Long-term exposure to sensory feed additives during the gestational and postnatal periods affects sows' colostrum and milk sensory profiles, piglets' growth, and feed intake¹

David Val-Laillet,^{†,2} J. Stephen Elmore,[‡] David Baines,^{||} Peter Naylor,^{||} and Robert Naylor^{||}

[†]INRA, INSERM, Univ Rennes, Nutrition Metabolisms and Cancer, NuMeCan, 35590 Saint-Gilles, France;

[‡]Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading, RG6 6AP, UK;

^{||}Inroads International Ltd., Wem, Shropshire, UK

ABSTRACT: This study investigated the effect of feed supplementation in sows and/or their progeny with 2 sensory feed additives (FA1: limonene and cinnamaldehyde; FA2: menthol, carvone, and anethole) on sows' feed intake, body weight, fat deposition, and colostrum/milk composition, as well as piglets' feed intake growth and feed efficiency from birth to slaughter at postnatal day 160 (PND160). During the last third of gestation and the whole of lactation, sows were subjected to a control diet (C) or the same diet containing FA1 or FA2 at 0.1% of complete feed content. Colostrum/milk samples were taken at days 1, 14, and 28 of lactation for gas chromatography-mass spectrometry analyses. After weaning, the progeny was subjected to a control diet (C) or experimental diets with a sweetener (0.015%) but no other additive (S), or to diets with a sweetener and the additive FA1 (FA1S) or FA2 (FA2S). There was no effect of dietary treatment on sows' feed intake, body weight, or adiposity ($P > 0.15$ for all), but the sensory characteristics of their colostrum/milk were modified by the diet and diet*time interaction. Limonene concentrations were higher in FA1 samples from PND1

to PND28, whereas carvone and anethole concentrations were higher in FA2 samples from PND1 to PND28. The concentration of these 3 compounds increased with time in the respective groups where they were mostly detected. Menthol concentrations were higher in FA2 samples at PND14 and PND28, but there was no time effect. Overall, cinnamaldehyde was always below the detection range. Piglets born from FA1 and FA2 sows had higher body weight ($P = 0.034$ at PND160), average daily gain (ADG; $P = 0.036$ for PND0-160), and average daily feed intake (ADFI; $P = 0.006$ for PND28-160) than piglets born from C sows. Overall, piglets that were never exposed to FA or only after weaning had lower ADG ($P = 0.030$ for PND0-160) and ADFI ($P = 0.016$ for PND28-160) than piglets that were exposed to FA only via the maternal diet, the condition combining both pre- and post-natal exposure being intermediary. In conclusion, FA1 and FA2 provided to gestating and lactating sows increased the progeny's feed intake and growth, suggesting nutritional programming and/or sensory conditioning during the perinatal period. Addition of FA only in the progeny's diet was not beneficial.

Key words: colostrum and milk sensory properties, feed additives, feed transition, nutritional programming, performance, sensory conditioning

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2018.96:3233–3248

doi: 10.1093/jas/sky171

¹The experimental work was funded by Inroads International Ltd. (Wem, UK). Inroads selected and provided the sensory feed additives.

²Corresponding author: david.val-laillet@inra.fr

Received April 25, 2018.

Accepted June 25, 2018.

INTRODUCTION

In pig production, sensory feed additives are commonly used in an attempt to improve feed palatability and zootechnical performance (Windisch et al., 2008; Franz et al., 2010; Jacela et al., 2010),

but discrepancies between studies are frequent (Jugl-Chizzola et al., 2006; Seabolt et al., 2010; Clouard et al., 2012; Michiels et al., 2012; Clouard and Val-Laillet, 2014; Val-Laillet et al., 2016). To improve the beneficial outcomes of feed additive exposure in piglets, one strategy would be to establish a sensory continuum by extending the exposure period to the perinatal environment and maternal diet during gestation and lactation, as suggested by previous authors through the concept of “fetal or sensory learning” (Mennella et al., 2001; Wells and Hepper, 2006; Oostindjer et al., 2010; Figueroa et al., 2013).

The aim of our study was to validate and compare the use of 2 different feed additives (FA) combining different phytochemical molecules, known to have behavioral and neurophysiological effects, to compare the impact of perinatal and/or postweaning exposure with the feed additives (compared one with another and with a control feed). In mammals, flavors from the maternal diet can reach the fetus before birth through the amniotic fluid (Mennella, 1995; Mennella et al., 1995; El-Haddad et al., 2005). To confirm that the compounds of interest in the feed additives can also reach the neonate through the maternal milk (Hausner et al., 2008), solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) analyses were performed on colostrum and milk samples from sows fed different diets with or without feed additives. Our hypotheses, in line with the aforementioned “sensory learning” concept, were that the active compounds of the feed additives would reach the neonate through the colostrum and milk, and that perinatal exposure might condition the piglets to develop an increased acceptance for feeds containing the same additives and consequently increase both feed consumption and growth. Moreover, we hypothesized that a continuum in the sensory exposure would potentiate the beneficial effects of the feed additives in terms of animal performance.

MATERIALS AND METHODS

The experiment presented in this paper was conducted in accordance with the current ethical standards of the European Community (Directive 2010/63/EU), Agreement No. A35-622, and Authorization No. 35-88. The whole protocol was submitted to the French Ministry of Research in December 2015. The Regional Ethics Committee in Animal Experiment of Brittany (France) has validated the entire procedure described in this paper and specifically approved this study (No. 2015121314449323).

