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ORIGINAL ARTICLE

Effects of different n-6 to n-3 polyunsaturated fatty acids ratio on reproductive performance, fecal microbiota and nutrient digestibility of gestation-lactating sows and suckling piglets

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ABSTRACT

This study was conducted to evaluate the effects of dietary ratios of n-6:n-3 polyunsaturated fatty acids (PUFA) on reproductive performance, fecal microbiota and nutrient digestibility of gestation-lactating sows and suckling piglets. Fifteen primiparous sows (Landrace × Yorkshire) were randomly allotted into three treatments. Fed diets contained different ratios of n-6:n-3 PUFA, including 20:1, 15:1 and 10:1. No differences were detected among the treatments for average daily feed intake (ADFI) of sows and the back fat levels during lactation ($P > 0.05$). Body weight (BW) loss of sows after farrowing to weanling was greater in the 10:1 treatment compared with 15:1 or 20:1 ($P < 0.05$). In piglets, a great significant difference for BW was observed at 4 weeks ($P < 0.01$). Furthermore, average daily gain (ADG) of piglets in the 10:1 treatment was higher ($P < 0.05$). No difference was observed among treatments in nutrient digestibility of sows ($P > 0.05$). A great significant difference for fecal microbiota was in the 10:1 treatment compared with 20:1 and 15:1 treatments ($P < 0.01$). In conclusion, altering the ratio of n-6:n-3 PUFA in gestation-lactating sow diet had no difference on nutrient digestibility in gestation-lactating sows, but it can partially improve reproductive performance.

Key words: *fecal microbiota, gestation-lactating sow, n-6:n-3 polyunsaturated fatty acids, nutrient digestibility, reproductive performance.*

INTRODUCTION

Essential fatty acids can be separated into two kinds of polyunsaturated fatty acids (PUFA), n-6 and n-3 PUFA; these two classes of essential fatty acids are not inter-convertible and often have important opposing physiological functions. The balance of essential fatty acids is very important for health and normal development (Calder 2003). Commercial pig diets are based on cereals and protein feed which hardly contain long-chain n-3 PUFA (Rooke *et al.* 2001). Actual requirements of the n-6 and n-3 families of PUFA in pig diets and the ideal ratio of n-6:n-3 are still a matter of research (Bryhnia *et al.* 2002). The n-6 and n-3 PUFA have distinct properties. It has been suggested a high n-6:n-3 ratio is considered to be a critical factor in both performance and nutrient digestibility in studies in man and animal models.

During the past years, studies had demonstrated that animal performance could be influenced by the level or the ratio of n-6 and n-3 PUFA in sows and dogs (Wander *et al.* 1997; Zanini *et al.* 2003). For instance, the study by Yao *et al.* (2012) demonstrated that altering the ratio of n-6:n-3 PUFA in the lactating sow diet had an effect on their performance and that of suckling piglets, and it tended to increase the litter average daily gain of piglets. Moreover, the amount and profile of PUFA in the diet had effects on lipid metabolism and the inflammatory system of animals, leading to the availability of more energy and nutrients for high performance and homeostatic pathways. (Grobas *et al.* 2001; Duan *et al.* 2014). Alterations of lipid content of the suckling piglet may affect its endogenous energy supply and its ability to endure the stress of weaning (Rooke *et al.*

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2000). Yao *et al*. (2012) reported that adding different sources of oil including maize, linseed or tuna oils to sow diets throughout pregnancy and lactation had a subsequent effect on piglet weight 7 days post-weaning. PUFA can benefit pregnant and lactating sows under catabolic conditions with outcomes of improving fetal growth, neonatal health and lactation performance (Kim *et al.* 2007). Previously, it was unknown whether a particular ratio of n-6:n-3 PUFA in maternal diet is beneficial to gestation-lactating sows' reproductive performance. However, it could be hypothesized that in the case of providing a diet for sows with a low n-6:n-3 ratio, reproductive performance and consequently postpartum feed intake would be improved, whereas diets with a high n-6:n-3 ratio would not exert these benefits (Quelen *et al.* 2010). On the other hand, nutrient digestibility is critical for sow survival and normal growth. Additionally, whether altering the ratio of n-6:n-3 PUFA that can improve sows' nutrient digestibility is unknown.

