

Physiological adaptation and lipid utilization efficiency in lactating fat-tailed Turki-Qashqaei ewes fed graded levels of cracked safflower

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Abstract

Dietary lipid supplementation is widely used in small ruminant nutrition to increase energy density and provide essential fatty acids, yet information on physiological adaptation and lipid utilization efficiency during lactation is limited, especially in fat-tailed breeds. This study examined the effects of graded dietary inclusion of cracked safflower seeds (0%, 3%, and 6% of dry matter) in lactating fat-tailed Turki-Qashqaei ewes ($n = 12$ per treatment). Nutrient intake (relative to metabolic body weight, $BW^{0.75}$), apparent total-tract digestibility (acid-insoluble ash method), rumen fermentation (pH, ammonia-N, total VFA, acetate:propionate ratio), blood metabolites (glucose, β -hydroxybutyrate, NEFA, triglycerides, total protein, albumin), and lipid utilization indices (milk fat yield per ether extract intake; milk long-chain fatty acid output per linoleic acid intake) were evaluated. Increasing safflower inclusion linearly elevated ether extract and linoleic acid intake ($P < 0.05$) but reduced digestibility of dry matter, organic matter, ether extract, neutral detergent fiber, and acid detergent fiber ($P < 0.05$). Rumen fermentation remained largely stable. At 6% inclusion, blood glucose decreased while NEFA and triglycerides increased ($P < 0.05$). Milk fat yield increased ($P < 0.01$), but lipid utilization indices showed nonlinear responses, reflecting adaptive metabolic regulation. These findings suggest ruminal biohydrogenation and post-absorptive constraints limit efficient use of elevated unsaturated fatty acids. In lactating fat-tailed ewes, lipid supplementation primarily influences nutrient utilization efficiency and physiological adaptation rather than linear productive gains, providing mechanistic insights into lipid metabolism and supporting physiologically based diet formulation for fat-tailed sheep breeds.

Introduction

Dietary lipid supplementation has long been employed in ruminant nutrition to increase dietary energy density, modify fatty acid supply, and influence metabolic efficiency, particularly during physiologically demanding stages such as lactation. In small ruminants, lactation represents a period of markedly elevated nutrient and energy requirements, during which dietary composition exerts a pronounced influence on nutrient partitioning between maintenance, milk synthesis, and body reserves (Cannas and Pulina, 2008). Lipids are of particular interest because they provide concentrated energy and supply essential fatty acids, yet their utilization in ruminants is substantially constrained by the unique ruminal environment, which differs fundamentally from the digestive physiology of monogastric species (Jenkins et al., 2008).

Unlike monogastric animals, ruminants are characterized by extensive microbial modification of dietary lipids in the rumen. Upon ingestion, dietary triacylglycerols and phospholipids undergo rapid microbial lipolysis, releasing free fatty acids that are subsequently subjected to biohydrogenation by rumen microorganisms (Harfoot and Hazelwood, 1997; Jenkins et al., 2008). This process leads to extensive saturation of unsaturated fatty acids, particularly linoleic (C18:2n-6) and linolenic (C18:3n-3) acids, thereby altering both the quantity and profile of fatty acids that escape the rumen and become available for intestinal absorption (Buccioni et al., 2012; Shingfield et al., 2013). Consequently, the physiological

response to increased dietary lipid supply in ruminants depends not solely on intake but also on the efficiency and pathways of ruminal and post-ruminal lipid metabolism.

Ruminal biohydrogenation is neither complete nor uniform, and its extent is influenced by lipid source, degree of unsaturation, physical form of the feed, and interactions with other dietary components such as fiber and starch (Jenkins et al., 2008; Lourenço et al., 2010). Oilseeds rich in polyunsaturated fatty acids, including safflower, represent a nutritionally relevant lipid source because of their high linoleic acid content and widespread availability in semi-arid regions. However, the high degree of unsaturation in safflower oil means that its ruminal metabolism may differ substantially from that of more saturated lipid sources, with potential consequences for fermentation and digestibility (Buccioni et al., 2012; Toral et al., 2024). These effects underscore the importance of evaluating lipid supplementation not merely in terms of productive responses but also through a physiological lens that accounts for digestive, ruminal, and metabolic adaptation.

In lactating ruminants, the relationship between dietary lipid intake and productive performance is further complicated by the metabolic priorities imposed by milk synthesis. Lactation requires a coordinated supply of glucose, amino acids, and fatty acids to the mammary gland, and alterations in dietary lipid supply can modify nutrient partitioning between milk production and body tissue accretion or mobilization (Bauman and Griinari, 2003; Shingfield et al., 2013). In dairy sheep, previous studies have demonstrated that dietary lipids can influence milk fatty acid composition and energy balance, yet responses in milk yield and composition are often nonlinear and highly variable (Toral et al., 2018; Toral et al., 2021). Such variability suggests that physiological regulation and metabolic constraints, rather than simple substrate availability, govern lipid utilization during lactation.

A central mechanism underlying these responses is the interaction between ruminal biohydrogenation and post-absorptive lipid metabolism. Fatty acids that escape ruminal biohydrogenation are absorbed in the small intestine and transported to peripheral tissues, including the mammary gland, where they may be oxidized, stored, or incorporated into milk fat (Palmquist and Jenkins, 2017). However, the capacity of these tissues to utilize increased supplies of long-chain fatty acids is limited, and excess unsaturated fatty acids may be preferentially oxidized or redirected away from milk fat synthesis (Bauman and Griinari, 2003; Shingfield et al., 2013). This phenomenon highlights the concept of lipid utilization efficiency, defined here as the proportion of ingested or absorbed fatty acids effectively used for productive functions such as milk fat synthesis.

Despite extensive research on dietary lipid supplementation, most studies in small ruminants have focused on production-oriented outcomes such as milk yield, milk fat percentage, or fatty acid profile, with comparatively less emphasis on integrative indices of nutrient utilization efficiency, rumen fermentation, blood metabolites, and metabolic adaptation (Cannas and Pulina, 2008; Toral et al., 2018). Moreover, the majority of mechanistic insights into lipid metabolism have been derived from dairy cattle, and their direct extrapolation to sheep may be inappropriate due to species-specific differences in digestive kinetics, mammary lipid synthesis, and body fat deposition patterns (Shingfield et al., 2013).

