Original Article

Effects of Lysophospholipids on Blood Parameters and Antioxidant Activity in Holstein Calves With a Diet Containing Saturated Fatty Acids

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ABSTRACT

Background: The digestive development of suckling calves poses significant challenges, primarily due to their limited ability to produce specific digestive enzymes, which hampers nutrient digestion and lipid absorption. Although fat digestion begins in the mouth, most fat hydrolysis occurs in the small intestine, where the action of pancreatic lipases and bile salts is crucial. Incorporating dietary emulsifiers, particularly lysophospholipids, can enhance fat digestion and absorption by promoting micelle formation and improving nutrient uptake.

Objectives: The aim of the present study was to investigate the effects of lysophospholipids on growth performance and blood parameters in Holstein calves with a diet containing saturated fatty acids.

Methods: A total of 48 calves (24 males and 24 females) with an age range of 3±1 days and an average birth weight of 39.4±4 kg were selected in a completely randomized design with 4 treatments and 12 replications for 80 days. The experimental treatments were: 1) Basic diet without additives, 2) Basic diet with 3% saturated fatty acid-rich fat, 3) Basic diet with 2 g of lysophospholipid daily, and 4) Basic diet with 2 g of lysophospholipid daily + 3% saturated fatty acid-rich fat. Lysophospholipids were added to the milk from days 6 to 40 and to the starter from days 41–80.

Results: The experimental treatments had no effect on metabolizable energy (ME) intake of the calves, nor did they significantly influence the final body weight. However, the ME/gain ratio was significantly affected by the treatments ($P<0.05$), with the groups receiving 3% fat showing the lowest ratio compared to the other experimental groups. Blood parameters, including glucose, cholesterol, triglycerides, blood urea, albumin, total protein, and beta-hydroxybutyrate, showed no significant alterations on days 5, 40, and 80. Furthermore, the levels of total antioxidant capacity (TAC), and malondialdehyde (MDA) were unaffected by the dietary treatments. Although fecal consistency was not impacted from 1 to 5 days, from 6 to 40 days, and 41 to 80 days, significant differences were found over the entire rearing period, with calves receiving lysophospholipid demonstrating better fecal consistency compared to the control group.

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Conclusion: In conclusion, energy intake, final body weight, and blood parameters were not affected by experimental treatments. However, calves on the 3% fat diet had the lowest ME/gain ratio. Future research should explore the long-term impacts of lysophospholipids on gut health, enzyme activity, and microbiota composition, and also evaluate different dosages and application periods to optimize their efficacy in calf nutrition.

Keywords: Antioxidant activity, Blood parameters, Calf, Lysophospholipids, Saturated fatty acids

Introduction

One of the fundamental challenges in milk-fed calves is the proper development of the hindgut. The lower sections, such as the cecum and large intestine are responsible for water reabsorption, fermentation of residual feed, and maintaining fecal consistency. These areas support microbial colonization, especially by fiber-degrading bacteria that produce volatile fatty acids, which are vital for intestinal health (Khan et al., 2016). However, milk-fed calves face limitations in digesting nutrients, especially fats, due to a deficiency in specific digestive enzymes. The activity levels of enzymes, such as pancreatic lipase, amylase, pepsin, and trypsin are low in early life and increase with age and dietary changes (Jones & Heinrichs, 2017). Although salivary lipase plays a limited role (approximately 3%) in the hydrolysis of milk fat in the mouth (Davis & Drackley, 1998), effective fat digestion mainly occurs in the small intestine with the help of pancreatic lipases, lysolecithin, and bile salts, which collectively increase the digestibility of milk fat to about 79% (Thornsberry et al., 2016). Given pancreatic insufficiency in enzyme secretion and the potential lack of stimulation of hormones, like secretin and cholecystokinin, the formation of mixed micelles in the digestive tract may be disrupted, resulting in reduced fat digestion (Haetinger et al., 2003). In this context, the use of dietary emulsifiers can be an effective solution to improve fat digestion and absorption processes.

