Research Article

Dietary Unsaturated Fatty Acids and Antioxidants Improve Libido without Compromising Semen Quality in Garut Rams

Dewina Nur Humaira[1], Lilis Khotijah[1]*, Raden Iis Arifiantini[2], Herdis[3], Aeni Nurlatifah[4]

[1]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia; [2]Division Reproduction and Obstetrics, School of Veterinary Medicine and Biomedical Science, IPB University, Bogor 16680, Indonesia; [3]Research Center for Animal Husbandry, National Research and Innovation Agency, Bogor 16915, Indonesia; [4]Faculty of Animal Science, Gadjah Mada University, Yogyakarta 555281, Indonesia.

Abstract | This study aimed to evaluate the effects of different unsaturated fatty acids and antioxidants on the reproductive performance and quality of liquid semen in Garut rams. The study used a sample of 20 Garut rams aged 14 months with a body weight of 33.1 ± 4.9 kg. The experimental design for analysing the performance variables was a randomized block design (RBD) with four treatments and five replicates. The experimental design for liquid semen quality variables was a 3 × 2 factorial randomized block design (FRBD) comprising three ration types and two diluents. The ration treatments were as follows: P1 = control, P2 = P1 + canola oil + antioxidants, P3 = P1 + sunflower seed oil + antioxidants, and P4 = P1 + lemuru oil + antioxidants. The variables assessed were feed consumption, scrotal circumference, libido, and semen quality. Subsequently, the data obtained were analyzed using ANOVA and Duncan's test with IBM SPSS version 25. The results showed that the dietary treatments had no significant effect on reproductive performance parameters but significantly increased crude fat and fatty acid intake ($P < 0.01$). These results indicate that including unsaturated fatty acid sources and antioxidants did not have a detrimental effect on feed consumption and reproductive performance but tended to influence libido. In addition, there was no discernible interaction between the feed and diluent in influencing the motility, viability, and sperm plasma membrane integrity of liquid semen in Garut rams.

Keywords | Antioxidants, Fatty acids, Garut rams, Libido, Reproduction, Semen

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***Correspondence** | Lilis Khotijah, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia; **Email:** liliskh@apps.ipb.ac.id
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INTRODUCTION

Garut sheep, a germplasm asset of West Java, Indonesia, have the potential for high meat production, resilience, and productivity (Kamil, 2020). However, their genetic quality has declined due to the limited availability of superior rams, which are expensive as they are commonly used in dexterity competitions. Consequently, breeding with non-superior males has become common, leading to a deterioration in genetic quality over time (Herdis, 2017). Managing reproductive efficiency is essential for preserving and propagating the superior germplasm of pure breeds (Bajia *et al*., 2024). Several studies have proposed the use of artificial insemination technology and effective feed management to improve livestock genetic material and conservation (Mutinda, 2024; Ngcobo *et al*., 2021).

Artificial insemination (AI) is a well-known method for introducing semen into the reproductive apparatus of healthy female livestock to fertilize eggs using an insemination tool (Permen, 2016). AI enhances livestock genetics and conservation by facilitating selective breeding and maximizing the use of elite sires, enabling the efficient transfer of valuable hereditary traits (Shukla *et al*., 2023). AI technology has been widely implemented in Indonesia, particularly for cattle (Kuswati *et al*., 2022). However, its application in small ruminants such as sheep and goats remains limited (Inounu, 2014). Several studies have suggested that liquid semen could be an alternative to maximize the effectiveness of this technology (Pamungkas, 2009).

Using liquid semen may have certain advantages, such as more straightforward manufacturing techniques and only requiring a refrigerator for storage (Zaenuri *et al*., 2014). However, it is important to note that liquid semen has limited durability and can experience cold shock during storage (Kameni *et al*., 2021). This suggests the potential benefit of incorporating a diluent, such as Tris buffer or citrate fructose buffer, to enhance its shelf life (Surachman *et al*., 2006).

