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RESEARCH PAPER

Influence of a spray-dried fat enriched with EPA and DHA on the fatty acid composition of sow milk

Mariola Grez¹, Mónica Gandarillas^{1,2}, Fernando González¹, and Einar Vargas-Bello-Pérez¹

¹Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Departamento de Ciencias Animales. Santiago, Chile, Casilla-306. P. O. Box 6904411.

²Universidad Austral de Chile, Facultad de Ciencias Agrarias, Instituto de Producción Animal. Independencia 641, Campus Isla Teja Valdivia. Postal Code: 5110566.

Abstract

M. Grez, M. Gandarillas, F. González, and E. Vargas-Bello-Pérez. 2016. Influence of a spray-dried fat enriched with EPA and DHA on the fatty acid composition of sow milk. Cien. Inv. Agr. 43(3):347-355. Supplementation with eicosapentaenoic and docosahexaenoic acids (EPA and DHA) through the addition of fish oils to mammal diets during lactation benefits milk production, litter growth and the litter immune system, but there is little evidence supporting the use of oils that have been dried using a spray-drying method designed to cool and crystallize fat particles. The objective of this study was to evaluate the effect of a spray-dried dietary fat enriched with EPA and DHA on the fatty acid composition of sow milk. Fifteen pregnant sows were assigned to three dietary treatments from day 100 of gestation until weaning (day 28). Control sows (CONT) were fed an unsupplemented basal diet, and supplemented sows were fed the basal diet plus 20 g (FOPF20) or 40 g (FOPF40) of a spray-dried powdered fat enriched with EPA and DHA. Milk and colostrum compositions and milk yield were similar among diets, and the main milk fatty acids (FA) were C14:0, C16:0, C18:0, C18:1 n-9 and C18:2 n-6. Supplementation with EPA and DHA (FOPF20 and FOPF40) decreased the saturated FA contents and increased the amount of polyunsaturated FA. In summary, the results indicated that supplementing sow diets with EPA and DHA could decrease the saturated fatty acid content and increase the polyunsaturated fatty acid content of milk.

Key words: Fatty acids, milk, powdered fat, sow, spray-dry.

Introduction

As in other mammals, n-6 and n-3 series polyunsaturated fatty acids (PUFA) are considered essential for swine because they cannot be endogenously synthesized and must therefore be obtained from the diet (Simopoulos, 1991). The

addition of fish oil [a preformed source of n-3 FA such as eicosapentaenoic (EPA; C20:5 n-3) and docosahexaenoic acids (DHA; C22:6 n-3)] to sow diets has been shown to be beneficial to sucking piglet growth (Luo *et al.*, 2013). EPA and DHA play an important role in the regulation of both immune and reproductive functions because they are precursors for the synthesis of different types of eicosanoids (Mateo *et al.*, 2009). Previous research (Fritsche *et al.*, 1993;

Rooke *et al.*, 2001; Mateo *et al.*, 2009; Luo *et al.*, 2013) found that supplementing sow diets during lactation with enriched sources of EPA and DHA increases the milk contents of these FAs, which are then transferred to suckling piglets and may increase their growth and improve their immune response. However, none of these previous studies have investigated the response of swine to fish oil that has been transformed into fat in the form of a solid powder. Therefore, the objective of this study was to evaluate the effect of spray-dried dietary fats enriched with sources of EPA and DHA on the fatty acid composition of sow milk.

Materials and methods

The animals used in this study were housed at the Centro de Investigación, Innovación Tecnológica y Capacitación para la Industria Porcina Nacional (CICAP) located in Pirque, Chile. Animal care procedures followed the guidelines of the Animal Care and Use Committee of the Facultad de Agronomía e Ingeniería Forestal of the Pontificia Universidad Católica de Chile.

