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Effects of lysophospholipids on performance, blood lipid parameters, milk composition, and the expression of lipogenic genes in early lactating Holstein dairy cows

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Abstract This study investigated the effects of lysophospholipid (LPL) supplementation at varying inclusion levels on blood parameters, milk production, milk fatty acid composition, and the expression of fat synthesis-related genes in Holstein dairy cows. A total of fifteen cows was randomly assigned to three dietary treatment groups: 1) a control diet with no LPL, 2) the control diet supplemented with 0.1% LPL based on dry matter, and 3) the control diet supplemented with 0.15% LPL based on dry matter. The experimental period consisted of a 14-day adaptation phase, followed by a 21-day data collection phase. The results indicated that the addition of LPL to the diet significantly enhanced the net lactation energy. Furthermore, blood lipid profiles improved with increasing LPL levels, suggesting enhanced lipid metabolism. The fatty acid composition of milk was significantly enriched in the 0.15% LPL group, particularly in terms of beneficial fatty acids. Moreover, the group receiving the higher LPL level showed upregulated expression of key lipogenic genes, including stearoyl-CoA desaturase (SCD) and fatty acid binding protein (FABP). Notably, daily feed intake and milk yield remained unaffected by LPL supplementation. Overall, the findings suggested that LPL inclusion, especially at 0.15% of dry matter, may improve metabolic efficiency and milk quality in early-lactation dairy cows. Further research is recommended to examine the long-term outcomes and economic benefits of LPL supplementation in dairy cows.

Keywords: blood parameter, early lactation, fatty acid, lipogenic gene, lysophospholipid

Introduction

Early lactation is a critical period in dairy cows, during which the high metabolic demands of milk synthesis can lead to metabolic stress and a negative energy balance. These physiological challenges can have adverse effects on both milk production and the overall health of the cow (Hippen et al., 2001). Fat supplementation in dairy cows improves milk production and composition, particularly during early lactation, when cows are most vulnerable to metabolic and nutritional stress (Abughazale et al., 2003). Lysophospholipids (LPLs) are bioactive lipids that can provide additional energy for milk production while also modulating the expression of genes involved in milk fat synthesis (Stoffel et al., 2003). Lysophospholipids can be produced by the hydrolysis of phospholipids via enzymes such as phospholipase A_2 (Ding et al., 2015). As a nutritional

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intervention in early lactation in dairy cows, LPL increased milk yield and milk fat content, altered the fatty acid (FA) profile of milk (Alharthi et al., 2018) and serum lipid parameters (Luo et al., 2019), increased serum triglycerides, and decreased serum cholesterol levels (Chen et al., 2021a).

Changes in serum lipid parameters can have implications for cow health and productivity, as high levels of serum triglycerides have been associated with metabolic disorders such as fatty liver syndrome. However, the effects of LPLs on early lactation are complex and can depend on several factors, such as the dose and duration of LPL supplementation (Chen et al., 2021b).

Synthesis of saturated FAs (SFAs) in milk constitutes a significant share of the energy required for milk production in cows (de Souza et al., 2019); for this reason, increasing the amount of SFAs in milk increases the energy requirements of cows (Hanigan et al., 2016). According to these studies, adding LPL to the diet of cows can reduce the share of energy required for milk production in cows. Additionally, LPLs can act as an energy source for cows and reduce energy consumption in milk production (Kim et al., 2017).

Lysophospholipids can affect the expression of lipogenic genes in mammary glands through several mechanisms (Hatch et al., 2014). For example, LPLs can increase the activity of glycerol-3-phosphate acyltransferase (GPAT), which is a key enzyme in the lipogenesis (Hatch et al., 2014). Lysophospholipids can also increase the expression of the gene encoding the transcription factors that activate the lipogenic genes, including PPARα, which is a key transcription factor in lipogenesis (Nordkrog et al., 2015). Furthermore, LPLs affect the expression of mammary lipogenic genes during early lactation in Holstein dairy cows (Zhang et al., 2015).

Lysophospholipids can affect the expression of key genes involved in milk fat synthesis, including FA synthase (FASN) and acetyl-CoA carboxylase, leading to changes in the milk FA profile (Li et al., 2014; Han et al., 2018; Zhang et al., 2019). Specifically, LPLs have been shown to increase the expression of FASN, which is the rate-limiting enzyme in milk fat synthesis, resulting in an increase in the proportion of saturated FAs in milk (Gao et al., 2019).

During this period, cows experience a negative energy balance, which can lead to a decrease in milk production and quality (He et al., 2020). Lysophospholipids can alleviate the negative effects of energy imbalance by increasing the expression of lipogenic genes, such as FASN and acetyl-CoA carboxylase, leading to increases in milk fat synthesis and milk yield (Zhang et al., 2019; Kellner and Parrish, 2020; Wang et al., 2022).

Overall, the effects of LPLs on milk production, the milk FAs profile, serum lipid parameters, and the expression of mammary lipogenic genes during early lactation in Holstein dairy cows are complex and multifaceted. However, understanding the effects of LPL supplements in early lactating dairy cows is highly important, as it can provide valuable insights into how to optimize the nutritional management of cows during this critical period. Therefore, this study was carried out to explore the effects of LPLs on milk production, milk FAs profile, serum lipid parameters, and expression of mammary lipogenic genes, fatty acid binding protein (FABP) and stearoyl-CoA desaturase (SCD), during early lactation in Holstein dairy cows.

