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The Effects of Lysophospholipid on Performance, Ruminal Bacteria and Some Blood Parameters in Lactating Holstein Dairy Cows

Research Article

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ABSTRACT

In order to examine the effects of feeding different levels of lysophospholipid (LPL) on early lactating Holstein cows, 15 cows were assigned in a completely randomized design with three experimental treatments including: 1) control ration; 2 and 3) control ration plus 0.1% and 0.15% of dry matter (DM) LPL, respectively. The experiment was conducted for 35 days (14 days for adaptation and 21 days for sample collection). Cows fed twice a day at 7:00 and 16:00 and received total mix ration (TMR). Daily feed intake and milk yield were measured. At the end of the experiment (day 35), the blood samples and rumen liquor were collected from each cow. The supplementation of LPL had no effect on DM intake and digestibility but increased digestibility of fat (P=0.0346). Addition of LPL to the diet slightly improved milk production (P=0.0673). The level of alanine aminotransferase (ALT) enzyme decreased with increasing LPL (P=0.0324) but activity of carboxymethyl cellulase enzyme activity increased (P=0.0421). Increasing LPL in the ration increased the amount of acetate (P=0.0452) and valerate (P=0.0033) produced in the rumen. The LPL significantly increased the population of cellulolytic bacteria (P=0.0386). In general, supplementing the ration of lactating cows with LPL, especially in the early lactation, increased feed efficiency in a dose-response manner, milk production and improved serum parameters, maintains liver health, improved ruminal bacterial population and fermentation parameters, and increased ruminal and hepatic enzymes activity.

KEY WORDS bacterial population, blood parameter, dairy cow, digestibility, early lactation, lysophospholipid.

INTRODUCTION

The negative energy balance (NEB) leads to mobilization of body reserves, especially fat mainly from adipose tissue to meet the energy requirements in early lactating of high-yielding dairy cows (Xing *et al*. 2018). The important proceedings in this period for providing the necessary energy are fat supplementation and ration enrichment. In addition to the beneficial effects of fat in providing energy, it also has adverse effects for ruminants that must be neutralized (Grummer *et al*. 2001). Researchers found that ruminal active fats decrease dry matter intake, rumen fermentation, neutral detergent fiber (NDF) digestibility, and decrease fat oxidation in the liver (Kim *et al*. 2004). A decrease in the rumen rate of passage of digestible substances by adding fat to the ration can increase the dilation of the rumen and stimulate stretch receptors in the rumen, the result of which is probably a decrease in dry matter intake (Allen *et al*. 2000). Therefore, by supplementing fat, its adverse effects should be minimized. In this case, the use of animal fats in livestock rations is more suitable because saturated fatty acids have less negative effect on rumen microbes than

unsaturated fatty acids (Zin *et al*. 1999). Emulsifiers are feed additives that can improve the digestion and absorption of fatty acids by acting as surfactants and helping to maintain the emulsion position of fatty acids in digesta (Neto and Moolenaar, 2011). Lysophospholipid (LPL) is an effective emulsifier that can improve fat digestion and absorption, leading to increased growth, feed efficiency (Mingret *et al*. 2011), and absorption of dietary nutrients in ruminants (Zhao and Kim, 2017). LPL are monoacyl-derivatives of phospholipids resulting from the action of phospholipase A1 or A2 (Joushi *et al*. 2006) and can improve the digestion and absorption of lipids (Boontiam *et al*. 2017). LPL has a balanced hydrophobic-hydrophilic part, can form protein channels in the membrane, and has a positive effect on emulsification, digestion, and absorption of fat and other parts of the feed (Loundbick *et al*. 2010; Boontiam *et al*. 2017). Few research has been conducted on use of LPL in the production and composition of milk, fatty acid profiles and blood parameters in ruminants (Lee *et al*. 2019; He *et al*. 2020), especially in early lactation of dairy cows. The hypothesis is that adding LPL along with fat powder to the diet of early lactation cows can increase eenergy release and have a positive effect on feed digestion and absorption and production performance of dairy cows. Therefore, in this experiment, the use of LPL in early lactation and its effects on digestibility, rumen and liver enzymes, rumen bacterial population and blood parameters in early lactation Holstein dairy cows were investigated.

