The Effects of Canola Oil, Vitamin E, and Selenium Supplementation in the Ration on Blood Metabolites Profile and Liquid Semen Quality in Fat-Tailed Rams

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(received 22-01-2025; revised 11-03-2025; accepted 17-03-2025)

ABSTRAK

Sutarno AP, Khotijah K, Arifiantini RI, Pamungkas FA, Nurlatifah A. 2025. Pengaruh suplementasi minyak kanola, vitamin E, dan selenium dalam pakan pada profil metabolit darah dan kualitas semen cair domba Ekor Gemuk jantan. JITV 30(1):42-51. DOI:http://dx.doi.org/jitv,v30.i1.3497.

Tujuan penelitian ini adalah mengevaluasi pengaruh pemberian minyak kanola, vitamin E, dan selenium dalam pakan pada profil metabolit darah dan kualitas semen cair pada domba ekor gemuk jantan. Ternak yang digunakan sebanyak 10 ekor domba ekor gemuk berumur 10 – 14 bulan dengan bobot awal 23.84±3.91 kg. Rancangan yang digunakan ialah rancangan acak kelompok (RAK) dan rancangan acak kelompok faktorial (RAKF) 2 x 2 dengan 2 perlakuan pakan (R0: ransum kontrol, R1: R0 + minyak kanola + vitamin E + selenium dan 2 jenis pengencer. Peubah yang diukur meliputi konsumsi nutrien, pertambahan bobot badan harian, efisiensi penggunaan pakan, metabolit darah, biokimia plasma semen, dan kualitas semen cair. Hasil penelitian menunjukkan bahwa pemberian minyak kanola dan antioksidan tidak mempengaruhi konsumsi bahan kering, pertambahan bobot badan harian, dan metabolit darah (P>0,05). Akan tetapi, terdapat peningkatan asupan nutrien berupa lemak kasar, asam lemak, efisiensi penggunaan pakan, kadar kolesterol total, dan kolesterol LDL darah (P<0,05). Pemberian minyak kanola dan antioksidan secara deskriptif juga menunjukkan peningkatan kadar trigliserida plasma semen dan LDL, sementara secara bersamaan mengurangi kadar glukosa dan kolesterol plasma semen. Pemberian minyak kanola dan antioksidan tidak memengaruhi viabilitas sperma (P>0,05), tetapi menunjukkan peningkatan motilitas sperma semen cair pada hari ke-3 (P<0,05). Sebagai simpulan, pemberian suplementasi minyak kanola, vitamin E, dan selenium dalam ransum domba jantan ekor gemuk mengakibatkan peningkatan kadar kolesterol total dan LDL dalam darah sekaligus mengurangi konsentrasi kolesterol dalam plasma semen. Disamping itu, suplementasi minyak kanola dan antioksidan meningkatkan asupan nutrien, efisiensi pakan, dan memperpanjang masa simpan semen cair.

Kata Kunci: Antioksidan, Domba Ekor Gemuk Jantan, Minyak Kanola, Lemak, Semen Cair

ABSTRACT

Sutarno AP, Khotijah K, Arifiantini RI, Pamungkas FA, Nurlatifah A. 2025. The effects of canola oil, vitamin E, and selenium supplementations in the ration on blood metabolites profile and liquid semen quality of Fat-tailed rams. JITV 30(1):42-51. DOI:http://dx.doi.org/jitv,v30.i1.3497.

This research aimed to assess the impact of dietary supplementation with canola oil, vitamin E, and selenium on blood metabolite profile and the quality of liquid semen in fat-tailed rams. The livestock consisted of 10 fat-tailed rams aged 10 to 14 months, with an average initial body weight of 23.84±3.91 kg. Randomized block design (RBD) and factorial randomized block design (FRBD) 2 x 2 were used for evaluating performance and liquid semen variables, with two feed treatments (R0: control diet, R1: R0 + canola oil + vitamin E + selenium). The variables measured included nutrient intake, daily body weight gain, feed efficiency, blood metabolites, seminal plasma biochemistry, and liquid semen quality. The results showed that canola oil and antioxidants did not influence dry matter intake, daily body weight gain, and blood metabolites (P>0.05). However, there was an increase in the nutrient intake of crude fat, fatty acids, feed efficiency, total cholesterol levels, and blood LDL cholesterol (P<0.05). In the ration, Canola oil and antioxidant supplementations were descriptively increased seminal plasma triglycerides and LDL. Additionally, supplementations of canola oil and antioxidants in the feed reduced seminal plasma glucose and cholesterol levels. The administration of canola oil and antioxidants did not affect sperm viability (P>0.05) but increased liquid semen motility on day 3 (P<0.05). In conclusion, supplementing canola oil, vitamin E, and selenium in the diet of fat-tailed rams increased total cholesterol and LDL levels in the blood while reducing cholesterol concentration in seminal plasma. Additionally, these dietary interventions improve nutrient intake and feed efficiency and extend the shelf life of liquid semen.