These gaps are particularly relevant for indigenous and fat-tailed sheep breeds, which possess distinct energy storage strategies that may influence lipid utilization during lactation.

Fat-tailed sheep breeds, such as the Turki-Qashqaei, are characterized by substantial adipose tissue deposition in the tail, serving as an energy reserve that can be mobilized during periods of nutritional stress or high physiological demand. This morphological and metabolic adaptation may modify the response to dietary lipid supplementation by altering lipid partitioning between milk synthesis, body reserves, and oxidation (Dolatpanah et al., 2008). However, empirical data describing lipid utilization efficiency, rumen characteristics, and metabolic adaptation to increased dietary unsaturated fatty acid supply in lactating fat-tailed ewes are scarce. Understanding these responses is essential for developing feeding strategies that optimize nutrient use efficiency without compromising rumen function or metabolic health.

Cracked safflower represents a practical dietary lipid source in sheep production systems, particularly in regions where oilseed processing residues are readily available. Its high linoleic acid content provides an opportunity to examine how graded increases in dietary unsaturated lipid supply influence intake, digestibility, rumen fermentation, blood metabolites, and metabolic handling of lipids in lactating ewes. Importantly, evaluating intake variables relative to metabolic body weight and integrating digestibility, rumen, and blood data allows for a more physiologically meaningful assessment of substrate availability and utilization than absolute intake measures alone (Cannas and Pulina, 2008). Such an approach facilitates the derivation of indices that reflect lipid utilization efficiency rather than simple production responses.

Therefore, the present study aimed to investigate the effects of graded dietary inclusion of cracked safflower on nutrient intake, apparent digestibility, rumen fermentation characteristics, blood biochemical parameters, and indices of lipid utilization efficiency in lactating fat-tailed Turki-Qashqaei ewes. By focusing on physiological adaptation and nutrient utilization rather than solely on productive outcomes, this study seeks to provide mechanistic insight into how increased dietary unsaturated lipid supply is processed within the rumen and handled at the systemic level during lactation. These findings are expected to contribute to a more nuanced understanding of lipid metabolism in sheep and to inform physiologically grounded feeding strategies for fat-tailed breeds.

Materials and methods

Experimental Animals and Ethical Approval

The experiment was conducted using lactating fat-tailed Turki-Qashqaei ewes maintained under controlled management conditions. A total of thirty-six multiparous ewes with similar days in milk (31 ± 3 days), body weight (54.5 ± 1.48 kg), and parity (2–4) were selected at the beginning of the experiment. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Yasouj University (Approval No. 4014441004) in accordance with national guidelines for

the care and use of agricultural animals (Iranian Council of Animal Care, 1995). Ewes were housed in individual pens equipped with free access to fresh water and mineral blocks throughout the experimental period. Animals were allowed an adaptation period of 12 days to experimental diets prior to data collection.

Experimental Design and Dietary Treatments

The experiment was arranged as a completely randomized design, with ewes randomly assigned to one of three dietary treatments (n = 12 per treatment). Treatments consisted of graded inclusion levels of cracked safflower incorporated into a basal diet formulated to meet or exceed the nutrient requirements of lactating ewes according to NRC (2007) recommendations. Cracked safflower was included at 0% (SS0), 3% (SS3), and 6% (SS6) of dry matter (DM), replacing equivalent proportions of barley grain to maintain approximate isonitrogenous and isoenergetic conditions. The ingredient composition and analyzed nutrient and fatty acid profiles of the three experimental diets are shown in Table 2. Diets were offered twice daily at 5% above expected intake to ensure ad libitum consumption, and orts were recorded daily.

Table 2

Ingredient and analyzed nutrient composition of experimental diets containing graded levels of cracked safflower.

Item	Treatment [†]		
	SS0	SS3	SS6
Ingredients (% DM)			
Alfalfa hay	20.32	20.28	20.30
Corn silage	20.58	21.62	21.17
Barley grain, crushed	22.92	18.87	16.28
Safflower seeds, cracked	0.00	3.00	6.00
Wheat straw	34.62	34.57	34.64
Mineral-vitamin premix [‡]	0.21	0.20	0.21
Salt	0.19	0.19	0.21
Sodium bicarbonate	0.57	0.58	0.61
Dicalcium phosphate	0.19	0.18	0.19
Limestone	0.39	0.39	0.40
Nutrient composition (mean ± SD)			
ME (Mcal/kg) [§]	2.23 ± 0.07	2.21 ± 0.04	2.24 ± 0.07
Crude protein (% DM)	13.38 ± 0.23	13.32 ± 0.12	13.19 ± 0.11
ADF (% DM)	23.33 ± 0.27	23.24 ± 0.23	23.08 ± 0.19
NDF (% DM)	36.02 ± 0.24	35.78 ± 0.16	35.67 ± 0.09
Ether extract (% DM)	3.96 ± 0.19	4.28 ± 0.27	4.51 ± 0.18
Ash (% DM)	5.10 ± 0.08	4.97 ± 0.07	4.91 ± 0.19
Fatty acids (% of ether extract)	85.28 ± 4.33	86.49 ± 6.04	87.81 ± 3.68
[†] Treatments: SS0 = basal diet with 0% cracked safflower (control); SS3 = basal diet with 3% cracked safflower; SS6 = basal diet with 6% cracked safflower (DM basis). Cracked safflower replaced an equivalent proportion of barley grain to maintain approximate isonitrogenous and isoenergetic conditions (NRC, 2007).			
[‡] Per kg: 193 g Ca, 97 g P, 76 g Na, 16 g Mg, 3 g Fe, 0.3 g Cu, 2.66 g Mn, 100 mg Co, 100 mg I, 0.1 g Se, 5 × 10 ⁶ IU vitamin A, 1 × 10 ⁶ IU vitamin D, 0.1 g vitamin E.			
[§] ME estimated from NRC (2007).			