Animals and Housing

A total of 40 Large White/Landrace sows (35 multiparous and 5 primiparous) and their piglets (Large White/Landrace × Pietrain), distributed in 3 consecutive batches ($N = 14$ in January 2016, $N = 13$ in February 2016, and $N = 13$ in March 2016) with homogenous body weight and parity among treatments and batches, were used for this study and reared at the experimental center of INRA (Saint-Gilles, France). Sows were housed in individual crates. Parturitions were not induced. Experimental piglets were suckled by their own mother and weaned at postnatal day 28 (PND28). After weaning, 160 piglets were included in the protocol, removed from the maternal crates, and housed in groups of 6 to 8 individuals of the same perinatal exposure (Figure 1). The smallest piglets were excluded from the experiment during this selection process. Piglets from sows that had received the same diets were mixed together, but piglets from sows that had received different diets were housed in different groups. All the animals were transferred to another building in groups of the same size and treatment at PND70 and until slaughter (Figure 1B). All the animals were slaughtered at PND160 according to the usual procedure in commercial pig husbandry.

Experimental Diets and Feed Additive Supplementation

Six maternal feeds were used for this study, all in accordance with the nutrient and energy needs of pregnant and lactating sows. They included a standard gestation feed and a standard lactation feed (Table 1), named the control diets ($C = 20$ sows), as well as the same standard feeds supplemented with either of 2 feed additives tested (named FA1 and FA2 diets, $N = 10$ sows per treatment). Groups were homogenized in terms of parity and body weight. Inroads International Ltd. (Wem, Shropshire, UK) provided the feed additives: FA1 contained limonene and cinnamaldehyde, whereas FA2 contained menthol, carvone, and anethole. Since both additives are part of a secret know-how, the exact composition cannot be divulged. These compounds were chosen on the basis of their biological effects on behavioral and neurophysiological functions (see Discussion). Sows in gestation were fed 2.5 to 3 kg of gestation feed per day. Sows in lactation were fed 3 kg (first day of lactation) to 9–11 kg (end of lactation) of lactation feed per day, with a progressive

Figure 1. Schematic representation of the experimental paradigm showing the (A) exposure periods to the different experimental feeds in sows and piglets (PND: postnatal day). Apart from the feed additives tested (FA1 and FA2), a sweetener was added in all piglets' diets excepting for a control group (C). The S diet corresponded to a control diet without feed additive but with the sweetener. (B) Distribution of the animals per batch (B1, B2, B3), experimental treatment and housing pen.

increase of the daily ration individually adapted to prevent excessive refusals. All the animals had free access to water during the whole experiment.

The feed additives were provided in the gestation and lactation feeds at 0.1% of complete feed content from the last third of gestation to the end of

Table 1. Composition of the animal feeds used in the study

	Gestation (GD)	Lactation (LD)	Pre-starter (PS)	Starter (ST)	Growth (GR)
Composition (%)					
Wheat	22.0	25.6		23.2	26.2
Corn	10.0	12.0		25.0	16.0
Barley	33.9	25.68	45.31	24.05	25.5
Wheat bran	15.0	10.0			5.0
Soybean meal	9.0	18.0	17.5	22.57	19.0
Soybean proteins			2.5		
Vegetal oil	2.0	2.0	2.3	0.45	2.0
Molasses		3.0			3.0
Beet pulp	5.0				
Mild lactoserum			20.0		
Fattened milk			8.0		
Carbonate calcium	1.74	1.2	1.41	1.13	1.29
Monocalcic phosphate			0.8	0.97	
Bicalcic phosphate	0.3	1.02			0.5
Salt	0.45	0.45		0.4	0.45
Vitamin complement	0.5	0.5	0.5	0.5	0.5
Lysine		+	++	++	+
Methionine		+	++	++	+
Threonine		+	++	++	+
Tryptophan			+	+	
Valine			+	+	
Acidifying agent	+	+	+	+	+
Phytase	+	+	+	+	+
Chemical composition, %					
Dry matter	87.58	86.94	89.92	86.99	
Mineral content	5.77	6.06	7.02	5.44	5.6
Crude Protein	13.32	16.45	18.99	18.0	16.5
Fat content	4.28	4.21	6.74	2.79	4.2
Crude fiber	5.14	4.09	2.97	3.62	3.8
Starch	40.5	38.9	24.5	43.5	40.9
Nutritional values					
Net energy, MJ/kg	9.25	9.41	10.63	9.67	9.67

The gestation and lactation feeds were provided to the gestating or lactating sows. The prestarter, starter, and growth feeds were provided to the piglets. ++ and + symbols indicate very small and infinitesimal quantities of compounds added in the diet.

lactation (28 d after farrowing), because it is commonly accepted that mammal fetuses are able to perceive flavors during the last third of gestation (Smotherman et al., 1991; Lecanuet and Schaal, 1996; Schaal et al., 2000; Oostindjer et al., 2010; Nicklaus, 2016a). During 10 d after weaning, the piglets received a prestarter feed and then a starter feed until PND70. A 3-d transition period was organized to familiarize piglets to the starter feed at the end of the prestarter period. After PND70, the animals received a growth diet until slaughter at PND160 (Table 1). Dietary treatments per group are summarized in Figure 1. Piglets born from control sows received control (C), sweetened control (S), FA1S, or FA2S diet ($N = 20$ per group). Piglets born from FA1 sows received either FA1S or S diet ($N = 20$ per group). Piglets born from FA2 sows received either FA2S or S diet ($N = 20$ per group).

The control diets (C) corresponded to the standard feeds described in Table 1 without any additive. FA1 and FA2 maternal diets corresponded to the gestation and lactation control feeds supplemented with 0.1% of feed additive 1 or 2. S piglets' diet corresponded to the prestarter, starter, and growth feeds supplemented with 0.015% of sweetener (High Intensity Sweetener, sodium-saccharin-based sweetener commercialized by Inroads International, Wem, Shropshire, UK). FA1S and FA2S piglets' diets corresponded to the prestarter, starter, and growth feeds supplemented with 0.015% of sweetener and 0.1% of feed additive 1 or 2. Except for 1 control group, the sweetener was added in all piglets' diets because it was expected to potentiate the effect of the other sensory feed additives. The control group without sweetener, compared with the control group with sweetener

alone, was aimed at discussing the specific impact of the sweetener, independently of the other additives. The experimental diets were produced at the feed mill of the INRA Saint-Gilles experimental facilities.