Lysophospholipids, as alternatives or complements to synthetic emulsifiers, have superior characteristics compared to other similar compounds in terms of function, structure, and physiological impact. Unlike common emulsifiers that are mainly used during the industrial processing of poultry feed, lysophospholipids have a targeted application in calf nutrition due to their multiple functions, including increasing the contact surface area of fat droplets, promoting the formation of smaller micelles, and improving nutrient transport in the intestine (Zhang et al., 2011; Zampiga et al., 2016). Previous studies have shown that the use of lysophospholipids in broiler chickens increases metabolizable energy (Melegy et al., 2010). Additionally, Zhang et al. (2022) showed that these compounds reduce the size of fat globules and increase absorption efficiency by creating smaller micelles and increasing the contact surface area. Furthermore, studies have (Haetinger et al., 2021; Huo et al., 2019) indicated the positive effect of lysophospholipids on increasing crude protein digestibility and modifying cell membrane permeability. From a biological perspective, lysophospholipids also play roles in regulating immune responses, cell proliferation, and glucose metabolism (Liu et al., 2020). Among their key components is choline, which helps improve intestinal function and growth in weaned piglets (Qiu et al., 2022).

In contrast to many studies that have focused on poultry or pigs, there is very limited information on the impact of lysophospholipids on milk-fed calves, especially under conditions where saturated fats are used. This study aimed to fill this scientific gap by investigating the effect of lysophospholipid supplementation on growth performance, blood parameters, and antioxidant activity in Holstein calves fed a diet containing saturated fatty acids.

Materials and Methods

The present study was conducted at the Taliseh Asil Agricultural and Livestock Company in Varamin County, Tehran Province. The study was initiated in early winter 2024 in Tehran province. For this purpose, 48 calves (24 males and 24 females) aged 3±1 days with an average birth weight of 39.4±4 kg were used in a completely randomized design with 4 treatments and 12 replications for 80 days. The calves received colostrum equivalent to 10% of their body weight from birth to 3 days of age. For the first two weeks after entering the study, they were fed 5.5 L of milk daily (twice a day), followed by 7.5 L of milk daily (twice a day) for 40 days. In the final week, they received 3.75 L of milk (once a day), and the calves were weaned at 65 days of age.

The experimental treatments included: 1) Control diet without additives, 2) Control diet with 3% saturated fatty acid-rich fat, 3) Control diet with 2 g of lysophospholipid daily, and 4) Control diet with 2 g of lysophospholipid daily + 3% saturated fatty acid-rich fat. Lysophospholipid was added to the milk from days 6 to 40 and to the starter from days 41 to 80.

The saturated fat supplement used in this research was Energizer RP10, a product of Malaysia. This supplement was prepared and used in the form of a solid, granulated white powder and had the following nutritional specifications: 99% pure fat, less than 0.5% moisture, free of plant fiber, with a melting point above 56 ˚C. The supplement contained at least 85% palmitic acid and at least 87% saturated fatty acids, with the total of other fatty acids estimated at a maximum of 13%. Its net energy for lactation (NEL) is equivalent to 6.1 Mcal/kg.

The lysophospholipid product used was Aqualyso, produced by Aruna. Aqualyso is a potent emulsifier and has the following composition: 3.5% moisture, 74% crude ash, 7.5% total phospholipids, 3.5% total lysophospholipids, and 1.65% lyso-phosphatidylcholine. The main components of this supplement include phosphatidylcholine (0.91%), lyso-phosphatidylcholine (1.65%), phosphatidylinositol (1.73%), lyso-phosphatidylinositol (0.6%), phosphatidylethanolamine (0.37%), lyso-phosphatidylethanolamine (0.78%), acyl-phosphatidylethanolamine (0.16%), phosphatidylglycerol (0.07%), phosphatidic acid (0.1%), and lyso-phosphatidic acid (0.47%).

The experimental diets were formulated using the NRC Dairy Cattle software (NRC, 2001) based on the nutritional requirements of a 41 kg calf, considering the chemical composition of the available feedstuffs. The chemical composition of the feedstuffs (crude protein, ether extract, neutral detergent fiber, acid detergent fiber, ash, calcium, and phosphorus) used in formulating the experimental diets was determined in the laboratory of the Research and Development Department of the Chaltasian Agricultural and Livestock Company (Table 1). Calves had access to a starter feed from day 5 of age. From day 20, chopped straw was mixed with the starter at a level of 7% of the total dry matter. Following weaning, the proportion of chopped straw in the starter mix was increased to 10% of total dry matter.

