Micellized conjugated linoleic acid as an immune modifier feed additive for suckling calves

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ABSTRACT

This study attempted to assess the effects of micellized conjugated linoleic acid (CLA) as a feed additive for suckling calves on their growth performance and blood metabolic, oxidative, and immune parameters. Forty-eight Holstein calves were divided among four experimental groups (12 calves/treatment), including 1) calves with no CLA supplementation (CON), 2) calves supplemented with 1 gr CLA/d as micellized form by dissolving 5 mL/d of a CLA-contained emulsion in milk (CLA1), 3) calves supplemented with 2 gr CLA/d as micellized form by dissolving 10 mL/d of a CLA-contained emulsion in milk (CLA2), and 4) calves supplemented with 3 gr CLA/d as micellized form by dissolving 15 mL/d of a CLA-contained emulsion in milk (CLA3). Calves in the CON group received 10 mL of the emulsion medium with no CLA. Feeding micellized CLA via whole milk resulted in a linear increase in blood glucose concentration on day 40 ($P=0.04$) and total protein ($P<0.01$), albumin ($P<0.01$), and triglyceride ($P=0.02$) concentrations on day 20 of the experiment. The inclusion of micellized CLA resulted in a linear decrease ($P<0.05$) in blood malondialdehyde concentration at both periods but had no effect on blood total antioxidant status. On day 20, tumor necrosis factor-alpha level in the blood of suckling calves exhibited a quadratic effect with micellized CLA inclusion; however, interleukin-6 concentration was not affected. The use of 3 g per day of micellized CLA, via daily milk has the potential to reduce inflammation in young calves during the pre-weaning period.

Introduction

Calf rearing is very challenging and the highest mortality in dairy cattle occurs from birth to the weaning period. Neonatal calves are born with no active immunity and rely only on passive immunity that comes from colostrum to protect them from environmental pathogens. Due to their higher susceptibility to both disease and death, improving the health and nutrition of dairy calves are important factors for maintaining viable and sustainable dairy farms. A better understanding of the immune system of calves will result in lower antibiotic use and the production of high-quality replacement heifers for the dairy herd (2, 25).

Conjugated linoleic acids (CLA), a class of LA isomers characterized by a conjugated double bond, are found mainly in milk and meat from ruminant animals due to their rumen microbial action on the LA. Cis-9, trans-11 CLA and trans 10, cis-12 CLA are the most important isomers, among CLA isomers, with some functional effects on human and animal health including body fat and serum cholesterol reduction, antioxidant activity, anticarcinogenic and immune-enhancing effects (6, 29). CLA feed supplements have been studied extensively in dairy cattle nutrition due to their effects on depressing milk fat, enhancing milk yield, and alleviating negative energy balance in early lactating dairy cows (6,

26). In addition, there are reports on the effects of dietary CLA on stimulating dairy cows' immune system by stimulating the ability of peripheral blood mononuclear cells (6) and reducing monocyte apoptosis (3). Maternal CLA supplementation has been reported to influence metabolic changes and factors related to neonatal insulin response and had some effects on the growth of intestinal mucosa in calves (29).

Emulsions are the mixture of two or more liquids that are naturally immiscible together and mix with each other by the process called emulsification. Emulsion systems have been used widely to deliver lipophilic bioactive compounds in food, cosmetic, and pharmaceutical products (22). Oil-in-water emulsions are the best medium for incorporating and delivering lipophilic nutraceuticals in milk and dairy products for domestic animals and humans (14). CLA and other lipophilic bioactive compounds cannot be administrated directly to suckling calves via milk due to their insolubility in milk. Naturally, milk is an oil-in-water emulsion and therefore, the use of emulsion systems to deliver lipophilic bioactive compounds via mixing in milk may be the best administration route.

There are efforts on micellizing CLA for use in human nutrition (31, 35, 19). However, there is no report focusing on feeding CLA in a micellized form to suckling calves. The present study aimed to evaluate an emulsion-based delivery system for administering CLA as a bioactive lipophilic compound via milk for suckling calves. This study was an attempt to assess the effects of micellized conjugated linoleic acid as a feed additive for suckling calves on their growth performance, and blood metabolic, oxidative and immune parameters.

