Journal of Animal Science, 2020, Vol. 98, No. 8, 1–11

doi:10.1093/jas/skaa227
Advance Access publication July 18, 2020
Received: 25 May 2020 and **Accepted:** 13 July 2020
Non Ruminant Nutrition

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Supplemental effects of dietary lysophospholipids in lactation diets on sow performance, milk composition, gut health, and gut-associated microbiome of offspring

Ki Beom Jang,[†] Jerry M. Purvis,[‡] and Sung Woo Kim[†,1]

[†]Department of Animal Science, North Carolina State University, Raleigh, NC 27695, [‡]N.G. Purvis Farm Inc., Robbins, NC 27325

[1]Corresponding author: sungwoo_kim@ncsu.edu

ORCiD number: 0000-0003-4591-1943 (S. W. Kim).

Abstract

Dietary lysophospholipids (LPL) would influence milk composition of sows, thus positively affect intestinal health of offspring. The objective of this study was to determine effects of dietary LPL fed to lactating sows on performance, milk characteristics, gut health, and gut-associated microbiome of offspring. Sixty pregnant sows were allotted to 2 treatments in a randomized complete block design with parity and BW as blocks on day 110 of gestation. Treatments were CON (no added LPL) and LPL (0.05% LPL; Lipidol-Ultra, Pathway Intermediates, Shrewsbury, UK). Sows were fed 2 kg/d from day 110 of gestation until farrowing and ad libitum after farrowing. Diets were formulated to meet NRC requirement for lactating sows. Colostrum and milk samples from 12 sows per treatment were collected to measure nutrients and immunoglobulins on days 1 and 18 of lactation, respectively. Twelve piglets per treatment (1 piglet per litter) were euthanized on day 18 to collect tissues to measure tumor necrosis factor-α, interleukin-8 (IL-8), malondialdehyde, protein carbonyl, IgA, histomorphology, crypt cell proliferation rate, and microbiota in the jejunum and colon. Data were analyzed using the MIXED procedure of SAS, and the mortality was analyzed using the GLIMMIX procedure of SAS. There was no difference in sow BW, parity, and litter size between treatments on day 0 of lactation. Sows fed LPL had increased ($P < 0.05$) litter BW gain (53.9 vs. 59.4 kg) and decreased piglet mortality (13.9% vs. 10.6%) on day 18 of lactation. Sows fed LPL had increased ($P < 0.05$) omega-6:omega-3 (22.1 vs. 23.7) and unsaturated:saturated (1.4 vs. 1.6) fatty acids ratios with increased oleic acid (29.1% vs. 31.4%) and tended to have increased ($P = 0.092$) IgG (1.14 vs. 1.94 g/L) and linoleic acid (17.7% vs. 18.7%) in the milk on day 18 of lactation. Piglets from sows fed LPL had increased ($P < 0.05$) IL-8 (184 vs. 245 pg/mg) and crypt cell proliferation rate (39.4% vs. 40.9%) and tended to have increased ($P = 0.095$) Firmicutes:Bacteroidetes ratio (1.0 vs. 3.5) in the jejunum. In conclusion, sows fed with LPL had milk with increased IgG, oleic acids, and linoleic acids without changes in BW and backfat during lactation. These changes could contribute to improved survivability and intestinal health of piglets by increasing IL-8 concentration, enhancing balance among gut-associated microbiome, and increasing enterocyte proliferation in the jejunum.

Key words: intestinal health, lactating sow, lysophospholipids, microbiome, milk

Abbreviations

BCA	bicinchoninic acid
BW	body weight
CD	crypt depth
CON	control
CP	crude protein
DM	dry matter
EE	ether extract
ELISA	enzyme linked immunosorbent assay
GE	gross energy
IL-8	interleukin-8
IgA	immunoglobulin A
IgG	immunoglobulin G
LPL	lysophospholipids
MDA	malondialdehyde
NRC	National Research Council
PBS	phosphate-buffered saline
SCD1	Δ9 stearoyl-CoA desaturase 1
SID	standardized ileal digestible
SOP	standard operating procedures
STTDP	standardized total tract digestible phosphorus
TNF-α	tumor necrosis factor-α
VH	villus height

Introduction

Lactating sows have been genetically improved to maximize productivity for swine production (Kim et al., 2013). However, hyperprolific sows suffer from highly stressful conditions including increased milk production, limited nutrient intake, increased tissue mobilization, and increased oxidative stress (Kim et al., 2009; Berchieri-Ronchi et al., 2011; Strathe et al., 2017). Improper nutrition to sows could cause negative impacts on the litter performance by decreasing milk production and litter growth (Kim et al., 1999; Kim and Easter, 2001; Kobek-Kjeldager et al., 2020).

Dietary manipulation should target to enhance nutrient intake and utilization in sows. Lysophospholipids (LPL) could be considered in lactation diets to enhance nutrient digestion and absorption. LPL are modified phospholipids removing 1 fatty acid by phospholipase A2 reaction (Burke and Dennis, 2009). This reaction increases the hydrophilic properties of the compound when compared with phospholipids and even with bile salts (Jansen et al., 2015). This process could be used to enhance the capacity of fat emulsification thus increasing the utilization of dietary fat (Armand et al., 1999; Reis et al., 2009). LPL could be considered as a potential compound to induce the remodeling of nutrient transportation (Zheng et al., 2017) in the cell membrane and proliferation of intestinal epithelial cells (Konno et al., 2019). LPL could also enhance nutrient utilization by altering the enterocyte membrane through increased fluidity of cell membrane and nutrient permeability (Lundbaek and Andersen, 1994; Maldonado-Valderrama et al., 2011).

Previous studies reported that dietary LPL supplementation improved lipid digestibility and growth of nursery pigs (Zheng et al., 2016) and broilers (Zhao and Kim, 2017). Wang et al. (2019) also reported that dietary LPL in lactation diets improved energy utilization of sows with increased immunoglobulin concentration in the milk as well as litter weight gain and piglet BW at weaning. However, a possible pathway of the maternal LPL supplementation on litter performance of sows in conjunction with intestinal health of the piglets is not yet clear.