MATERIALS AND METHODS

Animals and experimental design

The experiment was carried out at Mahdasht Dairy Farm Company, Sari, Mazandaran, Iran. Fifteen multiple dairy cows (with two and three parities) in early lactation (20±3 days of lactation, with an average milk production of 30±2 kg and body weight 580±45 kg) in separate pens in a completely randomized design. They were kept with 3 diets and 5 replications (cows) in each diet. The duration of the experiment was 35 days, which included 14 days of habituation to the new ration and 21 days of data collection. The ingredients and nutrient contents of the rations are listed in Table 1. Experimental rations included: 1) basal diet met the requirement of all nutrients according to NRC (2001) with 3 percent PRO-FAT powder (mixed fat, Ata co.), (control); 2) control ration with low level (0.1% of ration DM) of LPL (LLPL); and 3) control ration with high level (0.15% of ration DM) of LPL (HLPL). All diets were formulated to the requirement according to NRC (2001). The product of LPL used in the current study is hydrolyzed soy lecithin and includes phospholipids and free fatty acids as manufacturer (Behin Simorgh Darou Inc.Babolsar, Iran). All ingredients were thoroughly mixed, and it was given to the experimental cows as a total mixed ration (TMR) (all rations were prepared daily and fed to cows as TMR *ad libitum* (targeting 5% of refusal) with free access to water). LPL was extended in a milled corn carrier and added in a small batch vertical TMR mixer.

Measurements and sample collection

During the experimental period, the cows received TMR twice a day, at 7:00 a.m. and at 4:00 p.m. In order to measure digestibility, feed samples, feed residues, and feces were collected during the last 3 days. In addition, at the end of the experimental period (day 35), ruminal fluid was taken from the animal's rumen at 3 hours after feeding with an esophageal tube, smoothed out in a 4-layer cheese cloth, and immediately sent to the laboratory in a 39 ˚C flask for evaluation rumen enzymes and parameters. Also, blood was taken from the tail vein of the cows at the last day of experiment before milking, in the morning and it was transferred to the laboratory for evaluations in ethylene diamine tetra acetic acid (EDTA) tubes with ice.

Laboratory analysis

The digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF) of rations and feces samples was examined by AOAC (2000) method. In addition, acid detergent fiber (ADF) and NDF was analyzed by the method of Van Soest *et al*. (1991). According to this, ration and feces of samples were analyzed for DM, OM, CP, NDF, ADF and ether extract (EE). Crude protein was determined in the modified method of Kjeldahl (method 981.10-AOAC International, 2000) using an automatic nitrogen determination apparatus (model1225p; Behr Tech co., Berlin, Germany). Dry matter was determined by the loss of water during drying at 105 ˚C for 3 h (method 934.01; AOAC International, 2000). Ash was determined by samples heated until no smoking and then in a muffle at 550 ˚C for 4 h (method 942.05; AOAC International, 2000).

Ether extract was determined as dry matter loss after extraction in petroleum ether for 24 h at 30-60 ˚C (920.39 AOAC International, 2000). NDF and ADF were consecutively determined after boiling in 3% neutral detergent for 1 h (AOAC Official Method 674.26) and 2% acid detergent for 1 h (AOAC Official Method 973.18), respectively.

Heat-stable amylase (α-amylase) was used to estimate NDF and was expressed inclusive of residual ash. Also, the amount of nonfibre carbohydrate (NFC) in the feed was calculated through the equation (100–(%NDF+%ASH+%Fat+%CP)).