Materials and methods

Animals, feeding and treatments

The experiment was carried out at the Mahdasht Dairy Farm Company in Sari, Mazandaran, Iran. Fifteen multiparous dairy cows (second and third parities) in early lactation (within 2 weeks postpartum, with an average milk production of 30±2.55 kg and average body weight of 600±45.36 kg) were used in the experiment.

The cows were housed in individual pens and assigned to three dietary treatments of 5 cows each in a completely randomized design. The duration of the experiment was 35 days, which included a 14-day adaptation period followed by 21 days for data collection. The ingredients and nutrient contents of the diets are presented in Table 1.

The experimental diets were: 1) a basal diet that met the requirements of all nutrients according to NRC (2001) with 3% PRO-fat powder (mixed fat, Ata Co.), (control); 2) a basal diet with a low level [0.1% of diet dry matter (DM) basis] of LPL (LLPL); and 3) a basal diet with a high level (0.15% of diet DM) of LPL (HLPL). Lysophospholipid was as hydrolyzed soy lecithin, which contained phospholipids and free FAs (12%), according to the manufacturer's specification (Simorgh Behin Darou Inc. Babolsar, Iran).

All diets were prepared daily and fed as a total mixed ration (TMR) *ad libitum*, targeting 5% refusal, with free access to water. The LPL was diluted in a milled corn carrier and incorporated in the diet using a small-batch vertical TMR mixer. The cows were fed twice daily (at 7:00 a.m. and 7:00 p.m.) at 110% of the expected intake.

Milking was performed twice daily (5:00 a.m. and 5:00 p.m.) at weekly intervals and analyzed for fat, true protein, lactose, total solids and solids nonfat (SNF) by MilkoScan 134 A/B (Denmark).

Fatty acid profiles, gene expression, and blood constituents were measured at the end of the study (day 35). Milk urea nitrogen (MUN) was measured by acetyl monoxime (Iran-made treatment kit) using a 320 nm wavelength spectrophotometer (Wang et al., 2006).

Milk samples were analyzed for FA determination via GC as described by Rico and Havartine (2013). For this purpose, milk triglycerides were extracted using hexane: isopropanol and subsequently subjected to basic methylation (Antolín-Ametller et al., 2018).

Blood serum was separated by centrifugation at 3000× g for 15 minutes at 4 ˚C, and analyzed for triglyceride, cholesterol, LDL, HDL, nonesterified FA (NEFA), and urea nitrogen (SUN) using the Pars Azmoon Test Kit and spectrophotometry.

Table 1. Feed ingredients and chemical composition of the basal experimental diets

	Levels of lysophospholipid (% of dry matter)		
Item	0.0	0.1	0.15
Ingredients (%)			
Alfalfa hay	5.07	5.07	5.07
Barley grain	6.71	6.71	6.71
Corn grain	7.93	7.93	7.92
Soybean meal	4.57	4.57	4.57
Roasted soy	2.13	2.13	2.13
Wheat straw	1.13	1.13	1.13
Meat meal	1.67	1.67	1.67
Protein supplement	6.42	6.42	6.42
Fat powder	3.00	3.00	3.00
Alfalfa Silage	34.71	34.61	34.57
Urea	0.12	0.12	0.12
Sugar beet pulp	24.14	24.14	24.14
Cotton seed meal	0.76	0.76	0.76
Salt	0.09	0.09	0.09
Sodium bicarbonate	0.48	0.48	0.48
Carbonate calcium	0.15	0.15	0.15
Bentonite	0.09	0.09	0.09
Molasses	0.38	0.38	0.38
Magnesium oxide	0.17	0.17	0.17
Oilafor[1]	0.01	0.01	0.01
Toxin binder[2]	0.05	0.05	0.05
Vit. Premix[3]	0.11	0.11	0.11
Min. premix[4]	0.11	0.11	0.11
LPL[5]	_	0.10	0.15
Chemical composition (%)			
Dry matter	81.35	81.34	81.34
Organic matter	95.67	95.59	95.71
Crude protein	16.68	16.67	16.70
Neutral detergent fiber	26.55	26.54	26. 51
Acid detergent fiber	18.50	18.41	18.47
Non fiber carbohydrate	41.78	41.36	41.45
Ether extract	6.81	6. 83	6.83
Ca	0.83	0.83	0.83
P	0.41	0.41	0.41
NE_L (Mcal/kg)	1.62	1.63	1.64

[1] Organic form of zinc, manganese, copper and cobalt (Zinpro, Eden Prairie, MN) [2] Rooyan darou (Tehran,IRI) [3,4] Each kilogram of vitamin and mineral supplement contains 600,000 international units of vitamin A, 200,000 international units of vitamin D, 200 mg of vitamin E, 2,500 mg of antioxidants, 195 g of calcium, 80 g of phosphorus, 21 g of magnesium, and 2,200 mg of manganese. Three thousand milligrams of iron, 300 mg of copper, 3000 mg of zinc, 100 mg of cobalt, 120 mg of iodine and 20 mg of selenium were used. [5] Hydrolyzed soy lecithin containing lysophospholipids (Simorgh Behin Darou, Babolsar,IRI)

Gene expression assay

At the end of the experiment (after 35 days of treatment), milk samples were collected from each cow and subjected to RNA extraction and gene expression FABP and SCD genes. Following the protocol of Suárez-Vega et al. (2015), the udders were cleaned with water and soap and then disinfected with povidone-iodine. To maximize the number of somatic cells in milk, samples were collected approximately 1 h after morning milking (Gonzalo et al., 2004) and 10 min after the injection of oxytocin (10 IU/animal; Royan Darou, Iran). The samples were kept on ice and transferred immediately to the laboratory for RNA extraction.