Growth performance

Feed consumption was measured every 10 days for each calf using a method where a fresh feed bucket was provided along with a plastic bag to collect waste. Each morning, one hour after the morning milk feeding, the leftover feed from the previous day was collected, and fresh feed was provided to the calves. This procedure was repeated eight times for each calf throughout the experimental period. The feed intake was determined by calculating the difference between the amount of feed provided and the remaining feed (based on dry matter) for each calf over a 24-hour period (Hill et al., 2009). The calves were weighed at the beginning of the experiment and every 10 days until the end of the trial using a digital scale. All variables were measured at 2:00 PM throughout the entire study period. Metabolizable energy (ME) intake of calves from milk and starter was considered as total ME intake. The ME intake-to-gain ratio was calculated by dividing ME intake by each calf's average daily gain.

Blood sampling

Blood samples were collected from the calves using vacuum tubes at three time points: 5, 40, and 80 days of age, after the morning feeding, from the jugular vein. The collected samples were immediately transferred to the laboratory and centrifuged for 10 minutes at 3000 rpm. The samples were then stored at -20 ˚C until analysis. Two series of blood samples were collected: the first series was placed in tubes without anticoagulant, and the second series in tubes containing heparin as an anticoagulant, and then transported to the laboratory. The concentrations of biochemical parameters in the blood, such as glucose, total cholesterol, triglycerides, total protein, albumin, blood urea, and beta-hydroxybutyrate, were determined using a Biotecnica Instruments BT1500 autoanalyzer (Rome, Italy) and kits from Pars Azmoon.

To assess oxidative stress and hepatic function in calves, serum malondialdehyde (MDA), aspartate aminotransferase (AST), and total antioxidant capacity (TAC) were measured. MDA was measured as an indicator of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay. In this assay, serum was mixed with thiobarbituric acid reagent and incubated at 95 ˚C. The absorbance of the resulting colored complex was then read at 532 nm using a spectrophotometer. AST levels were assessed spectrophotometrically. In this assay, changes in absorbance due to NADH reduction at 340 nm were measured after adding the specific substrate (aspartate and α-ketoglutarate) to the serum sample. TAC of the serum was measured using a commercial kit (Randox Laboratories, Crumlin, UK).

Table 1. Feed ingredients and nutrient composition of experimental treatments 1 and 3

Feed Ingredients		Starter (% of DM)	Starter with Fat (% of DM)
Corn		44	41
Barley		18	18
Soybean meal		28	28
Full fat soy		4	4
Saturated fatty acid		0	3
Sodium bicarbonate		1	1
Calcium carbonate		1	1
Dicalcium phosphate		1	1
Salt		0.50	0.50
Vitamins and minerals premix		2	2
Toxy guard		0.5	0.5
Chemical composition	Dry matter	90.2	89.9
	Crude protein	20.8	20.6
	Ether extract	1.9	4.6
	Acid detergent fiber	6.4	6.6
	Neutral detergent fiber	22.1	20.9
	Ash	7.5	8.3
	Calcium	0.57	0.57
	Phosphorus	0.44	0.45

Statistical analysis

Statistical analysis was performed using mixed models in SAS software (version 9.1, 2003), considering the treatment effect as a fixed effect and initial weight as a covariate (initial weight was used as a covariate for the analysis of daily weight gain, feed intake, and feed conversion ratio). The covariate effect was not statistically significant and was removed from the statistical model. A significance level of 0.05 was considered. The results are reported as the mean of each parameter along with the standard error of the mean. The statistical model for the basic design was expressed as $Yij=\mu+Ti+eij$, where Yij represents the observed measurement (each observation); μ is the overall mean of the observations; Ti is the treatment effect; and eij is the experimental error.

Results

The results of calf starter consumption, dry matter intake, average daily weight gain, and feed conversion efficiency are presented in Table 2. The results indicated that calf ME intake was not affected by the experimental treatments. Additionally, the experimental diets did not have a significant effect on final body weight. However, the ME/gain ratio was significant among the experimental treatments ($P<0.05$), such that the groups receiving 3 % fat had the lowest ratio compared to the other experimental treatments. Body-weight change and ME intake over the rearing period are shown in Figures 1 and 2, respectively

Blood parameters

The results related to the effects of experimental treatments on blood parameters are presented in Table 3. Adding lysophospholipid to the diets, with or without saturated fatty acids, had no effect on the concentrations of the blood parameters glucose, cholesterol, triglycerides, blood urea, blood albumin, total protein, and beta-hydroxybutyrate on days 5, 40, and 80 of age.

