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Dietary Omega-3 Fatty Acid Supplementation Remodels the Seminal Biochemical Microenvironment and Sperm Metabolic Profile in Marwari Stallions

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ABSTRACT

Nutritional modulation of reproductive metabolism through omega-3 polyunsaturated fatty acids (PUFAs) has emerged as a promising strategy to improve male fertility; however, information on its effects on seminal plasma and sperm biochemistry in indigenous equine breeds remains limited. The present study evaluated the effect of dietary linseed oil, a rich source of α-linolenic acid (ALA), on the biochemical profile of seminal plasma and spermatozoa in Marwari stallions. Eight clinically healthy stallions were randomly allocated to either a control diet (T_1) or an isonitrogenous diet supplemented with 8% linseed oil (T_2) for 60 days. Seminal plasma and spermatozoa were separated following semen collection, and biochemical constituents, including metabolites, lipids, proteins, minerals and metabolic enzymes, were analysed using standardized commercial assay kits. Dietary linseed oil supplementation induced selective biochemical alterations without disturbing overall reproductive biochemical homeostasis. In seminal plasma, total lipid (364.14 ± 20.74 vs. 274.70 ± 29.27 mg/dL; P=0.05) and calcium (18.42 ± 1.33 vs. 7.29 ± 3.08 mg/dL; P = 0.02) concentrations increased significantly in supplemented stallions. In spermatozoa, cholesterol (142.16 ± 8.71 vs. 116.56 ± 4.36 mg/dL; P = 0.04) and lactate (64.29 ± 3.81 vs. 31.18 ± 3.98 mg/dL; P = 0.001) concentrations were significantly higher, while glucose showed an increasing trend (P=0.05). Other biochemical constituents, including triglycerides, phospholipids, proteins, urea, magnesium, LDH, SGOT and SGPT, remained unaffected. These findings demonstrate that dietary linseed oil selectively modulates lipid metabolism, calcium homeostasis and sperm energy metabolism while preserving overall biochemical stability, suggesting that omega-3 fatty acid supplementation may improve the reproductive metabolic microenvironment and provide a biochemical basis for enhancing stallion reproductive function.

HIGHLIGHTS

- Dietary linseed oil selectively modulated the biochemical profile of seminal plasma and spermatozoa in Marwari stallions.
- Linseed oil supplementation significantly increased seminal plasma total lipids and calcium, and sperm cholesterol and lactate concentrations.

Keywords: Marwari stallion, linseed oil, omega-3 fatty acids, seminal plasma, sperm biochemistry, reproductive metabolism

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Male fertility is determined by the coordinated interaction of sperm quality, seminal plasma composition and the reproductive tract environment. Although conventional semen evaluation parameters such as sperm concentration, motility and morphology are routinely used to assess breeding soundness, they often inadequately predict fertilizing potential because sperm function is regulated by complex molecular and metabolic processes. Consequently, contemporary reproductive research has shifted towards understanding the biochemical mechanisms that govern sperm functionality and fertility (Aitken, 2022).

Seminal plasma is now recognized as a biologically active reproductive fluid rather than merely a transport medium. It contains a diverse array of proteins, lipids, metabolites, antioxidants, ions and extracellular vesicles that regulate sperm maturation, membrane stability, capacitation, oxidative balance and sperm–female reproductive tract interactions (Rodríguez-Martínez *et al*., 2021). Therefore, alterations in seminal plasma composition can directly influence sperm physiology and fertilizing capacity, making it an attractive target for nutritional interventions aimed at improving male reproductive performance.

Reproductive efficiency in stallions is an important determinant of breeding success and genetic improvement in equine species. Semen quality and sperm functional competence are influenced by several physiological, nutritional and environmental factors that collectively regulate male fertility potential. In recent years, nutritional modulation has emerged as an important approach for improving reproductive performance through alteration of metabolic and biochemical pathways associated with spermatozoal function (Ngcobo *et al*., 2021). Among different nutritional interventions, dietary lipid supplementation has gained considerable attention because of its role in membrane architecture, endocrine regulation and oxidative homeostasis (Calder, 2015).