Animals and experimental treatments

The experiment employed a completely randomized design using 3 dietary treatments. Fifteen multiparous sows (Large white × Landrace, PIC genetics GP 1050, parity 8) were used in the study, which began at d 100 of pregnancy and continued until weaning (d 28 post farrowing). Sows were randomly allocated to dietary treatments (five sows per treatment) that differed in the EPA and DHA contents of the feed. The experimental diets contained 0 (CONT), 20 (FOPF20) and 40 (FOPF40) g of powdered fat that was high in EPA (2.7 g 100 g⁻¹) and DHA (1.8 g 100 g⁻¹) per kg of basal gestation or lactation diet, and the powdered fat supplement (FOPF) contained fish oil (15 g 100 g⁻¹), hydrogenated fat (80 g 100 g⁻¹) and soy lecithin (5 g 100 g⁻¹). The diets were designed to be isoenergetic and isonitrogenous by adding

powdered fat that was free of EPA and DHA (PF) to each diet to reach a total of 40 g powder fat kg⁻¹ of the basal diet; this powdered fat contained hydrogenated fat (95 g 100 g⁻¹) and soy lecithin (5 g 100 g⁻¹). The fatty acid compositions of both powdered fats are presented in Table 1. The gestation and lactation basal diets had a corn-soybean meal base, and their compositions are presented in Table 2. Diets were designed following NRC (2012) recommendations.

Table 1. Fatty acid composition of powdered fats containing EPA and DHA provided as a supplement to sows from day 100 of gestation to weaning (d 28).

Fatty acid	PF ¹	FOPF ²
C12:0	10.43	11.13
C14:0	4.53	6.55
C16:0	43.11	38.50
C18:0	0.01	0.20
C18:1 n-9	0.03	0.03
C18:2 n-6	37.35	30.73
C18:3 n-3	0.03	0.03
C18:3 n-6	0.02	0.06
C20:1 n-9	0.00	0.05
C20:5 n-3	0.01	4.09
C22:0	0.02	0.03
C22:1 n-9	0.00	0.01
C22:6 n-3	0.06	0.38
∑Saturated fatty acids	62.27	63.36
∑Monounsaturated fatty acids	0.18	0.45
∑Polyunsaturated fatty acids	37.55	35.62
Others	4.40	8.23

¹PF=powdered fat with hydrogenated fat (95 G 100 g⁻¹) and soy lecithin (5 g 100 g⁻¹) but without EPA and DHA.

²FOPF=powdered fat supplement containing fish oil (15 g 100 g⁻¹), hydrogenated fat (80 g 100 g⁻¹) and soy lecithin (5 g 100 g⁻¹).

Powdered fat production

Powdered fats were produced by atomization using a spray dryer (Compact Gas Model NR 28036, APV Anhydro SA, Denmark) that was

Table 2. Ingredients and chemical compositions of basal gestation and lactation diets.

Item	Gestation	Lactation
Ingredients (g kg ⁻¹)		
Corn	339.2	551.9
Sorghum	300.0	150.0
Rice bran	-	180.0
Wheat bran	250.0	55.0
Alfalfa	30.0	-
Yeast	2.0	-
Soybean meal	1.3	10.5
Soybean	16.0	12.0
Poultry oil	21.0	-
Lysine	5.6	1.6
Threonine	1.4	1.0
Methionine	1.0	1.0
Phytase	5.8	6.0
Calcium carbonate	16.0	6.0
Salt	6.0	-
Copper sulfate	0.5	-
Antioxidant	0.2	-
Vitamin-mineral mix	4.0	25.0
Chemical composition (%)		
Dry matter	88.6	88.9
Crude protein	12.6	17.8
Crude fiber	6.3	4.4
Ether extract	5.3	3.1
Ash	6.1	6.4
ME (Mcal /kg)	3.1	3.1
Fatty acids (g/100 g total fatty acids)		
C14:0	0.12	0.15
C16:0	0.02	0.01
C16:1	19.45	30.46
C18:0	0.06	0.08
C18:1 n-9	4.37	4.37
C18:2 n-6	44.43	54
C18:3 n-6	0.02	0.03
C18:3 n-3	0.71	0.56
C20:2	0.04	0.04
C20:3 n-6	0.02	0.01
C20:4 n-6	0.34	0.61
C20:5 n-3	0.07	0.25
C22:6 n-3	0.22	1.6
C23:0	0	0.03
C24:0	0.04	0.01
C24:1 n-9	0.3	0.08
∑Saturated fatty acids	29.67	7.68
∑Monounsaturated fatty acids	24.39	35.15
∑Polyunsaturated fatty acids	45.94	57.17
∑ n-6	44.79	54.65
∑ n-3	1.02	2.42
n-6/n-3 ratio	42	22.6

