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Impact of omega-3 fatty acid supplementation on hormonal profile in Mecheri ewes and lambs

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Abstract

The present study was undertaken to evaluate the effect of omega-3 fatty acid supplementation on the hormonal profile of Mecheri ewes and ram lambs. Rumen protected fat was prepared from linseed oil and palm oil and incorporated into four isocaloric experimental diets. In Trial I, forty Mecheri ewes were randomly allocated into four dietary groups, while in Trial II, thirty-six ram lambs were similarly grouped. The experimental diets consisted of a basal diet alone, basal diet with 3% palm oil protected fat, basal diet with 1.5% linseed oil plus 1.5% palm oil protected fat, and basal diet with 3% linseed oil protected fat. Blood samples were collected at regular intervals and analyzed for progesterone, thyroxine, and triiodothyronine using radioimmunoassay techniques. The results revealed a significant increase in progesterone concentration in ewes supplemented with 3% linseed oil, particularly on the 50th and 100th days of supplementation. Thyroxine levels were also significantly elevated in fat-supplemented ewes during the later stage of the experiment, whereas triiodothyronine levels remained unaltered. In ram lambs, supplementation did not significantly influence T₄ or T₃ concentrations throughout the experimental period. The findings suggest that omega-3 fatty acid supplementation, especially linseed oil protected fat, positively modulates reproductive hormonal activity in Mecheri ewes without adversely affecting thyroid hormone homeostasis.

Keywords: Linseed oil, palm oil, protected fat, progesterone; thyroxine, triiodothyronine, sheep

Introduction

Omega-3 fatty acids are essential nutrients that contribute to cellular structure, membrane functionality, absorption of fat-soluble vitamins, lipid metabolism, antioxidant defence, and overall physiological homeostasis, thereby supporting animal health and productivity (Kaur *et al.*, 2011) ^[10]. In ruminant nutrition, omega-3 fatty acids are derived from green forages, fish oils, marine feed ingredients, flaxseed, canola, and plant oils. Beyond their nutritional role, omega-3 fatty acids act as important nutraceuticals that enhance ovarian function and reproductive efficiency by supporting follicular maturation, ovulation, embryo development, and foetal survival (Thatcher *et al.*, 2011) ^[18]. They also influence the hypothalamo-hypophyseal-gonadal axis through modulation of reproductive hormones such as gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone, while their antioxidant properties protect cellular components from oxidative stress during pregnancy and lactation (Kaur *et al.*, 2011) ^[10]. Nutritional strategies such as flushing and supplementation with protected fats have therefore been adopted to improve reproductive performance in sheep (Dirandeh *et al.*, 2015) ^[7].

Flushing with energy-rich diets enriched with omega-3 and omega-6 fatty acids has been shown to improve ovulation rate, embryo survival, and reduce reproductive wastage in sheep (Scaramuzzi *et al.*, 2006) ^[15]. Supplementation of omega-3 fatty acids in small ruminants has also been associated with altered offspring sex ratio and increased live weight in lambs and growing goats (Teama and El-Tarabany, 2016) ^[17]. These effects are attributed to modulation of prostaglandin synthesis, especially reduced prostaglandin F_{2α} secretion, improved embryo survival, altered uterine environment, enhanced oocyte quality, sperm viability, and early embryonic development. Maternal supplementation with polyunsaturated fatty acids has further been reported to improve offspring energy metabolism (Carrenza-Martin *et al.*, 2025) ^[6], nutrient utilization efficiency, feed conversion ratio, and muscle accretion (Alshdaifat *et al.*, 2023) ^[2]. However, despite the recognized importance of omega-3 fatty acids, systematic studies evaluating their influence on growth, reproduction, hormonal profile, and

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biochemical responses in Mecheri sheep remain limited; therefore, the present study was undertaken to assess the effects of omega-3 fatty acid supplementation on hormonal profiles in Mecheri ewes and lambs.