Diet Composition and Feed Analysis

Representative samples of all feed ingredients and experimental diets were collected weekly and composited by period for chemical analysis. Samples were dried at 60–65°C for 48 h, ground to pass a 1 mm screen, and analyzed following standard procedures: dry matter (DM) by oven drying at 105°C; ash by combustion at 550°C; crude protein (CP) by the Kjeldahl method (AOAC, 2005; method 984.13); ether extract (EE) using petroleum ether extraction (AOAC, 2005; method 920.39); neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991) with heat-stable α -amylase and sodium sulfite. The fatty acid composition of cracked safflower and experimental diets was determined by lipid extraction followed by methylation and gas chromatography according to Folch et al. (1957) and ISO 12966-4 (2015). Individual fatty acids were expressed as g/100 g of total identified fatty acids. The chemical composition and detailed fatty acid profile of the cracked safflower seeds used in this study are presented in Table 1.

Table 1
Chemical composition and fatty acid profile of cracked safflower seeds used in the experimental diets

Component	Content (% DM unless noted)
Dry matter	93.03
Crude protein	19.52
NDF	38.13
ADF	29.74
Ether extract	40.20
Ash	1.21
Fatty acids [†] (% of ether extract)	97.53
C14:0	1.23
C16:0	5.41
C18:0	2.16
C18:1	14.19
C18:2	74.37
C18:3	0.28
Other FA [‡]	2.38
[†] FA reported as carbon:double bonds.	
[‡] Other FA = 100 - sum identified FA.	

Feed Intake and Nutrient Intake Calculations

Daily feed intake was calculated as the difference between feed offered and refusals on a DM basis. Nutrient intake was calculated by multiplying DM intake by the analyzed nutrient concentrations of the respective diets. To allow physiologically meaningful comparisons, intake variables were expressed relative to metabolic body weight ($\text{g/kg BW}^{0.75}$). Ether extract intake and linoleic acid intake were calculated to quantify dietary lipid substrate availability across treatments.

Apparent Digestibility Determination

Apparent total-tract digestibility of nutrients was determined using the acid-insoluble ash (AIA; Van Keulen and Young, 1977) internal marker method during the final 21 days of the experimental period. Fecal samples were collected twice daily (morning and afternoon), pooled by animal over 6 consecutive days, and subsampled for chemical analysis using the same procedures as described for feed samples. Digestibility coefficients were calculated as: $\text{Digestibility (\%)} = 100 \times (1 - (\text{AIA}_{\text{diet}} \times \text{Nutrient}_{\text{feces}}) / (\text{AIA}_{\text{feces}} \times \text{Nutrient}_{\text{diet}}))$.

Rumen Fluid Sampling and Analysis

Rumen fluid samples (~ 50 mL) were collected via stomach tube 4 h after the morning feeding on days 14, 18, and 21 of the collection period. Immediately after collection, pH was measured using a portable digital pH meter (Hanna Instruments HI-2002 Edge, Hanna Instruments, Italy). A subsample was acidified with 50% H_2SO_4 and stored at -20°C for ammonia-N determination by the phenol-hypochlorite method (Broderick and Kang, 1980). Another subsample was preserved with metaphosphoric acid for volatile fatty acid (VFA) analysis by gas chromatography.

Blood Sampling and Biochemical Analysis

Blood samples (~ 10 mL) were collected from the jugular vein into plain vacutainer tubes on days 0, 14, and 21 of the experimental period, 3 h after feeding. Serum was separated by centrifugation at $3000 \times g$ for 10 min at 4°C and stored at -20°C until analysis. Serum concentrations of glucose, β -hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), total protein (TP), and albumin (Alb) were determined using commercial enzymatic colorimetric kits (Pars Azmun Co., Tehran, Iran) on an automated biochemical analyzer (SELECTRA Prom, Elitech Group, France). Serum concentrations of glucagon (pg/mL), insulin (ng/mL), leptin (ng/mL), prolactin (ng/mL), adiponectin ($\mu\text{g/mL}$), and insulin-like growth factor-1 (IGF-1; ng/mL) were measured using species-specific enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, San Diego, CA, USA for glucagon, insulin, leptin, prolactin, and IGF-1; Elabscience, Houston, TX, USA for adiponectin) according to the manufacturers' instructions.

Assay precision was assessed by calculating intra-assay (within-run) and inter-assay (between-run) coefficients of variation (%CV) using pooled serum samples run in replicates. For the enzymatic colorimetric assays (Pars Azmun Co., Tehran, Iran), intra-assay CV ranged from 3.2–7.1% and inter-assay CV from 4.8–10.5%. For the ELISA kits, intra-assay CV was < 8% and inter-assay CV < 12% across all analytes (glucagon, insulin, leptin, prolactin, adiponectin, IGF-1).

Milk Collection and Analysis

Milk yield was recorded daily by hand milking twice per day (0600 and 1800 h). Proportional subsamples were collected weekly, preserved with potassium dichromate (as a bacteriostatic preservative to maintain sample integrity during storage), and analyzed for fat content using a MilkoScan™ FT + mid-infrared analyzer (FOSS, Hillerød, Denmark). Milk long-chain fatty acids (LCFA) were extracted, methylated, and quantified by gas chromatography following the same protocol used for feed fatty acids (Folch et al., 1957; with methylation and GC analysis as per ISO 12966-4:2015 or equivalent procedures validated for ruminant milk lipids).

Derived Indices of Lipid Utilization Efficiency

To evaluate physiological adaptation beyond absolute intake and digestibility, indices of lipid utilization efficiency were derived from intake and digestibility data. Ether extract intake and digestible ether extract supply were used as proxies for lipid availability, while productive responses (milk yield and milk fat output) were used cautiously as outcome variables without emphasizing performance per se.

These indices were employed to explore whether increasing dietary lipid supply resulted in proportional increases in utilization efficiency or reflected adaptive metabolic regulation during lactation, consistent with reported constraints on unsaturated fatty acid transfer in ruminants (Toral et al., 2018).

Statistical Analysis

All statistical analyses were performed using SAS software (Version 9.0; SAS Institute Inc., Cary, NC, USA). The experimental unit was the individual ewe.

