality, breeding season.

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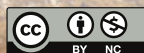
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INTRODUCTION

The hypothalamic-pituitary-testicular axis is influenced by nutrition through metabolic signals triggered by nutrients in the diet (Blache *et al.*, 2000; Ungerfeld, 2020), and therefore different strategies are being developed to include nutritional supplements and according



to the physiological state of the animal (Martin *et al.*, 2004). In sheep production systems, the increase of lambs per year is influenced by the reproductive capacity of females and males (Akhlaghi *et al.*, 2014).

Ginger (*Zingiber officinale*) a natural supplement with antioxidant, anti-inflammatory, and anti-apoptotic properties, has demonstrated positive effects on sperm quality across species, improving volume, concentration, motility, and viability while enhancing semen's antioxidant capacity, leading to increased fertility rates (Ali *et al.*, 2008; Jorsaraei *et al.*, 2008; Khaki *et al.*, 2009; Saeid *et al.*, 2011; Oyewo *et al.*, 2012; Akhlaghi *et al.*, 2014). However, its effects in sheep under production conditions remain largely unexplored. Given that rams exhibit seasonal variations in reproductive activity, supplementation strategies that optimize sperm production during the breeding season merit investigation.

Reproductive patterns vary among sheep breeds, particularly in photoperiod sensitivity. While Pelibuey, Dorper, and Damara breeds maintain prolonged reproductive activity with short anestrus periods, the East Friesian breed has a restricted breeding season (Porras *et al.*, 2003; Abecia *et al.*, 2024). Thus, evaluating ginger supplementation during the breeding season could enhance semen quality and reproductive efficiency in sheep (Rubianes and Ungerfeld, 2002).

This study assessed the impact of dehydrated ginger pellet supplementation on semen quality in rams of four breeds (Pelibuey, East Friesian, Damara, and Dorper) during the breeding season. We hypothesized that ginger supplementation would improve seminal parameters compared to a non-supplemented diet.

MATERIALS AND METHODS

Study site

The experiment was carried out at the Sheep and Goat Reproduction Laboratory (LaROCa) of the Colegio de Postgraduados, Campus Montecillo, located at Km. 36.5 Carretera México-Texcoco, Montecillo, Texcoco, Estado de México (19° 27' 38" N, 98° 54' 11" W) at an altitude of 2243 m according to Köppen, modified by García (2004), during the months of September to December (reproductive season, 16 weeks).

Animals and feeding

Thirty-one adult rams were used, eight Pelibuey, eight East Friesian, eight Damara and seven Dorper, weighing approximately 70 kg for Pelibuey and Damara, 80 kg for East Friesian and Dorper; aged between one and four years; clinically healthy, previously wormed, hoofless and vitaminised. For 16 weeks (September-December; breeding season) the rams were divided into four groups according to breed. The first eight weeks corresponded to the control period, they were fed a basic diet of 2 kg animal⁻¹ day⁻¹ based on commercial concentrate and minerals (Fosforysal Borrego[®] purine) from Ovina Reproductores 14, dehydrated lucerne, oats and water ad libitum, covering the nutritional requirements (NRC, 2007); the following eight weeks (from 9 to 16) corresponded to the experimental period, where 40 g of pelleted feed was added to the basic diet with a ratio of 1: 20 dehydrated ginger (2 g) animal⁻¹ day⁻¹, given in the morning on an empty stomach.

Scrotal circumference and determination of body weight

Scrotal circumference was measured weekly using a flexible tape measure and weighed on a weighing machine with a capacity of 250 kg \pm 100 g (Braunker model YP200S).

Semen collection and evaluation

Weekly semen collections were performed using an artificial vagina, and ejaculated volume, time of ejaculation, mass motility, percentage of live sperm, normal sperm (normality) and sperm concentration were determined according to the method described by Cortez-Romero & Gallegos-Sánchez (2014). A Carl ZEISS microscope, Primo Star, CP1145K06, Microimaging GmbH 37081, Göttingen, Germany, was used for the analysis of sperm concentration, normality and percentage of live spermatozoa. Series no: 3125001511; and for individual and progressive motility variables, an LW Scientific LW200, No. 506227 microscope equipped with a thermoplate (Vari-Warm LW Scientific) maintained at 37 °C was used.

Statistical analysis

A 2×4 factorial analysis was used for statistical analysis, where the factors were diet with and without ginger and the 4 breeds.