RNA isolation

Total RNA was extracted from 50 mL of fresh milk as described by Wickramasinghe et al. (2012), with some modifications described by Suárez-Vega et al. (2015). Briefly, samples were pelleted by centrifugation at 650 × g for 10 min at 4°C in the presence of a final concentration of 0.5 mM EDTA.

The cell pellet was washed with 10 mL of phosphate buffer saline (PBS) (pH 7.2 and 0.5 mM EDTA), followed by another centrifugation at 650×g for 10 min at 4 °C The washing and centrifugation procedure was repeated twice more with 2 or 1.5 mL of the same PBS mixture.

Total RNA was subsequently extracted and purified from the milk cell pellet with TRIzol (Invitrogen, Carlsbad, CA), as previously described (Suárez-Vega et al., 2015), and stored at −80°C until processed for RNA isolation.

The RNA was purified via a miRNeasy Mini kit (Yekta Tajhiz, Iran) and further digested on a column with RNase-free DNase (Yekta Tajhiz, Iran). The RNA was quantified via a Nanodrop spectrophotometer (Thermo Scientific ND 2000C, USA). The A260/A280 ratio was 1.8–2.0 for all the samples.

The RNA integrity was also confirmed by denaturing agarose gel electrophoresis using the method described by Tang et al. (2007). The cDNA was synthesized by using a first-strand cDNA synthesis kit (Yekta Tajhiz, Iran) according to the manufacturer's instructions.

Real-time quantitative polymerase chain reaction (PCR)

The genes responsible for fat synthesis were investigated for their differential expression, along with marker genes for epithelial cells. The relative expression of the lipogenic genes (SCD and FABP) was quantified via real-time PCR and analyzed with the ABI 7500 Software version 2.3.6 (Applied Biosystems).

The specific primers (Table 2) were selected from published references and were commercially synthesized by Sinaclon, Iran. The specificity of the primers was checked via the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

All real-time PCRs were carried out in 20 µL consisting of 10 µL of 1× real-time SYBR Green PCR master mix (SYBR Green, Yekta Tajhiz, Iran), 2.0 µL of cDNA, 1.0 µL of each primer (10 pM), and 6.0 µL of water. The PCR protocol involved a denaturation step (94°C, 15 s), annealing combined with an extension step (60°C, 30 s), and a cycling program followed by melt curve analysis was used for all genes.

A single sharp peak in the melt curve analysis and a single band in gel electrophoresis indicated specific amplification for each gene primer pair. A no template control was also included for each primer assay. Two biological replicates and three technical replicates were used for each sample.

The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was used as a reference index and for normalization. The quantitative cycle (Cq) values were calculated via Applied Biosystems 7500 software v2.0.5.

Relative mRNA levels were then calculated for each gene via the ΔΔCq method. The Cq values of the housekeeping genes were subtracted from the Cq value of each gene to obtain ΔCq (Pfaffl, 2001). The calibrator ΔCq was subtracted from each sample's ΔCq, and the relative mRNA value was subsequently calculated via the formula 2^−ΔΔCq (Livak and Schmittgen, 2001).

Table 2. Primers used for the expression of the desired genes (Sinaclon Company)

Gene	Accession number	Primers sequences(5'→3')	Sources	Orientation
FABP3	_	AGGGCAAGAACCCCAATTAAA	1	Forward
		CTCATTCCCATTCCTCTAGTTTTTG		Reverse
SCD	_	GCTGCTTGTGCGCAAACA	1	Forward
		TCGGCTCTTAGGTCGGATAAATTG		Reverse

1 = Bichi et al. (2013)

Statistical analysis

The Proc GLM (SAS 9.4; 2001; SAS Institute Inc., Cary, NC) was used for data analysis. Mean comparison was performed using the Duncan's multiple range test at a significance threshold of 95%.

Results

The LPL supplementation did not affect the DM intake (*P*=0.1217), milk yield (*P*=0.0673), 3.5% fat corrected milk (FCM) (*P*=0.0689), energy corrected milk (ECM) (*P*=0.3357), or feed efficiency (Table 3). Additionally, dietary LPL levels did not affect milk fat (*P*=0.4437), crude protein (*P*=0.6715), lactose (*P*=0.2283) or MUN (*P*=0.2283) contents.

Table 3. Effects of lysophospholipid on milk production and milk composition in dairy cows

	Diet[1]				
Item	CON	LLPL	HLPL	SEM	P value
Body weight (kg)	642	641	651	19.465	0.9347
Dry matter intake (kg/d)	22.57	23.03	23.38	0.775	0.1217
Milk yield (kg/d)	31.84	32.71	33.16	0.851	0.0673
Milk yield/DMI (kg/kg)	1.50	1.51	1.54	0.063	0.0641
3.5% FCM[2] (kg/d)	29.27	30.62	31.37	0.764	0.0689
3.5% FCM/DMI (kg/kg)	1.38	1.41	1.44	0.071	0.0699
ECM[3] (kg/d)	28.84	30.67	29.93	0.816	0.0597
ECM/DMI (kg/kg)	1.36	1.41	1.36	0.087	0.0686
Fat (%)	3.32	3.36	3.42	0.231	0.4437
True protein (%)	3.14	3.16	3.19	0.068	0.6715
Lactose (%)	4.92	4.97	4.94	0.038	0.2283
Total solid[4] (%)	12.69	12.86	13.09	0.195	0.2418
Solids non-fat [5] (%)	8.64	8.82	8.96	0.127	0.1624
Fat (kg/d)	1.07	1.12	1.18	0.071	0.1384
True protein (kg/d)	0.94	1.02	1.06	0.053	0.0661
Lactose (kg/d)	1.54	1.63	1.66	0.052	0.1402
Total solid (kg/d)	4.16	4.28	4.36	0.085	0.0834
Milk NE$_L$[6] (Mcal/d)	20.39^{b}	21.62^{ab}	22.30^{a}	0.583	0.0249
MUN (mg/dL)	11.84	11.62	11.39	0.394	0.1766

[1] CON = control; LLPL = 0.1% lysophospholipid; HLPL = 0.15% lysophospholipid.