It is hypothesized that feeding lactating sows with LPL would improve sow performance and influence milk compositions, thus improve the intestinal health of offspring. This study was conducted to determine supplemental effects of dietary LPL in lactation diets on performance, milk characteristics, and intestinal health of piglets in a commercial farm.

Materials and Methods

A protocol of this study was reviewed and approved by Institutional Animal Care and Use Committee at North Carolina State University (Raleigh, NC). The animal experiment was conducted at a commercial swine farm (NG Purvis Farm, Carthage, NC).

Animals and experimental design

Initially, a total of 60 sows (average parity: 3.7 ± 2.5) were identified, weighed, and moved to farrowing barn at day 110 of gestation. Sows were blocked based on the BW at day 110 of gestation (heavy, medium, and light) and parity (1st and 2nd parity vs. multiparity). Within each block, sows were randomly allotted to 2 dietary treatments based on a randomized complete block design with BW and parity as blocks. Dietary treatments were CON (no added LPL) and LPL (Lipidol-Ultra at 0.05% as a source of LPL; Pathway Intermediates, Shrewsbury, UK). The LPL complex (Lipidol-Ultra) used in this study contained LPL at 6% (including lysophosphatidylcholine, lysophosphatidylinositol, lysophosphatidylethanolamine, and lysophosphatidic acid) and other lipids at 33% (triglycerides and phospholipids) with carrier at 60% (calcium silicate).

Experimental diets were fed to sows from day 110 of gestation until weaning. Experimental diets were mainly composed of corn–soybean meal with animal-vegetable blend oil, poultry fat, and supplemental amino acids to meet NRC (2012) requirements (Table 1). According to standard operating procedures (SOP) of the farm (NG Purvis Farm, Carthage, NC), sows were fed 2 kg/d (twice per day at 0800 and 1400 hours) from day 110 of gestation until farrowing and ad libitum after farrowing. Water was freely accessible during the entire period. Eight sows were removed from this study due to pregnancy failure (3 sows) and low litter size (5 sows) on day 0 of lactation. The distribution of parity in each treatment is shown in Table 2.

Piglets were weighed individually (day 0: within 12 h after birth). On days 2 and 3 after farrowing, litters were processed based on SOP of the farm. When needed, cross fostering was done to balance the number of piglets within a treatment group and was only performed between litters farrowed on the same day. Cross fostering was completed by day 2 after farrowing before the onset of involution of unsuckled mammary glands could occur (Kim et al., 2001). The number of piglets was set near 13 to represent typical litter size of a modern highly prolific sow according to SOP of the farm. Body weight of piglets was also measured on days 0, 9, and 18. Twelve sows per treatment were selected based on their blocks for the collection of colostrum and milk. Samples of colostrum (40 mL) and milk (40 mL) were taken from 6 mammary glands (2nd, 3rd, and 4th pairs) on days 0 and 18 of lactation, respectively. Collection of milk was done after intramuscular injection of oxytocin (10 U). Colostrum and milk were frozen at –20 °C until further analysis.

Last day of BW measurement was set to day 18 of lactation. According to SOP, all litters were weaned at an average of 21 d of lactation (with a range of days 18 to 24 of lactation). Body

Table 1. Composition of experimental diets (as-fed basis)

Item	CON	LPL
Ingredient, %		
Corn, yellow	63.29	63.24
Soybean meal, 48% CP	27.73	27.73
Poultry fat	2.50	2.50
Animal-vegetable blend	2.50	2.50
L-Lys HCl	0.57	0.57
L-Thr	0.09	0.09
Dicalcium phosphate	1.60	1.60
Limestone	0.83	0.83
Trace mineral permix[1]	0.13	0.13
Vitamin permix[2]	0.07	0.07
Sodium	0.25	0.25
Phytases and xylanases	0.04	0.04
Choline chloride, 60%	0.30	0.30
Yeast	0.10	0.10
LPL[3]	0.00	0.05
Total	100.00	100.00
Calculated composition		
DM, %	89.7	89.3
ME, kcal/kg	3,510	3,508
CP, %	19.06	19.05
SID[4] Lys, %	1.30	1.30
SID Cys + Met, %	0.52	0.52
SID Trp, %	0.20	0.20
SID Thr, %	0.66	0.66
Ca, %	0.80	0.80
STTD P[5], %	0.40	0.40
Total P, %	0.66	0.66
Analyzed composition		
DM, %	87.75	87.41
CP, %	19.78	19.76
Fat, %	7.21	7.14
Mg, %	0.14	0.14
Mn, %	0.01	0.01
Fe, %	0.03	0.03
Zn, %	0.01	0.01
Cu, %	0.01	0.01

[1]The vitamin premix provided per kilogram of complete diets: 4,411 IU of vitamin A as vitamin A acetate, 794 IU of vitamin D_3, 26.5 IU of vitamin E, 1.76 mg of vitamin K as menadione sodium bisulfate, 0.02 mg of vitamin B_{12}, 3.32 mg of riboflavin, 11.01 mg of D-pantothenic acid as calcium panthonate, 1.82 mg of thiamine, 17.63 mg of niacin, 1.32 mg of folic acid, and 0.18 mg of biotin.
[2]The trace mineral premix contained calcium carbonate, manganous oxide, manganese methionine hydroxy analogue chelate, zinc oxide, zinc sulfate, zinc methionine hydroxy analogue chelate, Ferrous sulfate, copper sulfate, basic copper chloride, copper methionine hydroxy analogue chelate, calcium iodate, sodium selenite, selenium yeast, and chromium propionate.
[3]Lipidol (Pathway Intermediates, Shrewsbury, UK).

Table 2. Distribution of sow parity in this study

	Parity									
Item	1	2	3	4	5	6	7	8	9	Total
Sow, *n*										
CON	6	6	7	0	3	0	0	3	2	27
LPL[1]	4	7	7	0	3	1	0	2	1	25

[1]Sows were fed diets with 0.05% LPL during lactation.

