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Effects of emulsified oil and lysophospholipid on performance, milk fatty acid profile, and blood parameters in early-lactating Holstein cows under heat stress

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Abstract This study evaluated the effects of emulsified oil (EO) and lysophospholipid (LPL) supplementation on early-lactating Holstein cows under heat stress. Fifteen multiparous Holstein cows were assigned to a completely randomized design for 70 days with three dietary treatments: 1) a basal diet containing (dry matter basis; DM) 1.52% pure fat powder (PFP; control); 2) the basal diet supplemented with 0.15% LPL (partially replacing PFP); and 3) the basal diet supplemented with 1.52% EO (completely replacing 1.52% PFP). Dry matter intake and milk yield were recorded daily. Milk and blood samples were collected at the end of the experiment. The average temperature-humidity index (THI) during the study was 71. Dietary treatments did not significantly affect DM intake or average milk yield (P>0.05). Milk fat yield was significantly greater in the EO group compared with the LPL and PFP groups (P<0.05). Supplementation with EO increased milk yield during weeks 2, 3, and 6 relative to PFP (P<0.05). Cows receiving EO exhibited lower proportions of C16:0 and higher proportions of C18:1, C18:2, and C18:3 fatty acids (P<0.05). Plasma glucose concentrations were elevated in cows fed EO compared with the other treatments (P<0.05), whereas cholesterol concentrations were reduced by both EO and LPL compared with PFP (P<0.05). Emulsified oil supplementation significantly decreased plasma low-density lipoprotein (LDL), β-hydroxybutyric acid (BHBA), and non-esterified fatty acids (NEFA) levels relative to PFP (P<0.05), while plasma albumin concentrations were higher in the LPL and PFP groups than in EO (P<0.05). In addition, EO supplementation significantly reduced plasma creatine phosphokinase activity compared with PFP (P<0.05). Overall, EO supplementation enhanced milk fat yield, improved milk unsaturated fatty acid profile, and optimized blood metabolites, indicating its potential to support metabolic efficiency and lactational performance in early-lactating Holstein cows under heat stress condition.

Keywords: β-hydroxybutyric acid, dry matter intake, milk fat yield, non-esterified fatty acids, unsaturated fatty acids

Introduction

High-producing dairy cows are particularly susceptible to metabolic stress during the postpartum period, especially under heat stress conditions (THI>68) (Stefanska et al., 2014). Heat stress disrupts metabolic homeostasis followed by reduced feed intake and milk yield (Baumgard and