Thus, the aim of the trial was to investigate the effect of different ratios of dietary n-6:n-3 PUFA on the reproductive performance, fecal microbiota and nutrient digestibility in gestation-lactating sows and suckling piglets.

MATERIALS AND METHODS

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Dankook University, which is comparable to those laid down by the Canadian Council on Animal Care.

Source of n-6:n-3 PUFA

The omega-3 fatty acid used in this study was provided by a commercial company (Morningbio Co., Ltd., Cheonan, Korea). It was produced from linseed oil. It was protected using matrix coating technology. According to the information provided by the suppliers, the n-3 PUFA and n-6 PUFA contents were 56.71% and 14.79% of dry matter (DM; linseed oil), respectively.

Experimental design, animals and housing

A total of 15 multiparous sows (Landrace × Yorkshire), which were at day 107 of gestation, were assigned to three treatments, comprising of diets containing different ratios of n-6:n-3 PUFA, including 20:1, 15:1, 10:1, taking into account parity, body weight and expected farrowing date. There were five replicates per treatment. The experimental period started at day 107 of gestation and ended when the piglets were weaned at day 28 of lactation. The sows were housed individually in 2.1 × 1.8 m farrowing crates with solid concrete floors combined with slats of iron. The temperature in the farrowing house was maintained at a minimum of 20°C. Supplemental heat was provided for piglets using heat lamps. Piglets were treated according to routine management practices that included teeth clipping, tail docking, ear notching. The animal care and use protocol was approved by the Animal Care and Use Committee of Dankook University.

Diets and feeding

From day 108 of gestation until farrowing, sows were fed 2.5 kg/day of their respective experimental gestation diets. On the day of parturition, the sows were not offered feed. After farrowing, sows were fed their respective experimental lactation diets until weaning. During lactation, daily feed allowance was increased gradually until sows had *ad libitum* access to feed by week 2. All diets were provided in meal form twice daily and sows had free access to drinking water throughout the experimental period. The sow diets (Table 1) were based on corn-soybean meal and were formulated to meet or exceed the nutrient requirements recommended by the National Research Council (NRC) (2012). The ingredients in the formula were mostly the same in the three treatments, except the ratio of n-6:n-3 PUFA was modulated by changing the percentage of linseed oil which was mixed into the diet.

The fatty acid composition of the experimental diets is shown in Table 2. The fatty acid composition of the diets was determined according to the modified method of Kim *et al.* (2003). Lipid from the diets were extracted with hexane/isopropanol (3:2 v/v). The extracted lipids were mixed with 0.5 mL of toluene and 2 mL of 5% KOH-MeOH, and heated at 70°C for 8 min; and then 2 mL of 14% BF3-MeOH was added to the above mixture, and heated at 70°C for 2 min. The fatty acid methyl esters (FAME) were extracted with 3 mL of 5% NaCl and 1 mL of hexane. Samples were analyzed for total fatty acids, using an HP5890 gas chromatograph with a flame ionization detector (Hewlett Packard 5890 Series II; Palo Alto, CA, USA). The FAME were separated using a Supelcowax-10 fused silica capillary column (100 m × 0.32 mm × 0.25 μm; Supelco, Inc., Bellefonte, PA, USA), with a 1.2 mL/min helium flow. The oven temperature was increased from 220 to 240 C, at the rate of 2 C/min. Temperatures of the injector and detector were 240 and 250°C, respectively. The peak of fatty acids was identified by comparing the retention time and peak area of each fatty acid standard, respectively. Fatty acid content was expressed as a percentage of the total fatty acids.