Table 1 Feed ingredients and chemical composition of experimental rations

Item	Rations[1]		
	Control	Low LPL (0.1% lysophospholipids)	High LPL (0.15% lysophospholipids)
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 powder	1.67	1.67	1.67
Protein supplement	6.42	6.42	6.42
Fat powder	3	3	3
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
Common 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[2]	0.01	0.01	0.01
Toxin binder[3]	0.05	0.05	0.05
Vit. Premix[4]	0.11	0.11	0.11
Min. premix[5]	0.11	0.11	0.11
LPL[6]	_	0.1	0.15
Chemical composition			
DM, % as fed	81.3	81.3	81.3
OM	95.6	95.5	95.7
CP	16.6	16.6	16.7
NDF	26.5	26.5	26.4
ADF	18.5	18.3	18.4
NFC	41.78	41.367	41.45
EE	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] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
[2] Organic form of zinc, manganese, copper and cobalt (Zinpro, Eden Prairie, MN).
[3] Rooyan darou (Tehran, IRI).
[4] Each kilogram of vitamin supplement contains: vitamin A: 600000 IU; vitamin D: 200000 IU; vitamin E: 200 mg and Antioxidants: 2500 mg.
[5] Each kilogram of mineral supplement contains: Ca: 195 g; P: 80 g; Mg: 21 g; Mn: 2200 mg; Fe: 3000 mg; Cu: 300 mg; Zn: 3000 mg; Co: 100 mg; I: 120 mg and Se: 20 mg.
[6] Hydrolyzed soy lecithin containing lysophospholipids (Behin Simorgh Darou, Babolsar, IRI).
OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: nonfibre carbohydrate; EE: ether extract and NE_L: net energy for lactation.

Serum was taken from the collected blood in the laboratory, and then total protein measurement was done by biuret method (TPB-se), blood glucose and urea nitrogen measurement was done with Pars Azmoun kit by photometric method (Friedewald *et al*. 1972). Also, after preparing serum from blood samples in the laboratory, the activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was measured by spectrophotometric method (Stojević *et al*. 2005).

After preparing rumen fluid and measuring pH with a pH meter (wtw 330i), about 10 ml of rumen fluid sample was taken to determine ammonia nitrogen (NH_3-N) and volatile fatty acids (VFAs). Based on this, the assessment of NH_3-N was done by Conway's method (Conway, 1950) and the analysis of volatile fatty acids was done by Ottenstein and Bartley method (1971), with gas chromatography. Then cultured groups of viable bacteria were determined for identifying rumen bacterial groups (cellulolytic, proteolytic,

amylolytic and total viable bacteria) according to the method of Dehority (2003). First, tubes containing culture medium and gelatin powder were prepared. Then 5 grams of rumen juice sample was mixed with diluting liquid and inoculated into test tubes. The cultures were grown for 14 days at 39 ˚C. The number of cellulolytic, proteolytic, amylolytic and total viable bacteria was counted using most probable number (MPN) method.

Statistical analyses

Effect of treatment in this experiment was analyzed by analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS Institute Inc., Cary, NC (SAS, 2004). Comparison of means will be done using Duncan's test. Significance level was determined using 95% probability.

RESULTS AND DISCUSSION

As shown in Table 2 supplementation of the ration with LPL did not affect intake of DM, OM, CP, and NDF (P>0.1217). Increasing LPL in the ration didn't significantly effect (P>0.2681) on apparent digestibility of DM, OM, CP and NDF apparent digestibility. The digestibility of EE was significantly increased by adding LPL (P=0.0346). The highest EE digestibility was observed on HLPL treatment. Table 3 illustrates data on rumen bacteria. The results showed that the total bacterial population (P=0.0688), amylolytic (P=0.7225) and proteolytic (P=0.4176) bacteria did not make a significant difference with the addition of different levels of LPL. The cellulolytic bacteria population were highest when HLPL was supplemented (P=0.0386).

The effects of LPL on ruminal parameters is shown in Table 4. Ration treatments did not effect on ruminal pH (P=0.8244). Increasing LPL in the rations increased acetate as proportion of total VFA in a dose response manner (P=0.0452), but did not affect propionate (P=0.6817), and acetate to propionate ratio (P=0.5324). Furthermore, inclusion of LPL in the rations increased valerate (P=0.0033) without changes in other VFA composition (P=0.4175). In this regard, the highest value of valerate was for HLPL treatment which was significantly different from LLPL. Moreover, using LPL in the rations did not effect on ruminal NH_3-N (P=0.0843).

The effects of LPL on blood parameters and ruminal enzymes are shown in Table 5. The glucose (P=0.2389), blood urea nitrogen (P=0.3146), total protein (P=0.3357) and also the activity of the ALP (P=0.2655) and AST (P=0.0875) was not significantly affected by the experimental treatments. Alanine aminotransferase (ALT) activity was significantly different among treatments (P=0.0324). The highest level of ALT enzyme activity was for control, which had a significant difference with other treatments (P=0.0324).