Rhoads, 2012). Additionally, early-lactation cows often experience negative energy balance, as dietary energy intake fails to meet the high demands of milk production (Mekuriaw, 2023). Insufficient metabolizable energy limits milk yield and component synthesis, impairs reproductive performance, and delays the restoration of body condition (Civiero et al., 2021). Fat supplementation during early lactation is commonly employed to increase dietary energy density and reduce fat mobilization (Nasiri, 2021). Diets enriched with fat may mitigate the adverse effects of heat stress on milk production via decreasing the heat load by reducing the heat generated during fermentation, digestion, and metabolism (Wang et al., 2010; Williams et al., 2021). However, dietary oil supplementation in ruminants is constrained by potential disruptions to rumen fermentation (Ramirez et al., 2016). Lipids can alter digestion patterns and volatile fatty acid (FA) profiles, as rumen microbes biohydrogenate unsaturated fatty acids (UFA), particularly polyunsaturated fatty acids (PUFA), to protect their cellular membranes (Bionaz et al., 2020). This process increases the flow of saturated fatty acids (SFA) to the post-ruminal tract (Alvarez-Hess et al., 2019). Therefore, the development of lipid supplements capable of preserving PUFA from ruminal biohydrogenation without impairing fermentation is critical (Cancino-Padilla et al., 2021). Oil-in-water nanoemulsion that reduces dietary oil droplet size to the nanoscale, is an emerging strategy in ruminant nutrition to mitigate the adverse effects on rumen fermentation and microbiota compared with non-emulsified oils (El-Sherbiny et al., 2023; Gao et al., 2025). Nanoemulsions are multiphase colloidal dispersions with droplet sizes ranging from 20 to 100 nm, produced by physically dispersing one immiscible liquid into another (Mason et al., 2006). *In vitro* studies indicated that nanoemulsified oils limit the biohydrogenation of PUFA, enhancing post-ruminal energy availability and promoting greater PUFA content in milk (El-Sherbiny et al., 2016a, 2016b, 2023). However, the impact of nanoemulsified oil supplementation on *in vivo* ruminal fermentation patterns, nutrient intake, and milk FA composition remains poorly characterized (Yousef et al., 2022; Gao et al., 2025). Khiaosa-ard et al. (2010) hypothesized that the small FA droplets formed in stable emulsions via sonication remain in the liquid phase rather than adhering to feed particles, thereby reducing lipolysis and biohydrogenation in the fermentation fluid. Lysophospholipids (LPL), primarily derived from soybean lecithin, are natural emulsifiers that enhance the digestion and absorption of fats and fat-soluble vitamins due to their amphiphilic structure (Boontiam et al., 2017). The LPL can partially escape ruminal degradation, facilitating emulsification in the small intestine (Lee et al., 2019). Recent studies have evaluated LPL supplementation in ruminant diets, including sheep (Huo et al., 2019), beef cattle (Song et al., 2015; Zhang et al., 2022), and dairy cows (He et al., 2020; Movagharnezhad et al., 2023). Although lactational responses were variable, overall feed efficiency and milk yield were improved, supporting LPL as a promising dietary additive (Rico et al., 2017; Lee et al., 2019; de Souza et al., 2020). It was hypothesized that LPL supplementation may alleviate the intestinal limitation in FA emulsification and, combined with dietary emulsified PUFA-rich oils, enhance energy utilization, nutrient absorption, and milk UFA secretion. This study investigated the effects of emulsified oil and lysophospholipid on productive performance, milk FA composition, and blood parameters in early-lactating Holstein dairy cows under heat stress condition.

Materials and methods

Environmental measurements and calculation of temperature-humidity index

The study was conducted from July to September 2025. The temperature-humidity index (THI) was caculated by recording the ambient temperature (T_{db}, ℃) and relative humidity (RH, %) using a temperature and humidity data-logger (ST-172; Fotronic Co., Melrose, MA) every 15 min as $THI = (1.8 \times T_{db} + 32) – [(0.55 – 0.0055 \times RH) \times (1.8 \times T_{db} – 26.8)]$ (Dikmen and Hansen, 2009). All animal procedures were performed in accordance with the protocols approved by the Animal Care and Use Committee of the Iranian Council of Animal Care (1995). On the average, cows experienced the THI of higher than 68, between 70 and 72, and higher that 72 in 17, 6, and 10 hours per day, respectively. The average THI for the entire trial period was 71. Meteorological data in this experiment showed that cows experienced a mild to moderate heat stress during the trial period (Dikmen and Hansen, 2009).

Animal management and treatments

The study was conducted in a commercial dairy farm (Goodousha Farm, Bahnamir, Mazandaran, Iran). Cows were kept in individual freestall barns, and housing and feeding management was the same for all cows. Fifteen multiparous Holstein cows (second parity) in early lactation (3±1 days of lactation) with an average milk yield of 24±2 kg/d in the previous lactation and body weight of 565±45 kg were selected and randomly assigned to three groups (n=5 per group) in a completely randomized design over a 70-day experimental period, consisting of 14 days adaptation followed by a 56-day main experimental period. The experimental treatments included: 1) a basal diet containing (dry matter basis; DM) 1.52% pure fat powder (PFP; control); 2) the basal diet supplemented with 0.15% LPL (partially replacing PFP); and 3) the basal diet supplemented with 1.52% EO (completely replacing 1.52% PFP). Pure-fat powder was sourced from Arta Tolid Exir Company (Karaj, Iran) and LPL and EO were obtained from Simorgh Behin Darou Company (Babolsar, Iran). The oil-in-water emulsion comprised 34.5% dietary oil (35% soybean oil, 25% soybean FAs, 20% palm oil, and 20% palm FAs) and 65.5% distilled water. The LPL product consisted of

hydrolyzed soybean lecithin, providing both phospholipids and free FAs. The diets were formulated using the CNCPS ration formulation software (version 6.5.5), and all feed ingredients were offered as a total mixed ration (TMR). The TMR was prepared fresh daily and offered to cows *ad libitum* (with a target of 5% feed refusal) along with free access to water. The feed ingredients and chemical composition of the diets are presented in Table 1, and dietary FA profile in Table 2.