Table 3. Analyzed fatty acid composition of LPL complex[1]

Item	LPL	SEM
EE, %	40.24	0.32
Fatty acids, %		
Tridecylic, C14:0	0.20	0.03
Palmitic, C16:0	15.79	0.01
Palmitoleic, C16:1	0.10	0.01
Margaric, C17:0	0.10	0.00
Stearic, C18:0	3.77	0.03
Oleic, C18:1	18.24	0.26
Linoleic, C18:2, n-6[2]	54.69	0.13
α-Linolenic, C18:3, n-3	5.73	0.05
Arachidic, C20:0	0.25	0.00
Eicosenoic, C20:1	0.15	0.01
Behenic, C22:0	0.39	0.00
Erucic, C22:1	0.13	0.04
Docosenoic, C22:2, n-6	0.12	0.01
Lignoceric, C24:0	0.21	0.01
Total n-3	5.73	0.05
Total n-6	54.81	0.14
n-6:n-3 ratio	9.57	0.05
Unsaturated fatty acids	79.16	0.05
Saturated fatty acids	20.71	0.02
Unsaturated:saturated[3]	3.82	0.00

[1]Values are means of triplicate analyses.
[2]n-3, omega-3 fatty acid; n-6, omega-6 fatty acid.
[3]The ratio of unsaturated fatty acids to saturated fatty acids.

weight of sows was measured on day 110 of gestation and upon weaning. Due to complexity at a commercial farm, it was not allowed to measure BW of sows at farrowing. Sow BW at farrowing was calculated as subtracting litter weight on day 0 lactation from sow BW on day 110 gestation. The BW loss during lactation was estimated based on sow BW at farrowing and litter weight at farrowing. The thickness of backfat was measured using ultrasonic device (Renco Lean-Meater, Renco Corp., MN) on day 110 of gestation and days 0, 9, and 18 of lactation.

Twelve male piglets (medium BW piglet per litter) from previously selected 12 sows for colostrum and milk sampling were euthanized to collect tissues (10 cm) from the jejunum on day 18 of lactation. After gentle removal of digesta, a section (2 cm) was placed in formaldehyde, and the remaining section (8 cm) was used to take jejunal mucosa following Shen et al. (2014). A section (8 cm) of the colon was also taken to collect colonic mucosa.

Colostrum and milk composition

Colostrum and milk samples were stored at –20 °C. Fresh milk samples (20 mL) were sent to a commercial lab (DairyOne; Ithaca, NY) to analyze the composition of ether extract (EE; method 989.05; AOAC, 2019) and crude protein (CP) content was obtained using the Kjeldahl method (method 991.20; AOAC, 2019). The colostrum and milk samples (10 mL) were ovendried for 72 h at 55 °C and ground through a 1.0-mm screen. Dried samples were ground and analyzed for dry matter concentration (method 990.20; AOAC, 2019) and gross energy (GE) using a calorimeter (Model 6200, Parr Instrument Company). The remaining colostrum and milk samples were used to determine IgG and IgA concentrations in the milk samples by using commercial ELISA Kit (Bethyl Laboratories Inc., Montgomery, TX).

Fatty acid analysis

Compositions of total fatty acids were determined in LPL complex, colostrum, and milk samples. Fatty acid extraction and determination from the samples was followed as previous described by Lin et al. (2011). LPL complex (350 mg) and milk samples (50 μL) were transferred into 25 mL tubes with Teflon-lined screw caps. One milliliter of methanol and 3 mL of 3 mol/L methanolic–HCl were added. Tubes were capped tightly and refluxed in a water bath at 95 °C for 1 h. Eight milliliters of 0.88% NaCl (wt:vol) and 3 mL of hexane were added to each sample, vortexed, and centrifuged at 1,330 × *g* for 15 min at 4 °C. After centrifugation, the top layer was transferred to a 1.5-mL vial and evaporated to dry under nitrogen. Fatty acid methyl esters were dissolved in 25 μL hexane and analyzed on a percent basis of total fatty acids by gas chromatography-mass spectrometry using an Agilent 7890 B (Agilent Technologies, Palo Alto, CA) gas chromatograph and a column (30 m × 0.25 mm with 0.25 μm film thickness, Agilent Technologies).

Immune and oxidative stress status

Mucosa samples (0.5 g) from the jejunum and colon of piglets were homogenized (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL) on ice in 2 mL PBS. Sample preparation for analysis was followed as previous described by Chen et al. (2017). Homogenized samples were centrifuged at 15,000 × *g* for 15 min and then the supernatant was collected. Protein contents in supernatants were measured using Pierce BCA Protein Assay Kit (23225#, Thermo Fisher Scientific Inc.). Concentrations of tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) in the jejunal and colonic mucosa samples were determined using ELISA kits (R&D Systems, Minneapolis, MN) following the manufacturer's protocols. The concentrations of malondialdehyde (MDA) and protein carbonyl assay kits were measured by commercial kits (Cell Biolabs, Inc., San Diego, CA) following the manufacturer's protocols. Total concentrations of IgA in the jejunal and colonic mucosa of piglets were measured according to the method described by Shen et al. (2011) using an ELISA Kit for swine IgA (Bethyl Laboratories Inc., Montgomery, TX).

Histomorphology and immunohistochemistry

Jejunal tissue samples were fixed in 10% formalin buffer for 3 wk and sent to the Histology Laboratory of North Carolina State University (Raleigh, NC) for hematoxylin and eosin staining as well as immunohistochemistry for detecting Ki67+ cells as a biological maker for measuring the crypt cell proliferation. A total of 15 villi and 15 crypts in each slide were selected to measure villus height (VH), villus width, crypt depth (CD), and percent of Ki67+ enterocyte using a microscope (Olympus CX31 microscope). The ratio of VH:CD was calculated.

