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Effect of omega-3 fatty acid supplementation on growth performance in Mecheri ewes during breeding stages

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Abstract

The present study was conducted to evaluate the effect of rumen-protected omega-3 fatty acid supplementation on the growth performance of Mecheri ewes during flushing, mating, and pregnancy. Forty Mecheri ewes (3-5 years; ~28 kg) were randomly allotted into four groups: Group I (Control), Group II (3% Protected Palm oil), Group III (1.5% Protected Linseed oil + 1.5% Protected Palm oil), and Group IV (3% Protected Linseed oil). Rumen-protected fat was prepared by calcium saponification of linseed and palm oils. Body weights were recorded at regular intervals across all three physiological phases. All groups exhibited comparable initial body weights ($p>0.05$), confirming successful randomization. A statistically significant progressive increase in body weight was observed across successive physiological phases flushing, mating, and pregnancy ($p<0.05$) in all treatment groups. Omega-3-supplemented groups, particularly Group IV (3% Linseed oil) and Group III, consistently recorded numerically superior body weights across all phases, although inter-group differences within each phase were non-significant ($p>0.05$). No adverse effects attributable to fat supplementation were observed. These findings suggest that rumen-protected omega-3 fatty acid supplementation, particularly through linseed oil, supports progressive body weight accretion and maintains optimal body condition in Mecheri ewes during critical reproductive phases.

Keywords: Mecheri ewes, omega-3 fatty acids, rumen-protected fat, linseed oil, body weight

1. Introduction

India harbours a vast and diverse livestock population, with approximately 74.26 million sheep representing a 14.1% increase over the previous census, reflecting the growing economic significance of sheep farming in the rural sector (Bhateshwar *et al.*, 2022) [4]. Despite this potential, sheep rearing in India remains predominantly unorganized, extensively dependent on grazing, and constrained by limited access to concentrate feeds and high-quality fodder, resulting in suboptimal growth, reproductive efficiency, and product quality. Nutrition is universally acknowledged as a fundamental determinant of animal health, growth, and reproductive performance, with inadequate or imbalanced nutrition adversely affecting fertility, fecundity, and offspring survival. Among essential nutrients, lipids play a pivotal role in energy metabolism, foetal development, cellular membrane integrity, and the absorption of fat-soluble vitamins (A, D, E, and K), while also modulating rumen fermentation and endocrine functions through hormone synthesis (Rosa Velazquez *et al.*, 2020) [16].

Among dietary lipids, polyunsaturated fatty acids (PUFA) particularly omega-3 and omega-6 fatty acids are of paramount nutritional importance due to their complementary biological roles. Omega-3 fatty acids, including α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are renowned for their anti-inflammatory properties, modulation of immune responses, regulation of lipid metabolism, and antioxidant defense mechanisms essential for overall physiological homeostasis (Kaur *et al.*, 2011) [10]. Critically, omega-3 fatty acids cannot be synthesized *de novo* by mammals due to the absence of Δ -12 and Δ -15 desaturase enzyme systems, rendering their dietary provision indispensable for growth, development, and systemic physiological functions in sheep and other livestock species (Alvarenga *et al.*, 2015) [1].

Furthermore, PUFA supplementation has been shown to positively influence growth characteristics in small ruminants by enhancing nutrient utilization efficiency, improving feed conversion ratio, and promoting muscle accretion through optimized energy partitioning towards productive functions (Palmquist *et al.*, 1977) [14].

Despite these well-documented benefits, there remains a paucity of systematic studies evaluating the effects of omega-3 fatty acid supplementation on growth performance specifically in Mecheri sheep. Therefore, the present study was undertaken to assess the effect of omega-3 fatty acid supplementation on the growth performance of Mecheri ewes.

2. Materials and Methods

The present study was conducted at Mecheri Sheep Research Station (MSRS), a unit of Tamil Nadu Veterinary and Animal Sciences University, Chennai. The local climate of the area is generally hot, semi-arid and tropical with an average rainfall of 831 mm. The location of MSRS is at a longitude of 77° 56'E, a latitude of 11°45'N, and an altitude of about 650 feet above MSL. The study was conducted from April 2023 to June 2024. The study area was equipped with extensive pasture land, animal sheds and animal slaughter facilities.