Data were analyzed according to a completely randomized design using the MIXED procedure of SAS. The statistical model included dietary treatment as a fixed effect and animal as a random effect. For variables measured once during the experimental period (intake, digestibility, milk fat yield, lipid utilization indices, and mean rumen fermentation parameters), the following model was applied:

$$Y_i = \mu + T_i + A(T_i) + \varepsilon_i$$

where Y_i represents the dependent variable, μ is the overall mean, T_i is the fixed effect of dietary treatment ($i = \text{SS0, SS3, SS6}$), $A(T_i)$ is the random effect of animal within treatment, and ε_i is the residual error.

For blood biochemical variables collected on multiple sampling days, the mean value per animal across sampling days was calculated prior to analysis, and these mean values were used as the response variable in the same model structure described above. Baseline measurements (day 0) were evaluated and included as covariates only when statistically significant.

Normality of residuals was assessed using the Shapiro–Wilk test (PROC UNIVARIATE) and by visual inspection of residual plots. Homogeneity of variance among treatments was evaluated using Levene’s test. When necessary, data were examined for outliers based on studentized residuals. No data transformation was applied when assumptions of normality and homoscedasticity were satisfied. In cases where deviation from normality was detected, logarithmic transformation was evaluated; however, final analyses were performed on untransformed data when transformation did not materially alter statistical inference.

Orthogonal polynomial contrasts were used to test for linear and quadratic responses to increasing dietary inclusion levels of cracked safflower (0%, 3%, and 6% of dry matter). Treatment means were separated using Tukey’s adjustment for multiple comparisons. Least squares means are presented in the tables.

Results are expressed as least squares means with pooled standard error of the mean (SEM). Statistical significance was declared at $P < 0.05$, and tendencies were discussed when $0.05 \leq P < 0.10$.

Graphical representations of selected variables were prepared using GraphPad Prism (Version 8.0; GraphPad Software, San Diego, CA, USA).

Results

Nutrient intake, apparent total-tract digestibility, rumen fermentation, and lipid utilization efficiency

The effects of graded dietary inclusion of cracked safflower on nutrient intake (relative to metabolic body weight), apparent total-tract digestibility, rumen fermentation characteristics, and derived indices of lipid utilization efficiency are summarized in Table 3.

Table 3

Dry matter intake, nutrient intake, apparent total-tract digestibility, rumen fermentation characteristics, and derived lipid utilization indices in lactating fat-tailed Turki-Qashqaei ewes fed graded levels of cracked safflower.

Parameter	Treatment [†]			SEM	P-value
	SS0	SS3	SS6		
Intake					
DMI (kg/d)	1.51	1.45	1.45	0.020	0.07
DMI (g/kg BW ^{0.75})	74.78	72.28	72.28	1.201	0.25
Digestible DMI (per kg BW ^{0.75})	51.18 ^a	48.56 ^{ab}	47.11 ^b	1.094	0.04
EE intake (g/d)	58.51 ^c	62.41 ^b	65.32 ^a	1.026	0.02
EE intake (g/kg BW ^{0.75})	2.92 ^b	3.11 ^{ab}	3.25 ^a	0.079	0.02
Linoleic acid intake (g/d)	13.07 ^c	25.49 ^b	36.81 ^a	1.191	< 0.01
Linoleic acid intake (g/kg BW ^{0.75})	0.65 ^c	1.27 ^b	1.83 ^a	0.236	< 0.01
Digestibility					
Dry Matter (DM)	68.52 ^a	67.21 ^a	65.14 ^b	0.904	0.04
Organic Matter (OM)	71.08 ^a	69.27 ^a	67.77 ^b	0.839	0.03
Crude Protein (CP)	72.41	71.86	70.49	0.657	0.12
Ether Extract (EE)	85.56 ^a	82.27 ^b	78.92 ^c	0.918	< 0.01
Neutral Detergent Fiber (NDF)	55.23 ^a	53.84 ^a	50.08 ^b	1.268	0.02
Acid Detergent Fiber (ADF)	48.69 ^a	47.24 ^a	44.46 ^b	1.087	0.03
Rumen characteristics					
[†] Treatments: SS0 = basal diet with 0% cracked safflower (control); SS3 = basal diet with 3% cracked safflower; SS6 = basal diet with 6% cracked safflower (DM basis). Cracked safflower replaced an equivalent proportion of barley grain to maintain approximate isonitrogenous and isoenergetic conditions (NRC, 2007).					
Means within rows with different superscripts differ (P < 0.05; Tukey-adjusted). BW ^{0.75} = metabolic body weight; VFA = volatile fatty acids; LCFA = long-chain fatty acids; EE = ether extract.					
Statistical analysis was performed using PROC MIXED (SAS version 9.4; SAS Institute Inc., Cary, NC, USA). Treatment was included as a fixed effect and animal as a random effect. Reported means are least squares means with pooled SEM. Differences among treatments were declared significant at P < 0.05.					

Parameter	Treatment [†]			SEM	P-value
	SS0	SS3	SS6		
Intake					
pH	6.507	6.489	6.514	0.012	0.31
Ammonia-N (mg/dL)	4.97	4.88	4.43	0.270	0.33
Total VFA (mmol/L)	65.16	64.51	64.23	0.527	0.45
Acetate:Propionate Ratio	3.04	2.87	2.81	0.079	0.12
Efficiency					
Milk fat yield (kg/d)	0.15 ^b	0.16 ^b	0.19 ^a	0.010	0.02
Lipid utilization efficiency (milk fat / EE intake)	2.49 ^b	2.56 ^b	2.91 ^a	0.114	0.03
Linoleic Utilization Efficiency (g milk LCFA / g linoleic intake)	10.51 ^a	9.82 ^b	9.24 ^b	0.337	0.04
[†] Treatments: SS0 = basal diet with 0% cracked safflower (control); SS3 = basal diet with 3% cracked safflower; SS6 = basal diet with 6% cracked safflower (DM basis). Cracked safflower replaced an equivalent proportion of barley grain to maintain approximate isonitrogenous and isoenergetic conditions (NRC, 2007).					
Means within rows with different superscripts differ (P < 0.05; Tukey-adjusted). BW ^{0.75} = metabolic body weight; VFA = volatile fatty acids; LCFA = long-chain fatty acids; EE = ether extract.					
Statistical analysis was performed using PROC MIXED (SAS version 9.4; SAS Institute Inc., Cary, NC, USA). Treatment was included as a fixed effect and animal as a random effect. Reported means are least squares means with pooled SEM. Differences among treatments were declared significant at P < 0.05.					