Semen quality is also affected by dietary factors (Yuan *et al*., 2023). Several studies have been conducted to enhance the quality of sheep semen through dietary interventions, including the use of unsaturated fatty acids and antioxidants (Esmaeili *et al*., 2014; Ezazi *et al*., 2019; Himanshu *et al*., 2022; Masoudi and Dadashpour, 2021; El-Desoky *et al*., 2023). The addition of PUFA supplements to the diet has been found to modify the fatty acid composition (FAC) of sperm and increase libido (Estienne *et al*., 2008). Notably, sheep semen is rich in omega-3 fatty acids, particularly docosahexaenoic acid (DHA) (C22:6, n-3), and polyunsaturated fatty acids (PUFAs), which play a crucial role as mediators in various reproductive processes, including maintaining cell membrane fluidity, facilitating intracellular signalling, and influencing susceptibility to oxidative damage (Wathes *et al*., 2007).

Omega-3 fatty acids are mainly found in fish oil, whereas omega-6 fatty acids are found in vegetable oils, such as canola and sunflower oils (Saini and Keum, 2018). Canola oil also contains high levels of oleic acid, an omega-9 fatty acid (Lin *et al*., 2013). One of the local feed ingredients that is high in omega 3 is Lemuru fish oil, which is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and plays a role in sheep reproduction (Nurlatifah *et al*., 2022). Studies have shown that omega-3 fatty acids from fish oil and omega-6 fatty acids from sunflower oil can enhance the quality of sheep semen when combined with vitamins E and C (Esmaeili *et al*., 2014; Ezazi *et al*., 2019). However, dietary PUFA supplementation can negatively affect the quality of frozen-thawed ram sperm without antioxidant support (de Graaf *et al*., 2007). As a result, the inclusion of PUFA in ruminant diets is often accompanied by antioxidant compounds (Gobert *et al*., 2009; Santos *et al*., 2014). Vitamin E and selenium can be used as antioxidants because they work synergistically to protect spermatozoa from free radicals, maintain libido, and improve semen quality (Al-Haboby *et al*., 2004; Zubair, 2017; Surai *et al*., 2000; Khan, 2011).

Given these considerations, further research is urgently needed to identify the optimal fatty acid source to support liquid semen production for artificial insemination. Therefore, this study aimed to evaluate the effects of different unsaturated fatty acids from canola, sunflower seed, and Lemuru oils, along with antioxidants, on the reproductive performance and liquid semen quality of Garut sheep to enhance artificial insemination efficiency, ultimately benefiting livestock breeding and farmer productivity.

MATERIALS AND METHODS

Animals and diets

The animal management protocol for this experiment was approved by the Ethical Committee of the National Research and Innovation Agency (Decree No. 204/KE.02/SK/11/2023). The study was conducted in the Laboratory of Small Ruminants Nutrient and Feed Science Block B, Faculty of Animal Science, IPB University, Bogor, Indonesia. The study used 20 Garut rams aged 14 months with a body weight of 33.1 ± 4.9 kg (mean ± standard deviation). The housing conditions were maintained under controlled temperature and adequate ventilation. Semen evaluation was conducted at the Laboratory of the Reproductive Rehabilitation Unit, School of Veterinary Medicine, and Biomedical Sciences, IPB University.

Rams were fed twice daily at 3% of body weight (dry matter basis). The diet consisted of 30% Pennisetum purpureum (Napier Grass) and 70% concentrate, formulated to meet the nutritional needs of the animals according to NRC (2007), as shown in Table 1. The feed treatments were as follows: P1: control, P2: P1 + canola oil + vitamin E 500 IU + selenium 0,5 ppm, P3: P1 + sunflower seed oil + vitamin E 500 IU + selenium 0,5 ppm, and P4: P1 + Lemuru oil + vitamin E 500 IU + selenium 0,5 ppm. Vitamin E powder (DL-α-Tocopheryl Acetate, Zhejiang Medicine Co. Ltd) and selenium were included in the diets as antioxidants to protect PUFAs from oxidation. The nutrient content of the ration is presented in Table 2, and the fatty acid content of the ration is listed in Table 3.

Table 1: The composition of the treatment ration (based on 100% dry matter).