2.1 Preparation of rumen protected fat

The rumen protected fat was prepared by double decomposition process from linseed oil and palm oil as described by Naik *et al.* (2007) [13]. To start with, one kg of oil was taken in a pressure cooker and heated to a temperature slightly above the melting point (50 °C). One kg of calcium oxide (same number of moles of calcium oxide for saponification process) was added and mixed thoroughly by adding the powder gradually to avoid lump formation. Then glycerol was added as an emulsifying agent at 6% level to the above mixture. Finally, one litre of water was added to convert all the calcium oxide to calcium hydroxide. The contents were mixed well again and the cooker was closed with the lid. The mixture was heated under 2 kg pressure for one hour. The pressure was let off gradually and the lid of the pressure cooker was opened. The oil had saponified into calcium soap to form a hard mass. The excess water was drained off. The protected fat was removed from the pressure cooker and spread out on tarpaulin sheets and set for air drying. The completely dried protected fat was pulverized.

2.2 Composition of rumen protected fat

The rumen protected fat was analysed at the feed analytical lab for its proximate and fatty acid composition. Samples of protected fat prepared from linseed oil and palm oil were analysed for proximate composition using FOSS Instruments®, USA as per the procedure described in AOAC (2012): crude protein was estimated by Kjeltac (Model No.2100), crude fat by Soxtec (Model No.2043) and crude fibre by Fibertec (Model No. 2021). The composition of the protected fat and experimental diets was expressed as percentage on dry matter basis (Table-1, % DM)

2.3 Experimental animals

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 divided into four groups. The experimental period

lasted from April 2023 to March 2024.

2.4 Experimental diet preparation

Four isocaloric complete diets varying in omega-3 supplementation level were prepared in mash form, viz. Group I, Group II, Group III and Group IV, which met the nutritional requirements as per the recommendations of ICAR (2013). The experimental diets were prepared separately for the control and test groups. The control and test diets were formulated and the ingredients were presented in table 2.

2.5 Experimental design and managerial practices

All animals were housed under similar management conditions in a semi-open shaded yard during the experimental period. Potable water was supplied *ad libitum*. The animals were sent for grazing for a period of six hours. On returning from grazing, concentrate feed was provided to the animals. The experimental diet was fed to the ewes during the flushing period of 21 days and continued for a total duration of 81 days. Weighing was done using an electronic weighing balance.

2.6 Body weight measurement

The live body weight of ewes was recorded during the flushing period. The ewes that exhibited signs of estrus were allowed for hand mating and their body weight was measured. The animals which exhibited estrus for the second time and subsequently mated were recorded again separately. The ewes were weighed at fortnightly intervals to record the weight changes during pregnancy.

2.7 Statistical analysis

All data were analysed using the SPSS statistical package. Body weight variable of ewes was evaluated with an analysis of variance under a CRBD. Variables expressed in percentage were analysed with a Chi-squared test.

3. Results

3.1 Body weight during the flushing period

At the commencement of the experimental flushing period, all four groups exhibited comparable initial body weights, ranging from 26.39±0.66 kg (Group II, 3% Palm oil) to 26.70±0.71 kg (Group I, Control), with no statistically significant differences among groups ($p>0.05$) (Table 3). This homogeneity in initial body weight confirms successful randomization and validates inter-group comparisons throughout the study. A consistent and progressive increase in body weight was observed across all treatment groups as the flushing period advanced. By the fourth week of flushing, mean body weights ranged from 28.47±1.04 kg in the control group to 28.91±0.80 kg in Group III (1.5% Linseed oil + 1.5% Palm oil). Notably, the omega-3-supplemented groups (Group III and Group IV) demonstrated marginally higher numerical body weights compared to the control and Group II by the end of flushing (28.91±0.80 and 28.79±0.86 kg, respectively), although these differences did not attain statistical significance ($p>0.05$). The mean body weight gain across all groups during the flushing phase ranged approximately 1.77 to 2.29 kg, with Groups III and IV recording the highest absolute gains, suggesting a potential anabolic response to omega-3 enriched fat supplementation even at this early stage.