Materials and Methods

Emulsion preparation and evaluation: Emulsion preparation was based on the method described by Asghari et al. (2). In brief, 25 g of lecithin (Ehsan supplying group, Ardabil, Iran) was mixed with 200 mL of CLA oil (CLA oil 80%, 39.9% cis-9, trans-10 CLA, 39.4% cis-10, trans 12; CLA Suzhou Vit-ajoy Bio-Tech CO., Ltd. China) on a heater (55 ºC) equipped with a magnetic mixer until full mixing to make the lipid portion. To make the aqueous portion, 2 g of gum Arabic (Sigma-Aldrich, CAS Number, 9000-01-5) and 20 g of whey protein (Ehsan supplying group, Ardabil, Iran) were dissolved in 500 mL distilled water until full mixing on a laboratory heater (40 ºC). Then, the lipid portion was added slowly to the aqueous portion while mixing with a magnetic stirrer. After full mixing, distilled water was added to the emulsion to have a 1000 mL final volume. To avoid microbial contamination, sodium benzoate (0.1% w/w, 98.5% extra pure, Dr. Mojallali Industrial Chemical Complex Co.) and potassium sorbate (0.1% w/w, 99%, Mobtakeran Chemistry, Tehran, Iran) was added to the mixture. Butylated hydroxyl toluene (0.2% w/w, extra pure, supplied by CCIRAN Co.) was used as an antioxidant agent to protect the CLA fatty acids from oxidation. Thereafter, the emulsion was further mixed with a blender at 8000 rpm for 10 minutes and homogenized with a high-speed lab homogenizer (IKA T25, Germany) at 20000 rpm for 10 minutes. The final product, containing 200 g of CLA per L of the emulsion, was stored in a 1 L dark polyethylene bottle. A CLA-free emulsion was made and used for the control treatment.

After 3 weeks of storage at room temperature, the dynamic light scattering technique (DLS) was used to measure the droplet size of the micellized CLA (Nanopartica SZ-100V2 Series, HORIBA Scientific Instrument, Kyoto, Japan) and electrophoretic light scattering technique was applied to assess the surface charge density (Zeta potential) of the prepared emulsion droplets in triplicates. Atomic force microscopy (Core AFM Nano surf, Liestal, Switzerland) was used to take an image of the emulsion droplets (Figure 1). The samples were diluted to 1:1000 with distilled water before the measurements.

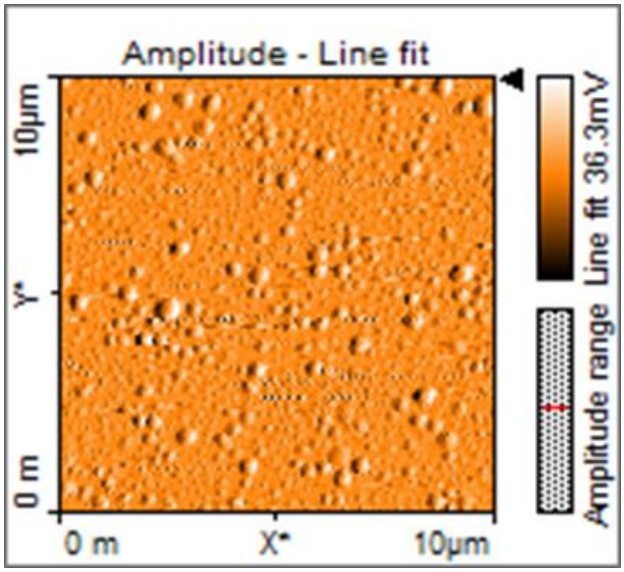

Figure 1. Atomic force microscopy image of the prepared emulsion sample.