Increasing dietary inclusion of cracked safflower seeds from 0% (SS0) to 6% (SS6) of dry matter had no effect on dry matter intake, which averaged 1.45–1.51 kg/d or 72.3–74.8 g/kg BW^{0.75} (P = 0.25; linear P = 0.12, quadratic P = 0.68). Digestible dry matter intake per unit metabolic body weight declined (overall P = 0.04; linear P = 0.02, quadratic P = 0.78), reaching the lowest value at the 6% inclusion level.

Ether extract intake increased linearly with safflower inclusion (overall P = 0.02; linear P = 0.008, quadratic P = 0.45), as did linoleic acid intake, both in absolute terms and relative to metabolic body weight (overall P < 0.01 for both; linear P < 0.001, quadratic P = 0.52 for g/d; similar for g/kg BW^{0.75}). Linoleic acid intake per kg BW^{0.75} increased by approximately 181.5% from SS0 to SS6.

Apparent total-tract digestibility responded negatively to graded safflower addition. Digestibility coefficients for dry matter, organic matter, ether extract, neutral detergent fiber, and acid detergent fiber all decreased (overall P = 0.04, 0.03, < 0.01, 0.02, and 0.03, respectively; linear P = 0.01–0.005 for all,

quadratic $P = 0.55\text{--}0.89$), with the greatest numerical reductions observed at the 6% inclusion level. Crude protein digestibility was unaffected (overall $P = 0.12$; linear $P = 0.06$, quadratic $P = 0.72$).

Rumen fermentation variables showed no treatment effects. Rumen pH averaged 6.503 ± 0.012 across treatments and was unaffected by safflower inclusion ($P = 0.31$; linear $P = 0.99$, quadratic $P = 0.88$), total volatile fatty acid concentration averaged $64.2\text{--}65.2$ mmol/L ($P = 0.45$; linear $P = 0.28$, quadratic $P = 0.91$), and acetate-to-propionate ratio did not differ significantly among treatments ($P = 0.12$). Ammonia-N concentration also did not differ statistically among treatments ($P = 0.33$; linear $P = 0.15$, quadratic $P = 0.48$).

Milk fat yield increased with safflower supplementation (overall $P = 0.02$; linear $P = 0.002$, quadratic $P = 0.41$). Lipid utilization efficiency, expressed as milk fat output per unit ether extract intake, rose linearly (overall $P = 0.03$; linear $P = 0.01$, quadratic $P = 0.32$), whereas efficiency of linoleic acid utilization for milk long-chain fatty acid output declined with increasing safflower level (overall $P = 0.04$; linear $P = 0.02$, quadratic $P = 0.89$).

Serum biochemical parameters

Graded dietary inclusion of cracked safflower seeds from 0% (SS0) to 6% (SS6) of dry matter induced significant alterations in several key serum biochemical parameters in lactating fat-tailed Turki-Qashqaei ewes (Table 4). Serum glucose concentration was markedly reduced at the highest safflower inclusion level (3.31 ± 0.12 mmol/L in SS6 vs. 3.72 ± 0.12 and 3.68 ± 0.12 mmol/L in SS0 and SS3, respectively; overall $P < 0.01$; linear $P = 0.003$, quadratic $P = 0.21$), representing an approximate 11% decrease relative to the control group. This decline was specific to the 6% treatment, with no difference between SS0 and SS3.

Table 4
Serum biochemical parameters in lactating fat-tailed Turki-Qashqaei ewes fed graded levels of cracked safflower.

Parameter	Treatment			SEM	P-value
	SS0	SS3	SS6		
Glucose (mmol/L)	3.72 ^a	3.68 ^a	3.31 ^b	0.098	0.01
β-hydroxybutyrate (mmol/L)	0.59	0.62	0.73	0.070	0.34
Free fatty acids (mmol/L)	0.14 ^b	0.18 ^b	0.29 ^a	0.039	0.03
Triglyceride (mmol/L)	0.21 ^b	0.26 ^b	0.39 ^a	0.049	0.04
Total Protein (g/L)	71.29 ^b	75.42 ^a	75.33 ^a	0.944	< 0.01
Albumin (g/L)	30.49 ^b	32.19 ^a	32.43 ^a	0.423	< 0.01
Means within rows with different superscripts differ ($P < 0.05$; Tukey-adjusted).					

Circulating non-esterified fatty acids (NEFA) and triglycerides (TG) concentrations responded positively to safflower supplementation (overall $P = 0.03$ and $P = 0.04$, respectively; linear $P = 0.01$ for NEFA and $P = 0.02$ for TG, quadratic $P = 0.28$ and $P = 0.35$). NEFA levels increased from 0.14 ± 0.06 mmol/L in SS0 to 0.29 ± 0.06 mmol/L in SS6, representing a 107% increase relative to SS0 at the highest inclusion rate. Similarly, TG concentrations rose from 0.21 ± 0.09 mmol/L in SS0 to 0.39 ± 0.09 mmol/L in SS6, an increase of approximately 86%. In both cases, the most pronounced elevations occurred at the 6% inclusion level, with SS0 and SS3 values statistically indistinguishable.

Serum total protein (TP) and albumin (Alb) concentrations were elevated in ewes receiving cracked safflower (overall $P < 0.01$ for both; linear $P = 0.004$ and $P = 0.003$, quadratic $P = 0.05$ and $P = 0.05$, respectively). TP increased from 71.29 ± 0.53 g/L in SS0 to 75.42 ± 0.53 g/L in SS3 and 75.33 ± 0.53 g/L in SS6 (~ 5.8% rise overall), while Alb rose from 30.49 ± 0.32 g/L in SS0 to 32.19 ± 0.32 g/L in SS3 and 32.43 ± 0.32 g/L in SS6 (~ 6.0–6.4% increase). For both parameters, the response plateaued between the 3% and 6% inclusion levels, with no further elevation observed beyond SS3 (evident in quadratic components).

In contrast, serum β -hydroxybutyrate (BHB) concentration remained stable across all treatments, averaging 0.59–0.73 mmol/L with no detectable effect of dietary safflower inclusion (overall $P = 0.34$; linear $P = 0.28$, quadratic $P = 0.41$).