For analysis of repeated measure over time, the following model was used:

$$Y_{ijk} = \mu + \beta_j + T_i + (T\beta)_{ij} + \varepsilon_{ijk}$$

Where: y_{ijk} =Response variable; μ =Mean effect; j =effect of level A (Control diet and diet containing ginger); T_i =Effect of level B (breeds, Pelibuey, East Friesian, Damara and Dorper); $(T\beta)_{ij}$ =Interaction effect; ε_{ijk} =Random error. Both models were run in SAS 9.1 (2003).

RESULTS AND DISCUSSION

Body Weight and Scrotal Circumference

The values obtained for body weight and scrotal circumference during the experiment are presented in Table 1. In the four evaluated breeds, a progressive increase in body weight was observed from week 1 to week 16, with values ranging from 66.2±4.9 kg to 71.1±4.9 kg in Pelibuey, 66.1±4.9 kg to 78.9±4.9 kg in Frisian East, 72.60±5.30 kg to 83.48±4.96 kg in Damara, and 64.26±5.30 kg to 70.7±5.3 kg in Dorper. Similarly, scrotal circumference increased, with values ranging from 31.5±0.9 cm to 33.9±0.9 cm in Pelibuey, 28.46±0.91 cm to 35.94±0.91 cm in East Friesian, 31.94±0.98 cm to 37.84±0.91 cm in Damara, and 28.16±0.98 cm to 31.93±0.98 cm in Dorper. These results are consistent with previous reports for these breeds (Chi *et al.*, 2009; Arellano-Lezama, 2015; Calderón-Leyva *et al.*, 2018).

Variations in body weight and scrotal circumference are indicators of sexual maturity and reflect neuroendocrine changes throughout the year (Lincoln, 1998). Environmental factors, such as photoperiod and nutrition, have been documented to influence these parameters (Martin *et al.*, 1994; Blache *et al.*, 2000). During the reproductive season, an

Table 1. Means and standard errors ($X \pm SE$) of the variables body weight and scrotal circumference evaluated in sheep of the Pelibuey, East Friesian, Damara and Dorper breeds.

Variable	Breed Week	Scrotal circumference				Body weight			
		Damara	Dorper	East Friesian	Pelibuey	Damara	Dorper	East Friesian	Pelibuey
Control	1	31.94±0.98	28.16±0.98	28.46±0.91	31.54±0.91	72.6±5.3	64.26±5.3	66.08±4.96	66.16±4.96
	2	32.96±1.02	29.4±1.24	29.81±1.35	31.69±0.15	76.1±3.5	65.03±0.77	68.58±2.5	66.55±0.39
	3	32.94±0.02	29.83±0.43	30.53±0.71	31.74±0.05	76.55±0.45	65.94±0.91	69.08±0.5	67±0.45
	4	33.43±0.49	29.3±0.53	31.05±0.53	31.86±0.13	77.15±0.6	66.23±0.29	69.68±0.6	67.39±0.39
	5	33.89±0.46	28.84±0.46	31.4±0.35	31.55±0.31	77.85±0.7	66.29±0.06	71.03±1.35	67.49±0.1
	6	34.23±0.34	29.51±0.67	31.53±0.13	32.2±0.65	78.48±0.63	66.6±0.31	71.58±0.55	67.73±0.24
	7	34.04±0.19	29.57±0.06	31.7±0.18	32.73±0.52	78.45±0.02	67.26±0.66	72.29±0.71	67.91±0.19
	8	34.55±0.51	28.81±0.76	31.91±0.21	33±0.27	78.58±0.13	67.57±0.31	73.15±0.86	68.03±0.11
Exp(ginger)	9	34.58±0.03	29.9±1.09	32.2±0.29	32.9±0.1	79.3±0.72	67.69±0.11	73.75±0.6	68.11±0.09
	10	35.13±0.55	30.07±0.17	32.56±0.36	32.5±0.4	79.13±0.17	67.77±0.09	74.35±0.6	68.26±0.15
	11	35.23±0.1	30.51±0.44	32.71±0.15	33.21±0.71	79.58±0.45	69.51±1.74	74.78±0.43	69.05±0.79
	12	35.18±0.05	30.54±0.03	33.09±0.38	33.18±0.04	79.88±0.3	69.51±0	75.38±0.6	70.25±1.2
	13	35.63±0.45	30.51±0.03	33.69±0.6	32.73±0.45	79.63±0.25	68.91±0.6	75.63±0.25	70.43±0.17
	14	36.33±0.7	30.74±0.23	33.94±0.25	32.43±0.3	80.5±0.88	69.77±0.86	76.18±0.55	70.69±0.26
	15	36.64±0.31	31.21±0.47	34.69±0.75	32.86±0.44	81.15±0.65	69.83±0.06	76.94±0.76	70.94±0.25
	16	37.84±1.2	31.93±0.71	35.94±1.25	33.95±1.09	83.48±2.32	70.74±0.91	78.93±1.99	71.1±0.16