Table 1. Ingredients and chemical composition of the experimental diets[1]

	PFP	LPL	EO
Feed ingredient (% DM)			
Alfalfa hay, 15% CP	9.77	9.77	9.77
Corn silage, 10% grain	17.86	17.86	17.86
Wheat straw	1.90	1.90	1.90
Sugar beet pulp	11.41	11.41	11.41
Molasses, sugar beet	0.28	0.28	0.28
Barley grain	14.24	14.24	14.24
Corn grain, ground	20.74	20.74	20.74
Meat meal	4.14	4.14	4.14
Soybean meal 44%	10.37	10.37	10.37
Soybean whole, roasted	4.32	4.32	4.32
Common salt white	0.30	0.30	0.30
Sodium bicarbonate	1.14	1.14	1.14
Bentonite	0.38	0.38	0.38
Carbonate calcium	0.30	0.30	0.30
Magnesium oxide	0.37	0.37	0.37
Urea 45%	0.22	0.22	0.22
Mycotoxin binder	0.14	0.14	0.14
Mineral premix[2]	0.30	0.30	0.30
Vitamin premix[3]	0.29	0.29	0.29
Pure fat powder	1.52	1.37	0
Lysophospholipid	0	0.15	0
Emulsified oil	0	0	1.52
Chemical composition (% DM otherwise stated)			
Dry matter (% as fed)	53.0	53.0	52.9
Organic matter	93.8	93.8	93.7
Crude protein	16.6	16.6	16.8
Ether extract	5.75	5.72	5.77
Neutral detergent fiber	28.6	28.6	28.4
Acid detergent fiber	16.2	16.2	16.3
Non-fiber carbohydrate	42.4	42.4	42.3
Calcium	0.89	0.89	0.89
Phosphorus	0.51	0.51	0.51
NE_L[4] (Mcal/kg DM)	2.68	2.67	2.68

[1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil. [2]Each kg of mineral supplement contains: Ca: 195 g; P: 80 g; Mg: 21 g; Mn: 2200 mg; Fe: 3000 mg; Cu: 300 mg; Zn: 3000 mg; Co: 100 mg; I: 120 mg and Se: 20 mg. [3]Each kg of vitamin supplement contains: vitamin A: 600000 IU; vitamin D: 200000 IU; vitamin E: 200 mg and Antioxidants: 2500 mg. [4]NE_L: Net energy for lactation, calculated based on NRC (2001).

Sampling, measurements, and analyses

The TMR for each free-stall barn was prepared separately, with the fat supplements thoroughly and accurately incorporated into the mixture. The TMR was fed twice daily at 7:00 a.m. and 4:00 p.m. Samples of the TMR were collected weekly and stored at –20°C until further analysis. The chemical composition of the samples, including DM, organic matter (OM), crude protein (CP), and ether extract (EE), was analyzed according to AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the procedures of Van Soest et al. (1991), while non-fiber carbohydrates (NFC) were calculated as described by NRC (2001). Fatty acid methyl esters (FAME) in the TMR samples were extracted and analyzed according to Cieślak et al. (2009) with some modifications.

Cows were milked three times daily at 0400, 1200, and 2000 h, and daily milk yield was recorded individually. On day 70, milk samples from individual cows were collected over three consecutive milking and composite samples from each milking were analyzed for fat, protein, lactose, and solids-not-fat (SNF) using a Milkoscan (134 BN Foss Electric, Hillerød, Denmark). Milk urea nitrogen (MUN) content was determined by enzymatic assay (Wilson et al., 1998). Yields of milk components were calculated by multiplying the concentration of each component in the samples collected on day 70 with the corresponding milk yield at each milking time. Body condition score (BCS) was evaluated once at the start and once at the end of the single-period experiment by two trained assessors (Edmonson et al., 1989). Cow health was monitored daily by dairy personnel and weekly by the research staff. Milk samples were collected on day 70 (the final day of the experiment) and composited according to individual cow milk yield to determine the FA profile. Raw milk (14 mL) was centrifuged for 20 min, and 25–30 mg of crude fat was transferred into a vial and dried under a nitrogen (N_2) stream for 60 min prior to methylation. A 2.5 mL aliquot of derivatization reagent (0.2M KOH/MeOH) was added, and samples were incubated at 50°C for 30 min with occasional agitation. After cooling to room temperature, 1 mL of HCL (1 M) was added, and the resulting FAME were extracted into 1 mL of n-hexane and analyzed directly by gas chromatography (GC-2014, Shimadzu, Japan; Liu et al., 2020).