Table 1 Composition of gestation-lactation sow diets (as-fed basis)†

Items	Gestation diet			Lactation diet		
	n-6:n-3 PUFA ratio			n-6:n-3 PUFA ratio		
	20:1	15:1	10:1	20:1	15:1	10:1
Ingredients (%)						
Corn	70.01	67.83	61.53	60.81	60.81	55.18
Wheat bran	2.50	2.50	2.50	2.50	2.50	2.50
Soybean meal (46% CP)	15.49	15.87	17.02	25.98	25.98	27.00
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	1.25	–	–	3.69	0.63	–
Molasses	2.00	2.00	2.00	2.00	2.00	2.00
Oat	2.00	2.00	2.00	–	–	–
L-lysine-HCl (78%)	0.14	0.14	0.14	–	–	–
Limestone	1.05	1.05	1.05	0.75	0.75	0.75
Dicalcium phosphate	1.73	1.73	1.73	1.64	1.64	1.64
Choline chloride (25%)	0.03	0.03	0.03	0.03	0.03	0.03
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix‡	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix§	0.10	0.10	0.10	0.10	0.10	0.10
Linseed oil (n-3)	1.20	4.25	9.40	–	3.06	8.30
Calculated composition						
ME (Mcal/kg)	3.25	3.25	3.25	3.35	3.35	3.35
Analyzed composition (%)						
Dry matter	89.42	91.72	90.08	91.73	91.48	92.60
Crude protein	14.00	14.00	14.00	17.50	17.50	17.50
Lysine	0.76	0.76	0.76	0.90	0.90	0.90
Methionine	0.24	0.24	0.23	0.28	0.28	0.28
Methionine + cystine	0.63	0.62	0.61	0.70	0.70	0.70
Calcium	0.87	0.87	0.87	0.76	0.76	0.76
Total phosphorus	0.64	0.63	0.63	0.65	0.65	0.65

†Dietary treatments were different ratios of n-6:n-3 polyunsaturated fatty acids (PUFA), including 20:1, 15:1 and 10:1. ‡Provided per kilogram of complete diet: vitamin A, 10 000 IU; vitamin D_3, 2000 IU; vitamin E, 48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; biotin, 0.2 mg; d-pantothenic, 17 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 μg. §Provided per kilogram of complete diet: Fe (as $FeSO_4 \times 7H_2O$), 90 mg; Cu (as $CuSO_4 \times 5H_2O$), 15 mg; Zn (as $ZnSO_4$), 50 mg; Mn (as MnO_2), 54 mg; I (as KI), 0.99 mg; and Se (as $Na_2SeO_3 \times 5H_2O$), .25 mg.

Sampling and measurements

Reproductive performance of sows

Individual sows were weighed and scanned for back fat thickness at day 108 of gestation, the day after farrowing and at weaning (26–28 days) to determine weight and back fat thickness loss. The back fat thickness of the sows (6 cm off the midline at the 10th rib) was measured using a real-time ultrasound instrument (Piglot 105; SFK Technology, Herlev, Denmark). The feed consumed during the gestation and lactation periods was recorded for each sow to calculate average daily feed intake. After farrowing, daily feed allowance increased 1 kg/day until day 6 postpartum, and then sows were given *ad libitum* access to feed and water.

Growth performance of piglets

During the experimental period, individual piglets were weighed within the first 24 h of farr-owing as well as at weaning (26–29 days). The numbers of piglets alive and death per litter were recorded to calculate survival ratio. To guarantee that all sows nursed a similar number of piglets (about 13 piglets per sow), litter sizes were adjusted by cross-fostering piglets within 24 h of birth (Zhou *et al.* 2012).