Seminal plasma represents a highly specialized extracellular reproductive milieu containing proteins, enzymes, lipids, antioxidants and energy substrates that are essential for sperm survival, motility and fertilizing ability (Mann and Lutwak-Mann, 1981). The biochemical composition of seminal plasma plays a crucial role in maintaining spermatozoal membrane stability, osmotic balance and mitochondrial bioenergetics (Rodríguez-Martínez *et al*., 2011). Alterations in seminal plasma constituents are closely associated with changes in sperm functionality and fertility outcomes in stallions (Katila and Kareskoski, 2006). Furthermore, sperm biochemical characteristics are considered important indicators of metabolic competence and structural integrity of spermatozoa (Storey, 2008).

Spermatozoa are particularly susceptible to oxidative stress because their plasma membranes contain high concentrations of polyunsaturated fatty acids (PUFAs), which are highly prone to lipid peroxidation (Aitken and Baker, 2006). Excessive generation of reactive oxygen species (ROS) may impair sperm membrane fluidity, mitochondrial activity and DNA integrity, thereby compromising fertility potential (Agarwal *et al*., 2014). Maintenance of seminal oxidative balance through nutritional strategies has therefore become an important area of reproductive physiology research. Dietary omega-3 fatty acids have been reported to improve membrane phospholipid remodeling, antioxidant defense mechanisms and sperm functional attributes in different livestock species (Abdulredha *et al*., 2026; Gürler *et al*., 2015).Among the factors governing sperm function, membrane lipid composition plays a central role. Mammalian sperm membranes are enriched with polyunsaturated fatty acids (PUFAs), phospholipids and cholesterol, which collectively determine membrane fluidity, permeability and signalling during capacitation and fertilization. However, this high PUFA content also increases susceptibility to oxidative damage, as mature spermatozoa possess limited antioxidant capacity and minimal ability to repair membrane injury (Aitken and Curry, 2011). Consequently, strategies that improve membrane lipid composition while maintaining redox homeostasis are expected to enhance sperm functional competence.

Linseed oil is an excellent source of α-linolenic acid (ALA), an essential omega-3 fatty acid known for its nutraceutical, antioxidant and anti-inflammatory properties. Typically, α-linolenic acid constitutes approximately 50–60% of the total fatty acids in linseed oil, making it one of the richest plant-derived sources of omega-3 fatty acids (Goyal *et al*., 2014). Supplementation of linseed oil has been shown to modulate lipid metabolism, enhance membrane flexibility and improve oxidative stability in biological systems (Parikh *et al*., 2019). In reproductive tissues, omega-3 fatty acids may influence sperm membrane dynamics, seminal biochemical architecture and reproductive metabolic

activity, thereby contributing to improved semen quality and fertility-associated traits (Burdge and Calder, 2005). Previous studies have demonstrated beneficial effects of omega-3 fatty acid supplementation on sperm quality, antioxidant status and fatty acid composition in livestock species (Singh *et al*., 2022).

Recent studies in livestock species have shown that dietary omega-3 supplementation improves sperm membrane integrity, cryotolerance and reproductive performance through modulation of lipid metabolism and cellular bioenergetics (Ngcobo *et al.,* 2021; Singh *et al.,* 2022). However, most investigations have focused on conventional semen quality traits or membrane fatty acid composition, while comparatively little attention has been given to the biochemical interplay between seminal plasma and spermatozoa. Moreover, information regarding the influence of dietary omega-3 supplementation on the reproductive biochemical profile of indigenous equine breeds, particularly the Marwari stallion, remains limited. We hypothesized that dietary linseed oil supplementation would selectively remodel the biochemical milieu of seminal plasma and spermatozoa by modulating lipid metabolism, calcium homeostasis and energy metabolism, thereby creating a more favourable environment for sperm function without disrupting physiological homeostasis. Therefore, the objective of the present study was to evaluate the effect of dietary linseed oil supplementation on the biochemical profiles of seminal plasma and spermatozoa in Marwari stallions and to elucidate its potential role in nutritional regulation of the reproductive microenvironment.