Key Words: Antioxidants, Canola oil, Fat, Fat-tailed Rams, Liquid Semen

INTRODUCTION

The lamb meat production and the sheep population in West Java have increased and decreased by 5.1% and 15.6%, respectively (BPS 2024). This condition raises concerns about the challenges in increasing meat production to meet the continuously rising market demand. The issue can be addressed by enhancing the population of fat-tailed rams as breeding stock. Fat-tailed rams are known as local meat sheep with good growth potential in this context. Optimizing the function of fat-tailed rams as breeding stock is achieved by managing nutrients and meeting feed requirements to improve reproduction. Feed plays a crucial role in the reproductive performance of sheep, particularly in providing sufficient energy sources.

Energy deficiency in males negatively impacts reproductive performance (De Souza et al., 2019). Alternative energy sources for livestock can be achieved through fat supplementation since the nutrient provides 2.24 times more energy than carbohydrates (Pramono et al. 2018). Fat plays an important role as an energy reserve and supports hormone synthesis, plasma membrane composition, and sperm motility function. The nutrient is included in the energy metabolism processes of sperm and seminal plasma (Van Tran et al. 2017; Alagawany et al. 2019; Fitriyah & Isyaturriyadhah 2021). Therefore, fat-source feed such as oil should be added to livestock rations.

According to Younis Talpur et al. (2009), canola oil contains 56.89% and 10.66% monounsaturated and polyunsaturated fatty acids. Oleic acid supplementation is reported to function as an energy source to enhance sperm motility (Zhu et al. 2020), maintain membrane fluidity, and improve sperm motility (Ferramosca et al. 2017). Linoleic acid supplementation enhances motility and acrosome integrity (Ezazi et al. 2019) and increases semen volume, concentration, total motility, viability, and plasma membrane integrity of sperm (Masoudi & Dadashpour Davachi 2021).

Feed is optimized by adding antioxidants such as vitamin E and the mineral selenium (Se) to reduce oxidative stress. Vitamins and minerals are crucial in livestock's growth and reproductive health (Zubair et al. 2015). Semen quality is a determining factor in optimizing the reproductive function of fat-tailed sheep for superior breeding stock. Sound quality is expected to increase the population of rams through artificial insemination (AI) or natural mating. AI can use liquid semen as an alternative because the process is fast and only requires the addition of diluent materials to fresh semen and storing at a temperature of 4-5°C (Zakiya et al. 2020; Rokana et al. 2022). In ruminant livestock, feeding high levels of fatty acids raises concerns about affecting consumption, which will affect semen quality because the addition of fatty acids in the diet causes a change in the fatty acid composition of the sperm membrane (Jafaroghli et al. 2014; Nassan et al. 2018). Therefore, this research aimed to assess the impact of dietary supplementation with canola oil, vitamin E, and selenium on blood metabolite profile and the quality of liquid semen in fat-tailed rams.

MATERIALS AND METHODS

Animals and diets

The Animal Maintenance has approved this research and Use Ethics Commission of the National Research and Innovation Agency (Number: 204/K.E.02. /SK/11/2023). The livestock used in this experiment consisted of 10 fat-tailed rams aged 10 to 14 months, with an average initial body weight of 23.84±3.91 kg divided into 2 treatments and 5 replications. The animals were maintained at the Meat and Work Animal Nutrition Laboratory, Pen Facility B, Faculty of Animal Science, IPB University, in individual pens equipped with feeding and drinking water facilities. Additionally, the experimental diet consisted of elephant grass *(Pennisetum purpureum)* and concentrate. The diet was provided at 3% of body weight with a forage-to-concentrate ratio 30:70 based on dry matter (DM). The formulation was isoenergetic and isoprotein, following the needs of male sheep, according to (NRC 2007). The experimental diets used were R0: control diet, R1: R0 + canola oil + vitamin E + selenium. Table 1 shows the nutrient composition of the concentrate used.