Colostrum and Milk Sampling and Analysis

Colostrum or milk samples (at least 60 mL) were collected from all sows in the morning of PND1 (PND0 corresponding to farrowing), PND14, and PND28, after an intramuscular injection of oxytocin (1 to 2 mL per sow). All samples were filtered and stored in 250-mL polyethylene sampling containers (Dutscher Brumath, France). The containers were stored at -20°C at the INRA of Saint-Gilles (France) before being shipped to the University of Reading (UK) for GC-MS analyses. DL-menthol (95+% purity), (R)-(+)-limonene (99+%), (*E*)-cinnamaldehyde (98+%), (S)-(+)-carvone (96+%), (*E*)-anethole (99%), triacetin (99+%), and 2,4,6-trimethylpyridine (99%) were purchased from Sigma-Aldrich.

Sample Preparation

Appropriate mixed standard solutions (from 0.1 to 20 mg/L) of menthol, limonene, cinnamaldehyde, carvone, and anethole were prepared in triacetin. A 20-mg/L solution of 2,4,6-trimethylpyridine (TMP) was also prepared in triacetin. These solutions were mixed in a 1:1 ratio to give the following set of calibration standards (each containing menthol, limonene, cinnamaldehyde, carvone, and anethole, plus 10 mg/L TMP): 0.05, 0.1, 0.25, 1, 2.5, and 10 mg/L. In addition, a 10-mg/L solution of TMP was prepared in triacetin to be added to the tested colostrum and milk samples as an internal standard.

Colostrum and milk samples were removed from the freezer and allowed to reach room temperature. The plastic bottles in which the colostrum and milk were stored were then shaken manually for 10 s to mix the contents. Samples were prepared by adding 5 mL of colostrum or milk along with 50 μL of 10-mg/L TMP internal standard solution to a 20-mL headspace vial with metal screw-cap and septum. To prepare a calibration curve for quantification of the compounds of interest, 50 μL of each standard solution was dissolved in 5 mL of a control sample from Batch 1 Day 1 in which none of the compounds of interest had been detected. All samples were analyzed in random order in one sequence and a calibration set was run both before and after the samples.

Three or 4 samples were analyzed from each diet (Control, FA1, FA2) at 3 collection points (days 1, 14, and 28) from each of 3 batches (1, 2, and 3), i.e., a total of 79 samples.

Solid-Phase Microextraction

Automated SPME was performed on an Agilent 5975 GC-MS system with GC Sampler 120. Samples were placed in the refrigerated tray of the autosampler (4°C). When the machine was ready, the sample was transferred to an incubated agitator at 60°C for 10 min, the agitator rotating at 500 rpm with an agitation cycle of 5 s on and 2 s off. After incubation, the headspace above the sample was extracted for 60 min at 60°C using an SPME syringe containing a 1-cm Stable-flex fiber coated with 50/30 μm divinylbenzene (DVB)/Carboxen on polydimethylsiloxane (PDMS) (Supelco Bellefonte PA). For both extraction and desorption, injection needle penetration was 32 mm and fiber exposure distance was 22 mm.

Gas Chromatography-Mass Spectrometry

After extraction, the fiber was desorbed in the injection port of the gas chromatograph at 250°C for 20 min onto a 30-m $\times$ 0.25-mm Stabilwax DA GC column (film thickness 0.50 μm ; Restek, High Wycombe, UK). The injection was splitless, the splitter opening after 0.75 min. Data acquisition commenced as soon as the desorption step began. The temperature of the GC oven was held at 40°C for 5 min before being raised at 4°C per minute to 260°C where the temperature was held for a further 5 min. Helium at a constant flow rate of 0.9 mL/min was used as the carrier gas.

The mass spectrometer operated in electron impact mode with an electron energy of 70 eV acquiring data in both scan and selected ion monitoring (SIM) modes simultaneously. In scan mode, the mass spectrometer scanned from m/z 38 to m/z 160. SIM mode was used for quantification. Four characteristic ion fragments were chosen for each compound of interest and the internal standard: 1 quantifying ion (shown in bold) and 3 qualifiers. Each ion was monitored for 50 ms. All 6 compounds measured were well separated by GC, so 6 separate SIM windows could be prepared, 1 for each compound. The ions measured in Window 1 (start time 0 min) were 68, 67, 121, and **136** (limonene); Window 2 (20 min) were **121**, 120, 126, and 79 (TMP); Window 3 (30 min) were

138, 81, 71, and 95 (menthol); Window 4 (33 min) were **82**, 150, 54, and 108 (carvone); Window 5 (35.5 min) were **148**, 147, 117, and 133 (anethole); and Window 6 (40 min) were 131, **132**, 103, and 104 (cinnamaldehyde). Quantifying peak areas of the compounds of interest were measured relative to the peak area of the quantifying ion of TMP in both the samples and standards, to calculate the concentrations of the compounds of interest in samples. Because some samples were used for method development, they were not available for the analysis. As a consequence, we analyzed 79 samples in total (colostrum samples: $N = 9$ C, $N = 10$ FA1, $N = 10$ FA2; milk samples at D14 and D28: $N = 9$ C, $N = 8$ FA1, $N = 8$ FA2).

Zootechnical Parameters

Sows were weighed at the onset of dietary treatment, at the beginning, and at the end of lactation. Sows' back fat thickness was measured by ultrasonography at the P2 site (Val-Laillet et al., 2010) a few days before farrowing and at the end of lactation.

Piglets were weighed immediately at birth and then weekly until weaning and every 2 wk until slaughter. The average daily weight gain (ADG g/d) was calculated for the suckling period (PND1 to PND28), for the postweaning period (PND28 to PND70), for the "growth" period (PND70 to PND160), from PND28 to PND160, and the whole experimental period. The average daily feed intake (ADFI g/d) and average feed efficiency (G:F) were calculated for the postweaning period (PND28 to PND70), for the "growth" period (PND70 to PND160), and from PND28 to PND160. ADFI and G:F data were averaged per group, since the feed consumption could not be measured individually.