Vitamins and minerals per kilogram of the diet provided by the premix: vitamin A: 3,300,000 IU; vitamin D₃: 550,000 IU; vitamin E: 2,560 IU; vitamin K₃: 1,400 mg; vitamin B₁: 1,100 mg; vitamin B₂: 3,300 mg; vitamin B₆: 1,460 mg; vitamin B₁₂: 1,460 mcg; biotin: 51 mg; folic acid: 256 mg; niacin: 14,600 mg; calcium pantothenate: 11,000 mg; choline: 11,000 mg; Mn: 15,000 mg; Cu: 5,000 mg; I: 183 mg; Zn: 43,333 mg; Fe: 58,333 mg; Se: 100 mg; calcium carbonate: 1,000,000 mg.

modified by incorporating a compressor to cool the atmospheric air to -3 ± 2 °C before it entered the atomization chamber, which was maintained at 0 ± 2 °C. A mixture of fat (and fish oil for FOPF) and emulsifier (soy lecithin) was melted at 80 °C and injected into the chamber through an atomizer disc (CF-100/28060, APV) rotating at 25000 rpm. A cyclone separator was used to separate the crystallized fat particles from the air, and using a centrifugal fan (CPE-315/50/R, Novenco), the air was then expelled from the drying equipment to collect the powdered fat. The synthesized fat was stored in 5-kg bags and maintained at room temperature until use.

Sow feeding and management

Each sow received 3 kg d⁻¹ of the experimental diets (with the gestation diet as a base) until farrowing (d 0), after which the feed delivery was increased (with the lactation diet as a base) by 0.5 kg d⁻¹ until d 3. From d 4 on, the sows had ad libitum access to feed. During lactation, feed intake was determined and recorded weekly. Animals had free access to water throughout the gestation and lactation stages. The sows stayed in individual gestation crates from d 100 to 111 and were moved to the farrowing unit on d 112. The farrowing room temperature was maintained at 17 ± 2 °C, but supplemental heat was provided for the piglets by heat lamps and floor heating (30 ± 2 °C).

Milk yield and chemical analysis

Milk production was estimated on d 7, 14, 21 and 28 of lactation using the weigh-suckle-weigh method (Speer and Cox, 1984). Litters were separated from their dams for 1 h, and the piglets were then weighed to obtain a pre-suckling litter weight (sum of weights of the individual piglets). They were then returned to their mothers and allowed to suckle until synchronized litter suckling ended; the piglets were then immediately weighed to obtain a post-suckling weight. This procedure

was repeated for four consecutive h. Hourly milk yield was a measurement of litter weight gain due to milk intake and was determined from the difference between the pre- and post-suckling litter weights. The daily milk yield was estimated by multiplying the mean hourly milk yield by 24.

Colostrum and milk samples were collected by manually milking all functional teats on both sides of the udder. Colostrum (50 mL) was collected 30 min after the birth of the first piglet, and milk (50 mL) was collected on d 7, 14, 21 and 28 after farrowing. Piglets were prevented from suckling for 1 h before the samples were obtained. Sows were milked for 3 min after receiving an intramuscular injection of 2 mL of oxytocin (10 UI mL⁻¹), and the samples were immediately frozen at -20 °C and stored until the analysis of total solids (16.032), crude protein (16.036; Kjeldahl N × 6.38), lactose (31.036) and ash (16.035) following AOAC (1984) procedures; fat content was determined by the Gerber method (British Standards Institution 696, 1969).