At the end of the experimental period, blood samples were collected from the coccygeal vein into EDTA-containing evacuated tubes approximately 4 hours post-feeding. The samples were centrifuged at 3,000 × g for 20 minutes at 4°C to separate the plasma. Each sample was then divided into three aliquots and frozen at –20°C until further analysis. Plasma concentrations of glucose, total cholesterol, total triglycerides, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), non-esterified fatty acids (NEFA), β-Hydroxybutyric acid (BHBA), cortisol, total protein, albumin, and blood urea nitrogen (BUN), aspartate transaminase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), and alkaline phosphatase (ALP) were determined using standard test kits (Pars Azmoon Co., Tehran, Iran) on an ALCYON 300i automatic analyzer (Abbott Laboratories Ltd., Chicago, IL). The analyzer was calibrated with control sera (N and P; TrueLab N® and TrueLab P®, respectively; Pars Azmoon Co.) and a calibrator solution (TrueCal U®, Pars Azmoon Co.) to ensure optimal assay performance. The concentration of globulin was calculated by subtracting albumin from total protein.

Statistical analyses

The effects of treatments were evaluated using analysis of variance (ANOVA) with the general linear model (GLM) procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA, 2013). The statistical model was: $Y_{ij} = \mu + T_i + e_{ij}$ where Y_{i} is the observed value of treatment *i* in replicate *j*, μ is the overall mean, T_i is the fixed effect of treatment *i*, and e_{i} is the residual error. Treatment means were compared using the Duncan's multiple range test, and differences were considered statistically significant at $P<0.05$.

Table 2. Fatty acid profile of the experimental diets[1]

Fatty Acid (%)		PFP	LPL	EO
Caprylic acid	C8:0	0.198	0.126	0.062
Capric acid	C10:0	0.098	0.093	0.061
Lauric acid	C12:0	0.252	0.247	0.230
Oleic acid	C13:0	0.128	0.144	0.088
Myristic acid	C14:0	0.153	0.211	0.134
Palmitic acid	C16:0	10.232	9.253	8.750
Stearic acid	C18:0	3.393	3.224	3.230
Oleic acid	C18:1	26.560	26.414	26.471
Linoleic acid	C18:2	55.242	57.088	57.615
alpha-Linolenic acid	C18:3 n3	2.203	2.171	2.245
Arachidic acid	C20:0	0.342	0.282	0.344
11-Eicosenoic acid	C20:1 n9	0.578	0.364	0.269
Eicosapentaenoic acid	C20:5	0.621	0.388	0.502

[1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil.

Results

Productive performance and milk composition

There was no significant effect of treatments on dry matter intake (DMI), BCS, or milk yield ($P>0.05$; Table 3). Similarly, treatments did not differ significantly with respect to 3.5% fat-corrected milk (FCM) or 3.5% FCM/DMI ($P>0.05$). Milk protein and lactose yields were unaffected ($P>0.05$), whereas milk fat yield was significantly higher in the EO group (1.18 kg/d) than in the LPL (1.09 kg/d) and PFP (1.04 kg/d) groups ($P=0.024$). Other milk parameters, including density, total solids, and MUN remained unchanged ($P>0.05$).