[2] 3.5% FCM = [milk fat (kg/d) × 16.218] + [milk yield (kg/d) × 0.4324] (Tyrrell and Reid, 1965).

[3] ECM (kg/d) = kg of milk × [(38.3 × % fat × 10 + 24.2 × % true protein × 10 + 16.54 × % lactose × 10 + 20.7)/3,140] (Sjaunja et al., 1990).

[4] Total solids (%) = [weight of dry residue (g)/weight of original sample (g)] * 100.

[5] Solids Non-Fat (%) = Total Solids (%) - Fat (%)

[6] Milk NE$_L$ (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).

[7] Milk Urea Nitrogen.

^{a,b} within row, means with common superscript(s) are not different (P>0.05).

The effects of LPL on serum lipid parameters are shown in Table 4. Serum cholesterol (*P*= 0.2631), triglyceride (*P*=0.1134) and NEFA (*P*=0.1064) contents were not affected by the experimental treatments. Blood LDL (*P*=0.0348) and blood urea nitrogen (*P*=0.0361) concentrations decreased with increasing LPL levels in the diet. In addition, the HLPL treatment caused the highest blood HDL concentration, which was not significantly different from that of the LLPL treatment but was significantly different from that of the control treatment (*P*=0.0247).

Table 5 displays the impact of LPL on the profile of milk FAs. The content of short-chain FAs (C4:0 to C18:0) was not affected by the level of LPL in the experimental diets. The contents of unsaturated FAs such as palmitoleic acid (C16:1) (*P*=0.0241), oleic acid (C18:1) (*P*=0.0336), linoleic acid (C18:2) (*P*=0.0143), and linolenic acid (C18:3) (*P*=0.0216) significantly increased as the level of LPL increased in the diet. However, no significant differences were observed in the amounts of other milk FAs among the treatments ($P<0.1437$).

Table 4. Effects of lysophospholipid on serum lipid parameters (mg/dL)

	Diet[1]				
Item	CON	LLPL	HLPL	SEM	P value
Cholesterol	76.41	76.09	75.68	0.671	0.2631
Triglycerides	39.68	40.25	40.53	0.362	0.1134
LDL[2]	39.74^{a}	38.27^{b}	38.06^{b}	0.245	0.0348
HDL[3]	22.53^{b}	23.27^{a}	23.79^{a}	0.218	0.0247
BUN[4]	40.26^{a}	39.54^{b}	39.08^{b}	0.206	0.0361
NEFA[5]	0.36	0.29	0.27	0.038	0.1064

[1] CON = control; LLPL = 0.1% lysophospholipid; HLPL = 0.15% lysophospholipid. [2] LDL=low-density lipoprotein; [3] HDL=high-density lipoprotein; [4] BUN=blood urea nitrogen; [5] NEFA=nonesterified fatty acid.
[a,b] within row, means with common superscript(s) are not different ($P>0.05$).

Table 5. Effects of different levels of lysophospholipid on the milk fatty acid profile (%)

	Diet[1]				
Item	CON	LLPL	HLPL	SEM	Pvalue
C4:0	0.38	0.32	0.30	0.082	0.1214
C6:0	0.61	0.53	0.52	0.076	0.1068
C8:0	1.11	1.04	1.02	0.053	0.0956
C10:0	1.92	1.85	1.84	0.037	0.0863
C12:0	2.63	2.57	2.46	0.096	0.0751
C14:0	10.09	10.01	10.02	0.054	0.0892
C16:0	26.95	26.88	26.81	0.085	0.1028
C17:0	9.56	9.47	9.43	0.068	0.0934
C18:0	11.24	11.17	11.15	0.071	0.0847
C16:1	2.16^{c}	2.34^{b}	2.51^{a}	0.034	0.0241
C18:1 cis9	23.81^{b}	24.66^{a}	24.73^{a}	0.067	0.0336
C18:2 cis9,12	2.17^{b}	2.38^{a}	2.40^{a}	0.063	0.0143
C18:3 cis9,12,15	0.48^{b}	0.57^{b}	0.61^{a}	0.041	0.0216
Others	6.89	6.21	6.20	0.183	0.1437

[1] CON = control; LLPL = 0.1% lysophospholipid; HLPL = 0.15% lysophospholipid.
[a,b] within row, means with common superscript(s) are not different ($P>0.05$).

Table 6 shows the effects of LPL on the expression of lipogenic genes (SCD and FABP) in the breast. Dietary supplementation with LPL, particularly at the higher level, resulted in a significant increase in the expression of the SCD and FABP genes ($P<0.05$). The HLPL treatment caused the highest SCD gene expression but was not significantly different from that of the control treatment, although it was higher than recorded for the LLPL treatment ($P=0.0288$). Furthermore, the data revealed that FABP gene expression in the HLPL treatment was significantly different from that in the control treatment but was not significantly different from that in the LLPL treatment ($P=0.0322$).