Determination of fatty acid composition

To determine fat composition, each colostrum and milk sample was thawed and mixed in a water bath at 40 °C, and the milk fat was separated using the method proposed by Feng *et al.* (2004). Ten mL of raw milk were centrifuged at 6000 rpm for 20 min at 4 °C in an Eppendorf 5417R centrifuge, and the fat layer was transferred to a microtube and left at room temperature for 30 min before being centrifuged at 6000 RPM for 20 min at 20 °C. After the second centrifugation, the top layer was removed and stored at -20 °C until FA analysis. Lipids were extracted from the powdered fats and basal diets (both gestation and lactation) by adapting the method of Bligh and Dyer (1959). The FA composition of the powdered fats, basal diets, colostrum and milk were analyzed by gas chromatography. Lipids were methylated following the method of Christie (1982) with the modifications by Chouinard *et al.* (1999), and all chemicals and solvents used for this method were

of analytical grade. A GC-2010 system (Shimadzu Scientific Instruments AOC-20s, Columbia, MD, USA) equipped with a 100-m column (100-m × 0.32-mm × 0.20- μ m Rtx column) was used with the following conditions. The oven temperature was initially set at 110 °C for 4 min after injection and then increased to 240 °C (20 °C min⁻¹) with an equilibration time of 2 min. The inlet and flame-ionization detector temperatures were 260 °C; the split ratio was 15:1; and a 2- μ L injection volume was used. The flow of the hydrogen carrier gas to the detector was 25 mL min⁻¹; airflow was 400 mL min⁻¹; and the flow of the nitrogen makeup gas was 40 mL min⁻¹. Fatty acid peaks were identified using a fatty acid methyl ester standard (FAME; Supelco 37 Component FAME mix, Bellefonte, PA, USA).

Statistical analysis

Data were analyzed as a completely randomized design using the GLM procedure in Statistical Analysis Systems software (SAS Institute Inc., Cary, NC, USA). The model included dietary treatment (0, 20, 40 g powdered fat high in EPA and DHA). The Student-Newman-Keuls (SNK) method was used for mean comparisons, and the level of significance was $P \leq 0.05$.

Results

Lactation performance

Total milk yield (176 ± 8 kg) and daily feed intake (6 ± 0.2 kg d⁻¹) were not affected by the treatments. Piglet characteristics including mortality ($19 \pm 3.3\%$), birth weight (1 ± 0.03 kg), and weaning weight (8 ± 0.2 kg) were also not affected by the treatments ($P > 0.05$) (Table 3).

Colostrum and milk composition

The dietary treatments did not affect colostrum and milk composition ($P > 0.05$) (Table 4).

Table 3. Influence of dietary eicosapentaenoic (EPA) and docosahexaenoic (DHA) on sow feed intake, milk yield and piglet performance throughout the 28 days of lactation¹.

Parameter	CONT ²	FOPF20 ³	FOPF40 ⁴	SEM ⁵	P-value
Sow characteristics					
Average daily intake (kg d ⁻¹)	5.52	5.80	5.93	0.274	0.836
Total milk yield (kg)	166.74	178.58	182.70	12.966	0.882
Piglet characteristics					
Piglet mortality (%)	17.78	18.80	19.52	3.313	0.979
Birth weight (kg)	1.47	1.31	1.37	0.036	0.218
Weaning weight (kg)	8.88	8.46	7.79	0.193	0.637
Average dairy gain (kg d ⁻¹)	0.26	0.26	0.26	0.006	0.752

¹There were 5 sows per dietary treatment.²CONT=control diet without FOPF (powdered fat with EPA and DHA).³FOPF20=diet with 20 g of FOPF per kg basal diet.⁴FOPF40=diet with 40 g of FOPF per kg basal diet.⁵SEM=standard error of the mean.**Table 4.** Influence of dietary eicosapentaenoic (EPA) and docosahexaenoic (DHA) on the colostrum and milk composition of sows supplemented from day 100 of gestation until weaning (d 28)¹.