Furthermore, in terms of ruminal enzyme activity, the amount of CMC activity was affected by treatments that had a significant difference with the control (P=0.0421). However, the highest CMC activity was observed in HLPL treatment.

As shown, the results related to milk production are given in Table 6, supplementation of the ration with different levels of LPL did not significantly changed on milk yield (P=0.0673), 3.5% FCM (P=0.0689), ECM (P=0.3357) and feed efficiency [milk, 3.5% FCM, and ECM (kg/kg of DMI)] (P=0.0686). Also, supplementing a ration with increasing LPL did not affect milk fat (P=0.4437), protein (P=0.6715), and lactose (P=0.2283) content. However, the net energy of lactation linearly increased (P=0.0249) with increasing LPL. Increasing LPL in the ration had no effects on MUN (P=0.1766).

According to the research done, very few studies are available in which LPL was investigated as a feed additive in dairy cows. Therefore, due to limited information on LPL in ruminants, especially in dairy cows, studies with dairy cows fed lecithin (a source of LPL) and studies with non-ruminant animals fed lecithin or LPL were used to discuss our results.

Hence, in this study, we focused on emulsification ability of LPL in the rumen ecosystem. We hypothesized that LPL supplementation could prevent the negative effects of fat supplementation by emulsifying the fat and improved the released energy of the feed.

The results of research by other researchers regarding the effect of using LPL in rations of ruminants and non-ruminants showed that daily feed intake in livestock was not affected by the addition of LPL. Numerous studies on *in vitro* digestion have shown that ration emulsifiers can modulate the direct contact of lipid substrates and lipase, and thus, promote lipid digestion (Lee *et al*. 2019). LPL could improve the nutrient digestibility in ruminant and non-ruminant animals, which can be mainly attributed to the emulsification characteristics there from (Rico *et al*. 2017). No studies are available that have examined effects of supplemental LPL on nutrient digestibility in dairy cows. Although in beef cattle, it has been reported that it increased the consumption of DM, OM and CP (Song *et al*. 2015; Zhang *et al*. 2010). In nonruminant animals, feeding LPL increased ration nutrient absorption and digestibility, which are the major positive effects of supplemental LPL (Zhang *et al*. 2010; Zampiga *et al*. 2016). It is also reported, high inclusion of lecithin (2 to 6% in ration DM) decreased fiber and OM digestion *in vitro* (Jenkins *et al*. 1998), which occurred due to increased polyunsaturated fatty acid (PUFA) in the rumen from supplemented lecithin.

Table 2 Effect of lysophospholipid levels on dry matter intake (DMI) and nutrient digestibility in lactating dairy cows

	Rations[1]				
Item	**CON**	**LLPL**	**HLPL**	**SEM**	**P-value**
Intake, kg/day					
DM	22.57	23.03	23.38	0.775	0.1217
OM	20.83	21.62	21.90	1.017	0.3165
CP	3.199	3.261	3.248	0.485	0.2441
NDF	5.745	5.822	5.871	0.364	0.4673
EE	2.063	2.128	2.184	0.426	0.1449
Digestibility, %					
DM	60.43	61.26	61.94	1.488	0.2454
OM	63.25	64.72	65.53	0.926	0.2681
CP	62.42	63.64	64.86	1.879	0.4439
NDF	44.26	44.77	45.14	2.148	0.6476
EE	78.37^{c}	82.21^{b}	84.39^{a}	0.643	0.0346

[1] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber and EE: ether extract.
SEM: standard error of the means.

Table 3 Effects of lysophospholipid levels on ruminal bacterial population in lactating dairy cows

	Ration[1]				
Item	**CON**	**LLPL**	**HLPL**	**SEM**	**P-value**
Total bacteria, cells/mL, x 10^{9}	4.68	4.93	5.18	0.465	0.0688
Cellulytic bacteria, CFY/mL, x 10^{7}	5.25^{c}	6.09^{b}	7.11^{a}	0.166	0.0386
Amylolytic bacteria, CFY/mL, x 10^{6}	7.58	7.53	7.46	0.247	0.7225
Proteolytic bacteria, CFY/mL, x 10^{6}	9.62	9.36	9.41	0.384	0.4176

[1] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of lysophospholipids on ruminal fermentation parameters