Discussion

Lysophospholipids are a class of lipids that affect milk production and composition in dairy cows. Cows fed diets containing LPLs showed higher milk production and milk fat than cows fed a control diet, as well as increased immunity (Lin et al., 2016; Lu et al., 2021; Zhao et al., 2020). Which may be attributed to the remarkable potential of LPLs to increase the assimilation and utilization of dietary fats. Additionally, these LPLs exhibit an impressive ability to stimulate mammary gland development while simultaneously increasing the secretion of milk fat. The beneficial effects of LPLs on milk production and the immune response may be due to their ability to modulate the inflammatory response in the mammary gland (Zhao et al., 2020).

Research carried out by De Souza et al. (2019), Zhao et al. (2017), and Lee et al. (2019) revealed that the impact of LPLs on milk yield, milk fat content, and milk protein content was not significant. The absence of an impact of LPLs on the production and composition of milk may stem from several factors, including the low levels of LPLs used in the study, individual variation in animal response, the high proportion of unsaturated fatty acids in some diets, and the physiological conditions of cows during early lactation (Chen et al., 2017; Lee et al., 2019; Zhao et al., 2017).

Table 6. Effect of lysophospholipid on the expression of lipogenic genes

		Diet[1]				
Gene symbol	Gene name	CON	LLPL	HLPL	SEM	P value
SCD	Stearoyl-CoA desaturase	1.01^{ab}	0.79^{b}	1.29^{a}	0.425	0.0288
FABP	Fatty acid binding protein	1.00^{b}	1.36^{ab}	1.58^{a}	0.449	0.0322

[1] CON = control; LLPL = 0.1% lysophospholipid; HLPL = 0.15% lysophospholipid.
[a,b] within row, means with common superscript(s) are not different ($P>0.05$).

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and blood urea nitrogen (BUN) are three important indicators of cardiovascular health and renal function (Oikonomou et al., 2015). Diets containing LPL caused a significant increase in the concentration of HDL and a decrease in the levels of LDL, triglycerides and blood cholesterol (Rezaei et al., 2020). Lysophospholipid increases the expression of the FASN gene, which encodes the key enzyme of FA synthesis called FA synthase. This important enzyme causes the production of myristic acid and other saturated FAs (Mashek et al., 2001). The increase in serum HDL and reduction in serum LDL and triglyceride levels, as well as the increase in saturated Fasin milk fat, can potentially be attributed to the amplification of FA synthesis within the mammary tissues (Abdollahi et al., 2020). Therefore, the main mechanism seems to be the effect of LPL on the expression of genes related to FA synthesis and FA synthase activity, which leads to changes in blood parameters and milk composition. However, some studies have shown a lack of effects of LPLs on cholesterol, triglyceride, and NEFA levels (Rezayi et al., 2020). Oikonomou et al. (2015) reported that a diet containing LPLs had no significant effect on cholesterol, triglyceride, or NEFA levels in the plasma or liver; however, it resulted in higher HDL and lower LDL levels in compared with the control diet. These findings suggest that the beneficial effect of LPLs on lipoprotein metabolism may be due to their ability to increase the absorption and utilization of dietary fat and promote the synthesis and secretion of lipoproteins in the liver. Furthermore, the absence of a discernible impact on lipid metabolism attributed to LPLs could be ascribed to several factors. These include the utilization of relatively small quantities of LPLs within the study, differences in experimental diet compositions, or inherent modifications occurring at the individual level in how cows respond to LPLs (Oikonomou et al., 2015).

The BUN concentration was significantly different among the treatment groups (Table 4). Blood urea nitrogen is a measure of the amount of nitrogen in the blood that results from the breakdown of urea, a waste product of protein metabolism (Yang et al., 2020). Elevated levels of BUN can indicate impaired renal function, dehydration, or other health problems. Oikonomou et al. (2015) and Chen et al. (2017) reported that cows fed a diet containing LPLs presented lower levels of BUN in their blood than did cows fed a control diet. These researchers suggested that the beneficial effect of LPLs on BUN levels may be due to their ability to increase the absorption and utilization of dietary protein and promote the elimination of nitrogenous waste products through the kidneys.

The concentrations of cholesterol, triglycerides, and NEFA in the blood were similar across all treatment groups. However, levels of LDL and BUN decreased. This suggests that LPLs may not significantly influence lipid metabolism in dairy cows, possibly because the cows were already receiving a diet rich in unsaturated fatty acids, which could have masked any potential effects of the LPL supplementation. The mechanism by which LPLs exert their beneficial effects on lipoprotein metabolism and renal function is not yet fully understood, but it is likely that they act through multiple pathways, including the enhancement of nutrient absorption and utilization, the modulation of lipid and protein metabolism, and the promotion of liver and kidney functions (Oikonomou et al., 2015).

The content of short-chain FAs (C4:0 to C18:0) was not affected by the level of LPL, but the levels of unsaturated FAs, including palmitoleic, oleic, linoleic, and linolenic acids, significantly increased as the level of LPS increased (Table 5). Ma et al. (2019) reported that cows fed a diet containing LPLs during the transition period presented higher levels of unsaturated FAs, including oleic and linoleic acids, in their milk than did cows fed a control diet. Researchers have suggested that the beneficial effect of LPLs on milk FA composition during the transition period may be due to their ability to increase the absorption and utilization of dietary fat and reduce the risk of metabolic disorders (Kellner and Parrish, 2020). In addition, Zhang et al. (2018) reported that feeding LPLs increased the concentrations of oleic acid, linoleic acid, and linolenic acid in milk from cows fed LPLs but had no effect on saturated FAs. However, Yang et al. (2020) reported that feeding LPLs had no significant effect on the concentration of USFAs in milk. However, other researchers reported a decrease in the concentration of stearic acid in milk from cows fed LPLs. Wang et al. (2019) reported that feeding LPLs increased the concentration of USFAs in milk (linoleic and linolenic acids) in goats fed LPLs. The milk FA profile, an important indicator of milk quality is influenced by various factors, including animal genetics, feed composition, and management practices (Bauman et al., 2011; Shingfield et al., 2013). Overall, these studies suggest that dietary LPLs can have a positive effect on the FA profile of milk in dairy cows and goats, including the modulation of USFA. The mechanism by which LPLs exert their beneficial effects on milk FA composition is not yet fully understood, but it is likely that they act through multiple pathways, including the enhancement of nutrient absorption and utilization, the modulation of lipid metabolism, and the promotion of mammary gland function (Lu et al., 2021).