Fecal microbiota of sows

On weaning day, fecal samples were collected from five sows per treatment by rectal palpation, and placed on ice for transportation to the laboratory, where microbial analysis was immediately carried out according to the method described by Zhao *et al.* (2015). Briefly, a 1 g fecal sample from each sow was diluted with 9 mL of 10 g/L peptone broth (Becton, Dickinson, and Co., Rutherford, NJ, USA) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), and lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany) to isolate the *E. coli* and *Lactobacillus*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C

Table 2 Fatty acid composition of the experimental diets (mg/g of diet)

Fatty acid	Gestation diet			Lactation diet		
	n-6:n-3 PUFA ratio			n-6:n-3 PUFA ratio		
	20:1	15:1	10:1	20:1	15:1	10:1
Myristic acid (C14:0)	0.065	0.040	0.061	0.137	0.058	0.060
Palmiyic acid (C16:0)	11.064	11.626	12.828	10.661	11.006	12.244
Palmitoleic acid (C16:1)	0.149	0.099	0.095	0.252	0.130	0.100
Stearic acid (C18:0)	2.142	2.655	3.874	2.416	2.630	3.798
Oleic acid (C18:1)	23.485	22.699	21.652	23.070	22.037	20.954
Linoleic acid (C18:2n-6)	43.442	42.687	40.397	42.590	42.696	40.659
α-Linoleic acid (C18:3n-3)	2.118	2.753	3.830	2.143	2.777	3.885
Octadectetraenoic acid (C18:4)	0.959	0.929	0.843	0.833	0.833	0.767
Arachidic acid (C20:0)	0.005	0.016	0.036	–	0.012	0.032
Arachidonic acid (C20:4n-6)	0.003	0.003	0.003	0.003	0.003	0.003
Eicosapentaenoic acid (C20:5n-3)	0.005	0.017	0.039	–	0.013	0.035
Docosapentaenoic acid (C22:5n-3)	0.007	0.024	0.054	–	0.017	0.048
Docosahexaenoic acid (C22:6n-3)	0.012	0.044	0.098	–	0.032	0.089
Saturated fatty acid, %	13.277	14.327	16.770	13.214	13.693	16.103
Monounsaturated fatty acid, %	23.634	22.797	21.747	23.322	22.166	21.054
PUFA, %	45.577	45.501	44.364	44.733	45.517	44.667
Total n-3 PUFA, %	2.142	2.838	4.021	2.143	2.839	4.057
Total n-6 PUFA, %	43.445	42.690	40.400	42.593	42.699	40.662
n-6:n-3 PUFA ratio†	20.282	15.042	10.047	19.875	15.040	10.023

†n-6:n-3 polyunsaturated fatty acids (PUFA) ratio calculated as (18:2 + 20:4, n-6)/(18:3 + 20:5 + 22:5 + 22:6, n-3). Results represent the average fatty acid profile of duplicate samples of each diet.

under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The *E. coli* and *Lactobacillus* were counted immediately after removal from the incubator.

Apparent total tract digestibility (ATTD) of nutrients in sows

To determine the ATTD for DM, nitrogen (N), and gross energy (GE), sows were fed diets containing chromic oxide (0.2%) as an indigestible marker for 5 days followed by fecal grab sampling from five sows per treatment via rectal palpation before farrowing, after farrowing and on weaning day. Before the chemical analysis, the fecal samples were thawed and dried in an oven at 60°C for 72 h, after which they were ground to pass through a 1 mm screen. Then, all the feed and fecal samples were analyzed, following the procedures outlined by the AOAC (2007). N was determined using a Kjeltec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden) and CP was calculated as N × 6.25. Gross energy (GE) was analyzed using an oxygen bomb calorimeter (Parr 1600 Instrument Co., Moline, IL, USA). Chromium was analyzed by UV absorption spectrophotometry (UV-1201; Shimadzu, Kyoto, Japan) according to the methods of Williams *et al.* (1962). The ATTD was then calculated using the following formula:

$$\text{Digestibility}(\%) = 1 - [(N_f \times C_d)/(N_d \times C_f)] \times 100,$$

where N_f is the nutrient concentration in the feces (% DM), C_d is the chromium concentration in the diet (% DM), N_d is the nutrient concentration in the diet (% DM) and C_f is the chromium concentration in the feces (% DM).