Gut-associated microbiota

The DNA was extracted from 250 mg of jejunal and colonic mucosa samples using a commercial DNA extraction kit (DNA Stool MiniKit, Qiagen, Germany). Extracted DNA samples were sent to Mako Medical Laboratories (Raleigh, NC) and prepared for template preparation on the Ion Chef instrument and sequencing was performed on the Ion S5 system. Variable regions V2, V3, V4, V6, V7, V8, and V9 of the 16S rRNA gene were amplified with the Ion 16S Metagenomics Kit (Thermo Fisher Scientific, Waltham, MA). Libraries were prepared from the amplified target regions with the Ion Xpress Plus Fragment Library Kit (Thermo Fisher Scientific). The IonCode Barcode Adapters 1–384 Kit (Thermo Fisher Scientific) was used for barcoding and multiplexing of the prepared libraries. The libraries were quantified with the Ion Universal Library Quantitation Kit (Thermo Fisher Scientific), and samples were diluted to equal concentration and pooled into multiplexed libraries for template preparation. Template preparation and chip loading were performed using the Ion Chef instrument and sequencing was performed on the Ion S5 system with the Ion 520 and Ion 530 Kit-Chef (Thermo Fisher Scientific) and the Ion 530 Chip Kit-4 Reactions (Thermo Fisher Scientific). The 16s rRNA sequences were processed using the Torrent Suite Software (version 5.2.2) to produce unaligned bam files for further analysis. For data analysis, GreenGenes and MiSeq databases were used to identify a taxonomic identification. Alpha-diversity and relative abundance of bacteria were analyzed by the Ion Reporter Software Suite (version 5.2) of bioinformatics analysis tools. The index of Chao1, Shannon, and Simpson were calculated to estimate microbial diversity. Chao1 index is used to estimate the bacterial species richness, whereas Shannon and Simpson estimators indicate the diversity in bacterial species. All samples had a depth of sequencing coverage > 1,000 ×. Sample preparation and analysis settings were performed under the manufacturer's recommended protocols and analysis settings.

Statistical analysis

Data from this study were analyzed based on a randomized complete block design using the Mixed model of SAS Software (Cary, NC). Sows (or litter) served as the experimental unit for reproductive performance parameters and piglets (1 per litter) were the experimental unit for other parameters. Treatment was the fixed effects. The sow BW and parity blocks were served as a random effect. Data of mortality were analyzed with the GLIMMIX of SAS, followed by previous studies (Hales et al., 2015; Guo et al., 2019). Statistical difference among treatment means was considered significant with $P < 0.05$, whereas $0.05 < P < 0.10$ was considered as tendency.

Results

Fatty acid composition of LPL

The fatty acid profile of the LPL is shown in Table 3. The LPL contained 40.2% EE and 14 different types of fatty acids. Palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2) were the main fatty acids in the LPL complex. The LPL contained higher amount of omega-6 (54.8%) and unsaturated fatty acids (79.2%) than omega-3 (5.73%) and saturated fatty acids (20.7%), respectively.

Sow and litter performance

Average parity and BW of sows at day 110 of pregnancy were not different between treatments (Table 4). Body weight and litter size of sows on the first day of lactation as well as BW change of sows during lactation were not different between treatments. Backfat thickness and ADFI during lactation were not different between treatments. However, litter weight and litter BW gain were increased ($P < 0.05$) in sows fed a diets with LPL on day 18 of lactation. Mortality of piglets from sows fed diets with LPL was decreased ($P < 0.05$) during lactation.

Table 4. Supplemental effects of LPL in lactation diets on the performance of sows and suckling piglets

Item	CON	LPL[1]	SEM	P-value
Sow parity	3.4	3.2	1.6	0.604
Sow BW, kg				
Day 110 of gestation	256.6	256.1	19.3	0.877
Farrowing	235.7	234.4	19.4	0.723
Weaning	219.6	222.3	19.9	0.606
BW loss, kg	-15.5	-11.8	4.0	0.323
ADFI, kg				
Days 0 to 9	6.45	6.61	0.35	0.512
Days 9 to 18	8.67	8.34	0.38	0.352
Total	7.58	7.25	0.36	0.160
Backfat thickness, mm				
Day 110 of gestation	10.9	11.4	0.9	0.521
Day 0	11.2	11.1	0.8	0.946
Day 9	10.6	10.6	0.6	0.875
Day 18	9.9	9.8	0.9	0.845
Backfat change	-1.3	-1.3	0.4	0.953
Litter size, head				
Day 0, liveborn	14.1	14.8	0.7	0.230
Day 1	13.7	14.3	0.5	0.227
Day 9	12.0	12.9	0.2	0.013
Day 18	11.9	12.8	0.2	0.006
Litter weight, kg				
Day 0, liveborn	20.9	21.7	0.9	0.464
Day 9	44.0	49.4	1.6	0.019
Day 18	75.0	81.2	2.6	0.047
Litter BW gain	53.9	59.4	2.8	0.043
Piglet BW, kg				
Day 0, liveborn	1.54	1.55	0.05	0.930
Day 9	3.67	3.85	0.12	0.260
Day 18	6.32	6.34	0.17	0.928
Mortality, %	13.9	10.6	1.3	0.004

[1]Sows were fed diets with 0.05% LPL during lactation.
[2]Sow BW at farrowing was calculated as subtracting litter weight on day 0 lactation from sow BW on day 110 gestation.

Table 5. Supplemental effects of LPL in lactation diets on colostrum and milk composition of sow

Item	CON	LPL[1]	SEM	P-value
Colostrum, day 0 of lactation[2]				
Dry matter, %	24.1	24.8	1.5	0.712
CP, % (DM basis)	48.3	42.3	4.7	0.375
EE, % (DM basis)	39.2	38.8	4.9	0.964
GE, kcal/kg (DM basis)	5,943	5,966	231	0.870
IgG, g/L	24.90	29.17	4.70	0.521
IgA, g/L	19.04	19.81	5.55	0.751
Milk, day 18 of lactation				
DM, %	19.9	20.2	0.9	0.510
CP, % (DM basis)	26.3	26.6	0.5	0.704
EE, % (DM basis)	37.7	40.4	2.8	0.176
GE, kcal/kg (DM basis)	5,977	5.926	99	0.713
IgG, g/L	1.14	1.94	0.38	0.092
IgA, g/L	2.85	2.91	0.24	0.760
Milk—colostrum				
DM, %	−4.2	−4.5	1.5	0.885
CP, % (DM basis)	−22.0	−15.7	4.8	0.363
EE, % (DM basis)	−1.4	1.7	6.3	0.735
GE, kcal/kg (DM basis)	25	−47	302	0.832
IgG, g/L	−23.76	−27.23	5.70	0.651
IgA, g/L	−16.10	−16.82	10.44	0.962

[1]Sows were fed diets with 0.05% LPL during lactation.
[2]Colostrum samples were collected within 24 h after farrowing.