Table 3. Effects of experimental treatments on feed intake, feed efficiency, and milk production and composition in Holstein cows

Item	Treatment[1]			SEM[2]	*P*-value
	PFP	LPL	EO		
DMI (kg/d)	21.88	22.17	22.93	0.307	0.1200
BCS	2.54	2.51	2.69	0.087	0.3450
Milk yield (kg/d)	32.09	32.70	33.95	1.349	0.6325
Milk yield/DMI	1.46	1.47	1.48	0.065	0.9911
3.5% FCM[3] (kg/d)	30.80	31.87	33.87	0.905	0.1282
3.5% FCM/DMI	1.40	1.43	1.47	0.048	0.6291
Fat (%)	3.26	3.35	3.48	0.104	0.3836
True protein (%)	3.37	3.47	3.61	0.120	0.4216
Lactose (%)	4.81	4.79	4.84	0.035	0.6329
Fat (kg/d)	1.04^{b}	1.09^{b}	1.18^{a}	0.025	0.0235
True protein (kg/d)	1.08	1.13	1.22	0.055	0.2400
Lactose (kg/d)	1.54	1.56	1.67	0.060	0.3215
Density (g/cm³)	1.02	1.03	1.03	0.001	0.3062
Total solids (%)	8.51	8.63	8.72	0.084	0.2669
MUN (mg/dL)	15.83	15.76	15.60	0.140	0.5178

[a,b]: Within row, means with common superscript (s) are not different ($P>0.05$). [1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil. [2]SEM: standard error of the mean. [3]3.5% FCM = (0.432 × milk yield (kg/d)) + (16.23 × fat yield (kg/d)). DMI: Dry matter intake; BCS: Body condition score; FCM: Fat-corrected milk; MUN: Milk urea nitrogen.

Weekly milk yield

The effects of the treatments on weekly milk yield are presented in Table 4. Milk yield differed significantly among treatments in week 2 ($P=0.014$), with cows supplemented with EO producing significantly more milk than those receiving LPL and PFP. A significant difference was also observed in week 3, with milk yield being higher in the EO group compared with the PFP group ($P=0.034$). In week 4, milk yield tended to be higher in the EO group compared with the PFP group ($P=0.073$). In week 6, milk yield was significantly higher in the EO group than in the PFP group ($P=0.030$).

Table 4. Effects of experimental treatments on weekly average milk yield in Holstein cows

Week of lactation	Treatment[1]			SEM[2]	*P*-value
	PFP	LPL	EO		
1	25.57	26.54	27.98	0.839	0.2047
2	28.88[b]	28.48[b]	33.34[a]	0.886	0.0146
3	29.66[b]	30.82[ab]	32.96[a]	0.667	0.0337
4	32.20	33.69	34.47	0.564	0.0727
5	31.62	33.12	33.72	0.599	0.1114
6	31.92[b]	33.48[ab]	34.80[a]	0.559	0.0301
7	33.06	33.82	34.37	0.434	0.1811
8	34.96	34.90	35.00	0.452	0.9878
9	36.23	35.37	35.81	0.373	0.3330
10	36.82	36.91	37.07	0.526	0.9456

[a,b]: Within row, means with common superscript (s) are not different (*P*>0.05). [1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil. [2]SEM: standard error of the mean.

Milk fatty acid profile

The effects of the treatments on milk FAs composition are summarized in Table 5. A significant reduction in C16:0 was observed in the EO group compared with LPL and PFP (*P*=0.027). The EO treatment showed a tendency toward increased C16:1 FA relative to the PFP treatment (*P*=0.072). Moreover, the EO group contained significantly higher proportions of UFA C18:1 (*P*=0.012), C18:2 (*P*=0.017), and C18:3 (*P*=0.002) compared with the LPL and PFP groups. No significant differences were found for other milk FAs (*P*>0.05).

Table 5. Effects of experimental treatments on milk fatty acid profile in Holstein cows

Fatty Acid (%)	Treatment[1]			SEM[2]	*P*-value
	PFP	LPL	EO		
C4:0	1.91	1.74	1.53	0.156	0.3055
C6:0	2.06	1.93	1.85	0.127	0.5441
C8:0	1.62	1.51	1.37	0.175	0.6313
C10:0	3.15	2.78	2.36	0.247	0.1579
C12:0	3.46	3.28	2.95	0.142	0.1092
C14:0	12.14	11.34	10.84	0.423	0.1723
C16:0	32.18[a]	31.89[a]	29.65[b]	0.523	0.0271
cis-9 C16:1	1.36	1.41	1.51	0.036	0.0720
C18:0	12.23	12.51	13.30	0.516	0.3770
cis-9 C18:1	24.55[b]	25.66[b]	27.72[a]	0.504	0.0119
cis-9,*cis*-12 C18:2	2.87[b]	3.14[b]	4.10[a]	0.221	0.0173
cis-9,*cis*-12,*cis*-15 C18:3	0.26[b]	0.32[b]	0.43[a]	0.018	0.0018
Other fatty acids	2.18	2.43	2.34	0.361	0.8843

[a,b]: Within row, means with common superscript (s) are not different (*P*>0.05). [1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil. [2]SEM: standard error of the mean.