Statistical Analyses

All the statistical analyses were performed with StatView (SAS Institute, Inc.). To compare the volatile profiles of the colostrum/milk samples, 2-way analysis of variance (ANOVA) with repeated measures was performed with maternal diet and batch as main factors. A first ANOVA was performed including all samples (colostrum at D1, milk at D14 and D28), and a second ANOVA was performed on milk samples only. Sows' feed intake, body weight, and fat deposition were analyzed with a 2-way ANOVA with repeated measures, with maternal diet and batch as main factors, and parity as a cofactor. Piglets' body weight, ADG, ADFI, and feed efficiency (growth:feed ratio, G:F) were

analyzed with different complementary statistical procedures depending on the question/objective:

- 1) Body weight was analyzed with a 2-way ANOVA with repeated measures on the whole dataset (from birth to PND160) with treatment (i.e., the association between a maternal diet and a progeny's diet: C/C, C/S, C/FA1S, C/FA2S, FA1/S, FA1/FA1S, FA2/S, FA2/FA2S) and batch as main factors, and sow/litter as a cofactor. The same strategy was then applied on the measures performed only before (from birth to PND28) and only after weaning (from PND28 to PND160).
- 2) Body weight was analyzed with 2 three-way ANOVA with repeated measures (before weaning and after weaning) on 2 different data subsets, i.e., FA1 or C sows $\times$ FA1S or S piglets, as well as FA2 or C sows $\times$ FA2S or S piglets (3 factors and 4 groups in each 3-way ANOVA), with maternal diet, progeny's diet and batch as main factors, and sow/litter as a cofactor. These analyses allowed evaluating the interaction between maternal and progeny's diets, contrary to the analyses performed on the whole dataset (including all groups and treatments) for which it was not possible to assess the interaction effect.
- 3) Body weight at PND1 (birth), PND28 (weaning), PND70 (transfer to another building), and PND160 (slaughter), as well as ADG, ADFI, and G:F were analyzed for each period of interest with a 2-way ANOVA on the whole dataset, with maternal diet and batch as main factors (3 groups compared: C, FA1, and FA2).
- 4) Body weight at PND1, PND28, PND70, and PND160, as well as ADG, ADFI, and G:F was analyzed for each period of interest with a 2-way ANOVA on the whole dataset, with progeny's diet and batch as main factors (4 groups compared: C, S, FA1S, and FA2S).
- 5) Body weight at PND1, PND28, PND70, and PND160, as well as ADG, ADFI, and G:F was analyzed for each period of interest with a 3-way ANOVA on 2 different data subsets, i.e., FA1 or C sows $\times$ FA1S or S piglets, as well as FA2 or C sows $\times$ FA2S or S piglets (3 factors and 4 groups in each 3-way ANOVA), with maternal diet, progeny's diet, and batch as main factors, and sow/litter as a cofactor. These analyses allowed evaluating the interaction between maternal and progeny's diets, contrary to the analyses performed on the whole dataset (including all groups and treatments).

- 6) Body weight at PND1, PND28, PND70, and PND160, as well as ADG, ADFI, and G:F was analyzed for each period of interest with a 2-way ANOVA on the whole dataset, with treatment and batch as main factors (4 groups: “No FA,” “Addition,” “Removal,” and “Continuity”), i.e., groups that never encountered FA (“No FA”: C/C and C/S), groups with a FA only added in the progeny’s diet after weaning (“Addition”: C/FA1S and C/FA2S), groups with a FA only added in the maternal diet (“Removal”: FA1/S and FA2S), and groups with a FA continuity between maternal and progeny’s diets (“Continuity”: FA1/FA1S and FA2/FA2S).

Data were expressed as mean $\pm$ standard error (SE), with a significance threshold set at $P = 0.05$ and a trend considered at $P < 0.15$.

RESULTS

Colostrum and Milk Analyses

The concentrations of the limonene, anethole, carvone, and menthol in the 79 samples are shown

in Table 2, Figure 2, Appendix 1. Four of the 5 compounds (FA1: limonene; FA2: menthol, carvone, and anethole) were present at relatively high concentrations in the colostrum/milk of sows receiving the corresponding treatment, although these compounds were often present at lower levels in the colostrum/milk from the 2 other diets. As limonene is ubiquitous, it sometimes gave high values in samples where it was not expected. Cinnamaldehyde could not be measured at a quantifiable level in any of the samples. Because the calibration standards were from 0.05 ppm upwards, and this concentration roughly corresponded to the detection limit for these 4 compounds, compounds present between 0.02 and 0.05 ppm were labeled trace, whereas those with values less than 0.02 ppm were labeled absent. Anethole and carvone were present in at least trace levels in all FA2 samples, whereas limonene was present in all FA1 samples but only a proportion of control and FA2 samples.

For the analysis including colostrum and milk samples, there was a significant interaction between diet and time of collection for limonene ($P = 0.0013$), carvone ($P = 0.0395$), and anethole ($P = 0.0246$), with all 3 compounds increasing with time (among D1, D14, and D28 of lactation) in the colostrum/milk of sows that, respectively, received

Table 2. Concentrations (ppm) of 4 target compounds in sows’ colostrum/milk (raw data are provided in Appendix 1)