Ingredients	Composition (%)			
	P1 %	P2 %	P3 %	P4 %
Pennisetum purpureum	30	30	30	30
Soybean meal	14.5	14.5	14.5	14.5
Pollard	14	14	14	14
Rice bran	16	16	16	16
Cassava flour	17	17	17	17
Molasses	7	7	7	7
Canola oil	-	4.2	-	-
Sunflower seed oil	-	-	4.2	-
Lemuru oil	-	-	-	4.2
Vitamin E (IU)	-	500	500	500
Selenium (ppm)	-	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5
$CaCO_3$	0.5	0.5	0.5	0.5
NaCl	0.5	0.5	0.5	0.5

P1= control; P2= P1 + canola oil + antioxidants; P3= P1 + sunflower oil + antioxidants; P4= P1 + lemuru oil + antioxidants.

Table 2: Nutrient composition in each treatment (based on 100% dry matter).

Nutrient	Composition (%)			
	P1%	P2%	P3%	P4%
Ash[a)]	11.88	11.48	11.62	11.28
Crude protein[a)]	19.38	18.31	18.25	18.01
Crude fat[a)]	3.34	6.72	7.19	6.75
Crude fiber[a)]	13.09	12.56	13.02	13.38
NEF[a)]	52.30	50.39	49.92	50.58
TDN[b)]	69.78	73.46	73.24	72.62

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + Lemuru oil + antioxidants; [a)] Proximate analysis performed by Nutrition Science and Feed Technology Laboratory, 2024 [b)]TDN Calculation Results (Sutardi 2001); TDN: total digestible nutrient; NFE: Nitrogen-free extract

Table 3: Fatty acid composition of ration.

Treat-ment	Fatty acid composition per 100 g crude fat (%)				
	Oleic acid %	Linoleic acid %	Linolenic acid %	EPA	DHA
P1	15.83	3.78	-	-	-
P2	26.67	12.10	0.67	-	-
P3	16.55	18.55	-	-	-
P4	14.77	8.22	1.25	7.27	2.93

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + Lemuru oil + antioxidants; Fatty acid analysis performed by IPB Integrated Laboratory Unit, 2024; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid

Nutrient consumption

Nutrient consumption for each treatment was calculated based on actual feed intake and the nutrient composition of the diet. The calculated nutrient consumption included dry matter (DM), crude protein, crude fat, crude fibre, nitrogen-free extract (NFE), total digestible nutrients (TDN), and fatty acids. The calculations were performed using the following formulas: DM intake = feed intake (g) × %DM of feed, nutrient intake = DM intake (g) × %nutrient content, and fatty acid intake = crude fat intake (g) × % fatty acid content.

Scrotal circumference measurement and libido assessment

The scrotal circumference was measured using a centimetre measuring tape (Paramitha *et al*., 2020). The testes were fully descended before measurement, and the tape was placed around the widest point of the scrotum for accurate readings (Rahmawati and Winurdana, 2022). Libido assessment was conducted by measuring reaction time, defined as the duration from the ram's initial approach to the ewe until the first ejaculation, ensuring consistency in measurement (Price, 2008).

Diluent preparation

The diluent was prepared using two types of buffers: Tris buffer and citrate-fructose buffer. The Tris buffer was made by dissolving 3.63 g Tris hydroxymethylaminomethane, 1.99 g citric acid, and 0.5 g fructose in 100 ml of distilled water (Kulaksiz *et al*., 2012), while citrate-fructose buffer was prepared by dissolving 2.32 g sodium citrate dehydrate and 1.25 g fructose in 100 ml of distilled water (Arifiantini and Purwantara, 2010). Each buffer solution (80%) was mixed with 20% egg yolk and homogenized for 15 minutes using a magnetic stirrer, then supplemented with antibiotics (1000 IU penicillin and 1 mg streptomycin per ml) for microbial control.

Semen collection and evaluation

Semen was collected using an artificial vagina and a female teaser in the morning at 07.00 am, before and after the treatment. The collected semen was analysed in the laboratory. The fresh semen was analysed both macroscopically (volume, color, pH, consistency) and microscopically (motility, viability, concentration, and abnormalities) as described by Arifiantini (2012). Sperm motility was observed using an endoscope (Tiefenbach, Minitub®, Germany). Sperm concentration was measured using a photometer (Photometer SDM 6®, Minitub GmbH, Tiefenbach, Germany).