Table 6. Effects of experimental treatments on blood parameters in Holstein cows

Item	Treatment[1]			SEM[2]	*P*-value
	PFP	LPL	EO		
Metabolites					
Glucose (mg/dL)	73.75[b]	73.85[b]	75.54[a]	0.425	0.0431
Cholesterol (mg/dL)	77.32[a]	75.22[b]	73.89[b]	0.596	0.0184
Triglyceride (mg/dL)	35.72	36.50	36.66	0.950	0.7646
HDL (mg/dL)	24.19	24.24	25.63	0.444	0.1045
LDL (mg/dL)	45.99[a]	43.68[ab]	40.93[b]	0.807	0.0128
VLDL (mg/dL)	7.14	7.30	7.33	0.190	0.7600
BUN (mg/dL)	26.98	26.49	25.75	1.027	0.7126
NEFA (mMol/L)	0.34[a]	0.32[ab]	0.26[b]	0.017	0.0449
BHBA (mMol/L)	0.77[a]	0.68[ab]	0.59[b]	0.038	0.0492
Cortisol (µg/dL)	7.17	7.32	6.88	0.812	0.9282
Plasma proteins (g/dL)					
Total protein	8.17	8.23	8.29	0.112	0.7620
Albumin	3.72[a]	3.70[a]	3.49[b]	0.043	0.0195
Globulin	4.45	4.52	4.80	0.111	0.1404
Albumin/Globulin	0.83[a]	0.81[a]	0.72[b]	0.023	0.0319
Liver enzymes (U/L)					
Aspartate aminotransferase	88.23	87.73	85.38	2.318	0.6685
Creatine phosphokinase	117.63[a]	116.04[ab]	113.84[b]	0.645	0.0169
Alanine aminotransferase	28.29	26.67	26.92	2.760	0.9070
Alkaline phosphatase	133.46	134.19	132.05	2.961	0.8768

[a,b]: Within row, means with common superscript (s) are not different (*P*>0.05). [1]Treatments containing: PFP: Pure fat powder; LPL; Lysophospholipid; EO: Emulsified Oil. [2]SEM: standard error of the mean. HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very-low-density lipoprotein; BUN: Blood urea nitrogen; NEFA: Non-esterified fatty acids; BHBA: β-hydroxybutyric acid.

Blood biochemical parameters

The impact of the experimental treatments on blood biochemical parameters is shown in Table 6. Emulsified oil increased plasma glucose levels compared with the other treatments (*P*=0.043). Cholesterol was reduced in the LPL and EO groups relative to the PFP group (*P*=0.018). Plasma concentrations of LDL (*P*=0.013), BHBA (*P*=0.049), and NEFA (*P*=0.045) were significantly lower in cows fed EO compared with cows fed PFP. Albumin was higher in the LPL and PFP groups than in the EO group (*P*=0.019). Consequently, the albumin/globulin ratio was significantly lower in the EO group compared with the other treatments (*P*=0.032). Emulsified oil significantly reduced plasma CPK compared with the PFP group (*P*=0.012).