increase in Sertoli cell volume promotes spermatogenesis, resulting in increased testicular size (Lincoln, 1998). In this study, the increase in scrotal circumference and body weight suggests greater reproductive activity, which may be related to the improvement in the evaluated seminal variables. The analysis of variance showed a significant effect of treatment ($p < 0.05$) on ejaculate volume, percentage of live sperm, sperm normality, and sperm concentration. A significant effect of breed ($p < 0.05$) was also detected on ejaculation time, ejaculate volume, mass motility, percentage of live sperm, sperm normality, and sperm concentration. Furthermore, the breed x treatment interaction had a significant effect ($p < 0.05$) on ejaculate volume, mass motility, individual motility, percentage of live sperm, sperm normality, and sperm concentration (Table 2). No significant differences ($p > 0.05$) were found between weeks in the temporal analysis.

During week 8 of the experiment, the highest values for all evaluated seminal variables were recorded. These findings suggest that supplementation with ginger pellets should be maintained for at least 60 days to maximize the reproductive response, in accordance with previous studies demonstrating that prolonged nutritional supplementation in males positively impacts spermatogenesis (Martin *et al.*, 2004).

Ejaculate volume

A significant interaction effect between treatment and breed ($p < 0.05$) was observed in ejaculate volume. A decrease was recorded in Pelibuey, Friesian East, and Dorper with

ginger administration, although the recorded values remained within previously reported ranges (Arellano-Lezama, 2015). Studies in domestic birds have not shown changes in ejaculate volume following ginger supplementation (Akhlaghi *et al.*, 2014), suggesting a species-specific response. However, in this study, the reduction in semen volume was accompanied by an increase in sperm concentration, indicating greater efficiency in sperm production.

Sperm concentration

Ginger supplementation significantly increased ($p < 0.05$) sperm concentration, from $381.87 \times 10^6 \text{ mL}^{-1}$ to $451.88 \times 10^6 \text{ mL}^{-1}$. This result aligns with findings in animal models such as mice (Khaki *et al.*, 2009; Oyewo *et al.*, 2012) and broiler chickens (Saeid *et al.*, 2011; Shanoon, 2011). The positive effect of ginger on spermatogenesis may be attributed to the presence of bioactive compounds such as gingerols, paradols, and shogaols (Faivre *et al.*, 2006), which possess antioxidant properties and reduce oxidative stress in testicular cells (Agarwal *et al.*, 2014). Additionally, ginger has been associated with modulation of glutamate receptors in the brain (Ali *et al.*, 2008; Kuete, 2017), influencing GnRH secretion and, consequently, LH and FSH production (Maffucci and Gore, 2009).

Mass motility and individual progressive motility

A significant interaction effect ($p < 0.05$) between treatment and breed was detected in mass and individual motility. During the control period, the Frisian East breed exhibited the highest mass motility (4.82 ± 0.09), while Pelibuey recorded the lowest values. After ginger supplementation, mass motility values were 4.36 ± 0.09 , 4.40 ± 0.09 , 4.67 ± 0.09 , and 4.74 ± 0.10 in Pelibuey, Frisian East, Damara, and Dorper, respectively, exceeding values documented in previous studies (Karagiannidis *et al.*, 2000; Cárdenas-Gallegos *et al.*, 2012; Arellano-Lezama, 2015). These findings support the hypothesis that the bioactive compounds in ginger can enhance sperm motility through antioxidant and hormonal mechanisms, highlighting its potential as a supplement to optimize seminal quality in sheep.

Table 2. Means and standard errors ($X \pm EE$) of the variables ejaculate time (ET), ejaculate volume (Vol); mass motility (Mass Mot); individual motility (Ind Mot); live sperm count (Live); normality (Norm) and sperm concentration (Con) evaluated in sheep of the Pelibuey, East Friesian, Damara and Dorper breeds.