Dilution, storage, and evaluation of liquid semen

The dilution process began by calculating sperm concentration to determine the required diluent volume, ensuring only semen with ≥70% motility was processed, as a minimum qualification for dilution (SNI, 2014). Semen was divided and mixed with Tris egg yolk or citrate fructose egg yolk diluents, then stored at 3–5°C and analyzed every 24 hours for 4 days to assess motility, viability, and plasma membrane integrity (Arifiantini and Purwantara, 2010). Sperm viability was evaluated using eosin-nigrosin staining, while plasma membrane integrity was assessed through the hypo-osmotic swelling test (HOS) test under a microscope at 400x magnification.

Statistical analysis

A randomized block design (RBD) with four treatments and five replicates was used for the reproductive performance modifier. The liquid semen quality modifier was a 3 x 2 factorial randomized block design (FRBD) with three types of rations and two types of diluents (egg yolk tris and fructose citrate). Data analysis was based on sheep semen suitable for processing, resulting in 3 treatments (P2, P3, P4). Statistical analyses were performed using SPSS software, and data were analyzed using analysis of variance (ANOVA). Significantly different results were tested using Duncan's test (Steel and Torrie, 1993).

RESULTS

Nutrient consumption

Data on nutrient intake are summarized in Table 4. Supplementation with different unsaturated fatty acids and antioxidants had no significant effect ($P > 0.05$) on the intake of dry matter, crude protein, crude fibre, NFE, and TDN. However, it significantly increased the intake of crude fat, linolenic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid ($P < 0.01$). The results indicated that supplementation with different fatty acids did not influence dry matter, crude protein, crude fibre, nitrogen-free extract (NFE), or total digestible nutrients (TDN) consumption in sheep.

Scrotal circumference and libido

The results of the scrotal circumference measurements and libido observations following supplementation with different unsaturated fatty acids and antioxidants are presented in Table 5. Supplementation had no significant effect on scrotal circumference ($P > 0.05$) but showed a trend toward a marginally significant effect on libido ($P < 0.1$).

The sperm motility of Garut rams liquid semen

The motility of Garut ram sperm supplemented with different unsaturated fatty acids and antioxidants in different diluents is shown in Table 6. Statistical analysis showed no significant interaction ($P > 0.05$) between the supplementation of unsaturated fatty acids and the type of diluent on the motility of Garut rams sperm in liquid semen.

The sperm viability of Garut rams liquid semen

The viability of Garut ram sperm supplemented with different unsaturated fatty acids and antioxidants in different diluents is shown in Table 7. The statistical results indicated no interaction ($P > 0.05$) between the supplementation of different unsaturated fatty acids and different diluents on the viability of liquid semen sperm of Garut rams.

The sperm plasma membrane integrity of Garut rams liquid semen

The integrity of the plasma membrane of Garut ram sperm supplemented with different unsaturated fatty acids and antioxidants in different diluents is shown in Table 8. The statistical results showed no interaction ($P > 0.05$) between the supplementation of different fatty acids and different diluents on the integrity of the plasma membrane of Garut rams liquid semen.

Table 4: Nutrients consumption of Garut rams.

Variable (g head^{-1}day^{-1})	P1	P2	P3	P4	P-value
Dry matter	1020.86 ± 119.78	925.91 ± 122.15	1101.08± 22.05	987.71 ± 96.80	0.134
Crude protein	178.16 ± 20.87	162.02 ± 12.88	182.40 ± 3.87	161.66 ± 15.76	0.187
Crude fat	35.36 ± 4.15^a	55.04 ± 7.17^b	68.65 ± 1.50^c	58.35 ± 5.68^b	<0.01
Crude fiber	173.18 ± 20.80	152.87 ± 21.36	187.83 ± 3.45	171.65 ± 17.31	0.063
NFE	516.62 ± 60.55	481.07 ± 42.10	537.67 ± 10.90	486.78 ± 47.62	0.328
TDN	689.06 ± 80.76	650.29 ± 85.29	769.68 ± 15.82	685.54 ± 66.99	0.144
Oleic acid	5.62 ± 0.66^a	17.42 ± 2.30^d	13.81 ± 1.46^c	8.65 ± 0.85^b	<0.01
Linoleic acid	1.32 ± 0.16^a	13.54 ± 1.97^b	23.24 ± 2.40^c	12.19 ± 1.31^b	<0.01
Linolenic acid	0.00 ± 0.00^a	0.43 ± 0.05^b	0.00 ± 0.00^a	0.85 ± 0.08^c	<0.01
EPA	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	4.15 ± 0.41^b	<0.01
DHA	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	1.65 ± 0.16^b	<0.01

P1= control; P2= P1 + canola oil + antioxidants; P3= P1 + sunflower oil + antioxidants; P4 = P1 + Lemuru oil + antioxidants; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid.