Expression of the lipogenic genes SCD and FABP differed between treatments (Table 6). These lipogenic genes are responsible for FA synthesis and the regulation of lipid metabolism. Several studies, concerning the effects of LPLs on lipogenic gene expression, reported that LPLs can affect the expression of these genes (Huang et al., 2018; Wang et al., 2019; Zhao et al., 2020). Huang et al. (2018) reported that treatment with LPLs resulted in the upregulation of several lipogenic genes, including stearoyl-CoA desaturase-1 (SCD1), FASN, and acetyl-CoA carboxylase alpha (ACACA). Wang et al. (2019) reported that, compared with cows fed a control diet, cows fed a diet containing LPLs presented increased expression of several FABP genes, including FABP3 and FABP4, in the mammary gland. These findings suggest that the beneficial effect of LPLs on FABP gene expression in dairy cows may be due to their ability to increase the synthesis and secretion of milk fat (Wang et al., 2019). Lysophospholipids can activate the PI3K/Akt/mTOR signaling pathway, which is known to regulate lipid metabolism and promote lipogenesis (Lu et al., 2021). This pathway may be activated by LPLs through the binding and activation of specific receptors on the cell surface, such as G protein-coupled receptors (GPCRs) and Toll-like receptors (TLRs; Huang et al., 2018). Another hypothesis is that LPLs directly interact with transcription factors that regulate lipogenic gene expression (AbuGhazaleh et al., 2010). For example, LPLs may interact with peroxisome proliferator-activated receptors (PPARs), which are nuclear receptors that play key roles in lipid metabolism and the regulation of lipogenic gene expression. LPLs may bind to PPARs and induce their activation, resulting in the upregulation of lipogenic genes (Wang et al., 2019). It has also been proposed that LPLs may affect lipogenic gene expression indirectly by altering the availability of substrates for lipid synthesis. Lysophospholipids are known to increase the uptake and utilization of FA by cells, which may provide the necessary substrates for lipogenesis. This, in turn, may lead to the upregulation of lipogenic genes (Zhao et al., 2020).

Overall, the exact mechanism by which LPLs affect the expression of lipogenic genes is likely complex and multifaceted. Further research is needed to fully understand the molecular mechanisms underlying the effects of LPLs on lipogenic gene expression and the potential applications of these findings in dairy cow production.

Conclusion

Lysophospholipids enhanced dairy production by improving milk FA profiles, particularly increasing beneficial USFA (e.g., oleic and linoleic acids), and upregulating lipogenic genes (SCD, FABP) via pathways such as PI3K/Akt/mTOR. They also improve metabolic health by elevating HDL, reducing LDL and BUN, and optimizing nutrient utilization. However, outcomes vary due to factors like LPL dosage, dietary composition, and individual cow physiology, with some studies reporting no significant effects on milk yield or fat content. While LPLs demonstrate promise for enhancing milk quality and cow health, further research is needed to fully elucidate their mechanisms and establish practical, economically viable applications in dairy nutrition.

Conflict of interest

We certify that there are no conflicts of interest with any financial organization regarding the material discussed in the manuscript.

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Informed consent statement

Not applicable.

Data availability statement

The data from this research can be sent without impediment upon reasonable request.

Ethics approval

This study was approved by the Animal Research Ethics Committee (JAN/016/2019) and complied with the guidelines of the Animal Welfare Declaration (code: MU. ECRA.2022.12).

Author contributions

All the authors contributed to the conception and design of the study. Materials preparation, collection and analysis were performed by [Matin Movagharnezhad] in collaboration with [Yadollah Chashnidel], [Asadollah Teymouri Yansari] and [Mohsen Gholizadeh]. The first draft of the manuscript was written by Matin Movagharnezhad, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

References

Abdollahi, M.R., Ekhlasi, H., Shivazad, M., Ghaffari, M.H., 2020.Inclusion of lysophospholipids in transition dairy cow diets: Impacts on production performance and blood metabolisms. *Italian Journal of Animal Science* 19(1), 677-690.

AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., 2010. Effects of lysophosphatidylcholine supplementation to transition dairy cows on milk yield, milk composition, and metabolic profiles.*Journal of Dairy Science* 93(5),2035-2042.

Alharthi, A.S., Zhou, Z., Lopreiato, V., Trevisi, E., Loor, J.J., Cardoso, F.C., 2018. Supplemental methionine and lysophospholipids on productive and reproductive performance of transition dairy cows. *Journal of Dairy Science* 101(1), 455-465.

Antolín-Ametller, J., Patrizio, C., Cruz-Hernandez, C., Shingfield, K.J., 2018. Determination of milk fat fatty acid composition: Evaluating the effect of sampling stage, storage conditions and extraction procedures. *Animal* 12(8), 1764-1773.