Nutrient consumption

Feed and nutrient intake measurements were conducted daily during the maintenance phase. The nutrients calculated included DM intake, crude protein (CP), crude fiber (CF), crude fat (CF), nitrogen-free extract (NFE), total digestible nutrients (TDN) intake, and fatty acid intake. The calculations for measuring feed intake and nutrient consumption can be performed using the formulas DM intake = feed intake (g) X % DM of feed, CP intake = DM intake (g) X % CP of feed, CF intake = DM intake (g) X % CF of feed, NFE intake = DM intake (g) X % NFE of feed, TDN intake = DM intake (g) X % TDN of feed, and fatty acid intake = Crude fat intake (g) x % fatty acid content.

Measurements of average daily weight gain

Body weight measurements in rams were conducted before and after the treatment. Meanwhile, weighing was performed using a digital scale with a capacity of 100 kg. The calculation of average daily gain (ADG) was obtained using the formula ADG= [(final weight – initial weight) / duration of maintenance (days)].

Table 1. The composition of ingredients concentrate, dosage of canola oil, vitamin E, selenium, and nutrient content (Fatty acids) of the treatment concentrate

Feed materials	Treatment	
	R0 (%)	R1 (%)
Canola oil	-	6.00
Soybean meal	20.71	20.71
Pollard	20.00	20.00
Rice bran	22.86	22.86
Cassava pulp	24.29	24.29
Molasses	10.00	10.00
Premix	0.71	0.71
$CaCO_3$	0.71	0.71
Salt	0.71	0.71
Vitamin E	-	500 IU
Selenium	-	0.5 ppm
Composition [(1)]	Treatment	
	R0 (%)	R1 (%)
Oleic acid	21.69	37.71
Linoleic acid	2.13	14.02
Linolenic acid	0.00	0.96

R0= control diet, R1= R0+ canola oil + vitamin E + selenium, * [(1)]= Integrated Laboratory of IPB University

Feed efficiency calculation

Feed efficiency was calculated based on the ADG and divided by feed intake before multiplying by 100%.

Blood metabolite analysis

Blood metabolite analysis was conducted using plasma blood samples. Plasma was obtained from blood centrifuged at 3000 rpm for 15 minutes. Subsequently, the obtained plasma was placed into tubes and analyzed using the enzymatic method. Total protein, glucose, triglycerides, cholesterol, HDL, and LDL were obtained using the Bio maxima, Greiner Glucose GOD-PAP, Greiner Triglycerides, Greiner Cholesterol, and Greiner HDL Cholesterol kits. The variables were analyzed using a 5010V+ photometer at the Bogor Center for Primate Studies Laboratory (PSSP).

Semen collection

Semen was collected towards the end of feeding treatment at 07:00 WIB using an electro-ejaculator. Preputial shaving and washing were performed with 3% sodium bicarbonate while drying was carried out before collection. The electrode of the electroejaculator (30 cm in length and 2 cm in diameter) was inserted into the rectum approximately 15 cm. Subsequently, low-voltage electrical stimulation was applied repeatedly with a voltage of 3-6 volts for 10 seconds, and the operator performed the electrical stimulation. The collected semen was gathered in tubes for evaluation.

Fresh semen plasma biochemical analysis

The plasma semen obtained from semen collection was separated by centrifuging fresh ram semen (3000 rpm, 20 minutes) and stored at -20°C until further analysis. The stored semen was thawed at room temperature and centrifuged at 10,000 rpm for 20 minutes. Subsequently, plasma biochemistry was analyzed using biological equipment, and the enzymatic colorimetric assay method was used with an ST-type spectrophotometer. The reagents were analyzed using a Chemistry Analyzer 220 V at the IRATco Laboratory Service, Dramaga, Bogor, Indonesia.