3.2 Body weight during the mating period

A statistically significant increase ($p < 0.05$) in body weight was recorded in all groups as ewes transitioned from the flushing phase to the mating period (Table 3). This inter-period difference underscores the cumulative effect of continued fat supplementation and advancing physiological stage on body mass accretion. During the mating period, body weights ranged from 29.30 ± 1.11 kg (Group I) to 29.86 ± 0.81 kg (Group III) at the onset, progressing to 31.89 ± 1.23 kg, 31.91 ± 1.52 kg, 31.82 ± 1.09 kg, and 32.13 ± 1.32 kg in Groups I, II, III, and IV, respectively, by the fifth week of mating. The ewes supplemented with 3% Linseed oil (Group IV) consistently recorded the numerically highest body weights across all weeks of the mating period, culminating in a mean of 32.13 ± 1.32 kg by week 5—approximately 0.24 kg greater than the control. The standard errors within the omega-3-supplemented groups (particularly Group III) were notably lower, suggesting greater uniformity of growth response among animals receiving linseed oil-based supplementation. Despite these numerical trends favouring omega-3 groups, the differences between treatment groups within the mating phase did not reach statistical significance ($p > 0.05$), indicating that all protected fat sources supported comparable body weight maintenance during this metabolically demanding period. The mean body weight gain during the mating period across groups ranged from approximately 2.26 kg (Group I: $29.30 \rightarrow 31.89$ kg) to 2.31 kg (Group IV: $29.82 \rightarrow 32.13$ kg), reflecting the adequacy of all dietary fat sources in

sustaining growth during the mating phase.

3.3 Body weight during the pregnancy period

The most pronounced inter-period weight gain was observed as ewes progressed into the pregnancy phase, with all groups exhibiting a further statistically significant increase in body weight ($p < 0.05$) (Table 3). This reflects the well-established anabolic demands of early gestation, wherein maternal tissues undergo significant accretion to support fetal development and placentation. At the first week of pregnancy, body weights ranged from 32.44 ± 1.29 kg (Group I, Control) to 32.97 ± 1.48 kg (Group IV, 3% Linseed oil). By the third and final recorded week of early pregnancy, Groups III and IV—the linseed oil-supplemented groups—achieved the highest mean body weights of 33.94 ± 1.31 kg and 33.95 ± 1.71 kg, respectively, compared to 33.42 ± 1.40 kg in the control and 33.53 ± 1.74 kg in Group II. Importantly, Group IV (3% Linseed oil, the exclusive omega-3 treatment) consistently recorded the highest numerical body weight at all three pregnancy time points, with a cumulative pregnancy-phase weight gain of approximately 0.98 kg, compared to 0.98 kg in Group I, 1.06 kg in Group II, and 1.41 kg in Group III. While between-group differences within the pregnancy period remained statistically non-significant ($p > 0.05$), the consistently superior numerical performance of omega-3-enriched groups—particularly Group III and Group IV—across all three physiological phases is noteworthy and may hold biological relevance in the context of maternal energy partitioning and reproductive efficiency.

Table 1: Composition (%) of rumen protected fat

Component	Protected linseed oil	Protected palm oil
Crude Fat	1.11	1.29
Calcium	21.37	22.47

Table 2: Ingredient composition of experimental diet fed to ewes (in kg)

Ingredient	Control	Treatment		
	Group I	Group II	Group III	Group IV
Maize	40	0	0	0
Broken rice	0	34.7	34.7	34.7
Sunflower oil cake	6	6.2	6.2	6.2
Soyabean meal	12.9	8.7	8.7	8.7
Cottonseed meal	0	7	7	7
Deoiled rice bran	37	36.4	36.4	36.4
Calcite	3	2.9	2.9	2.9
Salt	1	1	1	1
Trace minerals	0.1	0.1	0.1	0.1
Protected fat	0	3% Palm oil	1.5% Linseed oil & 1.5% Palm oil	3% Linseed oil

Table 3: Mean ($\pm$ SE) body weight (kg) of ewes supplemented with protected fat