Milk composition

Supplementation of LPL in the lactation diets did not affect DM, CP, EE, GE, and IgA in the colostrum or milk of sows (Table 5). However, supplementation of LPL in the lactation diets tended to increase ($P = 0.092$) IgG concentration in the milk collected on day 18 of lactation. Supplementation of LPL in lactation diets altered fatty acid composition in the milk on day 18 of lactation (Table 6). Milk from sows fed diets with LPL had lower ($P < 0.05$) myristic acid (C14:0) and palmitic acid (C16:0), whereas higher oleic acid (C18:1; $P < 0.05$), linoleic acid (C18:2; $P = 0.075$), eicosenoic acid (C20:1; $P = 0.059$), and eicosadienoic acid (C20:2; $P = 0.079$) compared with milk from sows fed diets without LPL. Milk from sows fed diets with LPL had higher ($P < 0.05$) unsaturated fatty acids and unsaturated fatty acids to saturated fatty acids ratio and lower ($P < 0.05$) saturated fatty acids compared with milk from sows fed diets without LPL.

Immune and oxidative stress status

Supplementation of LPL did not affect the concentration of TNF-α in the jejunal and colonic mucosa of piglets on day 18 of lactation (Table 7). However, supplementation of LPL in lactation diets tended to increase ($P = 0.058$) the concentration of IL-8 in jejunal mucosa of piglets on day 18 of lactation. Supplementation of LPL in lactation diets did not affect concentrations of MDA, protein carbonyl, or IgA in the jejunal and colonic mucosa of piglets on day 18 of lactation.

Histomorphology and immunohistochemistry

No differences in jejunal histomorphology of piglets were observed between treatments on days 1 and 18 of lactation (Table 8). Supplementation of LPL in lactation diets increased ($P < 0.05$) crypt cell proliferation rate in the jejunum of piglets.

Gut-associated microbiota

No differences in diversity estimates of microbiome in jejunal and colonic mucosa of piglets were observed between treatments on day 18 of lactation (Table 9). Supplementation of LPL in lactation diets tended to increase ($P = 0.095$) Firmicute to Bacteroidetes ratio in jejunal mucosa of piglets on day 18 of lactation. No differences in relative abundance of bacteria at the genus levels in the jejunal and colonic mucosa of piglets were observed between treatments on day 18 of lactation (Tables 10 and 11).

Discussion

Major challenge with lactating sows is related to the metabolic stress for milk production to support litter growth during lactation (Kim et al., 2009; Berchieri-Ronchi et al., 2011; Strathe et al., 2017). Newborn piglets are also highly sensitive to environmental factors due to their immature gastrointestinal tract and undeveloped immune system (Tourneur and Chassin, 2013; Splichalova et al., 2018). The newborn piglets should obtain passive immunity and nutrients through colostrum and milk from the sow to establish their defense system, including intestinal immunity and development (Hurley and Theil, 2011; Oliviero et al., 2019). In this study, the results indicate that inclusion of dietary LPL could support lactating sows without

Table 6. Supplemental effects of LPL in lactation diets on fatty acid profiles in colostrum and milk

Item	CON	LPL[1]	SEM	P-value
Colostrum, %				
Myristic, C14:0	0.80	0.67	0.09	0.229
Palmitic, C16:0	19.53	19.16	0.58	0.517
Palmitoleic, C16:1	3.80	3.91	0.19	0.685
Stearic, C18:0	5.80	5.30	0.34	0.281
Oleic, C18:1	36.75	38.22	1.17	0.384
Vaccenic, C18:1	2.45	2.54	0.13	0.580
Linoleic, C18:2, n-6[2]	27.23	27.15	1.08	0.950
ɣ-Linolenic, C18:3, n-6	0.13	0.13	0.08	0.922
α-Linolenic, C18:3, n-3	0.91	0.83	0.11	0.546
Arachidic, C20:0	0.04	0.04	0.02	0.947
Eicosenoic, C20:1	0.25	0.15	0.05	0.179
Eicosadienoic, C20:2, n-6	0.63	0.47	0.11	0.131
Eicosatrienoic, C20:3, n-3	0.20	0.20	0.07	0.992
Arachidonic, C20:4, n-6	1.17	1.08	0.16	0.654
Total n-3	1.12	1.22	0.13	0.641
Total n-6	29.15	28.82	1.03	0.807
n-6:n-3 ratio	26.29	23.97	1.50	0.276
Unsaturated fatty acids	73.68	74.79	0.93	0.225
Saturated fatty acids	26.27	25.26	0.89	0.258
Unsaturated:saturated[3]	2.84	2.98	0.14	0.276
Milk, %				
Myristic, C14:0	2.77	2.51	0.07	0.009
Palmitic, C16:0	34.82	32.09	0.86	0.010
Palmitoleic, C16:1	8.00	7.42	0.49	0.360
Stearic, C18:0	4.51	4.49	0.20	0.948
Oleic, C18:1	29.13	31.43	0.81	0.015
Vaccenic, C18:1	1.64	1.64	0.06	0.938
Linoleic, C18:2, n-6	17.73	18.67	0.45	0.075
ɣ-Linolenic, C18:3, n-6	0.00	0.01	0.01	0.329
α-Linolenic, C18:3, n-3	0.82	0.81	0.03	0.603
Eicosenoic, C20:1	0.05	0.16	0.07	0.059
Eicosadienoic, C20:2, n-6	0.14	0.26	0.09	0.079
Eicosatrienoic, C20:3, n-3	0.00	0.01	0.01	0.330
Arachidonic, C20:4, n-6	0.34	0.45	0.07	0.265
Total n-3	0.82	0.82	0.03	0.935
Total n-6	18.22	19.42	0.43	0.049
n-6:n-3 ratio	22.16	23.71	0.42	0.010
Unsaturated fatty acids	57.89	60.90	0.83	0.005
Saturated fatty acids	42.11	39.10	0.83	0.005
Unsaturated:saturated	1.38	1.57	0.05	0.007