Liquid semen evaluation

Preparing liquid semen starts with creating two types of extenders: Tris egg yolk and Citrate fructose egg yolk extender. The preparation of the egg yolk tris extender followed the method of Kulaksiz et al. (2012)

by dissolving 3.63 g, 1.99 g, and 0.5 g of tris(hydroxymethyl)aminomethane, citric acid, and fructose in 100 ml of distilled water. Meanwhile, the preparation of the fructose citrate extender was based on the method of Arifiantini & Purwantara (2010) by dissolving 1.25 g, 2.32 g, and 100 ml of fructose, sodium citrate dihydrate, and distilled water, respectively. Semen suitable for processing was fresh with motility greater than 70%.

The preparation of liquid semen starts with calculating the concentration of spermatozoa and determining the amount of extender needed, with a target concentration of 100 million/ml. Each fresh semen sample suitable for processing into liquid is divided into two glass test tubes. Each tube is supplemented with egg yolk tris or fructose citrate extender. In addition, the liquid semen is covered with plastic wrap and placed in a glass beaker without a water jacket. The sample is stored in a refrigerator at a temperature of 3-5ºC, analyzed periodically for 5 days, and evaluated microscopically for viability, motility, and intact plasma membrane.

Determining viability percentage is conducted by counting the number of live sperm and dividing it by the total sperm count, then multiplying the result by 100. Motility observation is performed using a microscope with a magnification of 400 times across five different fields of view, and the assessment is given on a scale of 0-100%. The percentage of intact plasma membrane can be calculated by dividing the number of spermatozoa that reacted by the total number of spermatozoa counted and multiplying the result by 100%.

Statistical analysis

Randomized block design (RBD) and factorial randomized block design (FRBD) 2 x 2 were used for performance and liquid semen variables, with two feed treatments (R0: control diet, R1: control diet + canola oil + vitamin E + selenium). The extenders used were egg yolk tris and fructose citrate based on body weight and sperm concentration. Data were analyzed using an Independent Sample T-Test, while the results of the plasma semen biochemistry were subjected to descriptive analysis. Data analysis was performed using IBM SPSS Statistics 25 software.

RESULTS AND DISCUSSION

Nutrient intake

Nutrient intake in rams treated with canola oil and antioxidants did not have a significant effect ($P > 0.05$) on DM, CP, CF, nitrogen-free extract (NFE), and TDN. However, the intake had a significant effect ($P < 0.05$) on crude fat and fatty acids (Table 2).

The similar nutrient content in the diets resulted in no significant differences in DM intake among treatments, indicating that feed sources like canola oil and antioxidants do not alter the aroma or texture. Fat-tailed rams fed ration supplemented with canola oil had higher crude fat and fatty acid intakes than the control group, likely due to the higher fat content. Maia et al. (2012) reported that adding canola, sunflower, or castor oil to the diets of Dorper x Santa Ines sheep did not affect DM intake but increased fat intake. Increased fat and fatty acid intake may improve semen quality, benefiting rams used for breeding. Díaz et al. (2016) highlighted the role of fatty acids in male reproductive function, particularly in improving membrane fluidity, motility, and viability of the sperm.

Average daily gain and feed efficiency

Average daily weight gain in fat-tailed rams treated with canola oil showed no significant effect ($P>0.05$). However, the variable improved feed efficiency ($P<0.05$), as reported in Table 3.

The variation in average daily weight gain between the control and treatment groups can be attributed to the absence of significant differences in nutrient intake. According to Riemas et al. (2021), supplementing unsaturated fatty acids in fat-tailed rams did not affect average daily weight gain. This result was because DM intake was similar, resulting in an equivalent nutrient supply to the animals. The addition of canola oil led to a significantly higher improvement in feed efficiency than the control. Therefore, rams consuming a diet of fatty acids from canola oil reported relatively better feed and energy efficiency. According to Parakkasi (1999) and Abrori et al. (2022), higher feed efficiency values showed better use in enhancing sheep growth.

Blood metabolites profile

Blood metabolites in fat-tailed rams treated with canola oil and antioxidants showed a significantly higher cholesterol level ($P < 0.05$) compared to the control. However, there was no significant effect ($P > 0.05$) on total protein, glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol (Table 4).