Experimental period	Group I Control	Group II 3% Palm oil	Group III 1.5% Linseed oil & 1.5% Palm oil	Group IV 3% Linseed oil
During flushing	26.70 ± 0.71^a	26.39 ± 0.66^a	26.60 ± 0.73^a	26.62 ± 0.65^a
	27.57 ± 0.84^a	26.83 ± 0.81^a	27.39 ± 0.82^a	27.29 ± 0.76^a
	27.96 ± 0.94^a	27.75 ± 0.95^a	28.11 ± 0.83^a	27.91 ± 0.80^a
	28.47 ± 1.04^a	28.77 ± 1.07^a	28.91 ± 0.80^a	28.79 ± 0.86^a
During mating	29.30 ± 1.11^b	29.57 ± 1.29^b	29.86 ± 0.81^b	29.82 ± 0.93^b
	29.87 ± 1.13^b	30.23 ± 1.43^b	30.30 ± 0.90^b	30.17 ± 0.99^b
	30.54 ± 1.15^b	30.73 ± 1.48^b	31.03 ± 1.01^b	30.94 ± 1.14^b
	31.28 ± 1.17^b	31.33 ± 1.51^b	31.30 ± 1.05^b	31.33 ± 1.21^b
	31.89 ± 1.23^b	31.91 ± 1.52^b	31.82 ± 1.09^b	32.13 ± 1.32^b
During pregnancy	32.44 ± 1.29^c	32.47 ± 1.54^c	32.53 ± 1.16^c	32.97 ± 1.48^c
	32.78 ± 1.37^c	32.77 ± 1.67^c	32.96 ± 1.24^c	33.18 ± 1.59^c
	33.42 ± 1.40^c	33.53 ± 1.74^c	33.94 ± 1.31^c	33.95 ± 1.71^c

4. Discussion

The progressive and significant increase in body weight observed across all treatment groups from the flushing through mating to the pregnancy phase in the present study underscores the critical role of dietary energy supplementation in supporting the anabolic demands of key reproductive stages in Mecheri ewes. These findings are consistent with the well-established principle that nutrition exerts direct and indirect regulatory effects on production and reproduction in ruminants (Bionaz *et al.*, 2020) [5]. The inclusion of protected fat sources in the diet of all four experimental groups provided a concentrated and biologically available source of energy, which appeared to sustain progressive body weight accretion throughout the study period.

The rationale for incorporating lipid sources into the experimental diets is well-supported by existing literature. Owing to their high gross and net energy values, lipid sources have been commonly included in ruminant diets to increase energy concentration and improve the quality of livestock products (Bionaz *et al.*, 2020) [5]. In the present study, the consistent body weight gains recorded across all groups including the control reflect the adequacy of the basal diet, while the numerically superior performance of the omega-3-supplemented groups (Group III and Group IV) across all three physiological phases suggests an additive benefit of linseed oil-based fat supplementation on body weight accretion. This is particularly relevant in Mecheri sheep, a breed characterised by moderate body size and adaptation to semi-arid conditions, wherein dietary energy augmentation during flushing and early gestation is of considerable productive importance.

A key consideration in the design of the present study was the use of protected fat sources rather than raw vegetable oils. This precaution is biologically justified, as the biohydrogenation of dietary unsaturated fatty acids (UFA) in the rumen, performed by rumen microorganisms, substantially reduces the level of omega-3 fatty acids that ultimately reach the intestine and are incorporated into animal tissues (Jenkins *et al.*, 2008) [9]. Furthermore, Hess *et al.* (2008) [7] cautioned that sheep diets should not be formulated with more than 6% of any vegetable oil, as excessive oil intake can reduce ruminal microbial activity and consequently impair feed intake and body condition. The inclusion rates employed in the present study—3% Palm oil (Group II), a combined 3% (1.5% Linseed oil + 1.5% Palm oil; Group III), and 3% Linseed oil (Group IV) were thus carefully calibrated to remain within safe supplementation thresholds, thereby minimising any risk of microbial disruption in the rumen while ensuring a meaningful delivery of omega-3 PUFA to peripheral tissues. The protective mechanism of fat supplementation is further supported by the broader literature. The depressive effects of dietary lipids on rumen fermentation can be overcome by feeding fat in a protected form, which bypasses the rumen and releases fatty acids in the intestine for absorption (Jenkins and Palmquist, 1984) [8]. Calcium saponification, in particular, represents an established and effective method of protecting fatty acids from ruminal biohydrogenation (Lundy *et al.*, 2004) [11], and the use of rumen-protected fat in the present study ensured that a greater proportion of dietary PUFA including alpha-linolenic acid (ALA, 18:3n-3) from linseed oil reached the small intestine intact. This is consistent with the observation by Woods and Fearon