	Control			FA1			FA2		
	PND1	PND14	PND28	PND1	PND14	PND28	PND1	PND14	PND28
Batch 1									
Limonene	1.85 $\pm$ 0.67	0.34 $\pm$ 0.18	1.34 $\pm$ 1.34	2.61 $\pm$ 1.14	6.31 $\pm$ 3.26	10.74 $\pm$ 4.21	3.54 $\pm$ 1.55	–	4.49 $\pm$ 3.66
Menthol	–	–	–	–	–	–	–	–	0.16 $\pm$ 0.16
Carvone	0.09 $\pm$ 0.04	trace	0.08 $\pm$ 0.05	0.06 $\pm$ 0.03	trace	trace	0.41 $\pm$ 0.08	0.47 $\pm$ 0.24	2.08 $\pm$ 1.45
Anethole	0.06 $\pm$ 0.01	–	trace	trace	–	trace	0.11 $\pm$ 0.05	0.13 $\pm$ 0.05	0.33 $\pm$ 0.01
Batch 2									
Limonene	2.54 $\pm$ 1.90	–	1.51 $\pm$ 1.51	6.46 $\pm$ 1.46	6.76 $\pm$ 2.60	12.66 $\pm$ 0.10	0.91 $\pm$ 0.79	0.33 $\pm$ 0.23	0.09 $\pm$ 0.07
Menthol	–	–	–	–	–	–	–	0.43 $\pm$ 0.18	–
Carvone	0.27 $\pm$ 0.18	trace	trace	–	trace	–	0.23 $\pm$ 0.06	0.29 $\pm$ 0.08	0.37 $\pm$ 0.01
Anethole	0.07 $\pm$ 0.03	trace	–	0.08 $\pm$ 0.06	trace	trace	0.14 $\pm$ 0.01	0.16 $\pm$ 0.02	0.36 $\pm$ 0.01
Batch 3									
Limonene	1.01 $\pm$ 1.00	0.60 $\pm$ 0.45	0.32 $\pm$ 0.32	2.87 $\pm$ 0.80	8.68 $\pm$ 0.95	6.36 $\pm$ 1.92	1.04 $\pm$ 0.81	0.42 $\pm$ 0.28	–
Menthol	–	–	–	–	–	–	–	0.17 $\pm$ 0.19	0.32 $\pm$ 0.07
Carvone	trace	trace	–	0.07 $\pm$ 0.03	trace	–	0.27 $\pm$ 0.09	1.39 $\pm$ 0.356	0.84 $\pm$ 0.08
Anethole	trace	trace	–	trace	–	–	0.16 $\pm$ 0.11	0.21 $\pm$ 0.07	0.11 $\pm$ 0.06
Total									
Limonene	1.80 $\pm$ 0.69	0.31 $\pm$ 0.16	1.06 $\pm$ 0.62	3.84 $\pm$ 0.83	7.31 $\pm$ 1.46	9.58 $\pm$ 1.97	1.75 $\pm$ 0.66	0.29 $\pm$ 0.14	1.15 $\pm$ 1.12
Menthol	–	–	–	–	–	–	–	0.19 $\pm$ 0.11	0.20 $\pm$ 0.06
Carvone	0.13 $\pm$ 0.07	trace	trace	trace	trace	0.06 $\pm$ 0.02	0.30 $\pm$ 0.05	0.89 $\pm$ 0.25	1.04 $\pm$ 0.41
Anethole	trace	trace	–	trace	trace	trace	0.14 $\pm$ 0.04	0.18 $\pm$ 0.04	0.23 $\pm$ 0.05

Samples with values lower than 0.05 ppm were labeled trace, whereas values lower than 0.02 ppm were labeled absent. Limonene and cinnamaldehyde were added to the FA1 diet, whereas menthol, carvone, and anethole were added to the FA2 diet. Cinnamaldehyde was always below the detection range. Data are expressed as mean $\pm$ SE.

Figure 2. Concentrations of 4 target compounds in the colostrum/milk of sows fed a control ($N = 9$), FA1 ($N = 10$), or FA2 ($N = 10$) diet. Limonene (A) and cinnamaldehyde were added to the FA1 diet, whereas menthol (B), carvone (C), and anethole (D) were added to the FA2 diet. Cinnamaldehyde was always below the detection range (0.05 ppm). Analyses were performed using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Data are expressed as mean $\pm$ SE.

these compounds in their diet. A batch*diet interaction was only detected for carvone ($P = 0.0014$). Limonene ($P < 0.0001$), carvone ($P = 0.0001$), and anethole ($P = 0.0019$) were significantly affected by the maternal diets; menthol did not show a significant effect (only a trend $P = 0.058$), probably as a result of it being absent from a large number of samples. Time of collection effect was only significant for limonene ($P = 0.0332$), whereas only carvone showed a batch effect ($P = 0.012$).

In the analysis including only milk samples, there was a significant interaction between diet and time of collection for limonene ($P = 0.049$) and anethole ($P = 0.019$), but not for menthol ($P = 0.872$) or carvone ($P = 0.833$). A batch*diet interaction was only detected for carvone ($P = 0.006$). Limonene ($P < 0.0001$), carvone ($P = 0.0009$), and anethole ($P = 0.0002$) were significantly affected by the maternal diets; menthol did not show a significant effect (only a trend $P = 0.058$). Time of collection effect was only significant for limonene ($P = 0.038$), whereas only carvone showed a batch effect ($P = 0.0178$).

Limonene, carvone, and anethole were all significantly higher in the milk of sows receiving the

diets to which they were added (FA1 for limonene, FA2 for carvone, and anethole).

Zootecnical Parameters

There was no difference between sows' groups in terms of parity (4 ± 0.3 ; $P = 0.915$) and body weight (at the onset of dietary treatment: 255 ± 5 kg, $P = 0.776$; early lactation: 279 ± 5 kg, $P = 0.752$; and end of lactation: 250 ± 5 kg, $P = 0.546$). There was an interaction between parity and batch on body weight ($P = 0.035$), but no significant effect of batch ($P = 0.099$) and no interaction with dietary treatment. There was no effect of group, batch, or parity, and no interaction between factors on litter size at farrowing (16.0 ± 0.5 piglets, $P > 0.1$), but there was an interaction between group and batch for the piglets' survival at weaning (12.2 ± 0.5 piglets, $P = 0.038$), with no remaining difference after pairwise comparisons. Over the 638 piglets that were born from the 40 sows of this study, there were 26 stillbirths and 64 additional piglets that died the day of farrowing. There was no difference between groups in terms of sows' feed consumption during lactation (216 ± 4 kg, $P = 0.447$). Sows'

back fat deposition did not differ between groups before farrowing (16 ± 1 mm, $P = 0.843$) and at the end of lactation (13 ± 1 mm, $P = 0.680$). There was a significant decrease of fat deposition for all groups between the end of gestation and the end of lactation ($P < 0.0001$), as well as a batch effect ($P < 0.0001$), but no group effect ($P = 0.610$) and no interaction between factors.