Total blood protein levels of fat-tailed rams remained within the normal range (Table 4). This condition is attributed to the lack of significant treatment effect on CP intake, resulting in similar absorption and metabolism. Siska and Anggrayni (2021) stated that the levels of total blood protein are influenced by the amount of dietary protein consumed. Blood glucose levels in the experimental rams treated with canola oil did not differ significantly from the control. This condition is due to insufficient treatment effect on feed intake. Similar results were observed by Jafaroghli et al. (2014), where

Table 2. Average nutrient intake in fat-tailed rams

Variables	Treatment		
	R0 (g head^{-1}day^{-1})	R1 (g head^{-1}day^{-1})	P-value
Total dry matter	787.74±117.98	708.34±71.58	0.23^{ns}
Crude protein	134.05±20.19	116.88±11.75	0.13^{ns}
Crude fiber	141.69±21.18	120.30±12.31	0.08^{ns}
Crude fat	27.61±4.14	41.44±4.15	0.00^{s}
Nitrogen free extract	396.44±59.61	350.45±35.36	0.17^{ns}
Total digestible nutrients	528.73±79.51	494.75±49.87	0.44^{ns}
Oleic acid	4.00±0.60	12.77±1.26	0.00^{s}
Linoleic acid	1.14±0.17	5.29±0.52	0.00^{s}
Linolenic acid	0.00±0.00	0.32±0.03	0.00^{s}

R0= control diet, R1= R0+ canola oil + vitamin E + selenium ^{ns}= Non significant (P>0.05), ^{S}= Significan*t* (P<0.05)

Table 3. Average daily gain and feed efficiency in fat-tailed rams

Variables	Treatment		
	R0	R1	P-value
Average daily weight (g head^{-1}day^{-1})	71.61±10.90	84.51±11.09	0.10^{ns}
Feed efficiency (%)	9.71±0.71	12.00±1.87	0.02^{s}

R0= control diet, R1= R0+ canola oil + vitamin E + selenium ^{ns}= Non significant (P>0.05), ^{S}= Significan*t* (P<0.05)

Table 4. Blood metabolites profile of experimental fat-tailed rams

Variables	Treatment		P-value	Normal range
	R1	R0		
Total protein (g dl^{-1})	4.58±0.30	4.60±0.37	0.85^{ns}	4.50-7.20$^{(1)}$
Glucose (mg dl^{-1})	32.68±8.50	21.48±19.39	0.27^{ns}	26.18-72.08$^{(2)}$
Triglycerides (mg dl^{-1})	30.82±7.05	37.62±7.62	0.18^{ns}	27.19-60.09$^{(2)}$
Total cholesterol (mg dl^{-1})	65.60±9.20	82.25±11.55	0.04^{s}	36,80-91,50$^{(3)}$
HDL cholesterol (mg dl^{-1})	34.40±5.59	39.75±2.87	0.12^{ns}	26,00-53,33$^{(3)}$
LDL cholesterol (mg dl^{-1})	25.03±2.66	35.15±7.64	0.02^{s}	15,60-39.25$^{(3)}$

R0= control diet, R1= R0+ canola oil + vitamin E + selenium, ^{ns}= *Non significant* (P>0.05), ^{S}= Significant (P<0.05), HDL= High density lipoprotein, LDL= Low density lipoprotein,$^{(1)}$ Mitruka 1981, $^{(2)}$ Eshratkhah et al. 2008, $^{(3)}$Sarmin et al. 2021

Moghani rams fed fish oil and vitamin C did not show any significant effect on blood glucose levels, and the same results by Khotijah et al. (2020) on sheep-fed vegetable oil ration did not have a significant effect either. Blood triglyceride levels in the experimental rams remained within the normal range (Table 4). This result is attributed to the nutrient content of feed meeting energy requirements and leading to differences in blood triglyceride levels. Triglycerides are broken down when insufficient dietary energy (Suharti et al. 2017; Ramadhina et al. 2019; Nurmalia et al. 2020). Adding canola oil and antioxidants increased sheep's blood and LDL cholesterol levels within the normal range (Table 4). This result is due to the significantly different intake of unsaturated fatty acids. According to Śpitalniak-Bajerska et al. (2020), increased cholesterol levels occur due to higher intake of unsaturated fatty acids. Susilowati et al. (2015) stated that the compound enhanced sperm membrane integrity. The elevated LDL levels correspond with the increased cholesterol concentration.