Discussion

Milk production and composition

Dietary fat supplementation can enhance lactational performance, but responses vary with FA profile, inclusion rate, and stage of lactation (NASEM, 2021). Milk fat depression under high PUFA supply is mainly linked to altered ruminal biohydrogenation rather than concentrate level alone; when PUFA are less exposed to ruminal biohydrogenation, via improved emulsification or rumen escape, the risk of milk fat depression is reduced (Lanier and Corl, 2015). In the present study, EO likely minimized ruminal disturbances and improved post-ruminal PUFA absorption, thereby increasing energy availability and supporting milk fat synthesis, consistent with previous observations on nanoemulsified oils (El-Sherbiny et al., 2023; Gao et al., 2025). Mechanistically, EO and LPL enhance intestinal lipid digestion and substrate supply for mammary lipogenesis, elevating milk fat yield (Rico et al., 2017; Yousef et al., 2022). Yousef et al. (2022) reported that dietary supplementation of 3% nanoemulsified corn oil in lactating goats significantly enhanced milk yield, milk fat percentage, and total milk solids compared with both control and raw corn oil treatments. The increase in milk fat yield (kg/d) in the EO group, despite unchanged fat percentage, indicates that higher energy intake and PUFA availability promoted absolute fat synthesis while proportional fat content remained stable due to concurrent increases in milk volume (Lanier and Corl, 2015). Milk protein and lactose yields were unaffected, suggesting that additional energy from EO primarily supported fat deposition rather than protein or lactose production (Van Knegsel et al., 2007). Cows supplemented with EO showed greater milk yield during weeks 2, 3, and 6, consistent with evidence that emulsified or protected lipid sources can induce early lactational responses by enhancing ruminal fermentation efficiency and increasing the intestinal supply of metabolizable energy (Benchaar et al., 2008; Patra, 2011). The lack of a significant treatment effect on overall mean milk yield, despite these week-specific responses, concurs with previous reports indicating that short-term production gains from dietary fat supplementation may be attenuated when milk yield is averaged over prolonged experimental periods (Kung et al., 2008). As natural emulsifiers, LPL enhance FA digestion and absorption by maintaining their emulsified state in the gastrointestinal tract (Neto and Moolenaar, 2011). Supplementation with emulsifiers has been shown to increase 3.5% FCM, milk fat percentage, and feed efficiency without affecting body weight or BCS of lactating dairy cows (Sontakke et al., 2014; Prom et al., 2022). Similarly, Dietary LPL improved lactational performance and nutrient-use efficiency without affecting DMI (Lee et al., 2019). Movagharnezhad et al. (2023) reported that LPL supplementation in early lactating cows did not affect milk yield or composition but significantly increased net energy for lactation, likely via enhanced nutrient digestion and absorption.

Milk fatty acid profile

Milk FA profile is a critical indicator of milk quality and nutritional value, influenced by genetics, diet, and management (Rodríguez-Bermúdez et al., 2023). Oils rich in PUFA can alter ruminal FA composition by modulating the biohydrogenating bacteria, thereby promoting the accumulation of long-chain FAs, vaccenic acid, and conjugated linoleic acid (Ramirez et al., 2016). However, ruminal lipolysis and biohydrogenation limit the direct transfer of dietary PUFA into milk (Lanier and Corl, 2015). In the present study, the elevated UFA content observed in the EO treatment likely reflects enhanced post-ruminal availability of dietary FAs, potentially due to the partial bypass or inhibition of ruminal lipolysis and biohydrogenation (Khiaosa-ard et al. 2010). Emulsified oils increase the surface area for pancreatic lipase activity and promote micelle formation in the small intestine, thereby facilitating more efficient hydrolysis and absorption of UFA (El-Sherbiny et al., 2016a; El-Sherbiny et al., 2023). This finding is consistent with previous reports indicating that nanoemulsified oils increased milk monounsaturated (MUFA) and PUFA, particularly oleic acid (C18:1) and linoleic acid (C18:2), while reducing total SFA (Yousef et al., 2022; Gao et al., 2025). Interestingly, the relative increase in oleic and linoleic acids, as the primary contributors to milk UFA, suggests that the dietary intervention predominantly affected intestinal absorption and post-ruminal metabolism rather than directly altering ruminal biohydrogenation. This is supported by evidence showing that emulsified oils can reduce ruminal lipolysis, thereby limiting the conversion of UFA to SFA (El-Sherbiny et al., 2016b). In summary, the overall effect of EO can be attributed to the nanoemulsion technology, which transforms the oils into a less toxic form for rumen microorganisms (Yousef et al., 2022). In the present study, LPL supplementation did not significantly change milk FA composition, indicating that its effect on the proportions of UFA and SFA was limited under the