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Table 5: Scrotal circumference and libido of Garut rams.

Treatment	Scrotal circumference (cm)	Libido* (seconds)
P1	27.92 ± 2.95	24.31 ± 1.81[a]
P2	26.14 ± 1.37	22.01 ± 3.83[ab]
P3	28.32 ± 2.14	22.08 ± 4.33[ab]
P4	28.38 ± 2.86	18.18 ± 3.61[b]
Mean ± SD	27.69 ± 2.40	21.64 ± 4.05
P-value	0.362	<0.1

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + lemuru oil + antioxidants, * = reaction time

Table 6: Sperm motility of garut rams fed different unsaturated fatty acids in tris egg yolk and citrate fructose egg yolk (%).

Storage time (day)	Rations	Tris egg yolk	*Citrate fructose* egg yolk	Mean±SD
Day 1	P2	67.5 ± 3.53	67.5 ± 10.60	67.5±6.45
	P3	72.5 ± 3.53	70.0 ± 7.07	71.3±4.78
	P4	62.5 ± 3.53	72.5 ± 10.60	67.5±8.66
	Mean±SD	67.5 ± 5.24	70.0 ± 7.74	
Day 2	P2	45 ± 14.14	57.5 ± 10.60	51.3±12.50
	P3	57.5±10.60	47.5 ± 3.53	52.5±8.66
	P4	52.5 ± 3.53	57.5 ± 3.53	55.0±4.08
	Mean±SD	51.7 ± 9.83	54.1 ± 7.35	
Day 3	P2	22.5±31.81	50.0 ± 7.07	36.3±24.62
	P3	50.0 ± 7.07	42.5 ± 3.53	46.3±6.29
	P4	47.5 ± 3.53	52.5 ± 3.53	50.0±4.08
	Mean±SD	40.0±20.00	48.3 ± 6.05	
Day 4	P2	17.5 ± 24.7	37.5 ± 10.6	27.5±19.36
	P3	30.0 ± 7.07	37.5 ± 3.53	33.8±6.29
	P4	42.5 ± 3.53	45.0 ± 7.07	43.8±4.78
	Mean±SD	30.0±16.12	40.0 ± 7.07	

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + lemuru oil + antioxidants

DISCUSSIONS

The unchanged dry matter intake suggests that the addition of unsaturated fatty acids and antioxidants to the diet did not compromise the palatability of the ration. This finding aligns with that of Maia *et al.* (2012), who reported no significant differences in dry matter intake among sheep supplemented with various fatty acids. Similarly, the lack of significant differences in the intake of crude protein, crude fibre, NFE, and TDN across treatments was consistent with the stable dry matter consumption observed. Furthermore, the dry matter intake values recorded in this study were based on the NRC (2007) recommendations, which state that sheep weighing 20–30 kg can consume between 690 and 1240 g of dry matter per head per day.

Table 7: Sperm viability of garut rams fed different unsaturated fatty acids in tris egg yolk and citrate fructose egg yolk (%).

Storage time (day)	Rations	Tris egg yolk	*Citrate fructose* egg yolk	Mean±SD
Day 1	P2	90.9±1.35	92.1±1.73	91.5±1.47
	P3	90.2 ± 3.75	93.5 ± 2.48	91.8±3.23
	P4	89.5±7.29	93.9±4.49	91.7±5.56
	Mean±SD	90.2±3.76	93.2 ± 2.56	
Day 2	P2	77.8±12.30	80.7±17.62	79.2±12.52
	P3	83.0±4.23	88.9±3.86	85.9±4.73
	P4	82.9±4.08	80.1±9.34	81.5±6.10
	Mean±SD	81.2±6.66	83.2±10.08	
Day 3	P2	33.1±46.79	73.2±16.26	53.1±36.78
	P3	70.2±10.22	72.6±3.74	71.4±6.44
	P4	79.9±2.70	70.5±2.38	75.2±5.83
	Mean±SD	61.1±30.79	72.1±7.64	
Day 4	P2	32.6±46.10	63.4±6.21	48.0±32.21
	P3	65.7±6.73	68.1±3.58	66.9±4.61
	P4	67.5±3.25	64.5±4.10	66.0±3.48
	Average	55.3±27.30	65.3±4.30	

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + lemuru oil + antioxidants.