Materials and Methods

The rumen protected fat was prepared by double decomposition process from linseed oil as well as palm oil as described by Naik *et al.* (2007) [13]. The prepared rumen protected fat was pulverized and stored. Four isocaloric complete diets varying in omega-3 supplementation level were prepared in mash form, viz. Group I (Basal diet), Group II (Basal diet + 3% palm oil protected fat), Group III (Basal diet + 1.5% linseed oil protected fat & 1.5% palm oil protected fat) and Group IV (Basal diet + 3% linseed oil protected fat), which met the nutritional requirements as per the recommendations of ICAR (2013) [9]. The experimental diets were prepared separately for the control and test groups.

The proposed objectives were studied in two trials and the trials were conducted separately. Trial-I was conducted in Mecheri ewes to assess the effect of omega-3 supplementation in the reproduction performance of ewes during flushing, mating, gestation and lactation. Forty Mecheri ewes at 3-5 years of age with an average live body weight of 28 kg in their second to fourth parity were randomly and equally allocated ($n = 10$) into four experimental diet groups. The ewes were mated with rams and the pregnancy was verified ultrasonographically on 45th day. Trial-II was conducted in Mecheri ram lambs to assess the effect of omega-3 supplementation from post-weaning period up to the marketing age. Thirty-six Mecheri ram lambs at 3 months of age (after weaning) and an average live body weight of 12 kg were selected and randomly divided into ($n = 9$) into four experimental diet groups. Standard housing, feeding and watering management were followed for both ewes and ram lambs.

Blood samples were collected on 0, 50 and 100 days of experiment in ewes and analysed for progesterone (P_4), thyroxine (T_4) and triiodothyronine (T_3). In ram lambs, blood samples were collected on 0, 60 and 120 days of experiment and analysed for thyroxine (T_4) and triiodothyronine (T_3).

Plasma was separated from blood samples and analyzed for the hormones by radioimmunoassay method. The hormone kits were procured from Beckman Coulter, Mumbai. The radioactivity in the samples was measured in a gamma counter (STRATEC, Germany) available at Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal.

The data obtained were subjected to one way analysis of variance (one way ANOVA) using the SPSS statistical package.

Results

Effect of omega-3 supplementation on progesterone levels in ewes

The concentration of progesterone hormone from the plasma samples is presented in Table 1 and the results revealed that, the progesterone concentration was similar in all the groups during the start of the experimental trial (0th day), whereas, during 50th day of supplementation, significant ($p < 0.05$) increase in the progesterone level was observed in ewes supplemented with 3% Linseed oil (2.57 ± 0.23 ng/mL) on

comparison to other treatment groups. Similarly, a significant ($p < 0.00$) increment in the progesterone concentration was noted in all the three groups of ewes supplemented with the protected fat as compared to control ewes received only the basal diet. Noteworthy, the ewes supplemented with the 3% Linseed oil revealed a significant increase in progesterone level (6.20 ± 0.23 ng/mL) when compared others during the 100th day of experimental trial.

Effect of omega-3 supplementation on Thyroxine (T_4) levels in ewes

Thyroxine hormone levels were unaltered in all the four experimental groups during the day 0 and 50 of the experimental trial period (Table 1). On 100th day of supplementation, the thyroxine concentration revealed a significant ($p < 0.00$) increase in the ewes supplemented with the protected fat as compared to control ewes. On comparing the thyroxine levels among the ewes supplemented with protected fat, the 3% Linseed oil (45.43 ± 0.40 ng/mL) as well as 3% Palm oil (45.05 ± 0.43 ng/mL) supplementation showed higher levels than 1.5% Linseed oil and 1.5% Palm oil (43.99 ± 0.48 ng/mL) supplementation.

Effect of omega-3 supplementation on Triiodothyronine (T_3) levels in ewes

Supplementation of ewes with omega-3 protected fat did not revealed any significant impact in their triiodothyronine levels during the entire experimental trial periods (0, 50 and 100 days) in all the three supplementation groups, when compared to the control ewes maintained only on the basal diet (Table 1).