	Ration[1]				
Item	**CON**	**LLPL**	**HLPL**	**SEM**	**P-value**
pH	5.82	5.86	5.93	0.253	0.8244
NH_3-N, mmol/L	7.17	7.25	7.23	1.072	0.0843
Total VFA, mmol/L	114	116	119	2.391	0.4175
% of total VFA					
Acetate	54.71^{b}	57.67^{ab}	63.98^{a}	2.934	0.0452
Propionate	19.82	19.73	19.45	1.092	0.6817
Isobutyrate	0.91	0.88	0.97	0.023	0.4677
Butyrate	10.18	11.60	12.17	0.897	0.4281
Isovalerate	0.74	0.92	0.88	0.066	0.4737
Valerate	1.73^{c}	1.81^{b}	1.89^{a}	0.288	0.0033
Acetate:propionate ratio	2.86	2.93	3.21	0.311	0.5324

[1] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Although the results of the present study showed that the addition of LPL causes a significant increase in fat digestion due to the fat supplement added to the ration. In the present study, an assumption was made that the digestibility of EE was improved due to LPL being able to effectively reduce the size of fat globules and form smaller micelles in the guts of animals, thereby increasing the larger surface areas of lipid droplets for pancreatic lipases to interact so that more fatty acids would be incorporated (Zhang *et al*. 2010; Lee *et al*. 2019).

Although, the amount of NDF decreased with the addition of LPL compared to the control. If NDF apparent digestibility was negatively affected by LPL in this study, it is not known how LPL decreased apparent digestibility of NDF (Zhang *et al*. 2010), because emulsifiers (e.g., Tween 80) can increase cellulolytic enzyme activities and enhance fiber degradation in rumen (Hwang *et al*. 2008). However, it is reported that, variability to response in some productive traits can return to different types of emulsifiers and its sources (Wieland *et al*. 1993).

Table 5 Effects of lysophospholipids on blood parameters and ruminal enzymes

Item	Ration[1]				
	CON	LLPL	HLPL	SEM	P-value
Blood parameters[2]					
Glucose, mg/dL	73.41	74.19	75.26	0.87	0.2389
BUN, mg/dL	16.78	15.76	15.19	0.648	0.3146
TP, mg/dL	71.06	71.49	72.34	1.163	0.3357
ALT, U/L	40.08[a]	33.36[b]	28.17	1.481	0.0324
ALP, U/L	134.73	134.89	135.18	12.72	0.2655
AST, U/L	57.48	55.76	54.86	1.744	0.875
Rumen enzymes[3]					
CMC, nmol min^{-1} mg^{-1}	50.92[b]	54.63[a]	55.81[a]	1.127	0.0421
MCC, nmol min^{-1} mg^{-1}	10.87	11.32	11.96	0.896	0.6899

[1] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
[2] BUN: blood urea nitrogen; TP: total protein; ALT: lanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase.
[3] CMC: carboxy methyl cellulose and MCC: micro crystalline cellulose.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of lysophospholipids on milk production and composition in experimental dairy cows

Item	Ration[1]				
	Con.	LLPL	HLPL	SEM	P-value
BW, kg	642	641	651	19.4	0.9347
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
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
Milk NE_L[4], Mcal/d	20.39[b]	21.62[ab]	22.30[a]	0.583	0.0249
MUN[5], mg/dL	11.84	11.62	11.39	0.394	0.1766