MATERIALS AND METHODS

Experimental animals

Eight healthy Marwari stallions of age 4-8 years with nearly similar body weight and conformation were selected from ICAR-National Research Centre on Equine, Bikaner campus. The horses were distributed randomly in two treatment groups of four each for conducting feeding trial for the period of sixty days. Each stallion was housed separately in their hygienic and well ventilated sheds having separate feeding and watering system. Prophylactic measures were taken against common parasitic infestations. All the experimental horses were offered feed separately in a measured quantity, with ad libitum access to clean drinking water.

Experimental feeds

The experimental diets were formulated to meet the nutrient requirements of adult horses as recommended by the Indian Council of Agricultural Research (ICAR, 2013). Stallions were randomly assigned to one of two dietary treatments: T_1 (control), receiving a low-fat concentrate without linseed oil, and T_2 (linseed oil supplemented), receiving a high-fat concentrate containing 8% linseed oil (w/w). Groundnut straw was offered as the sole roughage source, while the respective concentrate mixtures were provided according to the experimental treatment.

Food-grade, cold-pressed linseed oil procured from a commercial supplier was used as the dietary lipid source. The oil was top-dressed onto the concentrate mixture at 8% (w/w), corresponding to approximately 260 mL per stallion per day, and was offered during the morning feeding for 60 consecutive days. The inclusion level was selected based on the recommended safe dietary fat intake for horses and previous studies demonstrating the safety and efficacy of dietary linseed oil supplementation in equines (Delobel *et al*., 2008; Williams *et al*., 2017). Linseed oil is naturally rich in α-linolenic acid (ALA; C18:3 n−3), the principal plant-derived omega-3 polyunsaturated fatty acid. Typically, it contains 50–60% α-linolenic acid, 18–24% oleic acid (C18:1 n−9), 12–18% linoleic acid (C18:2 n−6), 5–7% palmitic acid (C16:0) and 2–5% stearic acid (C18:0), although the fatty acid composition may vary depending on cultivar, geographical origin and processing conditions (Goyal *et al*., 2014).

The ingredient composition of the experimental concentrate mixtures and the chemical composition of the roughage and concentrate mixtures are presented in Tables 1 and 2, respectively. Prior to the experimental feeding period, all horses were gradually adapted to their respective diets over 7 days to minimize the risk of diet-associated gastrointestinal disturbances. Throughout the experiment, each stallion was housed individually in a well-ventilated, hygienic stall equipped with separate feeding and watering facilities. Routine prophylactic measures, including deworming and parasite control, were implemented according to the standard management practices of the institute.

Table 1: Ingredient composition of experimental concentrate mixtures

Feed Ingredient	Low Fat Concentrate	High Fat Concentrate
Gram	10	10
Soybean meal	10	10
Barley	45	37
Wheat bran	32	32
Mineral mixture	3	3
Linseed oil	0	8
Total	**100**	100

Table 2: Chemical composition of groundnut straw and experimental concentrate mixtures (on DM basis)

Nutrient	Groundnut Straw	Low Fat Concentrate	High Fat Concentrate
Dry Matter (%)	89.03	91.36	91.15
Organic Matter (%)	90.77	93.33	93.64
Crude Protein (%)	8.47	15.88	15.60
Ether Extract (%)	1.49	4.13	9.82
Crude Fibre (%)	28.72	18.36	16.97
Nitrogen Free Extract (%)	52.09	54.96	51.28
Total Ash (%)	9.23	6.67	6.35
Neutral Detergent Fibre (%)	49.33	36.11	34.90
Acid Detergent Fibre (%)	30.00	14.83	13.89
Hemicellulose (%)	19.32	21.28	21.01