Effect of omega-3 supplementation on Thyroxine (T_4) levels in ram lambs

Thyroxine hormone levels of ram lambs were unaltered in all the four experimental groups received the protected fat supplementation during the entire experimental trial (0, 60 and 120 days) period as compared to the lambs maintained only on the basal diet (Grower feed) (Table 2).

Effect of omega-3 supplementation on Triiodothyronine (T_3) levels in ram lambs

Omega-3 protected fat supplementation to the ram lambs demonstrated, no significant impact in their triiodothyronine levels during the entire experimental trial periods (0, 60 and 120 days) in all the three supplementation groups, when compared to the control ewes maintained only on the basal diet (Table 2).

Discussion

The significant elevation of plasma progesterone in ewes received 3% linseed oil supplementation during day 50, as compared to other treatment groups attribute to the alpha-linolenic acid richness of the linseed oil, which rapidly incorporates into the systemic lipid pool and aids in progesterone synthesis. This result was in argument with Akhtar *et al.* (2024) [1], where, serum progesterone concentrations of linseed supplemented ewes differed significantly ($p < 0.05$) with control group as early as 15th of supplementation, with sustained incremental levels of progesterone during days 30, 45, and 60 of experimentation. The increased progesterone stimulating potency of linseed oil over palm oil protected fat on 100th day of

supplementation in ewes delineates the differential fatty acid composition between linseed oil and palm oil, wherein linseed oil is rich in alpha-linolenic acid (n-3, poly unsaturated fatty acid) but palm oil is predominantly enriched with saturated fatty acids (palmitic acid, C16:0) and oleic acid (C18:1 n-9) and has limited poly unsaturated fatty acid content (Golmakani *et al.*, 2024) [8].

Progesterone elevating effect of dietary omega-3 poly unsaturated fatty acid in multifactorial. In ruminants, omega-3 fatty acid competes with arachidonic acid as a substrate for cyclooxygenase enzyme activity, leading to the suppression of PGF2 α induced luteolysis of corpus luteum, resulting in elevated circulating progesterone concentrations (Mattos *et al.*, 2003; Akhtar *et al.*, 2024) [12, 1]. Noaman *et al.* (2011) [14] and Zeraatkar *et al.* (2026) [21] both attributed the elevated progesterone concentration following linseed oil supplementation to increased blood cholesterol levels. Addition of fat supplementation to the diet revealed increased plasma cholesterol and lipoprotein levels, which are primarily involved in regulating the ovarian steroidogenesis (Kia *et al.*, 2013) [11]. This was further corroborated Bianchi *et al.* (2018) [3], by demonstrating significantly elevated cholesterol, triglycerides, and progesterone levels in dairy sheep during day 60 and 120 of palm oil supplementation, supporting the function of dietary lipids in upregulating the ovarian steroid production mechanism.

The thyroxine concentration of ewes supplemented with protected fat remained unaltered across all experimental groups during 0 and 50 days of the supplementation, suggesting that short-term omega-3 fatty acid administration did not exert an immediate effect on thyroid gland secretory function. This result is comparable with the observation reported by Shunthwal *et al.* (2023) [16], who found that significant increase in serum thyroxine concentration in Beetal goat received linseed supplementation occurred progressively over time, rather than during the early phase of supplementation.

During 100th day of supplementation, a highly significant ($p < 0.00$) elevation in plasma T₄ concentration was recorded in all three protected fat supplemented groups compared to the control. The increase in plasma T₄ observed in fat supplemented ewes at 100th may be attributable to, dietary fat supplementation elevates circulating energy dense substrates and improves overall energy balance, which has been associated with upregulated hypothalamic-pituitary-thyroid axis activity, resulting in enhanced thyroidal T₄ synthesis and release. Furthermore, omega-3 poly unsaturated fatty acids, as bioactive lipid signaling molecules, have been shown to influence membrane phospholipid composition in thyroid follicular cells, potentially modulating receptor sensitivity and hormone secretion capacity (Calder, 2013) [4].