Statistical analysis

All data were subjected to the GLM procedures of SAS (2001) as a randomized complete block design (SAS Institute Inc., Cary, NC, USA). The individual sow or litter of piglets was used as the experimental unit. Differences among all treatments were separated by Duncan's multiple range test. A probability level of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Reproductive performance of sows

The effect of different ratios of n-6:n-3 PUFA on reproductive performance of sows is presented in Table 3. No differences were observed among treatments for sow back fat thickness and ADFI from gestation to lactation. After farrowing to weanling, sow BW loss in the 10:1 group decreased ($P < 0.05$) compared with 20:1 and 15:1 groups.

It is well known that the weight loss of lactating sows due to mobilization of fatty acids and protein can vary considerably between sows (Mullan & Williams 1990). This suggests that fatty acids-rich diets do not reduce mobilization of body stores. The n-6 and n-3 fatty acids are stored in phospholipids of cell and organelle membranes and in glycerides and

Table 3 Effect of different ratios of PUFA supplementation on reproductive performance in gestation-lactating sows†

Items	Dietary n-6:n-3 PUFA ratio			SEM‡	*P*-value
	20:1	15:1	10:1		
Parity	3.0	3.2	3.0	0.4	0.929
Body weight, kg					
Before farrowing§	220.8	219.4	220.0	2.3	0.889
After farrowing	194.8	192.1	193.7	2.5	0.753
Weanling	189.0	186.4	189.2	2.6	0.668
Body weight loss1¶	26.0	27.2	26.3	1.5	0.819
Body weight loss2¶	5.8[a]	5.7[a]	4.5[b]	0.3	0.031
Back fat thickness, mm					
Before farrowing§	20.2	20.6	21.0	0.5	0.522
After farrowing	20.0	20.2	20.8	0.5	0.434
Weanling	17.6	17.4	18.6	0.4	0.117
Back fat thickness loss1††	0.2	0.4	0.2	0.2	0.756
Back fat thickness loss2††	2.0	2.2	2.2	0.5	0.921
ADFI, kg					
Gestation	2.46	2.46	2.46	–	–
Lactation	8.87	8.94	8.92	0.02	0.698

†Dietary treatments were different ratios of n-6:n-3 polyunsaturated fatty acids (PUFA), including 20:1, 15:1 and 10:1. ‡Standard error of means. §Before farrowing: before 7 days. ¶Body weight loss: 1, before farrowing to after farrowing; 2, after farrowing to weanling. ††Back fat thickness loss: 1, before farrowing to after farrowing; 2, after farrowing to weanling. [a,b]Means in the same row with different superscripts differ ($P < 0.05$). ADFI, average daily feed intake.

phospholipids of lipid bodies. These fatty acids are released from phospholipids by phospholipase A_2 and are further metabolized to eicosanoids and other autacoids. AA (n-6)-derived eicosanoids are mostly proactive, whereas eicosapntemacnioc acid (EPA) (n-3)-derived eicosanoids are inhibitory (Schmitz & Ecker 2008).

Sow BW loss while after farrowing to weanling could be attributed to multiple causes: birth of a large litter of piglets, sows with a lower body condition mobilizing body stores to produce an adequate milk supply, or refusal of diet. Lauridsen and Danielsen (2004) reported that a better energy balance might be expected to decrease BW loss by sows during the lactation period. Dietary low n-6:n-3 fatty acid ratio in mice on a high fat diet, did not differ significantly in BW among the groups at any point throughout the study (Riediger *et al.* 2008). Growth and feed efficiency of male rats were not affected by diets varying from 1.2 to 23.8 in the ratio of (n-6)/(n-3) fatty acids (Watkins *et al.* 2000). According to Yao *et al.* (2012) no differences were detected among the treatments for the daily feed intake of sows or changes in sow weight and back-fat levels during lactation. Conversely, many previous studies demonstrated that fatty acids were added in the diet and had a positive effect on sows. Lauridsen and Danielsen (2004) showed that inclusion of fat acids at a level of 8% to lactation diets of sows improved their daily feed intake, enhanced the weight gain of the progeny from birth until weaning, and influenced the body weight loss of the lactating sow dependent on their parity. Furthermore, a lactation feed low in n-6:n-3 ratio administered from 8 days before farrowing ensures improved feed intake during the first days postpartum and was associated with a better metabolic change and inflammatory profile in sows in the periparturient period (Papadopoulos *et al.* 2008).