Plasma seminal biochemistry

Based on the descriptive analysis results, Table 5 presents the plasma seminal biochemistry of fat-tailed rams fed the control diet and the canola oil treatment. Plasma seminal glucose concentration in the present

experiment was lower than those reported by Almadaly et al. (2021) in Ossimi rams, and the reported levels ranged from 47.04 to 65.82 mg dl^{-1}. Meanwhile, the triglyceride concentration in the present experiment was lower than that of Hafez (2009), who reported values ranging from 229.71 to 242.76 mg dl^{-1} in Rahmani rams. The cholesterol levels in the plasma seminal of fat-tailed rams remained within the normal range compared to those reported by Gündoğan and Gündoğan (2006). The results showed that cholesterol levels in the plasma seminal of Akkaraman and Awassi rams were 111.00 mg dl^{-1}. The HDL and LDL cholesterol levels were higher compared to Motalebipour et al. (2022), that HDL and LDL levels in Afshari rams are between 14.50-19.00 mg dl^{-1} and 15.50-21.50 mg dl^{-1}, respectively. The total cholesterol and LDL concentration in seminal plasma was lower than in the control, while the opposite was observed in blood plasma (Table 5 and Table 4), suggesting that the lipids in seminal plasma may not originate solely from the blood, but that other factors may also be influencing this. According to the research by Lu et al. (2016), lipid levels in seminal plasma may not come directly from the blood. However, there is a possibility that they also originate from epithelial cells in the male reproductive system. Additionally, age affects lipid levels in male seminal plasma. As age increases, lipid levels tend to rise.

Quality of liquid semen

The quality of liquid seminal from the experimental fat-tailed rams fed with the control diet, canola oil, and two extender treatments showed no interaction in motility, viability, and plasma membrane integrity ($P > 0.05$), as reported in Tables 6, 7, and 8. There are no established quality standards for liquid semen in sheep in Indonesia. Therefore, the variable refers to the minimum quality requirements for frozen semen in goats and sheep (BSN 2023).

Sperm motility indicates sperm fertility in males Santoso et al. (2021). Sperm motility in the experimental fat-tailed rams fed with oil-based diets using extenders on day 3 showed a significant difference, maintaining

Table 5. Plasma seminal biochemistry in experimental fat-tailed rams

Variables	Treatment	
	R0	R1
Glucose (mg dl^{-1})	45.59	40.45
Triglycerides (mg dl^{-1})	152.89	171.36
Total cholesterol (mg dl^{-1})	127.21	113.00
HDL cholesterol (mg dl^{-1})	43.21	65.43
LDL cholesterol (mg dl^{-1})	48.25	38.43

R0= control diet, R1= R0+ canola oil + vitamin E + selenium, HDL= High density lipoprotein, LDL= Low density lipoprotein

Table 6. The percentage of sperm motility in fat-tailed rams fed with different diets varied between the tris egg yolk extender and the citrate fructose egg yolk

Storage time (Day)	Treatment	Extender	
		Tris egg yolk	Citrate fructose egg yolk
0	R0	77.40±11.10	77.40±11.10
	R1	84.00±6.55	84.00±6.55
1	R0	67.00±10.36	55.00±7.07
	R1	70.00±8.66	68.33±12.58
2	R0	47.00±7.58	47.50±3.53
	R1	51.67±2.88	55.00±10.00
3	R0*	31.00±18.50	40.00±7.07
	R1*	46.66±2.88	50.00±10.00
4	R0	9.00±8.98	20.00±16.35
	R1	38.33±5.77	36.67±12.58

R0= control diet, R1= R0+ canola oil + vitamin E + selenium; *= indicates a significant effect ($P<0.05$)

sperm motility above 40% (P<0.05). Furthermore, the sperm motility of experimental sheep on the control diet was lower than that of those fed rations with canola oil treatment in both extenders. This result is due to the diet's oleic, linoleic, and linolenic acids that sustain motility. Unsaturated fatty acids can maintain sperm motility (Ferramosca et al. 2017; Kogan et al. 2021). Although no effect was observed from the two extenders, the extenders provide nutritional contributions in the form of energy sources, specifically carbohydrates, to maintain sperm activity during storage. According to Arifiantini & Purwantara (2010), carbohydrates influence sperm motility in the extenders, and sperm motility ceases when carbohydrates are absent.