Ether extract intake and serum endocrine parameters

Graded inclusion of cracked safflower seeds in the diet elicited clear dose-dependent changes in ether extract (EE) intake and selected serum endocrine profiles in lactating fat-tailed Turki-Qashqaei ewes (Fig. 1). Ether extract intake increased linearly across treatments (overall $P < 0.001$; linear $P < 0.001$, quadratic $P = 0.82$; panel A), rising from approximately 58.5 ± 0.8 g/d in the control group (SS0) to 62.4 ± 0.9 g/d at 3% inclusion (SS3) and 65.3 ± 0.9 g/d at 6% inclusion (SS6), corresponding to an approximate 11.6% overall increase in lipid substrate availability.

Serum glucagon concentration showed a pronounced response to the highest safflower level (overall $P < 0.001$; linear $P < 0.001$, quadratic $P = 0.003$; panel B), with values remaining stable between SS0 ($\approx 81.7 \pm 0.3$ pg/mL) and SS3 ($\approx 90.5 \pm 0.4$ pg/mL) but increasing sharply to $\approx 134.0 \pm 0.4$ pg/mL in the SS6 group — an elevation of more than 60% relative to the control.

In contrast, serum insulin concentrations were unaffected by dietary treatment and remained stable across all groups (≈ 14.4 – 14.6 ng/mL; overall $P \geq 0.05$; linear $P = 0.48$, quadratic $P = 0.91$; panel C). Serum leptin levels also showed no significant treatment effect (≈ 3.35 – 3.52 ng/mL; overall $P \geq 0.05$; linear $P = 0.32$, quadratic $P = 0.76$; panel D). Similarly, serum prolactin concentrations exhibited only minor numerical variation (≈ 39.3 – 41.4 ng/mL) with no statistically detectable differences among treatments (overall $P \geq 0.05$; linear $P = 0.41$, quadratic $P = 0.85$; panel E).

Serum adiponectin displayed a subtle quadratic-like pattern (overall $P > 0.05$ but numerically highest at SS3; linear $P = 0.68$, quadratic $P = 0.09$; panel F), increasing from $\approx 7.23 \pm 0.19$ μ g/mL in SS0 to $\approx 8.12 \pm$

0.19 $\mu\text{g/mL}$ in SS3 before declining slightly to $\approx 7.62 \pm 0.20 \mu\text{g/mL}$ in SS6. Serum IGF-1 concentration increased with safflower inclusion (overall $P < 0.01$; linear $P = 0.004$, quadratic $P = 0.12$; panel G), rising from $\approx 158.5 \pm 5.1 \text{ ng/mL}$ in SS0 to $\approx 180.1 \pm 5.0 \text{ ng/mL}$ in SS3 and plateauing at $\approx 182.1 \pm 5.0 \text{ ng/mL}$ in SS6, representing an overall increase of $\approx 15\%$ that stabilized beyond the 3% inclusion level.

Discussion

The incorporation of graded levels of cracked safflower seeds as a lipid source in the diets of lactating fat-tailed Turki-Qashqaei ewes elicited nonlinear physiological responses, underscoring the complex interplay between ruminal biohydrogenation, post-absorptive metabolism, and endocrine regulation in ruminants adapted to semi-arid environments. Dietary lipid supplementation is a common strategy to enhance energy density and supply essential unsaturated fatty acids (UFA) during lactation, a period of heightened metabolic demand (Cannas and Pulina, 2008; Toral et al., 2018). However, in small ruminants, particularly fat-tailed breeds, the efficiency of lipid utilization is constrained by ruminal microbial modifications and systemic adaptive mechanisms (Buccioni et al., 2012; Carreño et al., 2019). The current findings align with recent studies demonstrating that increased UFA supply, such as linoleic acid from safflower, does not yield strictly proportional productive outcomes but instead provokes adaptive shifts in nutrient partitioning and metabolic homeostasis (Toral et al., 2019; Vargas-Bello-Pérez et al., 2025). These responses are particularly relevant for fat-tailed sheep, which exhibit distinct energy storage strategies in the tail adipose depot, enabling resilience to nutritional fluctuations (Xu et al., 2023; Mousaie et al., 2025).

The lack of effect on dry matter intake (DMI) despite elevated ether extract (EE) and linoleic acid intake is consistent with observations in lactating ewes fed lipid-supplemented diets, where moderate EE levels (up to 4.5% DM) do not depress intake due to minimal disruption of rumen function (Ma et al., 2025; Lunsin et al., 2020). In contrast, higher lipid inclusions often inhibit DMI through ruminal distension or altered palatability (Oguz et al., 2014). The linear increase in EE and linoleic acid intake, amounting to a 182% rise in linoleic acid per kg metabolic body weight from SS0 to SS6, reflects the intended escalation in lipid substrate availability without compensatory reductions in forage intake. This pattern supports the notion that fat-tailed sheep can accommodate moderate lipid increments without invoking hypophagic responses, possibly due to their evolutionary adaptation to variable feed resources (Gao et al., 2025; Ma et al., 2025). However, the decline in digestible DMI per metabolic body weight at higher safflower levels indicates a trade-off, where increased lipid supply impairs fiber utilization, a common consequence of UFA interference with rumen microbes (Buccioni et al., 2012; Toral et al., 2024).

Apparent total-tract digestibility exhibited negative responses to graded safflower inclusion, with reductions in dry matter (DM), organic matter (OM), EE, neutral detergent fiber (NDF), and acid detergent fiber (ADF) digestibility, particularly pronounced at 6% inclusion. These findings corroborate extensive evidence that dietary UFA, especially linoleic acid, inhibit fiber-degrading bacteria through micelle formation and membrane disruption, thereby depressing ruminal fermentation and digestibility (Jenkins et al., 2008; Buccioni et al., 2012). Recent meta-analyses in small ruminants confirm that lipid

supplementation at 2–6% DM linearly decreases NDF and ADF digestibility by 5–10%, aligning with the 9% and 8.7% reductions observed here for NDF and ADF, respectively (Carreño et al., 2019; Ma et al., 2025). The more substantial drop in EE digestibility (7.7% from SS0 to SS6) likely stems from incomplete ruminal biohydrogenation and soap formation, limiting micellar solubilization in the small intestine (Palmquist and Jenkins, 2017; Toral et al., 2019). Notably, crude protein digestibility remained unaffected, consistent with isonitrogenous diet formulation and minimal lipid-protein interactions in ruminants (Oguz et al., 2014; Vargas-Bello-Pérez et al., 2025). In fat-tailed breeds, this digestibility pattern may reflect an adaptive prioritization of energy conservation, where reduced fiber breakdown minimizes heat increment in semi-arid climates (Xu et al., 2023; Ettoumia et al., 2022).