[1]Sows were fed diets with 0.05% LPL during lactation.
[2]n-3, omega-3 fatty acid; n-6, omega-6 fatty acid.
[3]The ratio of unsaturated fatty acids to saturated fatty acids.

affecting BW and backfat loss and improve survivability of piglets by activation of inflammatory response and increased proliferation of enterocytes in the jejunum of suckling piglets. These benefits may be related to changes in IgG production and fatty acid composition in the milk during lactation. These findings are partly in agreement with a previous study (Wang et al., 2019), which showed an improvement in litter weight gain, nutrient digestibility, and overall health of sows and piglets with LPL. This study further investigated the effects of maternal LPL provision on milk quality and intestinal health of the piglets tested at a commercial environment.

The source of LPL used in this study is composed of 40% lipids and 60% calcium silicate. Lipids (40%) include LPL (6%) and other lipids (33%) including triglycerides and phospholipids which are possibly effective components to enhance the absorption of ingredients (Gimenez et al., 2011; Kennelly et al., 2018). Phospholipids take about 0.2% to 0.5% of typical sow feeds adapted from previous studies (Weihrauch and Son, 1983; Sharma et al., 2019) and the addition of other lipids at 0.02% to the lactation diets would not cause negative impacts on lipid absorption and health of sows (Cohn et al., 2008, 2010). Dietary inclusion of LPL (0.0003%), however, is shown to effectively modify cellular membrane in enterocytes. Previous studies showed that supplemental LPL were actively incorporated into cellular membrane of various types of cells (Tamura et al., 1985; Besterman and Domanico, 1992; Wongkajornsilp and Rosenberry, 1995). Interestingly, Parthasarathy et al. (1974) showed that exogenous radiolabeled LPL was first diffused to the outer membrane of mucosal cells, and then gradually translocated to the inner membrane. The composition of LPL in cellular membrane seems to increase as LPL intake increased (Tamura et al., 1985; Wongkajornsilp and Rosenberry, 1995). The increase in the concentration of LPL in the membrane of enterocytes is related to enhanced membrane permeability by increased membrane fluidity (Tagesson et al., 1985; Lundbaek and Andersen, 1994). In fact, Wang et al. (2019) showed that piglet BW and milk IgG concentration at weaning reached a plateau at 50 mg/kg inclusion of LPL during lactation. Zhao et al. (2017) also reported that supplementation of LPL at 30 mg/kg improved the sow performance and milk fat concentration during lactation. Therefore, continuously feeding diets with LPL at 30 mg/kg would be considerable for sows to improve the membrane permeability of enterocytes during lactation.

Eissen et al. (2003) showed that lactating sows would be predisposed to significant BW and backfat loss, because sows need to produce milk to support the growth of their piglets

Table 7. Supplemental effects of LPL in lactation diets on immune and oxidative stress status in jejunal and colonic mucosa of suckling piglets

Item	CON	LPL[1]	SEM	P-value
Jejunum				
TNF-α, pg/mg	1.04	1.29	0.16	0.195
IL-8, pg/mg	184	245	22	0.058
MDA, μM/mg	1.07	1.15	0.12	0.614
Protein carbonyl, μM/mg	6.56	7.31	0.61	0.389
IgA, mg/mg	2.07	2.42	0.79	0.742
Colon				
TNF-α, pg/mg	2.00	2.20	0.28	0.536
IL-8, pg/mg	10.03	11.53	3.02	0.704
MDA, μM/mg	1.17	0.98	0.12	0.279
Protein carbonyl, μM/mg	5.12	4.64	0.69	0.621
IgA, mg/mg	0.08	0.10	0.02	0.554

[1]Sows were fed diets with 0.05% LPL during lactation.

Table 8. Supplemental effects of LPL in lactation diets on jejunal morphology and crypt cells proliferation of suckling piglets

Item	CON	LPL[1]	SEM	P value
Jejunum				
Villus height, μm	484	493	11	0.513
Villus width, μm	94	93	3	0.875
Crypt depth, μm	139	145	5.83	0.374
VH:CD[2]	3.53	3.41	0.17	0.458
Crypt cell proliferation, %	39.4	40.9	0.4	0.027

[1]Sows were fed diets with 0.05% LPL during lactation.
[2]Villus height to crypt depth ratio.

Table 9. Supplemental effects of LPL in lactation diets on diversity estimates of bacteria in jejunal and colonic mucosa of suckling piglets

Item	CON	LPL[1]	SEM	P-value
Jejunum				
Chao1	113.50	109.63	18.84	0.777
Shannon	4.26	4.39	0.38	0.641
Simpson	0.83	0.89	0.05	0.204
Colon				
Chao1	51.69	52.47	6.12	0.919
Shannon	3.64	3.62	0.23	0.962
Simpson	0.84	0.84	0.02	0.952

[1]Sows were fed diets with 0.05% LPL during lactation.

Table 10. Supplemental effects of LPL in lactation diets on relative abundance of bacteria at the phylum level in jejunal and colonic mucosa of suckling piglets

Item	CON	LPL[1]	SEM	P-value
Jejunum				
Actinobacteria	0.2	0.2	0.1	0.954
Bacteroidetes	35.2	27.1	5.2	0.299
Firmicutes	44.5	53.0	6.4	0.178
Fusobacteria	0.6	0.8	0.2	0.185
Proteobacteria	13.4	12.1	2.3	0.708
F:B[2]	1.0	3.5	2.3	0.095
Colon				
Actinobacteria	0.1	0.2	0.1	0.969
Bacteroidetes	39.1	39.0	3.1	0.984
Firmicutes	38.3	38.3	4.2	0.992
Fusobacteria	0.2	0.2	0.1	0.605
Proteobacteria	10.2	13.2	1.9	0.292
F:B[2]	1.0	1.0	0.1	0.906

[1]Sows were fed diets with 0.05% LPL during lactation.
[2]Firmicutes to Bacteroidetes ratio.