Experimental design and animal management: Protocols applied for this experiment were approved by the Animal Ethics Committee of the University of Mohaghegh Ardabili (Ardabil, Iran) (Approval Number: IR.UMA.REC.1402.019) and a cooperation contract was signed between the University of Mohaghegh Ardabili and Moghan Agro-Industrial and Animal Husbandry company for the participation of the animals. Forty-eight male Holstein calves (6-day old; initial body weight of

39.43 ± 4.0 kg) were selected from farm-2 calf rearing station of (Parsabad, Ardabil, Iran) and assigned randomly to four groups (calves/treatment) in a completely randomized design. The experimental groups were: 1) the control group with no CLA supplementation (CON), 2) calves supplemented with 1 g of micellized CLA/d by dissolving 5 mL/d of the prepared emulsion in milk (CLA1), 3) calves supplemented with 2 g of micellized CLA/d by dissolving 10 mL/d of the prepared emulsion in milk (CLA2) and 4) calves supplemented with 3 g of micellized CLA/d by dissolving 15 mL/d of the prepared emulsion in milk (CLA3). Calves in the CON group received 10 mL of the CLA-free emulsion. The calculated amount of the emulsions was mixed with morning milk meal immediately before milk feeding for the first 42 days of the experiment and weaning was done on d 56.

Calves received 4 kg Colostrum after birth for 3 days and thereafter, a step-rise and step-down milk feeding program was applied for feeding calves. Briefly, 4 kg of whole milk was fed daily for the first 2 weeks, 6 kg from the third to sixth weeks, 4 kg for the seventh week in two equal meals at 8:00 and 18:00 and 2 kg of milk only for a morning meal for the eighth week to the weaning at day 56 (56 days). Milk feeding was performed by using plastic buckets and free access to a starter diet and water from d 7 was allowed. Ground chopped alfalfa hay (10%, w/w) was mixed with the starter diet from d 20. Calves were reared in individual pens (1 × 2.5 m) that were cleaned and re-bedded daily with dry straw. The ingredients and chemical composition of the starter feed, alfalfa hay, and milk (dry matter basis) are shown in Table 1.

Table 1. Ingredients and chemical composition of the starter feed, alfalfa hay and milk (dry matter basis).

	Starter feed	Alfalfa hay	Milk
Ingredients, g/kg			
Maize grain, grounded	350	-	-
Barley grain, grounded	220	-	-
Wheat bran	40	-	-
Soybean meal	360	-	-
Salt	5	-	-
Limestone	10	-	-
Vit and Min premix[1]	10	-	-
Dicalcium phosphate	5	-	-
Chemical composition, g/kg			
Dry matter	910	878	122
Crude protein	185	142	30.1
Ether extract	275	25.5	33.5
Neutral detergent fiber	166	561	-
Acid detergent fiber	81	380	-
Calcium	6.2	15	-
Phosphorus	5.2	4	-

[1] Vitamin Premix provided per kg of diet: vit A, 200000 IU; vit D, 300000 IU; vit E, 10000 IU; vit K, 2 mg; Butylated hydroxytoluene 1000 mg/kg. Mineral premix provided per kg of diet: Cu, 3300 mg/kg; Fe, 100 mg; Zn, 16500 mg/kg; Mn, 9000 mg; I, 120 mg/kg; Co, 90 mg/kg; Se, 90 mg/kg.

Sampling, data collection, and chemical analysis: Growth performance and body weight gain were done by serial weighing of all calves on d 14, 28, and 56 (weaning day) of the experiment. Daily solid feed intake was measured by recording daily feed offered and feed refused. The feed conversion ratio (FCR) was calculated by dividing daily feed intake by weight gain without considering the consumption of milk. On d 20 and 40, two blood samples were taken 3-4 hours after the morning meal from the jugular vein to obtain plasma and serum samples. Plasma and serum samples were taken by 15 min centrifugation at 3500 × g at 4°C and frozen at -20°C. On analysis day, plasma samples were thawed at room temperature and analyzed for blood metabolites such as glucose, triglycerides, cholesterol, total protein, albumin, and blood urea nitrogen (BUN) by using Pars Azmoon commercial kits (Pars Azmoon Co., Tehran, Iran). Ranbut assay was used for determining beta-hydroxybutyric acid (BHBA) concentration in serum samples (Ranbut assay, Randox Laboratories, Crumlin, UK). Liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in serum samples by using Pars Azmoon commercial kits. Total antioxidant status (TAS) and malondialdehyde (MDA) concentrations were determined in serum samples by Randox assay (Randox Laboratories, Crumlin, UK) and the method described by Moore and Robert (1998), respectively. Bovine tumor necrosis factor-alpha (TNF-α) and bovine interleukin-6 ELISA kits (Shanghai Crystal Day Biotech Co., LTD. Shanghai, China) were used for determining blood TNF-α and IL-6 concentrations, respectively.