Among the supplemented groups, ewes receiving 3% linseed oil demonstrated the highest T₄ levels (45.43 $\pm$ 0.40 ng/mL), followed by 3% palm oil (45.05 $\pm$ 0.43 ng/mL) and

the combined 1.5% linseed + 1.5% palm oil group (43.99 $\pm$ 0.48 ng/mL). These results align with the study of Todini *et al.* (2022) [19], where transition dairy ewes supplemented with extruded linseed reported time dependent transition in their plasma thyroid hormones, suggesting the greater bioavailability of alpha linolenic acid from protected linseed oil and its downstream effects on cell membrane composition and endocrine signaling.

Contrary to the results observed for thyroxine, plasma triiodothyronine concentrations in ewes supplemented with protected fat remained unaltered throughout the experimental period (0, 50, and 100 days) in all the three supplemented groups compared to the control. This result corresponds to the findings of Todini *et al.* (2022) [19], where plasma T₃ concentration did not significantly affected by dietary linseed supplementation in transition dairy ewes, with the dominant regulatory influence being physiological stage rather than diet. The absence of a significant T₃ response to omega-3 supplementation in ewes may be attributed to peripheral deiodination processes maintain circulating T₃ within normal ranges (Calder, 2012) [5].

In contrast to the significant increase of plasma thyroxine observed in ewes at day 100 of experimental trial, the present study found no significant alteration in T₄ concentrations in ram lambs across all four experimental groups during the entire supplementation period (0, 60, and 120 days). This age class difference in thyroidal responsiveness to dietary fat supplementation is noteworthy and may reflect fundamental differences in the regulatory dynamics of the hypothalamic-pituitary-thyroid axis between growing ram lambs and mature ewes. This finding is consistent with the results of Shunthwal *et al.* (2023) [16], they reported that the goat kids and ram lambs differ in metabolic rate, breed-specific thyroid sensitivity, dietary backgrounds, and their specificity to the type of linseed form used. Further, Yousif, (2019) [20] reported no significant change in the plasma T₄ concentration of lambs supplemented with linseed, suggesting that the T₄ unresponsiveness to dietary linseed supplementation may be a consistent pattern in growing lambs.

Consistent with the absence of a thyroxine response, supplementation with omega-3 protected fat also did not produce any significant variation in plasma triiodothyronine levels in ram lambs throughout the experimental period (0, 60, and 120 days) across all three supplementation groups as compared to the control. This finding mirrors the null T₃ response observed in ewes and reinforces that the omega-3 PUFA protected fat supplementation did not disrupted the euthyroid T₃ homeostasis in sheep, regardless of physiological status. The consistent non-significant T₃ pattern across the protected fat supplemented lambs in the present study suggests that the omega-3 protected fat supplementation does not induce a hyperthyroid state, excessive catabolism, or thermogenic dysregulation in sheep (Akhtar *et al.*, 2024) [1].

Table 1: Mean ($\pm$ SE) Plasma Progesterone, Triiodothyronine and Thyroxine of ewes supplemented with protected fat