Table 11. Supplemental effects of LPL in lactation diets on relative abundance of bacteria at the genus level in jejunal and colonic mucosa of suckling piglets

Item	CON	LPL[1]	SEM	P-value
Jejunum				
Acinetobacter	0.42	0.14	0.46	0.140
Actinobacillus	1.11	0.55	0.22	0.114
Alistipes	1.54	1.70	0.48	0.807
Bacteroides	6.39	8.59	2.81	0.458
Butyricimonas	1.08	1.31	0.42	0.672
Clostridium	8.18	7.44	0.70	0.429
Faecalibacterium	2.14	2.85	0.69	0.228
Fusobacterium	0.23	0.13	0.06	0.225
Lactobacillus	22.93	32.86	7.60	0.175
Parabacteroides	3.43	3.00	0.96	0.768
Phascolarctobacterium	0.43	0.64	0.12	0.224
Prevotella	14.71	9.30	3.91	0.229
Pseudomonas	0.73	0.50	0.38	0.516
Streptococcus	2.47	2.71	0.50	0.607
Colon				
Acinetobacter	0.96	0.86	0.06	0.916
Actinobacillus	1.15	0.47	0.26	0.120
Alistipes	3.21	2.19	0.59	0.250
Bacteroides	13.89	20.58	5.45	0.148
Butyricimonas	2.14	2.25	0.56	0.886
Clostridium	6.71	5.49	0.68	0.229
Desulfovibrio	1.51	1.55	0.43	0.923
Faecalibacterium	8.63	8.60	1.76	0.991
Fusobacterium	0.30	0.25	0.09	0.677
Lactobacillus	7.65	10.45	4.15	0.154
Parabacteroides	3.65	3.48	0.60	0.844
Phascolarctobacterium	3.66	3.87	0.68	0.831
Prevotella	20.24	19.31	4.52	0.889
Pseudomonas	0.01	0.06	0.06	0.295
Streptococcus	0.92	0.91	0.29	0.992

[1]Sows were fed diets with 0.05% LPL during lactation.

under insufficient feed intake. However, in this study, sows fed diets with LPL had higher number of piglets and litter weight gain during lactation without changes in feed intake and BW loss during lactation. The possible reason is that LPL may help to improve the utilization of energy and nutrients for lactating sows through fat emulsification (Armand et al., 1999) or altering nutrient transport across the cell membrane with changed size and number of membranous pores (Kelkar and Chattopadhyay, 2007; Lundbæk et al., 2010). Previous studies showed that supplementation of LPL improved nutrient digestibility of nursery pigs after weaning (Zheng et al., 2016) and sows during lactation (Wang et al., 2019). Therefore, supplementation of LPL possibly benefits lactating sows by supporting the nutrient and energy requirements of the sows to maintain their litter performance during lactation.

Immunoglobulin is the crucial factor in providing passive immunity protection for suckling piglets to help establish their intestinal immune system during the initial postnatal period (Hurley and Theil, 2011; Oliviero et al., 2019). It has been known that IgG in milk is derived from immune cells of the sow (Bourne and Curtis, 1973). However, the result in this study shows that supplementation of LPL in lactation diets increased the milk IgG concentration in late lactation. Wang et al. (2019) reported that increasing the supplemental level of LPL from 0 to 1,000 mg/kg linearly increased IgG concentration in milk of lactating sows. These results could be connected with three possible mechanisms how LPL could increase IgG in milk during lactation. Firstly, Gräler and Goetzl (2002) reported that LPL could modulate the immune response by activating the immune cells such as T-cells, B-cells, and macrophages. Secondly, LPL could support energy and nutrients for maintaining production IgG during lactation (Wang et al., 2019) because a considerable nutrient expenditure would be required for the synthesis of IgG in mammary glands (Woof, 2013). Finally, a high number of piglets may cause increased challenges for the immune system with an increased activity of lymphocytes and macrophages in mammary glands due to increased possibilities of bacterial infections and mammary lesions by suckling piglets (Hulten et al., 2003).

Fatty acids, especially long chain fatty acids, move into the mammary gland via transport proteins (CD36 and SLC27A3) and are utilized to progress the synthesis process and aid in secretion of fatty acids and triacylglycerols (Zhang et al., 2018). During the de novo pathway of lipid synthesis in the mammary gland, lysophosphatidic acid, one of the mediators in bioactive LPL (Shea and Tager, 2012), activated by glycerol-3-phosphate acyltransferase is required for forming the fat source in the milk (Yu et al., 2018). In this study, the results show that supplementation of LPL in lactation diets altered a fatty acid composition in the milk. Therefore, this study indicates that exogenous LPL could affect the de novo pathway for lipid synthesis in the mammary