Chemical analysis of starter and alfalfa hay was done to determine the contents of dry matter, crude protein, ether extract by methods AOAC (1), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (30) and calcium and phosphorous contents (AA-670, Shimadzu, and Tokyo, Japan). Chemical analysis of milk samples was performed by Delta dairy analyzer (CombiScope FTIR 600/300 Hp - Dairy Analyser, Delta Instruments, Drachten, and Netherland).

Statistical Analysis: Performance data (daily gain and feed intake) were analyzed as repeated measurements by using the Mixed Procedure of SAS (13) as a completely randomized design. CLA feeding effect as the treatment effect, time effect, and CLA and time interaction effect

were considered in the statistical model as fixed effects and individual effects of calves in treatments as random effects. Based on smaller Schwarz Bayesian criteria, the UN covariance structure was applied for the repeated measures analysis (13). The interaction between CLA feeding and time was not significant and therefore was excluded from the model. The effect of time was significant for all performance data ($P<0.01$). The orthogonal polynomial CONTRAST statement of SAS (13) was used for linear and quadratic contrasts among experimental groups. Statistical analysis of test-day data including blood parameters was performed by GLM procedure of SAS with the effect of CLA feeding as a fixed effect. The covariate effect of the body weight of calves at the onset of the experiment was included in the statistical model. Significant effects were declared at $P\leq0.05$ and $0.05<P<0.10$ was discussed as the significance trend.

Results

Emulsion characteristics: Emulsion characteristics measured three weeks after preparation are shown in Table 2. Based on dynamic light scattering (DLS) data, the micellized CLA had 423 nm mean particle size and 100 percent stability after two months of storage at room temperature. Consistent with high stability index, the micellized CLA had a high negative zeta potential values (-69.5 mV mean zeta potential).

Table 2. The CLA contained emulsions characteristics.

	Droplet size (nm)[a]	Z-average (nm)	Zeta potential (mV)	Stability (%)
Mean ± SD	423 ± 303	1109 ± 676	-69.5 ± 3.5	100[b]

[a] Measured by dynamic light scattering technique.
[b] measured as the percentage of creaming layer formed after two months.

Calves' growth performance: The final body weight of the claves was not affected by feeding the micellized CLA (Table 3). Supplementation with micellized CLA did not affect the average daily gain of calves on d 1−14. However, orthogonal contrast between the CON group and calves that received the micellized CLA showed a tendency for increased average daily gain on 14-28 of the experiment (P=0.09). The total average daily gain of calves was not influenced by increasing doses of the micellized CLA. Feeding different levels of the micellized CLA did not affect the daily feed intake of calves. However, a tendency for improved feed conversion ratio (FCR) was noted for calves supplemented with the micellized CLA compared with CON calves (P=0.07).

Blood metabolites: Feeding micellized CLA via milk increased the blood concentration of glucose on d 40 of the experiment (L; P=0.04) and CLA-fed calves also had higher blood glucose levels compared to the CON calves (P=0.03, Table 4). Blood concentrations of cholesterol, urea, and beta-hydroxybutyric acid were not affected by feeding micellized CLA. Total protein (P=0.001), albumin (P=0.002), and triglyceride (P=0.021) concentrations increased linearly on d 20 with increasing doses of the micellized CLA, however, after 40 days, their blood metabolite concentrations were similar. Blood levels of AST and ALT were not affected by CLA supplementation.