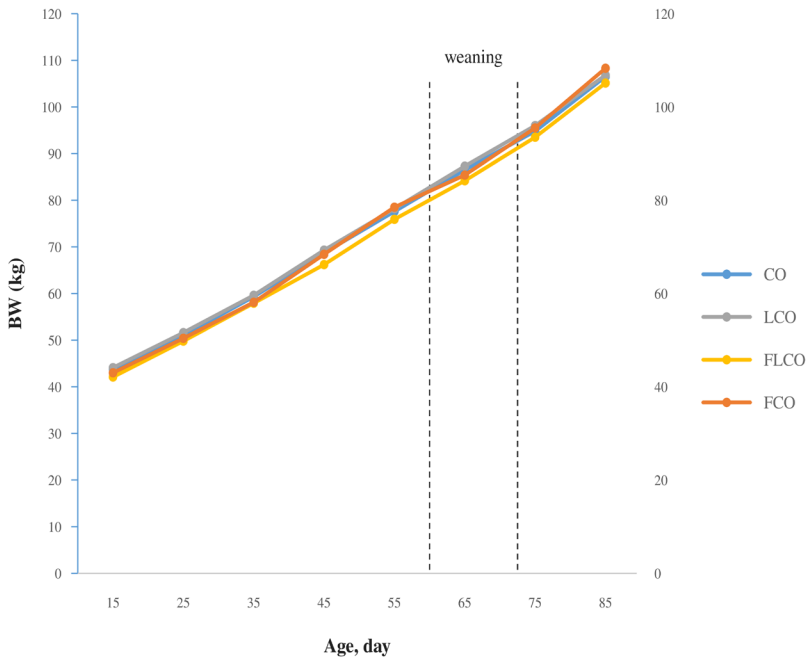

Figure 1. Body weight changes during the rearing period

Antioxidant activity

The results presented in Table 4 showed that adding lysophospholipid to the diets, with or without saturated fatty acids, had no significant effect on TAC. Additionally, the concentrations of MDA was also unaffected by the experimental treatments on days 5, 40, and 80 of age. At 5 days of age, there was no significant difference in AST concentrations among the experimental treatments. At 40 days of age, the addition of lysophospholipid to the diet significantly reduced AST concentrations ($P<0.05$); however, in the fat-containing diet, the addition of lysophospholipid caused no significant effect compared to the control group. At 80 days of age, the addition of lysophospholipid to both fat-containing and non-fat diets reduced AST concentrations compared to the control group ($P<0.05$).

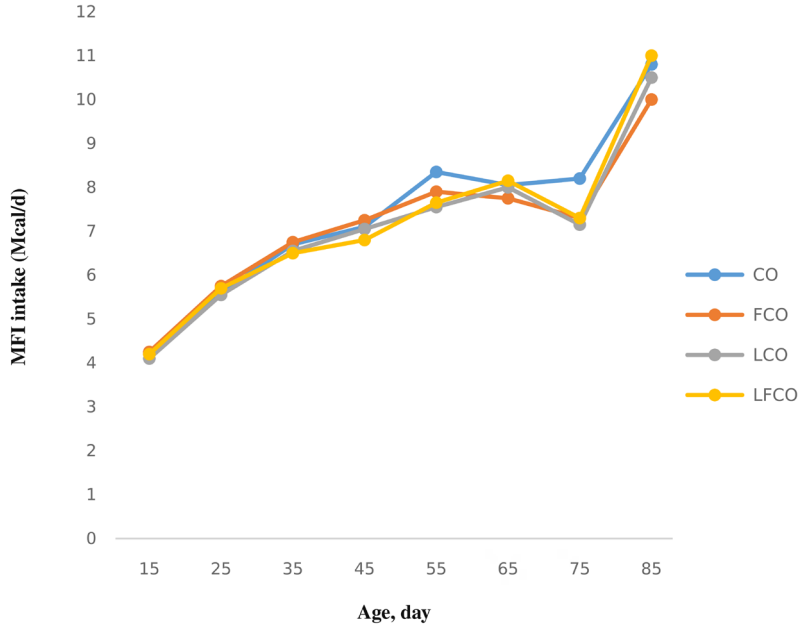

Figure 2. Metabolic energy intake of calf during the rearing period

Table 2. Effects of experimental treatments on ME intake and body weight of Holstein calves