Rumen fermentation parameters were largely unaltered, with stable pH, ammonia-N, total volatile fatty acids (VFA), and acetate:propionate ratio across treatments. This resilience contrasts with reports of UFA-induced shifts toward propionate production and reduced acetate due to inhibited cellulolytic bacteria (Buccioni et al., 2012; Toral et al., 2018). The cracked form of safflower may have mitigated rumen disruption by slowing lipid release, as physical processing influences biohydrogenation kinetics (Carreño et al., 2019; Ma et al., 2025). The numerical decline in acetate:propionate ratio (7.6% from SS0 to SS6) hints at subtle glucogenic shifts, potentially linked to partial biohydrogenation intermediates inhibiting acetate-producing microbes (Toral et al., 2019). In fat-tailed sheep, such stability underscores metabolic adaptation, where tail fat mobilization buffers against ruminal perturbations during lactation (Li et al., 2018; Gao et al., 2025). Recent genomic studies reveal enriched mitochondrial oxidative phosphorylation in fat-tailed adipose tissues, suggesting enhanced energy efficiency that complements ruminal homeostasis (Xu et al., 2023; Ma et al., 2025).

The increase in milk fat yield and lipid utilization efficiency (milk fat per EE intake) with safflower inclusion, coupled with decreased linoleic utilization efficiency (milk long-chain fatty acids per linoleic intake), highlights nonlinear metabolic regulation. Milk fat yield rose by 26.7% from SS0 to SS6, consistent with UFA supplementation enhancing *de novo* synthesis and preformed fatty acid incorporation in sheep milk (Toral et al., 2018; Lunsin et al., 2020). However, the nonlinear efficiency indices reflect ruminal biohydrogenation constraints, where extensive saturation of linoleic acid (up to 90%) limits mammary uptake of long-chain UFA (Buccioni et al., 2012; Toral et al., 2019). The continued increase in lipid utilization efficiency despite the decline in linoleic acid utilization efficiency suggests that a progressively smaller proportion of dietary linoleic acid was recovered in milk long-chain fatty acids, possibly due to enhanced ruminal biohydrogenation, preferential oxidation of absorbed UFA, or partitioning of fatty acids toward body lipid reserves rather than mammary secretion (Palmquist and Jenkins, 2017; Vargas-Bello-Pérez et al., 2025). In fat-tailed breeds, this may involve prioritized tail fat deposition as an energy reserve, aligning with genomic evidence of upregulated lipid biosynthesis genes in tail adipose (Li et al., 2018; Xu et al., 2023). Recent studies in Awassi ewes show similar nonlinear milk fat responses to safflower, attributing them to biohydrogenation intermediates like conjugated linoleic acid (CLA) modulating mammary lipogenesis (Carreño et al., 2019; Ma et al., 2025).

Serum biochemical alterations provide insight into systemic metabolic adaptation. The 11% glucose reduction at SS6, without BHB changes, indicates enhanced lipid mobilization without ketosis, typical in early lactation ruminants (Nafikov et al., 2013; Mousaie et al., 2025). Increased NEFA (107%) and TG (86%) at higher safflower levels reflect lipolysis and hepatic VLDL export, respectively, as adaptive responses to UFA overload (Li et al., 2019; Galsgaard et al., 2019). Elevated total protein and albumin (5.8–6.4%) suggest improved hepatic function or reduced inflammation, consistent with UFA's anti-inflammatory effects (Bradford et al., 2008; Heppner et al., 2010). In fat-tailed sheep, these profiles may facilitate tail fat utilization during energy deficits, as evidenced by stable BHB despite glucose decline (Ettoumia et al., 2022; Gao et al., 2025).

Endocrine responses further elucidate adaptive mechanisms. The 15% linear increase in EE intake corroborates digestibility findings, while the 60% glucagon surge at SS6 implies threshold activation for lipolysis and gluconeogenesis, countering glucose decline (Li et al., 2018; Galsgaard et al., 2019). Stable insulin aligns with ruminant lipid metabolism, where glucagon dominates hepatic regulation (Heppner et al., 2010; Nafikov et al., 2013). Unchanged leptin and prolactin suggest minimal adiposity signaling shifts, despite increased lipid supply (Bradford et al., 2008). The quadratic adiponectin pattern (peak at SS3) may reflect transient insulin sensitization, while the 15% IGF-1 increase plateauing at SS3 supports mammary proliferation and lactation persistence (Scatà et al., 2010; Zheng et al., 2025). In sheep, IGF-1 correlates with milk yield during energy challenges (Simonov et al., 2021), and its stabilization here indicates optimized nutrient partitioning in fat-tailed breeds (Forsyth et al., 1992).

Fat-tailed sheep's metabolic adaptations are evident in these responses. Tail fat serves as a dynamic energy buffer, with genomic studies showing enriched ECM remodeling and lipid metabolism genes for rapid mobilization (Li et al., 2018; Xu et al., 2023). The nonlinear patterns observed—e.g., digestibility declines without rumen disruption, efficiency plateaus—highlight resilience, where biohydrogenation limits systemic UFA overload, and endocrine shifts (glucagon, IGF-1) prioritize survival over maximal production (Ma et al., 2025; Gao et al., 2025). Compared to thin-tailed breeds, fat-tailed ewes exhibit lower inflammation and stable homeostasis under lipid stress (Alves et al., 2013; Ettoumia et al., 2022). These traits inform sustainable feeding in arid regions, where safflower residues offer economical lipid sources (Vargas-Bello-Pérez et al., 2025; Mousaie et al., 2025).