Hormone	Day of experiment	Group I	Group II	Group III	Group IV	P Value
Progesterone (ng/mL)	0 day	0.20 ^a $\pm$ 0.01	0.19 ^a $\pm$ 0.01	0.19 ^a $\pm$ 0.01	0.21 ^a $\pm$ 0.01	0.451
	50 days	2.05 ^{ab} $\pm$ 0.09	2.17 ^{ab} $\pm$ 0.09	2.02 ^a $\pm$ 0.08	2.57 ^b $\pm$ 0.23	0.029
	100 days	3.91 ^a $\pm$ 0.06	5.62 ^b $\pm$ 0.12	5.79 ^b $\pm$ 0.15	6.20 ^b $\pm$ 0.23	0.000
Triiodothyronine (T ₃) (ng/mL)	0 day	1.35 ^a $\pm$ 0.11	1.48 ^a $\pm$ 0.09	1.51 ^a $\pm$ 0.04	1.58 ^a $\pm$ 0.07	0.285
	50 days	1.37 ^a $\pm$ 0.05	1.39 ^a $\pm$ 0.07	1.54 ^a $\pm$ 0.09	1.61 ^a $\pm$ 0.09	0.122
	100 days	1.38 ^a $\pm$ 0.09	1.40 ^a $\pm$ 0.10	1.57 ^a $\pm$ 0.48	1.59 ^a $\pm$ 0.08	0.303
Thyroxine (T ₄) (ng/mL)	0 day	47.11 ^a $\pm$ 0.44	45.77 ^a $\pm$ 0.45	46.28 ^a $\pm$ 0.66	45.52 ^a $\pm$ 0.64	0.216
	50 days	46.60 ^a $\pm$ 0.61	46.19 ^a $\pm$ 0.49	45.92 ^a $\pm$ 0.54	44.76 ^a $\pm$ 0.85	0.225
	100 days	42.27 ^a $\pm$ 0.95	45.05 ^b $\pm$ 0.43	43.99 ^{ab} $\pm$ 0.48	45.43 ^b $\pm$ 0.40	0.003

Table 2: Mean ($\pm$ SE) plasma Triiodothyronine and Thyroxine of ram lambs supplemented with protected fat

Hormone	Day of experiment	Group I	Group II	Group III	Group IV	P Value
Triiodothyronine (T ₃) (ng/mL)	0 day	1.35 ^a $\pm$ 0.08	1.57 ^a $\pm$ 0.11	1.47 ^a $\pm$ 0.09	1.38 ^a $\pm$ 0.08	0.361
	60 days	1.52 ^a $\pm$ 0.09	1.45 ^a $\pm$ 0.11	1.46 ^a $\pm$ 0.10	1.47 ^a $\pm$ 0.09	0.970
	120 days	1.62 ^a $\pm$ 0.08	1.43 ^a $\pm$ 0.08	1.43 ^a $\pm$ 0.08	1.38 ^a $\pm$ 0.08	0.225
Thyroxine (T ₄) (ng/mL)	0 day	44.69 ^a $\pm$ 0.64	44.69 ^a $\pm$ 0.78	45.21 ^a $\pm$ 0.60	44.49 ^a $\pm$ 0.53	0.878
	60 days	44.25 ^a $\pm$ 0.80	43.79 ^a $\pm$ 0.44	42.61 ^a $\pm$ 0.28	44.00 ^a $\pm$ 0.39	0.143
	120 days	46.82 ^a $\pm$ 0.47	47.55 ^a $\pm$ 0.42	45.99 ^a $\pm$ 0.51	47.35 ^a $\pm$ 0.96	0.327

Conclusion

The present study demonstrated that supplementation of omega-3 fatty acid protected fat, particularly 3% linseed oil protected fat, positively influenced the hormonal profile of Mecheri ewes by significantly increasing plasma progesterone and thyroxine concentrations during the later stages of supplementation. The enhanced progesterone levels indicate improved reproductive endocrine function, which may contribute to better reproductive efficiency in ewes. However, triiodothyronine concentrations remained unaffected, suggesting maintenance of normal thyroid hormone balance. In contrast, omega-3 supplementation did not significantly alter the thyroid hormone profile of growing ram lambs, indicating physiological differences in endocrine responsiveness between mature ewes and lambs. Overall, the study concludes that dietary supplementation with omega-3 rich protected fat can be effectively utilized as a nutritional strategy to improve reproductive hormonal status in Mecheri ewes without causing adverse metabolic disturbances.

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