Item	CO	FCO	LCO	LFCO	SEM	P
Final body weight (Kg)	106.05	109.17	105.34	106.1	1.74	0.06
ME intake (Mcal/d)	10.97	10.05	10.5	10.96	0.43	0.39
ME/gain ratio	13.21[a]	11.53[b]	12.89[a]	13.21[a]	0.45	0.03

Note: SEM: Standard error of the mean. CO: Control. FCO: Control plus 3% saturated fatty acid-rich fat; LCO: Control plus 2 g lysophospholipids daily; LFCO: Control plus 2 g lysophospholipids daily and 3% saturated fatty acid-rich fat. Within a row with different superscript letters differ significantly (P<0.05).

Table 3. Effects of experimental treatments on blood parameters of suckling calves

Parameter	Time Point (days)	CO	FCO	LCO	LFCO	SEM	P
Glucose (mg/dL)	5	105.6	100.2	111.6	122	10.74	0.53
	40	122.5	126.3	120.5	119.2	4.23	0.65
	80	103.2	105.8	98.5	105.8	4.82	0.66
Cholesterol (mg/dL)	5	76.33	60.66	57	67.66	8.23	0.38
	40	107	106.5	125.5	87.83	14.63	0.37
	80	66	84.33	69.16	73.33	7.67	0.37
Triglycerides (mg/dL)	5	66.33	50.16	53.66	44.33	9.72	0.45
	40	57.66	62.5	43.5	45.5	8.33	0.32
	80	25	26.66	23.16	34.5	5.05	0.42
Urea (mg/dL)	5	43.33	32.33	28.33	27.83	4.94	0.12
	40	24.33	17.16	16.5	18.5	4.01	0.51
	80	23	17	20	19.16	2.15	0.29
Beta-hydroxy-butyric acid (mmol/L)	5	0.071	0.085	0.095	0.07	0.01	0.34
	40	0.105	0.111	0.118	0.09	0.016	0.64
	80	0.24	0.303	0.26	0.255	0.035	0.63
Albumin (g/dL)	5	2.88	2.66	2.7	2.6	0.09	0.2
	40	2.91	3.05	3.01	3.08	0.08	0.53
	80	3.06	3.08	2.93	3.2	0.13	0.58
Total protein (g/dL)	5	9.08	8.98	8.41	8.93	0.26	0.32
	40	7.93	7.95	7.46	7.7	0.3	0.72
	80	8.05	8.41	8.35	8.41	0.27	0.75

Note: CO: control: calf starter without supplementation; FCO: control treatment plus 3% fat rich in saturated fatty acids; LCO: control treatment plus 2 g of lysophospholipids daily; LFCO: control treatment plus 2 g of lysophospholipids daily + 3% fat rich in saturated fatty acids.

Table 4. Effects of experimental treatments on antioxidant activity in Holstein calves

Parameter	Time Point (days)	CO	FCO	LCO	LFCO	SEM	P
Malondialdehyde (nm/dL)	5 days	2.78	3.08	2.95	2.35	0.45	0.69
	40 days	1.28	1.46	1.16	1.2	0.14	0.50
	80 days	1.18	1.16	1.25	1.48	0.13	0.35
Total antioxidant activity (µmol/L)	5 days	0.448	0.408	0.405	0.36	0.039	0.48
	40 days	0.31	0.333	0.231	0.335	0.019	0.77
	80 days	0.333	0.375	0.36	0.401	0.024	0.10
Aspartate amino-transferase (U/L)	5 days	26.45	25.32	24.01	25.02	2.48	0.17
	40 days	32.45^{a}	34.2^{a}	21.01^{b}	27.02^{ab}	3.58	0.05
	80 days	51.21^{a}	52.02^{a}	40.58^{c}	44.54^{b}	4.48	0.03

Note: CO: control, calf starter without supplementation; FCO: control treatment plus 3% fat rich in saturated fatty acids; LCO: control treatment plus 2 g of lysophospholipids daily; LFCO: control treatment plus 2 g of lysophospholipids daily + 3% fat rich in saturated fatty acids.