Var	Period		EEM	Breed				EEM	P>F		
	Control	Exp(ginger)		Pelibuey	EF	Damara	Dorper		Control	Breed	Per* Breed
ET	0.71	0.52	0.10	0.51 ^{ab}	0.34 ^b	0.88 ^a	0.74 ^{ab}	0.14	0.2094	0.0266	0.4985
Vol	0.90 ^a	0.76 ^b	0.21	0.82	0.78	0.83	0.89	0.03	<0.0001	0.0051	0.0006
Mass Mot	5.53	4.55	0.05	4.19 ^b	4.61 ^a	4.72 ^a	4.66 ^a	0.10	0.9081	<0.0001	0.0033
Ind Mot	95.94	96.18	0.61	94.67 ^b	96.13 ^a	98.34 ^a	95.00 ^b	0.88	0.5989	0.0815	0.0009
Live	66.38 ^b	69.40 ^a	0.65	74.59 ^a	68.48 ^b	61.92 ^c	66.32 ^b	0.93	0.0021	<0.0001	0.0100
Norm	91.78 ^b	93.52 ^a	0.40	92.46 ^{ab}	93.63 ^a	93.66 ^a	90.61 ^b	0.56	0.0017	0.0007	0.0059
Con	381.87 ^b	451.88 ^a	6.80	394.08 ^b	394.33 ^b	451.4 ^a	429.9 ^a	9.71	<0.0001	<0.0001	0.0002

Means with different literals in the same row are different ($p < 0.05$).

Percentage of live spermatozoa

When analysing the variable percentage of live spermatozoa, a treatment effect was observed ($p < 0.05$) that increased the percentage of live spermatozoa per ejaculate, a similar effect has been previously reported in rat (Khaki *et al.*, 2009; Oyewo *et al.*, 2012) and broilers (Saeid *et al.*, 2011; Shanoon, 2011). The improvement recorded in the percentage of live spermatozoa in the present experiment is probably due to its antioxidant property, due to the presence of gingerols, paradols and shogaols in ginger (Faivre *et al.*, 2006). This antioxidant property helps to protect the sperm membrane and counteracts the peroxidation that occurs in semen over time, thereby increasing the percentage of live sperm. At the brain level, ginger has been shown to have effects on glutamate receptors (Ali *et al.*, 2008; Kuate, 2017), which in turn influence GnRH secretion (Maffucci and Gore, 2009), so it can be suggested that ginger influences the increased production of LH and FSH, leading to an increase in sperm and testosterone production; not surprisingly, an increase in sperm concentration was reported in this study. It is therefore suggested that a 60-day course of ginger pellets will increase the percentage of live sperm per ejaculate.

Sperm normality

When analysing the sperm normality variables, a treatment effect was observed ($p < 0.05$). This effect of ginger intake has been reported in rat (Khaki *et al.*, 2009; Oyewo *et al.*, 2012) and broilers (Saeid *et al.*, 2011; Shanoon, 2011). The sperm normality in sheep reported in the present study during the period with ginger was 93.52%, a value higher than those previously reported with other diets (Arellano-Lezama, 2015; Cadena-Villegas, 2015). This increase in sperm normality could be due to the presence of gingerols, paradols and shogaols, which support spermatogenesis (Faivre *et al.*, 2006).

When analysing the interaction effect between treatment and breed, it was found that there were significant differences ($p < 0.05$) in the interaction for the normality variable. The Pelibuey breed showed the highest increase in normality (94.63 ± 0.77), which may be due to the fact that it is a breed that is less susceptible to the effect of photoperiod (Porrás *et al.*, 2003) and may be a breed that is more responsive to changes in the amount of nutrients supplied (De Waal and Combrinck, 2000). However, it is important to carry out further studies on this subject, including the reproductive rest period, in order to be able to recommend the use of ginger during the rest period and to be able to maintain a constant semen quality throughout the year or whenever the producer wishes to breed.

CONCLUSIONS

The inclusion of ginger in the diet of rams at a rate of 2 g per day per animal has a positive effect ($p < 0.05$) on the variables volume, percentage of live spermatozoa, sperm normality and sperm concentration. Therefore, we can conclude that the inclusion of ginger in the diet of rams during the reproductive season is a viable option to improve sperm quality. It would be interesting to repeat this experiment during the inactive reproductive period and to compare the results between the two periods.

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