[1] CON: control; LLPL: Low LPL (0.1% lysophospholipids) and HLPL: high LPL (0.15% lysophospholipids).
[2] 4% FCM= [milk fat (kg/d) × 16.218] + [milk yield (kg/d) × 0.4324].
[3] ECM (kg/d)= kg of milk × [(38.3×% fat×10+24.2×% true protein×10+16.54×% lactose×10+20.7) / 3.140].
[4] Milk NE_L (Mcal/d)= kg of milk × (0.0929×% fat+0.0563×% true protein+0.0395×% lactose) (NRC, 2001).
[5] MUN: milk URE nitrogen.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The major changes observed in ruminal fermentation with add of LPL in rations in this study caused increased proportion of acetate in total VFA with no differences in propionate proportion, resulting in a tendency for increasing the ratio of acetate to propionate, butyrate and isobutyrate. In this regard, Zhang *et al*. (2010), reported that the addition of LPL decreased acetate production as well as the acetate-propionate ratio in dairy cows, which contradicts the results of this experiment. However, in that experiment use of LPL were lower (0.05-0.075% of dry matter of the ration). Studies that examined effects of LPL on ruminal fermentation are extremely limited. When alfalfa hay supplemented with soy lecithin was incubated, inconsistent results of acetate:propionate ratio were observed from 5 *in vitro* incubations (Jenkins *et al*. 1998). In that study, when purified phospholipids were incubated with alfalfa hay, proportion of propionate linearly increased with increasing phospholipids where decreased NDF digestibility was observed. In the current study, the numerical increase in acetate proportion and the ratio of acetate to propionate may have been associated with a slight increase in apparent digestibility of NDF. Also, in this experiment, the amount of valerate increased with increasing levels of LPL. These results were somewhat close to the experimental results of Zhang *et al*. (2010) regarding the slight increase in amount of valerate increased proportion of valerate with increasing LPL might be partially increased in NDF digestion and proportion of acetate in the rumen because valerate is re-

quired by cellulolytic bacteria to stimulate fiber digestion (Andries *et al*. 1987). In addition, increasing the amount of valerate in cows feeding with rations containing LPL as an emulsifier source may affect the growth of cellulytic bacteria. However, other branched chain VFAs were not significantly affected. Overall, changes in acetate and valerate concentrations without altering total VFA concentration indicate that LPL might effect on ruminal bacterial proportion. However, the degree of changes in microbial proportion by LPL was not according to no changes in ruminal pH, NH_3 and the degree of changes in VFA by LPL, which is in agreement with an *in vitro* study by Sontakke *et al*. (2014). In this study, LPL was produced from soybean lecithin and used for incubation where the effects of LPL on rumen fermentation were negligible. The effect of emulsifiers supplementation on rumen fermentation is varied depending on type and saturation of fat and type of rations (Brooks *et al*. 2017; Kim *et al*. 2004).

Relatively small effects of LPL on ruminal fermentation are also supported by minimal changes in bacterial populations observed in current study. The population of bacteria that were altered by LPL was very slight. Only the population of cellulolytic bacteria increased with increasing LPL content in the ration. Also, LPL supplemented treatments tended to increase the total bacterial population linearly. Although, the populations of amylolytic and cellulytic bacteria did not change. In this regard, even though some changes were observed, the degree of changes in populations by LPL was likely trivial to contribute to significantly altering ruminal fermentation. It seems that LPL supplementation at the levels used could not reduce the antimicrobial effects of consumed fat on amylolytic and proteolytic bacteria. However, the results of Zhang *et al*. (2010) were against the study of the effect of LPL on population of ruminal bacteria. They reported that to considering that *Treponema bryantii* causes cellulolytic fiber degradation (Kudo *et al.* 1987) and the proportion of this bacterium increased from 1.19 to 1.69% with increasing LPL, however, the proportion of cellulolytic bacteria did not increase (Zhang *et al.* 2022). In another *in vitro* study conducted by Kim *et al.* (2020) reported that a significant decrease in the relative proportions of cellulolytic (*Fibrobacter succinogenes* and *Ruminococcus albus*) and lipolytic (*Anaerovibrio lipolytica* and *Butyrivibrio proteoclasticus*) bacteria was observed with increasing levels of LPL supplementation. On the other hand, Kamande *et al*. (2000) stated that the use of emulsifiers (Tween 60 and Tween 80) may increase rumen microbial cellulase activity and increase cellulose degradation instead of improving the binding ability of fibrolytic bacteria. Considering previous results, it is possible that LPL supplementation could increase the enzymatic activity of cellulolytic bacteria similar to that by other emulsifiers (Tween 60 and Tween 80) in the rumen under high lipid conditions (Kim *et al*. 2004).