Bauman, D.E., Perfield, K.L., Harvatine, K.J., 2011. Milk fat globules and their surrounding membrane: Potential implications in the mammary gland and the dairy industry. *Journal of Dairy Science* 94(6), 2538-2551.

Bichi, E.P., Frutos, P.G., Toral, D., Keisler, G., Hervas, P., Loor, J.J., 2013. Dietary marine algae and its influence on tissue gene network expression during milk fat depression in dairy ewes. *Animal Feed Science and Technology* 186, 36-44.

Chen, X., Zhang, H., Li, X., Wang, J., 2017. Effect of lysophospholipids supplementation on milk production, nutrient digestion and serum lipid profile in early lactating dairy cows. *Animal Science* 49, 112-128.

Chen, X., Zhang, H., Zhang, Y., Ding, L., Sun, Y., Wang, J., 2021a. Lysophospholipids supplementation during early lactation improves milk production and quality in Holstein cows. *Livestock Science* 244(2), 104-125.

Chen, X., Zhang, H., Zhang, Y., Ding, L., Sun, Y., Wang, J., 2021b. Lysophospholipids supplementation during early lactation regulates lipid metabolism and improves hepatic function in Holstein cows. *Journal of Dairy Science* 104(6), 6554-6566.

de Souza, J., Batistel, F., Harvatine, K.J., Allen, M.S., Bicalho, R.C., 2019. Lysophosphatidylcholine supplementation increases milk production and modifies milk fatty acid profile in dairy cows. *Journal of Dairy Science* 102(7), 6307-6318.

Ding, L., Li, X., Zhang, H., Chen, X., Wang, J., 2015. Effects of lysophospholipids supplementation to transition cows on performance, metabolic response, and hepatic gene expression. *Journal of Dairy Science* 98(9), 6198-6210.

Gao, X., Zhang, H., Zhang, L., Li, X., Wang, J., 2019. Effects of lysophospholipids on the expression of lipogenic genes in mammary gland of dairy cows during early lactation. *Journal of Dairy Science* 102(2), 1480-1489.

Gonzalo, C., Carriedo, J.A., Beneitez, E., Juárez, M., De La Fuente, M.A., 2004. Factors affecting the somatic cell count of small ruminant milk: A review. *Journal of Dairy Science* 87(6), 1711-1726.

Han, I.H., Lee, S.W., Kim, H.G., Sung, S.Y., Lee, S.S., Lee, H., Hong, Y.C., Kwak, K., Ha, J.K., 2018. Effects of cellulolytic enzymes on nutrient digestion and cellulolytic bacterial adhesion. *Asian-Australasian Journal of Animal Science* 24(3), 1561-1573.

Hanigan, M.D., Tucker, C.A., Jacobs, K.E., 2016. Effect of increasing dietary saturated fatty acids on milk production, energy balance, and methane emissions of lactating dairy cows: A meta-analysis. *Journal of Dairy Science* 99(5), 3665-3682.

Hatch, C., Hagen, J., Christensen, D., Nordkrog, M., Andersen, J., 2014. Effect of dietary supplementation with lysophospholipids on milk production, fat composition, and fatty acid profile in lactating dairy cows. *Journal of Dairy Science* 97(1), 233-241.

He, Y., Chen, X., Li, X., Wang, J., 2020. Effects of lysophospholipids on the expression of lipogenic genes in liver of transition dairy cows. *Journal of Dairy Science* 103(1), 767-776.

Hippen, A.R., Kalscheur, K.F., Schingoethe, D.J., Casper, D.P., 2001. Supplemental dietary fat sources for dairy cattle: Effects on lactation performance, plasma metabolites, and hepatic gene expression. *Journal of Dairy Science* 84(3), 1008-1019.

Huang, Y., Zhang, Y., Zhu, X., Liu, J., Li, W., 2018. Lysophosphatidic acid enhances lipid synthesis and secretion in bovine mammary epithelial cells via an autocrine mechanism. *Journal of Cellular Physiology* 233(8), 6145-6155.

Kellner, T.A., Parrish, F.C., 2020. Effects of lysophospholipids on the production, composition, and fatty acid profile of milk from Holstein cows during early lactation. *Journal of Dairy Science* 103(4), 3551-3562.

Kim, J., Park, C.S., Lee, S.H., Hong, S.M., Lee, H.S., Kim, K.H., 2017. The effects of dietary lysophospholipid supplementation on milk production and composition, rumen fermentation, and nutrient digestibility in lactating dairy cows. *Journal of Dairy Science* 100(3), 757-770.

Lee, C., Morris, D.L., Copelin, J.E., Hettick, J.M., Kown, I.H., 2019. Effects of lysophospholipids on short-term production, nitrogen utilization, and rumen fermentation and bacterial population in lactating dairy cows. *Journal of Dairy Science* 97(6), 3589-3600.

Li, H., Zhang, H., Li, X., Chen, X., Ding, L., Wang, J., 2019. Effects of lysophospholipids on milk production, milk composition, and metabolic status in dairy cows during early lactation. *Animal Feed Science and Technology* 248(2), 79-88.

Lin, K., Cheng, L., Wu, T., You, P., Shen, S., Li, Y., He, Y., Tian, W., Li, R., Li, J., Song, B., Wang, C., Sun, X., 2016. Rationary Supplementation of Lysophospholipids Affects Feed Digestion in Lambs. *Animals* 9, 791-805; doi:10.3390/ani9100805.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4), 402-408.

Lu, J., Zheng, N., Lin, X., Cai, Y., Guo, X., 2021. Dietary lysophosphatidylcholine supplementation enhances milk production and alters milk fatty acid profile in lactating dairy cows. *Animal Feed Science and Technology* 277(4), 114-142.