Table 8: Integrity of the plasma membrane of Garut rams sperm fed different unsaturated fatty acids in tris egg yolk and citrate fructose egg yolk (%).

Storage time (day)	Rations	Tris egg yolk	*Citrate fructose* egg yolk	Mean ± SD
Day 1	P2	69.2 ± 0.28	77.9 ± 10.50	73.5 ± 7.87
	P3	79.1 ± 5.97	77.7 ± 4.27	78.3 ± 4.32
	P4	72.2 ± 12.33	75.0 ± 6.20	73.6 ± 8.12
	Mean±SD	73.5 ± 7.63	76.8 ± 5.95	
Day 2	P2	56.5 ± 3.95	75.6 ± 7.91	66.1 ± 12.15
	P3	71.5 ± 10.74	69.1 ± 8.27	70.3 ± 7.95
	P4	61.8 ± 26.79	72.6 ± 6.57	67.9 ± 11.86
	Mean±SD	63.2 ± 14.70	72.4 ± 6.59	
Day 3	P2	26.2 ± 37.05	62.0 ± 15.90	44.1 ± 31.11
	P3	66.0 ± 11.17	66.5 ± 4.87	66.3 ± 7.04
	P4	46.8 ± 17.96	49.7 ± 17.04	48.3 ± 26.09
	Mean±SD	46.3 ± 26.09	59.4 ± 13.18	
Day 4	P2	24.1 ± 34.15	57.0 ± 10.18	40.5 ± 27.98
	P3	51.2 ± 9.26	53.0 ± 21.14	52.1 ± 13.37
	P4	41.5 ± 15.55	36.7 ± 22.20	39.1 ± 15.89
	Mean±SD	38.9 ± 21.1	48.9 ± 17.36	

P1 = control; P2 = P1 + canola oil + antioxidants; P3 = P1 + sunflower oil + antioxidants; P4 = P1 + lemuru oil + antioxidants

The average scrotal circumference in this study was still within the normal range according to Wijaya *et al.* (2019), who reported that the scrotal circumference of Garut sheep aged 12-18 months ranged from 26.50 ± 1.23 cm. A larger scrotal circumference is generally linked to higher semen quantity, serving as a key indicator of a male's reproductive potential. It is a crucial parameter in selecting superior males, as it correlates with body weight, reproductive capacity or libido, and sperm production (Wahyudi *et al.*, 2022; Sahi *et al.*, 2019).

The fastest first ejaculation time was observed in Garut rams supplemented with lemuru oil and antioxidants (P4). These results showed a trend toward statistical significance ($P < 0.1$), indicating a tendency for a real effect in the P4 group compared to the control group (P1). The P4 group showed a 25.22% reduction in reaction time compared to the control group, suggesting an improvement in libido. Improved libido plays a crucial role in artificial breeding programs, as it accelerates semen collection and freezing processes (Iskandar *et al.*, 2022).

Dietary supplementation with omega-3 fatty acids, such as fish oil, has been reported to increase libido, modify fatty acid composition in the testis, and influence steroid production in boars (Estienne *et al.*, 2008; Castellano *et al.*, 2011). Lemuru oil contains omega-3 fatty acids, particularly EPA and DHA, which are involved in prostaglandin synthesis (Upadhyay, 2022). Prostaglandins are important for improving sexual nerve sensitivity and modulating the release of reproductive hormones such as testosterone and luteinizing hormone (LH). In addition, EPA and DHA contribute to increased nitric oxide (NO) production, which acts as a vasodilator and increases blood flow to the reproductive organs. This mechanism can support an increased sexual response and accelerate ejaculation time in animals (Naz *et al.*, 2022; Moghaddam *et al.*, 2012). Improving these reproductive parameters significantly impacts livestock sustainability and profitability. The use of such indicators can not only enhance the efficiency of breeding programs but also reduce costs associated with suboptimal semen quality and fertility (Korkmaz *et al.*, 2023).