The level of glucose, total protein, and urea nitrogen in the blood indicate hepatic function (Bobe *et al.* 2004) and a slight increase in their concentration may indicate the absorption of ration fatty acids in the presence of LPL (Gallo *et al.* 2019). This potential changes with addition of emulsifier (LPL) on the liver function may have beneficial effects on the metabolism of animal and effect on the production of milk. The measurement of hepatic enzymes is used to diagnose the health of the liver and check its function and metabolism. One of the main abnormalities that increase the level of liver enzymes in the blood is the occurrence of fatty liver. Therefore, an increase in amount of alanine aminotransferase (ALT) in blood indicates liver problems (Stojević *et al*. 2005). Rico *et al.* (2017) reported that lecithin supplementation (used at levels of 10 g/day) had no effect on liver enzymes at the levels used. However, ALT may affect lipid metabolism, but the mechanism[s] are not clarified. Also, the amount of fat supplement in the ration may play a different role in different periods of lactation in dairy cows. In another experiment conducted by Huo *et al*. (2019), it was reported that LPL increases the concentration of liver enzymes, especially AST enzyme in blood, which is different from results of the current study.

In agreement with published results (Wettstein *et al*. 2001), the activity of microbial enzymes (CMCase and MCCase) were higher in rations supplemented with fat and lecithin compared with the control. This may indicate that LPL can moderate the harmful effects of fat addition in rumen, which may lead to increased effects of particle-associated bacteria, which are mainly responsible for the activity of fibrolytic enzymes and are mainly involved in fiber digestion (Stojević *et al*. 2005). Rumen microbial enzyme activities are a qualitative reflection of rumen microbes involved in digestion of feed (Raghuvansi *et al*. 2007; Kamra *et al*. 2010). In the present study, the activity of fibrolytic enzymes (CMCase and MCCase) improved when dairy cow's rations containing fat supplements, as the source of energy, was supplemented with LPL. Indeed, Gallo *et al*. (2019) observed that a ration containing oil supplemented with lysolecithin improved the activity of ruminal enzymes compared to a ration without lysolecithin and the control.

LPL increases the absorption of feed nutrients and improved feed efficiency by consuming similar feed among different groups (Zampiga *et al.* 2016; Zhao and Kim, 2017; Lee *et al*. 2019). In lactating sows fed a ration with LPL (0.03% in ration DM), increased milk fat, protein, and lactose concentrations were found (Zhao and Kim, 2017).

The current study with dairy cows also observed positive effects of LPL on production where milk yield, feed effi-

ciency, and milk protein yield were increased and milk fat yield tended to be increased compared with control treatment which agreed with the results of Lee *et al*. (2019). They Examining the effects of LPL on short-term production in experimental dairy cows, and reported that Supplementation of LPL to the ration did not alter DM intake but linearly increased milk yield, resulting in increases in feed efficiency (milk yield/DM intake), milk protein and fat yields. In a study by Rico *et al*. (2017), however, dairy cows fed a ration with LPL (10 g/d; ~0.035% of ration DM) did not increase milk yield, although milk fat content was increased. The discrepancy between Rico *et al*. (2017) and the current study is difficult to explain. However, it could be partially attributed to different products of LPL used in the studies. Depending on the sources of phospholipids and process of enzymatic hydrolysis of phospholipids to produce LPL, proportion of LPL in the product can vary. In addition, the tested cows breed, the amount of production, the stage of its use in the ration of dairy cows and the amount of LPL consumption in the present study were different from the study of Rico *et al*. (2017) and Lee *et al*. (2019). In non-ruminant animals, LPL has been widely investigated as feed additives, and production responses to LPL have been quite consistent (growth rate and feed efficiency; Zhao and Kim, 2017). This suggests that the degree of ruminal bypass of LPL may be critical for consistent positive production responses to LPL in dairy cows [Jenkins *et al*. (1998); Escape from rumen degradation of part of phospholipids (source of LPL) was observed in continuous culture study].

CONCLUSION

According to the results, the apparent digestibility of DM and OM did not show a significant increase with increasing the level of LPL. Nevertheless, the ration with LPL did improve the activity of fibrolytic enzymes, rumen fermentation and cellulytic population in experimental cows. Supplementation of a lactation ration with LPL increased milk yield and feed efficiency in a dose–response manner. It seems that the use of LPL along with fat in the feeding of dairy cows is economically beneficial in terms of meeting energy needs, especially in the early stages of lactation. Therefore, further studies are needed to confirm our results and explore the mode of action of LPL in dairy cows and in different stages of lactation.

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