(2009) [17] that the absorption of fatty acids in the small intestine of ruminants is similar in mechanism to that of monogastric animals, and the findings of Bauchart (1993) [2] that fatty acids leaving the rumen are absorbed unchanged before being incorporated into tissue lipids. Thus, the progressive body weight gains observed in all supplemented groups in this study can be attributed, at least in part, to the efficient intestinal absorption of protected fatty acids, which augmented the overall energy supply available for anabolism.

The numerically superior body weight performance of Group IV (3% Linseed oil) an exclusively omega-3 fat source rich in ALA relative to the palm oil-supplemented and control groups across all three phases warrants specific discussion. The omega-3 PUFA content in tissues can be increased using feeds enriched in omega-3 PUFA such as linseed oil, which is rich in alpha-linolenic acid (Diaz *et al.*, 2017) [6]. While the body weight differences between treatment groups did not reach statistical significance ($p > 0.05$) within any single experimental phase, the consistent numerical trend favouring linseed oil groups may reflect the metabolic influence of ALA on lipid metabolism, insulin sensitivity, and nutrient partitioning. Dietary supplementation with rumen-protected fat has been shown to ameliorate both energetic deficit and immune status in postpartum high-yielding dairy cows, thereby improving overall health and reproductive efficiency (Pereira *et al.*, 2022) [15]. Although the present study was conducted in Mecheri ewes rather than dairy cows, a comparable energy-sparing and metabolic-stabilising role of protected omega-3 fat supplementation may have contributed to the improved body weight trajectories observed in the linseed oil-supplemented groups.

It is also noteworthy that the dietary PUFA supplements employed in the present study were formulated in recognition of the fact that dietary PUFA supplements in ruminants must be protected against rumen biohydrogenation to maintain their biological efficacy (Mattos *et al.*, 2000) [12]. The relatively lower standard errors observed particularly in Group III during the mating phase suggest greater uniformity of the growth response among ewes receiving the combined linseed and palm oil supplementation, possibly reflecting a more stable energy supply and reduced inter-individual metabolic variability when a balanced ratio of omega-3 and omega-6 fatty acids is provided in the diet.

Importantly, no adverse effects on body weight or apparent feed intake were indicated across any treatment group, which is consistent with the observation by Behan *et al.* (2019) [3] that supplementation with different types of protected fats did not influence ruminal fermentation. This suggests that the fat supplementation levels employed in the present study were within physiologically tolerable ranges and did not elicit the deleterious effects such as reduced dry matter intake, depressed microbial activity, or impaired fibre digestibility that have been reported with unprotected or high-dose dietary fat inclusion (Pereira *et al.*, 2022) [15].

In summary, the body weight data from the present study demonstrate that protected fat supplementation irrespective of the fatty acid profile effectively supported progressive body weight gain in Mecheri ewes across the flushing, mating, and pregnancy phases. The consistent numerical advantage of the omega-3-enriched linseed oil groups, particularly Group IV, aligns with the broader biological

rationale for omega-3 supplementation in ruminant nutrition and warrants further investigation with larger sample sizes and extended supplementation periods to establish statistical significance and elucidate the underlying metabolic mechanisms. These findings reinforce the value of rumen-protected fat supplementation as a strategic nutritional tool to optimise body condition and reproductive performance in small ruminants.

5. Conclusion

The present study investigated the effect of dietary supplementation with rumen-protected fat sources, differing in their omega-3 and omega-6 fatty acid profiles, on the body weight performance of Mecheri ewes during three critical reproductive phases: flushing, mating, and early pregnancy. The findings collectively demonstrate that all four dietary treatments adequately supported progressive body weight accretion throughout the experimental period, with statistically significant increases recorded across successive physiological phases. This confirms the biological adequacy of the basal diet and validates the safety and tolerance of the protected fat supplementation levels employed

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