In our study, we observed reduction ($P < 0.05$) on sow BW loss in the 10:1 treatment, after farrowing to weanling. Although BW was comparable among groups in the present study, food intake was not. At present, we cannot explain this apparent paradox. However, it is possible that either the absorption of dietary fatty acids was impaired, therefore, unabsorbed fatty acids was excreted, or the interactions between high levels of n-3 fatty acids and other fatty acids may prevent additional BW gain. One may also speculate that these formulations may induce the β-oxidation system, preventing fat accumulation and BW gain. The action of PUFA in adult animals is not well studied, and a possible explanation for the reduction of the sow BW loss during the suckling period could be the increased feed intake.

As reported by Thacker (2013), sows' back fat could reflect nutrient digestibility and potential reproductive efficiency. The sows needed to increase the metabolic rate of body reserves to maintain health and survival, which leads to an increased back fat thickness loss (Muhlhausler *et al.* 2011). In addition, it was likely that parity also had effect on back fat thickness loss during the gestation period. In our study, no differences were found on sow back fat thickness loss among dietary treatments. Also, Li *et al.* (2015) reported that the n-6:n-3 PUFA ratios had no effect ($P < 0.05$) on back fat thickness in finishing pigs.

Growth performance of piglets

The effect of different ratios of n-6:n-3 PUFA on growth performance of suckling piglets is presented in Table 4. The number of piglet survivals, total piglets, live piglets and stillbirths were unaffected ($P > 0.05$) by treatments, but piglet BW at 4 weeks was increased ($P < 0.01$) in the 10:1 treatment compared with the other two treatments. As expected, piglets nursing sows fed the 10:1 treatment had greater ADG (229 g/day) during lactation when compared to the 20:1 treatment and 15:1 treatment (210 g/day, 216 g/day, $P < 0.05$).

Table 4 Effect of different ratios of PUFA supplementation on growth performance in suckling piglets†

Items	Dietary n-6:n-3 PUFA ratio			SEM‡	*P*-value
	20:1	15:1	10:1		
Litter size, *n*					
Total born	12.4	12.6	11.8	0.8	0.790
Born alive	11.4	11.6	10.8	0.9	0.781
Stillborn, %	8.3	7.4	8.0	4.6	0.976
Weaned	11.0	11.2	10.6	0.7	0.842
Survivability, %	97.1	96.4	98.6	2.1	0.784
Piglets					
Initial weight, kg	1.367	1.368	1.436	0.079	0.747
Weaning weight, kg	7.248[b]	7.426[b]	7.845[a]	0.110	0.003
ADG, g/day	210[b]	216[ab]	229[a]	5	0.040

†Dietary treatments were different ratios of n-6:n-3 polyunsaturated fatty acids (PUFA), including 20:1, 15:1 and 10:1. ‡Standard error of means. [a,b]Means in the same row with different superscripts differ ($P < 0.05$). ADG, average daily gain.