Semen collection and seminal plasma separation

Semen was collected early in the morning and estrus mare was used as a dummy as per the methods previously described by Talluri *et al.* (2012). Semen collection was done using an autoclaved Colorado Model Artificial Vagina (AV) which was lubricated with liquid paraffin and pre-warmed to 45- 50ºC and fitted with an inline filter to separate the gel fraction. The semen was collected over a four-day period before to the start of the actual experiment. Two days of sexual rest were given to the stallions before the starting of the experiment. Each stallion was given one false mount before actual collection. Thereafter, two ejaculates were collected from each stallion. Soon after collection, ejaculates were transported to the laboratory and kept at 37ºC for until processing. Gel portion of the semen was removed by filtration and immediately the seminal plasma and sperm sediment as pellet form at the bottom was separated by centrifugation at 3000 rpm for 30 min at 4°C and stored at −20°C until further analysis. Biochemical parameters of seminal plasma and sperm biochemicals, various enzymes and metabolites were estimated with the help of Semi-Autoanalyzer as per the protocols given in the respective standard kits manufactured by SPINREACT (Ark Diagnostics Pvt. Ltd., Mumbai).

Statistical procedure

Data were analyzed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Individual stallions were considered the experimental unit, and all data are presented as mean ± standard error of the mean (SEM). Prior to statistical analysis, the assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests, respectively. Variables satisfying these assumptions were compared between the control (T_1) and linseed oil-supplemented (T_2) groups using an independent-samples Student's t-test. For variables violating normality or homogeneity assumptions, the Mann–Whitney U test was applied. Differences were considered statistically significant at $P < 0.05$, whereas $0.05 \leq P < 0.10$ was interpreted as indicating a statistical trend. All statistical tests were two-tailed.

RESULTS

Seminal plasma biochemistry

The mean ± S.E values of various biochemical parameters in seminal plasma have been presented in Table 3. All the biochemical parameters of seminal plasma during the present study are almost in normal ranges reported by Pal *et al.* (2009), Talluri *et al.* (2012) and Songara *et al.* (2020). The effect of dietary linseed oil supplementation on seminal plasma biochemical parameters in Marwari stallions is presented in Table 3. Among the various biochemical constituents evaluated, total lipids and calcium concentration differed significantly ($P<0.05$) between the treatment groups. Stallions supplemented with linseed oil

Table 3: Effect of linseed oil on biochemical parameters of seminal plasma and sperm in different treatment groups

Attributes	Treatment groups		Significance
	T1	T2	(P Value)
Glucose (mg/dL)	6.26 ± 2.11	38.11 ± 15.18	0.08
Cholesterol (mg/dL)	54.98 ± 2.68	53.06 ± 1.22	0.53
Triglycerides(mg/dL)	157.01 ± 29.99	180.16 ± 36.57	0.64
Total Lipids (mg/dL)	274.7 ± 29.27[a]	364.14 ± 20.74[b]	0.05*
Phospholipids (mg/dL)	90.715 ± 6.518	109.24 ± 16.50	0.33
Urea (mg/dL)	79.89 ± 2.18	71.53 ± 6.14	0.25
Lacate (mg/dL)	35.60 ± 22.88	74.35 ± 7.85	0.16
LDH (IU/L)	169.12 ± 23.08	137.46 ± 58.63	0.63
SGOT (U/L)	186.84 ± 6.18	103.20 ± 5.50	0.35
SGPT (U/L)	25.05 ± 16.05	29.11 ± 20.46	0.88
Total Protein (g/dL)	2.89 ± 0.35	3.23 ± 0.47	0.57
Albumin (g/dL)	0.22 ± 0.05	0.38 ± 0.13	0.3
Calcium (mg/dL)	7.29 ± 3.08[a]	18.42 ± 1.33[b]	0.02*
Magnesium (mg/dL)	3.025 ± 0.36	3.97 ± 0.49	0.17

Note: Means with different superscripts in a row differ significantly from each other (P<0.05).