Discussion

In the present study, the addition of lysophospholipid to diets with and without fat did not have a significant effect on dry matter intake, body weight gain, or feed efficiency (Table 2). Farahmandpour et al. reported that the addition of 0.75% lysophospholipid to lipid-containing diets increased feed intake and body weight gain and improved feed conversion ratio (Farahmandpour et al., 2021). Reis et al. found that the inclusion of lyso-phospholipids as a feed additive in milk replacers at a dose of 4 grams per day improved average daily gain and feed efficiency, which contrasts with the results of the current study (Reis et al., 2021). Although the present findings showed improved nutrient absorption and fecal consistency, the lack of improvement in growth performance might be attributed to limitations in metabolic utilization or energy partitioning. It is possible that enhanced digestion did not translate into increased tissue accretion due to physiological constraints in young calves or suboptimal energy availability (Sordillo, 2013; Jang et al., 2020). According to studies by Reiss et al. (2021) on dairy cows and Gallo et al. (2019) on lambs, the consumption of lyso-phospholipids improved feed efficiency and growth performance. Researchers also demonstrated that the use of fat supplements increased starter feed intake compared to the control group (Mohtashami et al., 2021).

In another study, Azadshahraki et al. (2019) examined the effect of palmitic acid supplementation on Holstein calves and found that starter intake and growth rates were not influenced by fat consumption. These researchers reported that the addition of palmitic acid to the starter feed did not have a beneficial effect on the performance of pre-weaning calves. Conflicting results among various studies may be due to the source of the lyso-phospholipid supplement (Lee et al., 2019), the dosage of lysophospholipid (Song et al., 2019), the duration of supplementation (Lee et al., 2019), and the breakdown of the lysophospholipid supplement in the rumen (Huo et al., 2019). Incorporating emulsifiers, such as lysophospholipids or bile salts into the diet can generally improve the digestion and absorption of fats and growth performance (Maisonnier et al., 2003).

The efficacy of lysophospholipid supplementation may vary significantly depending on both the source and the dosage used, which could partially explain the inconsistent effects on growth performance observed in various studies. Different commercial lysophospholipid products contain varying proportions of active components, particularly lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), and other phospholipid derivatives, which influence their emulsifying potential and biological activity (Lee et al., 2019; Haetinger et al., 2021). For instance, lysophospholipids are known to enhance micelle formation and fat absorption, while LPI may modulate immune signaling and gut integrity (Liu et al., 2020). In the present study, the lysophospholipid product contained only 1.65% LPC and a total of 3.5% lysophospholipids, delivered at a fixed dose of 2 g/day. However, studies using higher inclusion rates have shown more pronounced effects. Reis et al. (2021), for example, used 4 g/day in dairy calves and reported improved daily weight gain and

feed efficiency. Similarly, Farahmandpour et al. (2023) observed enhanced rumen fermentation and microbial populations in lambs supplemented with 3 to 6 g/day. This dose-response relationship highlights the importance of optimizing lysophospholipid levels according to animal age, physiological stage, and dietary fat content. Furthermore, the stability of lysophospholipids in the rumen environment can differ depending on their structure and encapsulation method. Huo et al. (2019) demonstrated that ruminal degradation of unprotected lysophospholipids may limit their effectiveness, suggesting that bypass strategies or enteric-coated forms might improve bioavailability. Additionally, lysophospholipids derived from soy lecithin may differ in function compared to synthetic or marine sources (Zhang et al., 2022), making source standardization critical for reproducibility. Therefore, by supplementing with lysophospholipids, it was expected that the efficiency of fat utilization in the diet would increase. However, in experiments related to this hypothesis, no improvement in the performance of dairy calves was observed using lysophospholipid supplements.

In the present study, the addition of lysophospholipid supplementation with and without saturated fatty acids to the diet did not have a significant effect on the concentration of blood parameters (Table 3). In this regard, Farahmandpour et al. (2022) demonstrated that the inclusion of lysophospholipid in a lipid-containing had no effect on the concentrations of total protein and albumin, but the concentrations of urea increased with lysophospholipid supplementation.