This research demonstrates that supplementing a sow diet with n-6 and n-3 PUFA during gestation will result in incorporation of these fatty acids into the developing piglets. Although the increase in fetal weight during late gestation in sows reaches a plateau (McPherson *et al.* 2004), it appears that the ratio of n-6 and n-3 PUFA change during the last days of gestation can affect piglet weight. This effect might be due to an increased transfer of energy and nutrients to the piglets *in utero* (Gabler *et al.* 2009). The apparent digestibility of fatty acids by suckling piglets is high (96%), as reported by Cranwell and Moughan (1989), and it may therefore be assumed that a rise in sow milk fat acids would be effectively used by the suckling piglet. In addition, Friend (1974) reported an increased body lipid content in piglets at weaning in association with increased milk fat acids content, thus indicating that most of the lipids from the milk are deposited in the piglet's body. As we know, arachidonic acid (ARA) is important for ensuring normal growth (Carlson *et al.* 1993). The piglets of n-6 and n-3 PUFA fed sows, had relatively higher percentages of eicosapentaenoic acid and dipropylacetic acid and a lower percentage of ARA in tissues at birth, and 28 days of age, suggesting that the supplemented n-6 and n-3 PUFA could cross the placenta and be incorporated into growing tissues (Meers *et al.* 2006).

Previous studies have also shown that the consumption of dam's milk with high fat content by the piglets has a beneficial effect in their growth. For instance, Rooke *et al.* (2001) found that piglets from sows fed diets containing tuna oil (which is a source rich in n-3 PUFA) had a more active suckling behavior immediately after birth, which contributes to their enhanced growth during the entire lactation period. Likewise, Mateo *et al.* (2009) reported that n-3 PUFA alone during lactation improved the growth of nursing piglets, and may have improved piglet birth weight in the subsequent litter. Indeed, Yao *et al.* (2012) demonstrated that altering the ratio of n-6:n-3 PUFA in lactating sow diet had an effect on performance of suckling piglets, and it tended to increase the litter ADG when dietary ratio of n-6:n-3 PUFA was 9:1. In contrast, Li *et al.* (2014) reported that 3% n-3 PUFA in the weanling piglet's diet did not result in significant improvement of ADG, ADFI or gain-to-feed ratio (G:F) in weanling pigs. In our study, altering maternal dietary ratio of n-6:n-3 PUFA greatly affected the litter average daily gain from day 0 to day 28, and it was greater in the 10:1 group compared with the other two groups. Also, BW of piglets was increased in the 10:1 group. Because the content of fatty acids and energy in milk play an important role in keeping piglets alive, and the improvement in litter weight by adding fatty acids to sow diets has been observed previously (Kim *et al.* 2007).

Fecal microbiota of sows

There was a significant increase ($P < 0.05$) in fecal *Lactobacillus* counts and a decrease ($P < 0.05$) in fecal *E. coli* counts associated with the inclusion of different ratios of n-6:n-3 PUFA in diets (Table 5). Sows fed the 10:1 treatment and 15:1 treatment had decreased ($P < 0.05$) fecal *E. coli* counts than those fed the 20:1 treatment. Moreover, the 10:1 treatment and 15:1 treatment had increased ($P < 0.05$) fecal *Lactobacillus* counts compared to those fed the 20:1 treatment.

The n-3 and n-6 PUFAs are well known modulators of the inflammatory process. The PUFA have competing roles in inflammatory pathways where a high n-3 PUFA intake can reduce the production of proinflammatory eicosanoids derived from n-6 PUFA, partially because n-3 fatty acids are the preferential substrates for enzymes involved in eicosanoid

Table 5 Effect of dietary different ratios of PUFA supplementation on fecal microbial shedding in gestation-lactating sows†

Item, log10 cfu/g	Dietary n-6:n-3 PUFA ratio			SEM‡	*P*-value
	20:1	15:1	10:1		
Lactobacillus	7.14[b]	7.38[a]	7.45[a]	0.04	<0.001
Escherichia coli	6.31[a]	6.13[b]	6.11[b]	0.03	0.003

†Dietary treatments were different ratios of n-6:n-3 polyunsaturated fatty acids (PUFA), including 20:1, 15:1 and 10:1. ‡Standard error of means. [a,b]Means in the same row with different superscripts differ ($P < 0.05$). cfu, colony-forming units.