Luo, J., Wang, C., Li, Y., Liang, X., Chen, H., 2019. Effects of lysophospholipid supplementation on milk production, milk composition, and metabolic parameters in dairy cows during early lactation. *Journal of Dairy Science* 102(6), 5230-5241.

Ma, X., Li, C., Sun, P., Zhang, L., Jiang, L., 2019. Effects of dietary lysophospholipids on milk production, milk composition, and fatty acid profile in dairy cows. *Journal of Dairy Science* 102(12), 1409-1418.

Mashek, D.G., Li, L.O., Coleman, R.A., 2001. Long-chain acyl-CoA synthetases and fatty acid signaling. *Lipids* 36(7), 697-706.

Nordkrog, M., Hagen, J., Hatch, C., 2015. Effect of lysophospholipids on expression of lipogenic genes in bovine mammary gland. *Journal of Dairy Science* 98(2), 653-660.

NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th revised edn., National Research Council, National Academy Press, Washington, DC, USA.

Oikonomou, G., Arsenos, G., Tzora, A., Katsoulos, P.D., 2015. Effects of dietary lysophospholipids supplementation on blood biochemical and hematological parameters in dairy cows. *Journal of Dairy Science* 98(7), 4627-4638.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Resource* 29(9), 45-52.

Rezayi, M., Nazifi, S., Mirzaei, M., Moheghi, M., 2020. Incorporation of dietary lysophospholipids modulates blood metabolites and milk components in transition dairy cows. *Animal Nutrition* 14(2), 38-51.

Rico, D.E., Harvatine, K.J., 2013. Milk fat synthesis in the dairy cow: a complex and highly regulated process. In Energy and protein metabolism and nutrition in sustainable animal production. *Animal Production* 3(6), 165-178.

Shingfield, K.J., Bonnet, M., Scollan, N.D., Lee, M.R.F., 2013. Invited review: recent advances in biohydrogenation and its role in ruminant nutrition. *Animal* 7(11), 267-274.

Stoffel, C.M., Crump, P.M., Armentano, L.E., 2003. Lysophosphatidylcholine concentration in ruminal digesta and milk of dairy cows fed fat. *Journal of Dairy Science* 86(12), 3937-3945.

Suárez-Vega, A., Gutiérrez-Gil, B., Klopp, C., Robert-Granié, C., Tosser-Klopp, G., Arranz, J.J., 2015. Characterization and comparative analysis of the milk transcriptome in two dairy sheep breeds using RNA sequencing. *Scientific Reports* 5(1), 1-14.

Tang, C., Qi, J., Li, H., Zhang, C., Wang, Y., 2007. A convenient and efficient protocol for isolating high-quality RNA from latex of Hevea brasiliensis (para rubber tree). *Journal of Biochemical and Biophysical Methods* 70(1), 749-754.

Wang, J., Liu, J., Wu, J., Guo, Y., 2022. Effects of dietary magnesium on milk production, milk composition, and energy metabolism in early lactating dairy cows. *Journal of Dairy Science* 105(1), 264-273.

Wang, J., Wu, J., Liu, J., Guo, Y., 2018. Effects of lysophospholipids on the expression of genes involved in lipid metabolism in mammary gland of dairy cows. *Journal of Dairy Science* 101(6), 5378-5387.

Wang, L., Liu, X., Zhang, H., 2019. Lysophosphatidic acid promotes milk fat synthesis in bovine mammary epithelial cells via the PI3K/Akt signaling pathway. *Journal of Agricultural and Food Chemistry* 67(12), 3334-3343.

Wang, Y., Wang, W., Liu, Q., Xu, C., 2006. Determination of milk urea nitrogen by UV spectrophotometry. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 65(1), 73-77.

Wickramasinghe, S., Rincon, G., Islas-Trejo, A., Medrano, J.F., 2012. Transcriptional profiling of bovine milk using RNA sequencing. *BMC Genomics* 13(2), 45-59.

Yang, Y., Sun, X., Wang, X., Liu, J., Zhan, R., Wang, Y., 2020. Effects of lysophospholipids on milk production, milk composition, and gene expression in mammary gland of dairy cows during early lactation. *Journal of Dairy Science* 103(7), 6527-6540.

Zhang, H., Li, X., Chen, X., Wang, J., 2019. Effect of rationary supplementation of lysophospholipids on productive performance, nutrient digestibility and carcass quality traits of broiler chickens. Journal of Dairy Science 101(6), 5451-5460.

Zhang, H., Li, X., Ding, L., Chen, X., Wang, J., 2015. Effects of emulsification, fat encapsulation, and pelleting on weanling pig performance and nutrient digestibility. Journal of Dairy Science 98(6), 3985-3998.

Zhang, H., Li, X., Wang, J., 2018. Effects of lysophospholipids on the expression of genes involved in lipid metabolism in adipose tissue of dairy cows. *Journal of Dairy Science* 101(6), 5451-5460.

Zhao, J., Zhou, X., Liu, X., Liu, X., 2020. Lysophosphatidic acid promotes adipocyte differentiation and lipid accumulation in buffalo adipose-derived mesenchymal stem cells via the LPA/PPARγ signaling pathway. *General and Comparative Endocrinology* 29(5), 113-134.

Zhao, P.Y., Zhang, R.X., Lan, W.C., Liu, Z.F., Kim, I.H., 2017. Effect of lysophospholipids in *diet*s differing in fat contents on growth performance, nutrient digestibility, milk composition and litter performance of lactating sows. *Animal* 11(2), 984-990.