In another study, Lough et al. (1992) showed that a diet containing 4.9% deoiled soy lecithin altered cholesterol and fatty-acid composition in carcass tissues of growing ram lambs. In a study, Movagharnezhad et al. (2024) demonstrated that diets containing lysophospholipid in dairy cows led to a reduction in blood urea nitrogen levels. However, the concentrations of total protein, triglycerides, total cholesterol, non-esterified fatty acids, and beta-hydroxybutyrate were not affected by the lysophospholipid supplementation. Salari et al. (2023) demonstrated that the addition of lysophospholipid to diets containing fat reduced the triglyceride concentration compared to diets without lysophospholipid supplementation. Hosseinzadeh et al. (2023) reported that the use of 1% active lysophospholipid in calves increased total blood protein levels and decreased blood urea levels. In contrast, blood cholesterol levels tended to increase with the use of this supplement. In contrast to the present study, Zhang et al. (2022) observed a linear increase in the total protein and albumin concentration with the supplementation of lysophospholipids, indicating that protein metabolism and liver function were influenced by this supplement. Additionally, Reis et al. (2021) stated that the addition of lysolysin as a milk replacer could lead to an increase in plasma protein concentration in dairy calves. Explaining the contradictions observed among studies is challenging. However, it is noteworthy that the exact and definitive mechanism by which lysophospholipids affect plasma lipids has not yet been identified, and further studies are needed to determine how emulsifiers influence blood parameters (Zhang et al., 2022).

In the present study, the adding lysophospholipid to the diet had no significant impact on TAC values or MDA concentrations over different time points. At 40 days of ag, lysophospholipid supplementation reduced AST levels significantly. At 80 days of age, both fat-containing and non-fat diets with lysophospholipid reduced AST levels. Oxidative stress resulting from an imbalance of reactive oxygen species (ROS) can lead to tissue damage and the loss of normal cellular function in cattle (Sordillo, 2013). Some studies have indicated that excess dietary fat can result in an imbalance in energy metabolism, the deposition of harmful lipids, and lipid peroxidation, ultimately leading to liver damage, inflammation, apoptosis, and the production of ROS (Dai et al., 2019; Lee et al., 2019).

Previous studies have shown that bile acid supplementation, which functions similarly to lysophospholipids, can enhance antioxidant activities and decrease the antioxidant system damage caused by high fat levels (Ding et al., 2020). Moreover, Huang et al. found that milk phospholipids can significantly improve antioxidant activity and delay the oxidation of fatty acids in vitro (Huang et al., 2020). In the present study, adding lysophospholipids to the fat-containing diet resulted in a non-significant increase in antioxidant capacity (Table 4). The antioxidant effects of lysophospholipids may be related to their choline component, which reduces oxidative stress by modulating cellular redox status and inhibiting the inflammatory response (Mehta et al., 2009) MDA is a product of lipid peroxidation in tissues, and as a biomarker, indicates the peroxidation of poly unsaturated fatty acids and the existence of oxidative stress (Asghari et al., 2021). In the present study, adding fat to the diet caused a numerical increase in MDA concentrations, which was reduced by adding lysophospholipids (Table 4). Cai et al. (2016) found that there is a negative correlation between dietary lecithin and MDA levels; as the phospholipid content increases, the MDA content decreases.

In general, the increase in AST levels in animals due to the addition of fat supplements to the diet may indicate liver damage, which can potentially lead to a reduction in liver weight (Bianchi et al., 2014; Carlson, 1996). However, in contrast to the current study's results, Farahmandpour et al. (2023) reported that the addition of lysophospholipid to the diet of lambs did not significantly affect AST concentrations. A study has shown that adding 5% soybean lecithin supplement to the diet does not alter the concentrations of hepatic enzymes, such as alanine aminotransferase and AST (Carlson, 1996). Another study demonstrated that the inclusion of canola and soybean oil in the diet resulted in an increase in the concentrations of these enzymes (Parvar et al., 2017). Furthermore, in another study, the addition of 6% lecithin supplement to the diet increased AST levels in male fattening lambs (Rahmani et al., 2012).

Conclusion

It is concluded that the experimental treatments did not significantly affect ME intake, final body weight, blood parameters, or antioxidant indices, except AST. However, calves on the 3% fat diet had the lowest ME/gain ratio.

Ethical Considerations

Compliance with ethical guidelines

This study was conducted at the Taliseh Asil Agricultural and Livestock Company in Varamin County. All animal operations were carried out in compliance with protocols approved by the Research Ethics Committees of the University of Mohaghegh Ardabili. Animal procedures were carried out according to protocol No. 19293, approved by the Iranian Council of Animal Care.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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