Blood oxidative and immune markers: The blood total antioxidant status (TAS) of the calves was not affected by feeding the micellized CLA in milk. However, CLA supplementation reduced blood malondialdehyde concentration (MDA) in d 20 (L; P=0.01) and d 40 (L; P=0.03) of the experiment. On d 20, tumor necrosis factor-alpha (TNF-α) level in the blood of suckling calves decreased (L; P=0.001) with micellized CLA inclusion, however, interleukin-6 (IL-6) concentration was not affected (Table 5).

Table 3. Effects of feeding micellized CLA on feed intake and growth performance of suckling calves.

	CON	CLA1	CLA2	CLA3	SEM	P-value		
						O	L	Q
Initial weight (kg)	39.4	39.3	39.5	39.3	1.62	0.982	0.986	0.969
Final weight (kg)	87.1	90.3	89.1	91.6	2.14	0.211	0.216	0.885
Feed intake (g/d)								
1-14 days	168.5	175.6	142.1	192.2	15.76	0.935	0.597	0.183
14-28 days	454.8	496.1	494.6	419.8	44.83	0.330	0.599	0.357
Total	881.7	835.8	818.4	856.0	26.4	0.420	0.773	0.238
ADG (g/d)								

1-14 days	401.7	471.2	435.7	480.3	37.21	0.176	0.247	0.744
14-28 days	539.3	548.2	503.5	575.0	51.36	0.090	0.122	0.715
Total	734.6	784.6	762.5	804.8	35.02	0.234	0.241	0.914
Total FCR[2]	1.18	1.09	1.08	1.07	0.046	0.068	0.138	0.370

CON = control group with no CLA supplementation, CLA1= calves received 1 g CLA/d; CLA2 = calves received 2 g CLA/d; CLA3= calves received 3 g CLA/d. ADG = average daily gain. Total FCR = total feed conversion ratio calculated as total feed intake divided by total ADG. O = orthogonal contrast between the control group and CLA receiving groups, L = linear effect of feeding emulsified CLA, Q = quadratic effect of feeding emulsified CLA. SEM = standard error of the mean.

Table 4. Effects of feeding micellized CLA on blood metabolites of suckling calves.

	CON	CLA1	CLA2	CLA3	SEM	P-value		
						O	L	Q
Glucose (mg/dl)								
d 20	117.0	99.3	114.4	110.8	9.25	0.416	0.939	0.448
d 40	57.2	68.1	82.3	73.4	6.77	0.035	0.048	0.155
Cholesterol (mg/dl)								
d 20	95.1	93.8	101.4	111.9	7.73	0.412	0.601	0.911
d 40	104.6	103.9	85.0	105.6	10.29	0.591	0.733	0.308
Triglyceride (mg/dl)								
d 20	14.6	15.5	18.9	19.8	1.714	0.095	0.021	1.000
d 40	22.0	27.0	22.7	28.9	3.179	0.263	0.260	0.856
BUN (mg/dl)								
d 20	36.6	36.5	42.1	42.0	3.72	0.412	0.203	1.000
d 40	10.6	17.2	21.6	12.1	2.95	0.073	0.509	0.011
Albumin (g/dl)								
d 20	3.4	3.6	4.3	4.2	0.20	0.011	0.002	0.498
d 40	3.6	3.6	3.7	3.5	0.08	0.777	0.772	0.773
Total protein(g/dl)								
d 20	6.5	6.7	7.1	7.4	0.14	0.001	0.001	0.760
d 40	7.2	7.0	6.9	6.9	0.21	0.221	0.224	0.666
BHBA (mg/dl)								
d 20	0.14	0.11	0.11	0.12	0.02	0.135	0.273	0.338
d 40	0.30	0.27	0.39	0.27	0.04	0.849	0.904	0.262
AST (U/ml)								
d 20	23.0	23.0	20.5	24.3	3.21	0.911	0.931	0.562
d 40	64.0	58.5	63.3	63.4	2.93	0.501	0.824	0.343
ALT (U/ml)								
d 20	26.1	25.5	28.1	25.4	3.63	0.961	0.982	0.772
d 40	20.7	21.6	20.8	20.3	0.90	0.879	0.579	0.420