Sperm viability in liquid semen treated with canola oil showed no significant difference (P > 0.05) compared to the control (Table 7). Even though there was no significant difference, viability was maintained up to day 4 in the canola oil treatment (R1). This result was influenced by the high fat intake, affecting the increase in blood cholesterol and plasma seminal levels within the normal range. According to Motalebipour et al. (2022),

Table 7. The percentage of sperm viability in the experimental fat-tailed rams fed with different diets varied between the tris egg yolk extender and the citrate fructose egg yolk

Storage time (Day)	Treatment	Extender	
		Tris egg yolk	Citrate fructose egg yolk
0	R0	86.51±4.90	86.51±4.90
	R1	90.60±3.14	90.60±3.14
1	R0	80.59±4.06	80.44±3.57
	R1	87.60±4.02	85.99±2.10
2	R0	70.61±6.25	72.86±5.57
	R1	79.94±3.58	76.77±5.65
3	R0	50.96±12.85	53.03±17.31
	R1	67.88±15.51	67.92±6.50
4	R0	11.70±11.68	37.23±19.68
	R1	58.69±7.68	64.73±6.37

R0= control diet, R1= R0+ canola oil + vitamin E + selenium; *= indicates a significant effect (P<0.05)

Table 8. The percentage of intact plasma membrane integrity in the experimental fat-tailed ram sperm fed with different diets varied between the egg yolk tris extender and the citrate egg yolk extender

Storage time (Day)	Treatment	Extender	
		Tris egg yolk	Citrate fructose egg yolk
0	R0*	82.94±5.75	82.94±5.75
	R1*	70.61±16.16	70.61±16.16
1	R0*	74.36±3.43	73.74±4.96
	R1*	65.43±12.53	64.66±7.77
2	R0	64.87±9.28	63.81±8.12
	R1	59.47±11.31	57.40±12.48
3	R0	46.70±12.19	42.72±25.38
	R1	55.22±11.97	51.02±12.47
4	R0	11.84±11.83	31.53±16.82
	R1	45.81±0.96	45.11±14.46

R0= control diet, R1= R0+ canola oil + vitamin E + selenium; *= indicates a significant effect (P<0.05)

sperm viability is influenced by the increased fat levels in the blood and semen to improve plasma membrane integrity and viability.

The plasma membrane integrity of sperm in the experimental fat-tailed rams fed with the control diet was significantly higher on days 0, 1, and 2 ($P<0.05$). On day 3, the treatment diet showed higher results than the control, as reported in Table 8. The maintenance of intact plasma membranes is influenced by the high levels of blood cholesterol, vitamin E, and selenium in the diet to reduce ROS during storage. High cholesterol concentrations in the blood also affect sperm membranes from cold shock (Motalebipour et al. 2022). Prolonged storage increases ROS levels and impacts energy metabolism, motility, viability, and plasma membrane integrity (Abdel-Khalek et al. 2016; Monova & Ducha 2019; Naz et al. 2022).

Maintaining motility, viability, and plasma membrane integrity was supported despite no interaction between the diet and extenders. This fact is evidenced by the motility, viability, and plasma membrane integrity of sperm used for AI up to day 3. The egg yolk tris and fructose citrate egg yolk were reported by Arifiantini and Purwantara (2010) and Rather et al. (2017) to be equally effective in preserving liquid semen in Holstein Friesian cattle and ram; this is because tris and citrate egg yolk contain phospholipids and lecithin to protect sperm from cold shock.

CONCLUSION

In conclusion, the supplementation of canola oil, vitamin E, and selenium in the diet of fat-tailed rams increased total cholesterol and LDL levels in the blood while reducing cholesterol concentration in seminal plasma. Additionally, these dietary interventions improved nutrient intake, feed efficiency, and the shelf life of liquid semen.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Education, Research, Culture, and Technology, who provided the basic research scheme 2024 with a contract number 027/E5/PG.02.00.PL/2024 to fund and support the research.

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