The 2-way ANOVAs with repeated measures performed on the whole dataset revealed an overall significant increase of the progeny's body weight along time ($P < 0.0001$). After weaning, there was an interaction between time and treatment ($P < 0.0004$), and between time and batch ($P < 0.0001$), as well as a significant time effect after weaning ($P < 0.0001$), but no significant effect before weaning ($P > 0.15$ for all). The batch effect was significant after weaning ($P < 0.0001$), but not before ($P = 0.464$). Overall, body weight evolution of piglets was significantly

influenced by the interaction between maternal diet and time ($P = 0.0001$) and by the maternal diet in itself ($P = 0.035$), but not by the piglets' diet ($P = 0.563$), nor by the mother identity ($P = 0.505$). The 3-way ANOVAs with repeated measures performed on the 2 data subsets (FA1 and FA2, respectively) revealed no interaction between the maternal and progeny's diets, from birth to PND160 (FA1: $P = 0.178$, FA2: $P = 0.344$), and either before weaning (FA1: $P = 0.730$; FA2: $P = 0.345$) or after weaning (FA1: $P = 0.172$; FA2: $P = 0.797$).

Piglets' birth body weight significantly differed between groups of maternal diet (C: 1.48 ± 0.02 kg; FA1: 1.62 ± 0.03 kg; FA2: 1.56 ± 0.03 kg; $P = 0.002$), with a significant difference after pairwise comparisons between C and FA1 ($P = 0.005$), a trend between C and FA2 ($P = 0.059$), and no difference between FA1 and FA2 ($P = 0.186$) (Figure 3A). These differences disappeared at weaning (9.26 ± 0.09 kg; $P = 0.623$). The ratio between piglets' birth weight

Figure 3. Impact of the maternal diet on the progeny's body weight (A), average daily gain (B), and average daily feed consumption (C) at different ages and periods from birth to slaughter (PND: postnatal day). C sows were subjected to a control diet during the whole trial. FA1 and FA2 sows were subjected to the control diet with a feed additive (FA1 or FA2) during the last third of gestation and whole lactation period. Data are expressed as mean $\pm$ SE. Two different letters indicate a significant difference at $P < 0.05$.

and weight at weaning significantly differed between groups (C: 6.37 ± 0.09 kg; FA1: 5.96 ± 0.13 kg; FA2: 6.19 ± 0.14 kg; $P = 0.027$), with a lower ratio in FA1 compared with C ($P = 0.008$), FA2 being intermediary. There was no difference between groups in terms of body weight at PND70, but a significant effect of maternal diet was observed at PND160 with piglets born from FA1 (118.5 ± 1.6 kg; $P = 0.034$) and FA2 (118.6 ± 1.7 kg; $P = 0.034$) sows being heavier than piglets born from C sows (113.7 ± 1.3 kg) (Figure 3A). The 3-way ANOVAs performed on the 2 data subsets (FA1 and FA2, respectively) at critical stages revealed a significant effect of FA1 maternal diet at birth ($P = 0.0013$) as well as a trend at slaughter (PND160, $P = 0.080$); it also revealed a significant effect of FA2 maternal diet at birth ($P = 0.016$), PND70 ($P = 0.020$), and at slaughter (PND160, $P = 0.022$), but only a trend at weaning (PND28, $P = 0.088$).

Overall at the group level, there was no significant effect of maternal diet, piglets' diet, and crossed dietary treatments on piglets' feed consumption for the different periods or the whole duration of the experiment ($P > 0.15$ for all comparisons). There was no effect either on the feed intake during the first 2 wk of access to solid feed, or during the 3 d of transition between the prestarter and starter diet ($P > 0.015$). However, feed consumption was significantly different between batches ($P < 0.001$ for all comparisons), with decreased overall group consumption along repetitions (Batch 1 > Batch 2 > Batch 3).

The comparison between both control groups (C/C vs. C/S) revealed no difference in terms of piglets' growth ($P = 0.777$ at PND160). Merging data from both feed additives and investigating the impact of no FA/addition/removal/continuity in terms of feed additive exposure between the pre- and post-weaning periods, significant differences appeared between situations for body weight at PND160 ($P = 0.026$), with piglets subjected to FA only before weaning having a higher body weight than piglets exposed to the FA only after weaning (PND160: $P = 0.054$) or not exposed to FA at all (PND160: $P = 0.003$). There was also a trend for piglets exposed to FA before and after weaning to have a higher body weight than piglets that were not exposed to FA at all ($P = 0.067$). These differences already existed for the birth body weight ($P = 0.003$), i.e., before the onset of postweaning dietary treatment (Figure 3A).

Overall, there was an effect of the maternal diet and transition condition between the pre- and post-weaning periods on ADG and ADFI, but not on G:F, whereas no effect of the piglets' diet was

observed on these variables (Table 3). The cofactor "mother identity" had no significant effect on these variables ($P > 0.15$ for all). A significant effect of maternal diet for both ADG and ADFI was observed for PND70-160, PND28-160, and PND0-160 periods, with piglets born from C sows having lower ADG and ADFI in comparison to piglets born from FA1 and FA2 sows (Figure 3B and C). ANOVAs performed on the FA1 and FA2 data subsets revealed no interaction between the maternal diet and progeny's diet ($P > 0.15$ for all). A significant effect of transition condition between the pre- and post-weaning periods for both ADG and ADFI was observed for PND70-160, PND28-160, and PND0-160 periods. Pigs exposed to FA before weaning, with or without postweaning exposure, had higher body weight at birth and PND160 than pigs with no exposure at all (Figure 4A). Pigs exposed to FA before weaning only had higher ADG than piglets exposed to no FA at all for PND70-160, PND28-160, and PND0-160 (Figure 4B). Piglets exposed to FA before and after weaning had higher ADG than piglets exposed to no FA at all for PND70-160. Piglets exposed to FA before weaning only had higher ADFI than piglets exposed to FA after weaning only, or no FA at all (for PND70-160 and PND28-160) (Figure 4C). Moreover, piglets exposed to FA before and after weaning had higher ADFI than piglets exposed to no FA at all for PND28-160.