Conclusion

Graded dietary inclusion of cracked safflower seeds (0–6% DM) in lactating fat-tailed Turki-Qashqaei ewes induced dose-dependent metabolic and endocrine adaptations without substantially disrupting voluntary intake or rumen fermentation stability. Moderate supplementation improved milk fat yield and lipid utilization efficiency, while higher inclusion reduced nutrient digestibility and altered systemic metabolites, reflecting enhanced lipid mobilization. The nonlinear nature of these responses underscores the importance of accounting for both ruminal biohydrogenation and post-absorptive constraints when formulating lipid-supplemented diets for fat-tailed breeds during lactation. Further

research should address the role of tail fat mobilization and evaluate longer-term effects on energy balance and lactation performance.

Declarations

Ethics approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Yasouj University (Approval No. 4014441004) in compliance with the Iranian Council of Animal Care (1995) guidelines. Animal welfare was maintained under professional veterinary and scientific supervision, with all handling procedures designed to minimize injury and stress.

Conflict of interest

The authors affirm that there are no financial, personal, professional, or intellectual conflicts of interest that could have influenced the outcomes or interpretation of this work. No funding or financial support was received from any entity that could gain or lose from the publication of these results. Furthermore, no authors have any affiliations with or involvement in organizations with a financial or non-financial interest in the subject matter discussed. All authors declare that the research was conducted and the manuscript was prepared with complete scientific objectivity and independence.

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Author contributions

S.F.: Investigation, Data collection, Resources.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

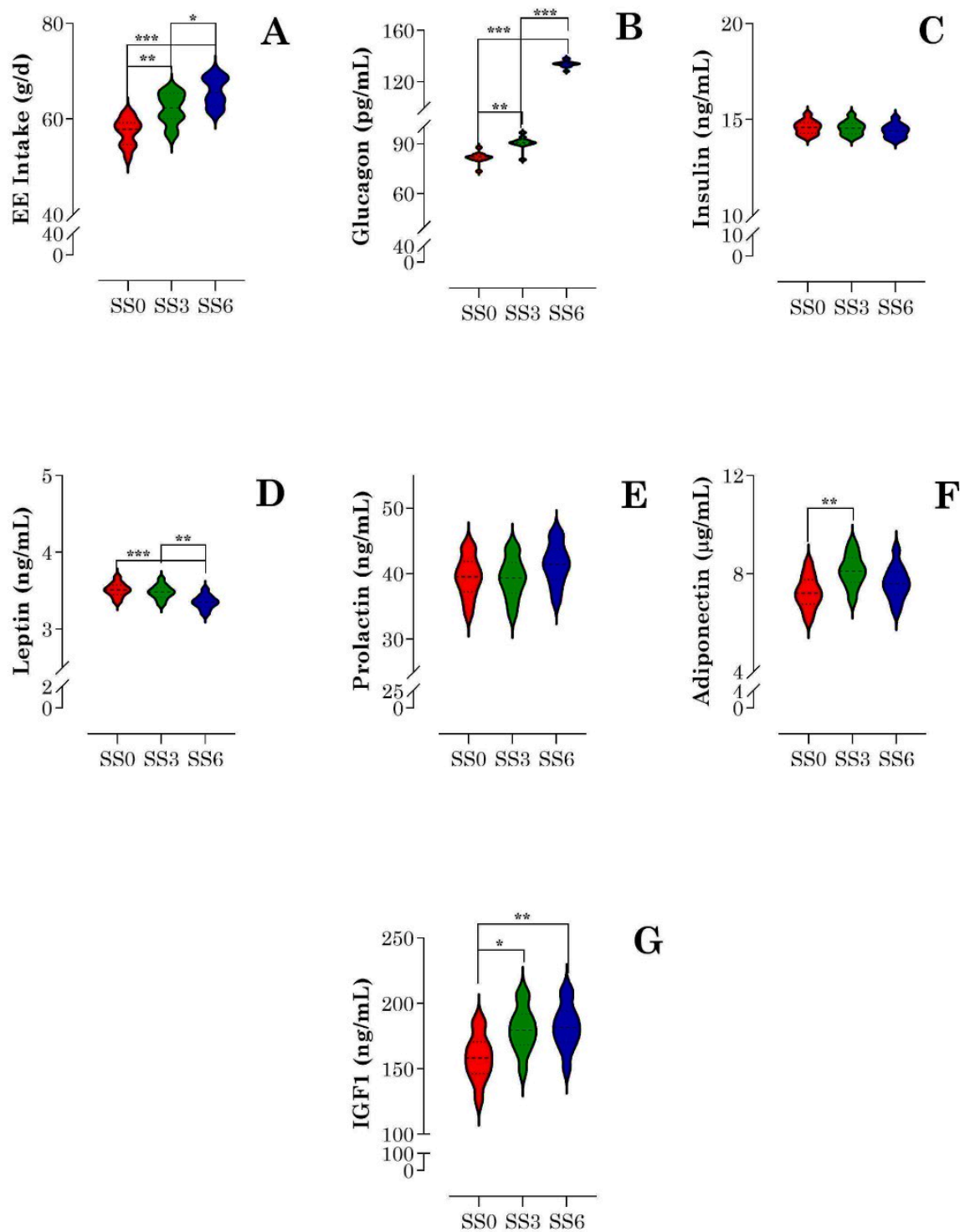


Figure 1

Effect of graded dietary inclusion of cracked safflower (0% [SS0], 3% [SS3], and 6% [SS6] of dry matter) on selected intake and serum endocrine parameters in lactating fat-tailed Turki-Qashqaei ewes. Panels show violin plots of (A) ether extract (EE) intake (g/d), (B) serum glucagon (pg/mL), (C) serum insulin (ng/mL), (D) serum leptin (ng/mL), (E) serum prolactin (ng/mL), (F) serum adiponectin (μ g/mL), and (G) serum IGF-1 (ng/mL). Violins represent the distribution of individual ewe values (n = 12 per treatment),

with embedded box plots or means as appropriate. Asterisks above violins indicate significant treatment effects from orthogonal polynomial contrasts: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; absence of asterisks denotes non-significant effects ($P \geq 0.05$). Values are least squares means $\pm$ pooled standard error of the mean (where applicable) or raw distributions.