CON = control group with no CLA supplementation, CLA1= calves received 1 g CLA/d; CLA2 = calves received 2 g CLA/d; CLA3= calves received 3 g CLA/d. BHBA = beta-hydroxybutyric acid, BUN = Blood urea nitrogen, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase. O = orthogonal contrast between the control group and CLA receiving groups, L = linear effect of feeding emulsified CLA, Q = quadratic effect of feeding emulsified CLA. SEM = standard error of the mean.

Table 5. Effects of feeding micellized CLA on blood oxidative and immune parameters of suckling calves.

	CON	CLA1	CLA2	CLA3	SEM	P-value		
						O	L	Q
TAS (mmol/l)								
d 20	0.55	0.60	0.59	0.66	0.06	0.358	0.249	0.873
d 40	0.56	0.55	0.54	0.52	0.03	0.543	0.280	0.713
MDA (mmol/l)								
d 20	2.29	2.38	1.49	1.30	0.16	0.006	0.001	0.396
d 40	2.83	3.03	2.09	2.26	0.26	0.23	0.035	0.963

TNF-α (ng/l)								
d 20	183.5	90.8	139.4	116.6	5.80	0.001	0.001	0.001
IL-6 (ng/l)								
d 20	31.8	38.9	35.3	38.5	3.02	0.115	0.242	0.512

CON = control group with no CLA supplementation, CLA1= calves received 1 g CLA/d; CLA2 = calves received 2 g CLA/d; CLA3= calves received 3 g CLA/d. MDA = Malondialdehyde, TAS = Total antioxidant status, TNF-α = Tumor necrosis factor-alpha, IL-6 = Interleukin-6. O = orthogonal contrast between the control group and CLA receiving groups, L = linear effect of feeding emulsified CLA, Q = quadratic effect of feeding emulsified CLA. SEM = standard error of the mean.

Discussion and Conclusion

The incorporation of lipid-based nutrients and bioactive components in aqueous-based foods and drugs is faced with some challenges due to their hydrophobic nature and water insolubility. The application of emulsion-based delivery systems is the best route to overcome the problem of administering lipophilic bioactive compounds such as conjugated linoleic acid and other functional fatty acids, herbal essential oils, fat-soluble vitamins, etc (2, 22). Due to increased digestion, absorption, stability, and bioavailability, emulsion-based delivery systems are used not only for delivering lipophilic bioactive compounds but also for delivering water-soluble micronutrients such as vitamin C (9).

Milk is an oil-in-water emulsion with its fat globules dispersed in an aqueous medium along with other nutrients such as protein, carbohydrates, vitamins, and minerals (2). In the present study, CLA, as a lipidic bioactive molecule, was incorporated in an oil-in-water emulsion to deliver via milk to suckling calves. Milk fat micelles (globules) had a wide range of diameter from 100 to 10000 nm with an average diameter of around 4000 nm (34). The micellized CLA droplet size was similar to milk fat micelles and their incorporation into calves' daily milk intake had no digestive problems during the experiment period. In addition, the droplets of the micellized CLA had a large negative surface charge (-69.5 mV) that shows excellent stability. Zeta-potential values of greater than ±60 mV are classified as having excellent stability and emulsions with values greater than ±30 mV are classified as having good stability (12).