	Day of lactation	CONT ²	Sow diet FOPF20 ³	FOPF40 ⁴	SEM ⁵	P-value
Crude protein (%)						
Colostrum	0	14.96	15.65	14.62	0.596	0.779
Milk	7	5.11	4.81	4.93	0.159	0.755
	14	5.11	4.91	4.94	0.157	0.854
	21	5.05	5.67	4.47	0.273	0.306
	28	5.15	5.39	5.24	0.101	0.660
Lactose (%)						
Colostrum	0	ND ⁶	ND ⁶	ND ⁶		
Milk	7	4.70	4.70	4.67	0.118	0.992
	14	4.83	4.45	4.47	0.140	0.501
	21	4.98	4.65	5.65	0.144	0.120
	28	4.70	4.60	4.60	0.052	0.706
Fat (%)						
Colostrum	0	5.78	4.25	4.80	0.360	0.267
Milk	7	6.73	7.25	6.23	0.300	0.473
	14	7.47	8.80	8.30	0.351	0.335
	21	7.35	7.82	7.00	0.247	0.473
	28	7.27	7.20	6.20	0.741	0.801

¹There were 5 sows per dietary treatment.²CONT=control diet without FOPF (powdered fat with EPA and DHA).³FOPF20=diet with 20 g of FOPF per kg basal diet;⁴FOPF40=diet with 40 g of FOPF per kg basal diet;⁵SEM=standard error of the mean;⁶ND=not detected.

Milk fatty acid composition

As expected, the concentrations of EPA and DHA in the milk increased with the supplemented diets compared to the control diet ($P \leq 0.05$). As the concentrations of EPA and DHA increased, the concentration of saturated FA decreased while that of monounsaturated FA increased (Table 5).

Discussion

Modern pig production systems demand highly prolific sows with a high milk production, and adding fat with essential fatty acids as a dietary supplement supports these requirements (Rosero *et al.*, 2016). Moreover, lactating sow diets without essential fatty acid supplementation can result in a negative balance of linoleic and linolenic acids

(Rosero *et al.*, 2016). Thus, the objective of this study was to evaluate the effect of spray-dried dietary fats enriched with sources of EPA and DHA, an alternative fat supplementation approach during lactation, on the fatty acid composition of sow milk.

Fat supplementation at the level of 10% of the diet improves litter weight and piglet survival (Seerley *et al.*, 1981). In terms of lactation performance and consistent with the results of Mateo *et al.* (2009), milk components such as lactose, protein and fat were not affected by the dietary treatments in this study. However, Rosero *et al.* (2015) found greater lipid excretion in the milk of fat-supplemented sows relative to non-supplemented sows, whereas Eastwood *et al.* (2014) found no improvement in performance when adding EPA to a sow diet. However, the increases in the milk EPA and DHA

Table 5. Influence of dietary eicosapentaenoic (EPA) and docosahexaenoic (DHA) on milk fatty acid composition¹.

Fatty acid (g 100 g ⁻¹ of fatty acids)	CONT ²	FOPF20 ³	FOPF40 ⁴	SEM ⁵	P-value
C14:0	2.74	2.68	2.52	0.405	0.190
C16:0	25.27	24.69	23.65	0.573	3.872
C16:1	0.89	1.04	1.31	0.451	0.031
C18:0	2.34	1.60	1.12	0.372	0.256
C18:1 n-9	22.10	19.27	20.58	2.782	0.438
C18:2 n-6	38.11	35.13	24.10	2.963	0.112
C18:3 n-6	0.46	0.79	0.46	0.310	0.421
C18:3 n-3	0.76	1.50	1.18	0.298	<0.001
C20:2	0.91	1.15	1.73	0.708	0.152
C20:3 n-6	0.44	0.45	0.99	0.353	0.579
C20:5 n-3	0.98	1.33	2.41	0.652	<0.001
C20:6 n-6	0.83	0.64	0.78	0.302	0.044
C22:6 n-3	0.90	6.75	13.21	2.977	<0.001
C23:0	1.48	1.02	1.52	0.721	0.658
C24:0	0.48	0.43	0.64	0.335	0.059
C24:1 n-9	1.40	1.48	3.76	1.178	<0.001
ΣSaturated fatty acids	32.30	30.40	29.42	1.587	<0.001
ΣMonounsaturated fatty acids	24.38	21.79	25.66	2.737	0.299
ΣPolyunsaturated fatty acids	43.32	47.81	44.92	3.368	<0.001

¹There were 5 sows per dietary treatment.