(T_2) exhibited significantly higher total lipid concentration (364.14 ± 20.74 mg/dL) compared to the control group (274.70 ± 29.27 mg/dL). Similarly, calcium concentration was significantly increased (P<0.05) in the linseed oil supplemented group (18.42 ± 1.33 mg/dL) as compared to the control group (7.29 ± 3.08 mg/dL).

Numerically higher values of glucose, triglycerides, phospholipids, lactate, total protein, albumin and magnesium were observed in the supplemented group; however, the differences were statistically non-significant (P>0.05). The concentrations of cholesterol, urea, lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were not significantly affected by dietary linseed oil supplementation (P>0.05).

Overall, supplementation of linseed oil resulted in significant alterations in total lipid and calcium concentrations, while the remaining seminal plasma biochemical parameters remained comparable between the treatment groups.

Sperm Biochemistry

The influence of dietary linseed oil supplementation on sperm biochemical parameters of Marwari stallions is presented in Table 4. Supplementation with linseed oil resulted in significant alterations in selected biochemical constituents of spermatozoa. Sperm cholesterol concentration was significantly higher (P<0.05) in the linseed oil supplemented group (142.16 ± 8.71 mg/dL) compared with the control group (116.56 ± 4.36 mg/dL). Likewise, sperm lactate concentration exhibited a marked increase (P<0.01) in supplemented stallions (64.29 ± 3.81 mg/dL) relative to the control animals (31.18 ± 3.98 mg/dL).

A substantial numerical increase in sperm glucose concentration was also observed in the supplemented group (249.57 ± 11.00 mg/dL) compared to the control group (187.51 ± 23.08 mg/dL), with the difference approaching the level of statistical significance (P=0.05). Similarly, triglycerides, total lipids, urea, SGOT, SGPT, albumin and calcium concentrations tended to be higher in stallions receiving linseed oil supplementation; however, these differences were not statistically significant (P>0.05).

The concentrations of phospholipids, LDH, total protein and magnesium remained unaffected by dietary treatment and did not differ significantly between the groups (P>0.05). Overall, dietary supplementation of linseed oil induced significant changes in sperm cholesterol and lactate concentrations, indicating alterations in

Table 4: Effect of linseed oil on biochemical parameters of sperm in different treatment groups

Attributes	Treatment groups		Significance
	T1	T2	(P Value)
Glucose (mg/dL)	187.51 ± 23.08	249.57 ± 11.00	0.05
Cholesterol (mg/dL)	116.56 ± 4.36a	142.16 ± 8.71b	0.04*
Triglycerides (mg/dl)	190.96 ± 13.75	229.51 ± 33.69	0.33
Total Lipids (mg/dL)	741.79 ± 87.74	937.80 ± 44.97	0.09
Phospholipids (mg/dL)	292.87 ± 43.72	300.20 ± 54.40	0.92
Urea (mg/dL)	27.63 ± 6.74	72.34 ± 39.50	0.31
Lacate (mg/dL)	31.18 ± 3.98a	64.29 ± 3.81b	0.001**
LDH (IU/L)	459.06 ± 60.90	307.52 ± 91.23	0.21
SGOT (U/L)	258.82 ± 21.15	268.45 ± 10.87	0.72
SGPT (U/L)	56.53 ± 9.24	89.12 ± 22.91	0.23
Total Protein (g/dL)	5.21 ± 0.36	5.09 ± 0.34	0.82
Albumin (g/dL)	0.64 ± 0.08	0.71 ± 0.04	0.45
Calcium (mg/dL)	3.72 ± 0.39	4.56 ± 0.20	0.10
Magnesium (mg/dL)	1.70 ± 0.06	1.68 ± 0.05	0.89

Note: Means with different superscripts in a row differ significantly from each other (P<0.05).

sperm biochemical milieu following omega-3 fatty acid supplementation.