metabolism (Larsson *et al.* 2004). For this reason, the balance of n-6 and n-3 PUFA in the diet may have stronger effects on fecal microflora than individual PUFA. Experimental data suggest that the balance between n-6 and n-3 PUFA can alter the behavior of fecal microflora. According to Calder (2013), the anti-inflammatory effects of PUFA suggested they may be useful as therapeutic agents in disorders with an inflammatory component. Ringo *et al.* (1998) reported an increased content of *Lactobacilli* under n-3 PUFA consumption and a lower content under n-6 PUFA consumption in fish. Moreover, Yu *et al.* (2014) found that fish oil with a high content of n-3 and n-6 PUFA are capable of producing significant changes in the gut microbiota. Similarly, in our study, significant effect ($P < 0.05$) on fecal *E. coli* counts and fecal *Lactobacillus* counts were observed by altering the n-6:n-3 PUFA ratio in the diet.

The n-3 and n-6 ratio in the diet strongly affects autacoid production and increased consumption of fatty acids leads to reduced synthesis of inflammatory eicosanoids from n-6 fatty acids, but elevated production of anti-inflammatory autacoids from n-3 fatty acids (Schmitz & Ecker 2008). Thus, sows fed the PUFA are likely to excrete faces resembling their gut microflora, for example fewer pathogenic germs and high numbers of bacilli spores. These feces are spread in the farrowing pen, probably creating a better (or even helpful) environment for the newborn pig, to colonize in the sterile gut already in advance of delivery and before the consumption of creep feed (Oliviero *et al.* 2010). The above hypothesis was strongly indicated where sows fed PUFA resulted in reduction of pathogen challenge of the neonatal environment.

ATTD of nutrients in sows

After farrowing and weanling of sows, no differences ($P > 0.05$) were observed in ATTD of nutrients among treatments (Table 6).

Fatty acids of animal origin are normally considered to have a lower digestibility and hence lower energy value than fatty acids of vegetable origin, owing to the higher content of saturated fatty acids in the former, which have lower nutrient digestibility than the unsaturated fatty acids (Jobrgensen *et al.* 1992; Overland *et al.* 1994). This could partly explain the lower energy intake for sows fed the vegetable fat acids. Cho and Kim (2013) reported that n-3 PUFA have no effect on nutrient digestibility in finishing pigs. Because there is not enough research about the dietary ratio of n-3:n-6 PUFA in gestation-lactating sows, no comparisons could be made with other studies. In our study, we demonstrated that altering the ratio of n-6:n-3 PUFA has no effect on nutrient digestibility in gestation-lactating sows.

Table 6 Effect of different ratios of PUFA supplementation on ATTD (%) of nutrients in gestation-lactating sows†

Items	Dietary n-6:n-3 PUFA ratio			SEM‡	*P*-value
	20:1	15:1	10:1		
Farrowing					
Dry matter	72.06	72.22	73.24	1.42	0.860
Nitrogen	71.62	72.29	70.66	2.18	0.891
Gross energy	73.72	72.45	73.66	1.41	0.839
Weanling					
Dry matter	70.64	71.38	71.96	0.93	0.612
Nitrogen	71.35	70.92	71.93	1.91	0.936
Gross energy	70.68	71.97	72.98	1.07	0.323

†Dietary treatments were different ratios of n-6:n-3 polyunsaturated fatty acids (PUFA), including 20:1, 15:1 and 10:1. ‡Standard error of means. [a,b]Means in the same row with different superscripts differ ($P < 0.05$). ATTD, apparent total tract digestibility.

Conclusion

In summary, our study demonstrated that fecal microbial and reproductive performance components, including sow BW loss, litter ADG and piglet BW were well affected by altering the ratio of n-6:n-3 PUFA in gestation-lactating sow diets. It increased litter ADG from day 0 to day 28 when dietary ratio of n-6:n-3 PUFA was 10:1. Further research might examine whether there is an interaction between dietary energy level and the ratio of n-6 and n-3 PUFA on reproductive performance of gestation-lactating sows and their suckling piglets.

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