CLA isomers, including cis-9, trans-11 and cis-10, trans-12, have been studied extensively due to their potential effects on immune system modulation, disease prevention, lipid and glucose metabolism, and body composition alteration in animals (6, 23, 24). In this study, feeding CLA isomers as the micellized form to suckling calves did not improve daily weight gain which resulted in better FCR values for supplemented calves compared to CON ones. This observation was consistent with their higher blood glucose, albumin, and total protein concentrations and lower tumor necrosis factor-α (TNF-α) levels. The highest and lowest FCR values were recorded for calves in the CON and CLA3 groups, respectively. Increased blood glucose level by feeding micellized CLA observed in the present study is consistent with previous results reported by Moloney et al. (17) and Risérus et al. (21). The effect of CLA on blood glucose was attributed to reduced insulin sensitivity (12). Cantwell et al. (4) reported that incubating hepatocytes with mixed CLA isomers resulted in increased protein synthesis and this report is consistent with our results on the positive effects of CLA supplementation on blood protein parameters with higher albumin and total protein concentrations after 20 days of feeding the micellized CLA. Previous studies in animals and humans have reported CLA effects on energy, lipid, and glucose metabolism (7, 8, 11, 33). Increased triglycerides (TG) concentration in the blood of CLA-received claves could have been caused by reduced insulin sensitivity (17), increased lipolysis (7), and induction of adipocyte apoptosis (8). Increased TG level with micellized CLA supplementation, observed in the present study, was in agreement with some earlier works (7, 33).

In farm animals, stimulated immune function coincides with decreased production performance due to inflammatory functions and conversely, increased growth rate and milk production suppresses the immune system (5, 20). Inflammatory cytokines are produced when the immune system is stimulated to increase the proliferation of immune cells to attack pathogens. However, these cytokines have some extra catabolic effects on body tissues and can result in a decrease in feed intake and the breakdown of skeletal muscle (32). CLA has been recognized as an anti-inflammatory compound that can decrease the production of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines (27). Miller et al. (16) reported that feeding mice with CLA prevented anorexia and weight loss induced by bacterial lipopolysaccharide injection. Therefore, decreased blood TNF-α level by CLA supplementation, in this study, is consistent with other reports (16, 27) and can be attributed to the suppressive effect of the micellized CLA fed to the calves. IL-6 is a pro-inflammatory cytokine that causes the induction of the hepatic acute phase protein response and stimulation of B- and T-cell responses (15). TNF-α, produced by white blood cells, especially macrophages, is one of the most important pro-inflammatory cytokines and is involved in vasodilatation and edema formation, and

contributes to oxidative stress in sites of inflammation (36). Therefore, a reduction in TNF-α production may be associated with higher cell integrity and lower tissue peroxidation. This matter may explain the lower malondialdehyde (MDA) concentration in the blood of calves that received the emulsified CLA supplement. MDA is a good marker for the tissue oxidative stress produced from the peroxidation of polyunsaturated fatty acids. The hypothesis that the effects of CLA could be mediated by preventing the production of some pro-inflammatory cytokines, particularly TNF-α and the activation of peroxisome proliferator-activated (PPAR) receptors have been proposed in some previous studies (10, 23, 28).

This study was an attempt to use an emulsion-based delivery system to make CLA soluble in an aqueous medium like milk and to design a CLA feed additive for suckling calves. The application of this method introduces a new administration route for feeding lipophilic nutrients like CLA for suckling calves. The micellized CLA fed to the calves was effective in causing metabolic changes such as enhancing blood glucose and protein and decreasing blood TNF-α levels. Based on the current results, micellized CLA can be introduced as an anti-inflammation feed additive for suckling calves, especially for recovery periods after infectious diseases. The results suggest that 3 g per day of the micellized CLA can be used for suckling calves via daily milk to maximize its positive effects.

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Conflict of Interest

The authors declare that when conducting their search, there was no business or financial rela-tionships that may be interpreted as constituting a conflict of interest.

Author Contributions

BK, HAB, JS designed the experiment, carried out the research and laboratory analysis. The re-search was conducted under the supervision of HAB, JS, and MRZ. The final version of the manuscript was submitted by agreement of all authors.

Data Availability Statement

All data and materials are available.

Ethical Statement

Protocols applied for this experiment were approved by the Animal Ethics Committee of the University of Mohaghegh Ardabili (Ardabil, Iran) (Approval Number: IR.UMA.REC.1402.019) and an cooperation contract was signed between University of Mohaghegh Ardabili and Moghan Agro-Industrial and Animal Husbandry company for the participation of the animals.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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