DISCUSSION

The present investigation evaluated the influence of dietary linseed oil supplementation on seminal plasma and sperm biochemical characteristics in Marwari stallions. The findings demonstrated that supplementation with linseed oil selectively modified specific biochemical constituents associated with lipid metabolism, mineral homeostasis and cellular energy metabolism, while the majority of biochemical parameters remained within physiological limits. Significant increases in seminal plasma total lipid and calcium concentrations, together with elevated sperm cholesterol and lactate concentrations, indicate that dietary omega-3 fatty acid supplementation altered the reproductive biochemical milieu without disrupting overall metabolic stability.

Linseed oil is one of the richest plant sources of α-linolenic acid (ALA), a precursor of long-chain omega-3 polyunsaturated fatty acids (PUFAs). Dietary omega-3 fatty acids have attracted considerable attention in reproductive nutrition because of their ability to influence membrane lipid composition, cellular signalling pathways and metabolic activity of spermatozoa. The significant increase in seminal plasma total lipid concentration observed in the supplemented stallions suggests an alteration in reproductive lipid metabolism and increased availability of lipid substrates within the seminal environment. Seminal plasma lipids play an important role in maintaining sperm membrane integrity, protecting spermatozoa from environmental stress and supporting normal reproductive function. Previous studies conducted by Ngcobo *et al*., 2021; Singh *et al.,* 2022 have demonstrated that dietary omega-3 supplementation can modify lipid composition of reproductive tissues and semen, thereby influencing sperm physiology and fertility-related traits.

The significant elevation in sperm cholesterol concentration further supports the hypothesis that linseed oil supplementation influenced membrane-associated lipid metabolism. Cholesterol is a critical structural component of the sperm plasma membrane and contributes to membrane stability, permeability and fluidity. Maintenance of appropriate membrane cholesterol levels is essential for preserving sperm viability and regulating capacitation-related events. Although omega-3 fatty acids do not directly increase cholesterol synthesis, dietary lipid supplementation may alter membrane

lipid remodeling and modify cholesterol distribution within sperm membranes. Enhanced membrane stability resulting from optimized lipid organization may improve sperm resistance to environmental and oxidative stress. Similar improvements in sperm membrane characteristics following dietary omega-3 supplementation have been reported in several livestock species by Gürler *et al*., 2015 and Ngcobo *et al*., 2021. The numerical increase observed in sperm total lipids and phospholipids, although not statistically significant, also supports the possibility of membrane lipid remodeling in response to dietary linseed oil supplementation.

Another interesting finding was the significant increase in sperm lactate concentration in the supplemented group. Lactate is increasingly recognized as an important component of sperm bioenergetics rather than merely a terminal product of anaerobic metabolism. Spermatozoa rely on a complex interaction between glycolysis and mitochondrial oxidative phosphorylation to meet their energy requirements. The elevated lactate concentration observed in supplemented stallions may indicate enhanced glycolytic activity and increased metabolic turnover associated with improved energetic status of sperm cells. This interpretation is further supported by the numerical increase in sperm glucose concentration observed in the supplemented group. Together, these findings suggest that linseed oil supplementation may have influenced sperm energy metabolism by increasing substrate availability and metabolic activity. Previous studies have reported that omega-3 fatty acids can improve mitochondrial function, cellular energy utilization and sperm functional competence through alterations in membrane composition and cellular metabolism (Ngcobo *et al*., 2021; Abdulredha *et al*., 2026).

Calcium homeostasis appeared to be another important target of dietary linseed oil supplementation. The significant increase in seminal plasma calcium concentration observed in supplemented stallions is of particular biological relevance because calcium plays a central role in sperm motility, hyperactivation, capacitation and acrosome reaction. Calcium-mediated signaling pathways regulate several critical events required for successful fertilization. Increased calcium availability within the seminal plasma may therefore provide a more favourable extracellular environment for sperm function. Dietary lipids have been reported to enhance intestinal calcium absorption and mineral utilization, which may partially explain the elevated calcium concentration observed in the present study (Naz *et al*., 2022). Although sperm calcium concentration was not significantly altered, the increased availability of calcium within the seminal environment may nevertheless contribute to improved reproductive physiology.