DISCUSSION

According to our data, feed supplementation with FA1 or FA2 in the sows' diet during the last third of gestation and the whole lactation period improved the daily feed intake and growth of the progeny from weaning to slaughter at PND160. The sensory properties of the sows' colostrum and milk were modified by their diet, since chemical compounds of the FA were transferred into the colostrum and milk; the nature and the amount of these compounds depended on the FA formulation but also on the lactation stage and type of sample (colostrum or milk). There was no significant effect of the progeny's diet on their feed intake and growth, and no interaction between the maternal and progeny's diets contrary to our initial hypothesis speculating a positive impact of a sensory continuum between the pre- and post-weaning periods in the progeny. As a consequence, the higher growth and feed intake of piglets/pigs exposed to the FA during the gestation, lactation, and postweaning periods is probably due to the preweaning than the postweaning exposure to FA. Moreover, the group

Table 3. Pigs' average daily gain (ADG), average daily feed intake (ADFI), and growth:feed ratio (G:F) depending on the treatment (sow's diet/progeny's diet, e.g., C/C C/S, etc.) and time period (PND postnatal day)

	ADG					ADFI			G:F		
	PND0-28	PND28-70	PND70-160	PND28-160	PND0-160	PND28-70	PND70-160	PND28-160	PND28-70	PND70-160	PND28-160
C/C	294 ± 7	505 ± 22	905 ± 18	781 ± 14	696 ± 12	806 ± 16	1578 ± 30	1344 ± 25	0.63 ± 0.02	0.58 ± 0.02	0.58 ± 0.01
C/S	296 ± 7	511 ± 17	893 ± 24	774 ± 18	691 ± 15	807 ± 7	1557 ± 18	1330 ± 12	0.63 ± 0.02	0.57 ± 0.02	0.58 ± 0.01
C/FA1S	298 ± 9	512 ± 24	932 ± 22	801 ± 20	713 ± 17	861 ± 27	1594 ± 36	1373 ± 32	0.60 ± 0.03	0.59 ± 0.02	0.59 ± 0.02
C/FA2S	292 ± 7	474 ± 31	937 ± 28	793 ± 26	705 ± 21	716 ± 11	1591 ± 20	1325 ± 17	0.66 ± 0.04	0.59 ± 0.02	0.60 ± 0.02
FA1/S	305 ± 10	529 ± 23	983 ± 18	842 ± 15	748 ± 13	855 ± 22	1760 ± 66	1485 ± 49	0.62 ± 0.02	0.57 ± 0.02	0.58 ± 0.02
FA1/FA1S	309 ± 12	485 ± 26	936 ± 19	796 ± 17	711 ± 15	782 ± 26	1593 ± 74	1348 ± 58	0.62 ± 0.03	0.62 ± 0.04	0.61 ± 0.03
FA2/S	304 ± 9	516 ± 32	957 ± 19	820 ± 22	730 ± 18	799 ± 28	1636 ± 57	1382 ± 38	0.64 ± 0.03	0.60 ± 0.03	0.61 ± 0.03
FA2/FA2S	300 ± 9	531 ± 24	956 ± 18	823 ± 17	732 ± 15	844 ± 34	1700 ± 75	1485 ± 36	0.63 ± 0.02	0.58 ± 0.02	0.56 ± 0.02
Maternal diet effect	0.298	0.571	0.024	0.049	0.036	0.419	0.039	0.006	0.817	0.847	0.839
C progeny	295 ± 4	500 ± 12	917 ± 12	787 ± 10	701 ± 8	798 ± 10	1580 ± 13	1343 ± 11	0.63 ± 0.01	0.58 ± 0.01	0.59 ± 0.01
FA1 progeny	307 ± 8	508 ± 17	960 ± 13	819 ± 12	730 ± 10	820 ± 18	1678 ± 50	1418 ± 39	0.62 ± 0.02	0.59 ± 0.02	0.59 ± 0.02
FA2 progeny	302 ± 6	524 ± 20	956 ± 13	822 ± 14	731 ± 12	822 ± 22	1669 ± 47	1435 ± 27	0.64 ± 0.02	0.59 ± 0.02	0.58 ± 0.02
Progeny's diet effect	0.787	0.814	0.411	0.531	0.522	0.255	0.467	0.388	0.589	0.752	0.810
Transition effect	0.541	0.701	0.009	0.039	0.030	0.468	0.054	0.016	0.999	0.830	0.947
No FA	295 ± 5	508 ± 14	899 ± 15	778 ± 11	693 ± 9	806 ± 8	1567 ± 17	1337 ± 14	0.63 ± 0.02	0.58 ± 0.01	0.58 ± 0.01
Addition	295 ± 6	493 ± 19	934 ± 18	797 ± 16	709 ± 13	789 ± 18	1592 ± 20	1349 ± 18	0.63 ± 0.02	0.59 ± 0.01	0.58 ± 0.01
Removal	304 ± 7	522 ± 19	970 ± 13	832 ± 13	739 ± 11	828 ± 18	1699 ± 44	1435 ± 32	0.63 ± 0.02	0.59 ± 0.02	0.59 ± 0.02
Continuity	304 ± 8	508 ± 18	946 ± 13	810 ± 12	722 ± 10	814 ± 22	1648 ± 53	1418 ± 35	0.63 ± 0.02	0.60 ± 0.02	0.58 ± 0.02

C = control diet; S, FA1S, FA2S = diets with sweetener; FA1 = diet with feed additive 1; FA2 = diet with feed additive 2. *P*-values for the maternal diet, progeny's diet, and transition effects are indicated for each parameter and time period. Data are expressed as mean ± SE. Significant values (*P* < 0.05) are indicated in bold and italic.