gland, leading to an altered fatty acid composition in the milk during lactation. Interestingly, the results in this study also showed that sows fed diets with LPL had increased the ratios of omega-6:omega-3 fatty acids and unsaturated:saturated fatty acids with decreased myristic acid (C14:0), palmitic acid (C16:0) and increased oleic acid (C18:1), linoleic acid (C18:2), eicosenoic acid (C20:1), and eicosadienoic acid (C20:2) in the milk during lactation. Two types of enzyme, elongases and desaturases, are involved in the fatty acid metabolism to regulate the degree and length of fatty acids in mammalian cells. Oleic acid is one of the most abundant fatty acids in the mammalian cell and it is synthesized by de novo fatty acid synthesis from palmitic acid through elongation and desaturation to stearic acid followed by Δ9 stearoyl-CoA desaturase 1 (SCD1) (Guillou et al., 2010). The SCD1 would be activated by a proliferation of the mammary epithelial cell to compensate for lipids in the cells under challenges with increasing metabolic rates during lactation (Igal, 2010; Shao and Zhao, 2014). Previous studies reported that lysophosphatidic acid could be a key factor for the proliferation and differentiation of mammary epithelial cells through activations of the G protein-coupled receptors and mitogen-activated protein kinase signaling pathway (Radeff-Huang et al., 2004; Panupinthu et al., 2010; Yuh, 2011). On the other hand, linoleic acid should be obtained from dietary sources for the biosynthesis of eicosadienoic acid by fatty acid elongase-5 (Guillou et al., 2010). In this study, linoleic acid in LPL would be supplemented at 0.027% in the diets during lactation. According to the fatty acid composition in the milk, the proportion of linoleic acid in milk was increased by 0.9%, and eicosadienoic acid, which would be synthesized from linoleic acid by fatty acid elongase was also increased much higher than the inclusion level of LPL in the diets. Thus, the fatty acid composition in LPL could not be directly related to the production of milk fatty acid in lactating sows. It has been known that exogenous LPL could be incorporated into the membrane of mammalian cells with improving the membrane permeability (Tagesson et al., 1985; Besterman and Domanico, 1992; Lundbaek and Andersen, 1994). In particular, linoleic acid is considered as a preferred substrate for the synthesis of triglycerides (Minich et al., 1997) due to the high affinity to acyl-CoA:lysophospholipid-acyltransferase (Bakken and Farstad, 1992), which is involved in the triglyceride synthesis and remodeling of phospholipids in the cell membrane (Ayyash et al., 2014; Yamashita et al., 2014). According to previous studies, oral administration of LPL increased retention and absorption of linoleic acids through incorporation into phospholipids in the intestine (Viola et al., 1993; Minich et al., 1997). Therefore, supplementation of LPL would change the fatty acid composition in the milk through improving proliferation and permeability of mammalian gland epithelium during lactation.

The IL-8 is a pro-inflammatory cytokine and represents the mediator of intestinal inflammation (Li et al., 1998; Andrews et al., 2018). In this study, IL-8 concentration was increased in the jejunum of piglets from sows fed diets with LPL. An increase of IL-8 in the jejunum of piglets could be related to an increased oleic acid, linoleic acids, and omega-6 to omega-3 ratio in the milk from the sows. Oleic acid as one of the omega-9 fatty acids also shown to activate immune responses by inducing transformation of lymphocytes in gut-associated lymphoid tissues of rats (Miura et al., 1993; Yoshida et al., 2001). Tanaka et al. (2001) also reported that oleic acid induced IL-8 production in Caco-2 cells representing human intestinal epithelial cells under in vitro condition. Linoleic acid upregulated the production of IL-8 in the intestine from patients with Crohn's disease (Alzoghaibi et al., 2003). Yao et al. (2012) also reported that when sows fed diets with different fat sources, the altered ratio of omega-6:omega-3 in milk could influence the immune status of suckling piglets during lactation. This study could suggest a possible mechanism of IL-8 release in the jejunum of piglets consuming milk with increased oleic acid, linoleic acid, and omega-6 to omega-3 ratio from sows fed with LPL.

Newborn suckling piglets have intensive cellular proliferation in the intestine during lactation (Wang et al., 2018) requiring massive nutrients to support the intestinal growth and development (Zhou et al., 2018). This study showed that supplementation of LPL in lactation diets increased crypt cell proliferation in the jejunum of piglets. The altered fatty acid composition in milk from sows fed with LPL could be related to increased enterocyte proliferation in the jejunum of piglets. A previous study showed that dietary oleic and linoleic acids are preferred substrates for energy metabolism in pigs (Bruininx et al., 2011). Milk with high oleic and linoleic acids maybe an effective energy source for enterocyte proliferation in suckling piglets.

Suckling piglets could be exposed to harmful microorganisms and antigens from the surrounding environments, leading to increased mortality (Sangild, 2003). In this study, sows fed with LPL improved litter BW gain and survivability of piglets during lactation. Increased IgG and altered fatty acid compositions in milk could also contributed to improved survivability of piglets. According to Kielland et al. (2015), IgG in the milk from sows could be a key element to provide passive immunity for the survival of suckling piglets during the initial postnatal period. High oleic and linoleic acids in the milk also be effective energy sources for growth and survivability of pigs (Bruininx et al., 2011). Lim et al. (2013) reported that oleic acid could also be anti-inflammatory and protective against metabolic diseases such as insulin-resistance and atherosclerosis with an effective fatty acid oxidation in the skeletal muscle. Linoleic acid is required to synthesize longer chain fatty acids which are biologically relevant in the pigs (Dugan et al., 2004). Linoleic acid is shown to attenuate mucosal damages induced by colitis (Hontecillas et al., 2002). According to Skrzypczak et al. (2015), linoleic acid in the milk was correlated to survivability and BW of suckling piglets. These previous findings support and explain the results in this study indicating supplementation of LPL improved the survivability of piglets by enhancing their passive immunity through the milk with increased IgG, oleic acid, and linoleic acid.

Firmicutes to Bacteroidetes ratio has been used to indicate the overall gut-associated microbiota balance (Mariat et al., 2009; Niu et al., 2015; Adhikari et al., 2019) and the mucosal immune activation (Sui et al., 2018). In this study, the result shows that supplementation of LPL in lactation diets increased Firmicutes to Bacteroidetes ratio in the jejunum of piglets. This result could be connected with increased omega-6 fatty acids in the milk from sows fed with LPL. High omega-6 fatty acids could activate the immune responses to improve host ability to protect enteric pathogens infection (Quin and Gibson, 2019).

In summary, supplementation of LPL in lactation diets effectively increased IgG concentration in milk and also altered fatty acid composition, especially oleic and linoleic acids, in milk without affecting BW and backfat loss in sows. Furthermore, dietary LPL could potentially help piglets to improve intestinal health by increasing IL-8 and crypt cell proliferation in the jejunum, with increased Firmicutes to Bacteroidetes ratio. These findings support the hypothesis that

supplementation of LPL in lactation diets could enhance milk composition, leading to improved survivability of piglets with an activation of immune response, enterocyte proliferation, and enhancing the balance of gut-associated microbiome in the jejunum.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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