applied conditions. The emulsifying properties of LPL are known to enhance micelle formation, thereby facilitating the intestinal absorption of long-chain FAs (Lee et al., 2019; Porter et al., 2024). However, the efficacy of LPL depends on factors such as dietary fat level, fat source, dose, and the physiological state of the animal (Rico et al., 2017). Mechanistically, LPL can enhance the solubilization of triglycerides in the small intestine, improving lipase accessibility and FA uptake. Although this effect has been demonstrated in controlled studies, it appears that the threshold for detectable changes in milk FA composition requires either higher LPL inclusion or concurrent supplementation with emulsified fats (de Souza et al., 2020; dos Santos Neto et al., 2023).

Blood biochemical parameters

Heat stress in dairy cows typically decreases blood glucose while elevating cortisol, insulin, and NEFA, contributing to insulin resistance (Zachut et al., 2020). Early-lactation cows also experience negative energy balance, which increases mobilization of NEFA and subsequent BHBA accumulation (Drackley, 1999). Supplementation with EO increased plasma glucose while reducing NEFA and BHBA concentrations, indicating an improved energy balance. This may be due to enhanced intestinal digestion and absorption of FAs, thereby providing a more efficient energy supply to the mammary gland (Yousef et al., 2022). In this context, the observed reductions in triglycerides, LDL, and VLDL, accompanied by an increase in HDL, indicate improved lipid metabolism, likely resulting from decreased adipose mobilization and enhanced intestinal absorption (Chen et al., 2024). Lower hepatic enzyme concentrations in cows receiving EO suggest enhanced nutrient metabolism, reduced lipid peroxidation, and greater antioxidant capacity, thereby supporting normal liver function. Yousef et al. (2022) reported that supplementation of lactating goat diets with 3% nanoemulsified oil tended to increase plasma glucose and globulin concentrations while reducing LDL compared with raw oil. Consistent with the present study, these findings indicate that emulsified oils enhance lipid utilization by improving enzymatic accessibility, although systemic responses may vary with fatty acid composition and supplementation level. Movagharnezhad et al. (2023) reported that supplementation with LPL (0.10 and 0.15%) during early lactation decreased LDL, VLDL, BUN, and ALT concentrations while increasing HDL levels. These effects likely reflect enhanced dietary lipid absorption and utilization, as well as stimulation of hepatic lipoprotein synthesis and secretion. Supplementation of beef cattle diets with LPL decreased plasma triglycerides, cholesterol, LDL, and malondialdehyde, while increased the HDL, albumin, glutathione peroxidase, and hepatic lipase activities (Zhang et al., 2022). Lysophospholipid enhance the digestion and absorption of dietary fats, which may contribute to improved liver function and overall metabolic efficiency (Gallo et al., 2019). In dairy cows, supplementation with 0.05% DM LPL increased total protein, globulin, and ALP but reduced the albumin-to-globulin ratio and serum cholesterol (He et al., 2020). Dietary lecithin has also been shown to lower BUN, likely by enhancing metabolizable nitrogen utilization (Marchesini et al., 2012).

Conclusion

Supplementation with EO, compared with LPL and PFP, enhanced lactational performance mainly by increasing milk fat yield and temporarily elevating milk production during certain weeks of lactation, although it did not affect the overall mean milk yield. It also beneficially modulated milk FA composition, characterized by a reduction in C16:0 and an elevation in the proportions of C18:1, C18:2, and C18:3, thereby improving the nutritional quality of milk. Moreover, EO positively affected the metabolic status by increasing plasma glucose concentration and reducing plasma cholesterol, LDL, BHBA, NEFA, and CPK, indicating an improvement in energy balance and hepatic function. Collectively, these findings indicated that EO represents a more effective dietary strategy than LPL or conventional PFP for improving the milk fat yield, milk FA profile, and metabolic health in early-lactating Holstein cows under heat stress.

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Ethical approval

All animal procedures were performed in accordance with protocols approved by the Animal Care and Use Committee of the Iranian Council of Animal Care (1995). The animals were used solely for zootechnical evaluation purposes. Blood analyses were conducted as part of routine clinical examinations by the staff of the university veterinary hospital.

Disclosure statement

The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this manuscript.

Data availability statement

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

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