²CONT=control diet without FOPF (powdered fat with EPA and DHA).

³FOPF20=diet with 20 g of FOPF per kg basal diet.

⁴FOPF40=diet with 40 g of FOPF per kg basal diet.

⁵SEM=standard error of the mean.

contents of sows fed the FOPF20 and FOPF40 treatments compared with the unsupplemented diet in this study are consistent with previous reports (Jensen *et al.*, 2000; Mateo *et al.*, 2009). The main milk FAs found in this study were C14:0, C16:0, C18:0, C18:1 n-9 and C18:2 n-6, which is consistent with Lauridsen and Danielsen (2004), and our dietary treatments also decreased the saturated FA contents while increasing polyunsaturated FA. Eastwood *et al.* (2014) observed increased EPA in sow milk following ALA supplementation. Yao *et al.* (2012) demonstrated that altering the PUFA ratio from n-6:n-3 to 9:1 in the diet of lactating sows influenced immunoglobulin and cytokine levels and tended to increase litter performance and piglet immune status.

The average daily gain by the piglets and the feed intake by the sows were not affected by the dietary treatments; this concurs with the findings reported by Smits *et al.* (2011), who supplemented sow diets with 2.9 g d⁻¹ of EPA + DHA from d 107 of gestation until weaning (d 19) and found no change in piglet BW gain. However, those authors observed interesting effects in the subsequent parity. Additionally, Leonard *et al.* (2010) found no changes in the average daily gain in piglets when sows were fed 39 g d⁻¹ of EPA and 24 g d⁻¹ of DHA from d 109 of gestation until weaning (d 26). However, a positive response to higher doses was found by Luo *et al.* (2013), who reported that supplementing sow diets with 7.41 g kg⁻¹ of EPA and 4.38 g kg⁻¹ of DHA 10 d before farrowing

until 28 d of lactation increased the BW gain in weanling pigs (d 35 to 70) compared to sow diets without n-3 supplementation. This study focused on milk fatty acid composition, so more research is needed on the effect of sow diet supplementation with EPA and DHA on piglet performance parameters in a larger number of animals.

The main conclusion of this study is that supplementing sow diets with EPA (2.7 g 100 g⁻¹) and DHA (1.8 g 100 g⁻¹) in the form of powdered fats could decrease milk saturated fatty acid contents and increase polyunsaturated fatty acid contents without affecting milk composition. Studies involving higher concentrations of EPA and DHA in sow diets and a larger number of both sows and piglets are required to detect changes in the performance of weanling pigs.

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Resumen

M. Grez, M. Gandarillas, F. González y E. Vargas-Bello-Pérez. 2016. Influencia de EPA y DHA dietarios sobre el perfil de ácidos grasos de leche de cerda. Cien. Inv. Agr. 43(3): 347-355. El objetivo de este estudio fue evaluar el efecto de fuentes dietarias enriquecidas con EPA y DHA (Ácidos eicosapentaenoico y docosahexaenoico) sobre el perfil de ácidos grasos de leche de cerda. Quince cerdas preñadas fueron repartidas en tres tratamientos desde el día 100 de gestación hasta el destete de lechones (día 28). Las cerdas del grupo control (CONT) fueron alimentadas con una dieta basal sin suplemento de EPA y DHA. Las cerdas alimentadas con dietas suplementadas recibieron una dieta basal con 20 g (FOPF20) o 40 g (FOPF40) de grasa en polvo enriquecida con EPA y DHA. La composición de leche y calostro y la producción de leche de las cerdas fueron similares entre tratamientos. Los principales ácidos grasos en

leche fueron: C14:0, C16:0, C18:0, C18:1 n-9 y C18:2 n-6. La suplementación con EPA y DHA (FOPF20 y FOPF40) disminuyó el contenido de ácidos grasos saturados e incrementó poliinsaturados. En resumen, los resultados indicaron que la suplementación de dietas de cerdas con EPA y DHA puede disminuir el contenido de ácidos grasos saturados e incrementar contenido de ácidos grasos poliinsaturados.

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