Interestingly, despite significant alterations in selected biochemical constituents, most seminal plasma and sperm parameters remained unaffected by dietary treatment. Cholesterol, triglycerides, phospholipids, urea, lactate, LDH, SGOT, SGPT, total protein, albumin and magnesium concentrations in seminal plasma did not differ significantly between groups. Likewise, sperm triglycerides, phospholipids, total protein, albumin, urea, LDH, SGOT, SGPT, calcium and magnesium remained comparable between treatments. The stability of these biochemical markers suggests that linseed oil supplementation did not induce metabolic disturbances or cellular damage within the reproductive tract. In particular, the absence of significant changes in LDH activity indicates preservation of membrane integrity, as leakage of this intracellular enzyme is commonly associated with cellular injury. Similarly, unchanged SGOT and SGPT activities indicate that supplementation did not adversely affect cellular metabolism. Maintenance of protein concentrations within physiological limits further suggests that secretory activity of the accessory sex glands remained largely unaffected. In contrast, phospholipid concentration, total protein, albumin, calcium, magnesium, urea, LDH, SGOT, and SGPT did not differ significantly between groups. The absence of substantial variation in these parameters indicates that dietary supplementation did not markedly influence sperm protein metabolism or intracellular enzyme activity. Overall, the sperm biochemical response was characterized by significant increases in cholesterol and lactate concentrations, together with numerical increases in glucose and total lipid concentrations, suggesting a selective effect on membrane-associated and energy-related metabolites. The pattern of results indicates that dietary linseed oil supplementation exerted selective rather than generalized effects on reproductive biochemistry. The observed alterations were primarily associated with pathways related to lipid metabolism, membrane organization, mineral homeostasis and cellular energetics. Such targeted responses are consistent with the

known biological functions of omega-3 fatty acids, which act not only as structural membrane components but also as regulators of cellular metabolism and signalling pathways. Importantly, the absence of adverse changes in enzyme activities or protein metabolism suggests that the supplementation strategy was metabolically safe and well tolerated by the stallions.

From a reproductive perspective, the simultaneous increase in seminal plasma total lipids and calcium together with elevated sperm cholesterol and lactate concentrations may indicate a more favourable biochemical environment for maintenance of sperm structure and function. These biochemical modifications could potentially contribute to improved sperm performance through enhanced membrane stability and metabolic competence. The present study demonstrates that dietary supplementation with linseed oil, a rich source of α-linolenic acid (ALA), selectively remodelled the biochemical microenvironment of seminal plasma and spermatozoa in Marwari stallions. Rather than inducing generalized metabolic alterations, omega-3 supplementation primarily influenced pathways associated with lipid metabolism, calcium homeostasis and sperm energy metabolism, while maintaining the stability of protein metabolism and intracellular enzyme activities. These findings indicate that dietary omega-3 fatty acids act as metabolic modulators that optimize the reproductive milieu without compromising physiological homeostasis.

In conclusion, dietary linseed oil supplementation selectively modulated the biochemical profile of seminal plasma and spermatozoa in Marwari stallions. The supplementation enhanced seminal plasma total lipid and calcium concentrations and increased sperm cholesterol and lactate levels while maintaining overall biochemical homeostasis. These findings support the potential role of omega-3 fatty acid supplementation in modulating reproductive metabolism and provide a basis for future investigations focusing on membrane fatty acid composition, oxidative status, sperm functional attributes and fertility outcomes in stallions.

GENERATIVE AI DECLARATION

The author(s) confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI. The authors developed and verified all scientific content, data analysis, interpretation of results, and conclusions. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

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