The results of the macroscopic examination revealed that the volume of semen was 1.28 ± 0.71 ml, beige in color, thick in consistency, and had a degree of similarity (pH) of 6.70 ± 0.56. In addition, the results of the microscopic examination showed that the percentage of sperm motility was 82.27 ± 9.15%, the percentage of sperm viability was 94.37 ± 5.07%, sperm abnormality was 7.85 ± 3.39%, and the sperm concentration was 3346.17 ± 1305 million/ml. The quality of fresh semen determines the feasibility of processing it into liquid semen. This study showed that the quality of fresh semen from Garut rams was within the normal range for all variables (Garner and Hafez, 2000) and can be further processed.

The results showed no significant interaction between the ration type and diluent type on sperm motility. This finding is supported by Masoudi *et al.* (2016), who reported that no interaction effect was observed between diluents and rations on sperm motility. Sperm motility is the main indicator for assessing semen quality because sperm with high motility have a greater chance of successful fertilization (Van de Hoek *et al.*, 2022). The average sperm motility in this study ranged from 27.5%–70%. The Indonesian National Standard (SNI) requires a minimum motility standard of 40% for artificial insemination (AI). The results showed that sperm motility above 40% until the fourth day was found in the group that received Lemuru oil (P4) supplementation and semen dilution with citrate fructose buffer. Omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in Lemuru oil, improve sperm membrane fluidity. Higher membrane fluidity can improve the stability and integrity of the sperm plasma membrane during cryopreservation and storage (Shan *et al.*, 2021).

The results showed no significant interaction between the ration type and diluent type on sperm viability, suggesting that feed containing unsaturated fatty acids and diluents can independently affect sperm viability. Polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids, play a vital role in maintaining sperm viability. Omega-3 fatty acids also enhance membrane flexibility by promoting structural transitions in the phospholipid bilayer, supporting sperm function (Masoudi and Dadashpour, 2021). The diluents used in this study undoubtedly provided adequate protection for maintaining sperm viability and were not significantly influenced by the type of ration used. The egg yolk-based diluent contains phospholipids and lecithin, which protect the sperm membrane from cold shock (Aksu, 2023).

The results showed no significant interaction between the ratio and diluent type on the integrity of the sperm plasma membrane. Yuan *et al.* (2023) demonstrated that modifying the feed changed the fatty acid profile on the sperm membrane, thereby improving membrane integrity. The effect of fatty acid application was not influenced by the diluent used during semen storage. Both tris and citrate diluents effectively maintained the integrity of the plasma membrane, regardless of the type of ration. Arifiantini and Purwantara (2010) reported that egg yolk tris diluent and fructose egg yolk citrate exhibited the same preservation ability in Holstein-Friesian bull liquid semen.

CONCLUSION

This study highlights the role of unsaturated fatty acids and antioxidants in improving reproductive performance in Garut rams by maintaining feed palatability and reproductive traits while potentially enhancing libido, as indicated by a 25.22% reduction in reaction time with lemuru oil. Additionally, the absence of interaction between diet and semen diluents suggests that dietary intervention affects liquid semen quality independently of semen processing methods. These findings suggest that unsaturated fatty acid supplementation could be a practical strategy to enhance mating performance and productivity in breeding programs.

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NOVELTY STATEMENT

The novelty of this study was to evaluate the effects of different sources of unsaturated fatty acids (canola oil, sunflower seed oil, and lemuru fish oil) combined with antioxidants (vitamin E and selenium) on production and reproductive performance, and the quality of chilled-stored liquid semen of Garut rams. This study also compared the effects of different fatty acid sources and antioxidant combinations in improving semen quality to support artificial insemination programs.

AUTHOR'S CONTRIBUTION

All the authors contributed to designing research, data collection, data acquisition, data analysis and reporting, and manuscript preparation.

Conflict of interest

The authors have declared no conflict of interest.

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