Figure 4. Impact of the transition type between the sows' diet and progeny's diet on the progeny's body weight (A), average daily gain (B), and average daily feed intake (C) at different ages and periods (PND: postnatal day). The "No FA" condition corresponded to sows and their progeny subjected to a diet without feed additive, the "Addition" condition corresponded to the situation where only the progeny was subjected to a diet with a feed additive (FA1 or FA2), the "Removal" condition corresponded to the situation where only the sows were subjected to a diet with a feed additive (FA1 or FA2), the "Continuity" condition corresponded to the situation where both sows and their progeny were subjected to a diet with a feed additive (FA1 or FA2). Data are expressed as mean $\pm$ SE. Two different letters indicate a significant difference at $P < 0.05$.

that better responded was that exposed to the FA through the maternal diet only. This highlights the importance of the maternal diet for programming further feed intake and growth in the progeny, even in the absence of body weight and adiposity differences between sows. The batch effect (i.e., 3 repetitions of the paradigm in January, February, and March 2016) observed for feed consumption was probably related to increasing temperature, leading to a slight decrease in feed intake and weight gain. Though, there was no major batch effect on the colostrum and milk sensory profiles.

Even though our results did not support our initial hypothesis of a favorable sensory continuum, they are quite in line with several studies (Langendijk et al., 2007; Oostindjer et al., 2009; Oostindjer et al., 2010; Oostindjer et al., 2011; Blavi et al., 2016), demonstrating that prenatal exposure to some flavors affects eating behavior and growth of piglets and growing pigs. Similar to Oostindjer

et al. (Oostindjer et al., 2009; Oostindjer et al., 2010; Oostindjer et al., 2011), we showed that postnatal exposure only did not enhance feed intake after weaning and that prenatal exposure in combination with postnatal exposure during the lactation period had beneficial effects. We did not specifically investigate health and welfare criteria in our study and cannot tell whether the differences observed in terms of feed intake and daily weight gain were accompanied by other behavioral or physiological effects. Interestingly, the group that better performed was that exposed to the FA during gestation and lactation, but not after weaning. This suggests that the increased growth and feed intake observed were not induced by some kind of habituation or facilitation process regarding the sensory characteristics of piglets' feed in comparison to what was showed in previous studies (Langendijk et al., 2007; Oostindjer et al., 2009; Oostindjer et al., 2010; Oostindjer et al., 2011). On the contrary, the

beneficial effects observed in our piglets exposed to FA during gestation and lactation were independent of the perception of these specific flavors later on, which is partly in line with a recent study published by [Blavi et al. \(2016\)](#). They demonstrated that the positive reward associated with the flavor included in the sows' diet was stronger when piglets were offered a nonflavored creep feed, suggesting that early exposure of pigs' fetuses to maternal dietary clues at the end of gestation might allow for conditioning pigs after weaning. Though, contrary to our own results, [Blavi et al. \(2016\)](#) also showed that supplementing the prestarter and starter diets with the flavor increased feed intake early after weaning.

Different hypotheses can be advanced to explain the beneficial effects of FA exposure through the maternal diet. First, FA exposure in sows might have induced metabolic effects that we did not assess in this study and that could have provided their progeny with an adaptive advantage from birth, leading to better growth and/or appetite. Second, the growth/appetite advantage of piglets born from FA sows might be directly related to what they were exposed to during gestation and lactation. Limonene, cinnamaldehyde, menthol, carvone, and anethole are the active compounds used as additives in this study. They are extracted from fruits, spices, and other aromatic plants for use in aromatherapy and alternative medicine, and have various functional effects that are unequally documented in the scientific literature, as described below.

Citrus aromas or extracts such as limonene can reduce heart rate, arterial pressure, and cortisol ([Lehrner et al., 2000](#); [Chang and Shen, 2011](#); [Goes et al., 2012](#); [Jafarzadeh et al., 2013](#)), as well as anxiety symptoms ([Morrone et al., 2007](#); [Faturi et al., 2010](#); [Saiyudthong and Marsden, 2011](#); [Goes et al., 2012](#)) in humans and animal models. They can even normalize neuroendocrine hormone levels and immune functions in some instances ([Komori et al., 1995](#)), and influence the dopaminergic and serotonergic brain turnover in the prefrontal cortex and striatum ([Komiya et al., 2006](#)). Sweet orange extracts supplementation can also increase learned and spontaneous feed preferences in lambs and piglets ([Simitzis et al., 2008](#); [Clouard and Val-Laillet, 2014](#)), and specifically modulate brain regions involved in appetite, feed pleasure, and motivation in piglets ([Val-Laillet et al., 2016](#)). Concerning cinnamaldehyde, [Yang et al. \(2010\)](#) showed that supplementing cattle with the main active compound of cinnamon oil improved feed intake, although it had a reduced impact on weight gain or carcass traits. On the other hand, some studies showed in mice fed a high-fat diet that cinnamaldehyde could increase

adipose tissue lipolysis, decrease fasting-induced hyperphagia, feed intake, and/or gastric emptying rates, modulate secretion of leptin and ghrelin, and reduce inflammation ([Camacho et al., 2015](#); [Khare et al., 2016](#)). Interestingly, [Blavi et al. \(2016\)](#) showed that piglets that were born from sows exposed to a feed additive containing cinnamaldehyde during gestation and lactation consumed more feed and gain more weight. Both limonene and cinnamaldehyde were active compounds of the FA1, and the GC-MS analyses demonstrated that limonene was successfully transferred into the maternal colostrum and milk, meaning that piglets were exposed to it during all the lactation period and probably also during the gestation phase through the amniotic fluid, as already demonstrated for cinnamaldehyde by [Blavi et al. \(2016\)](#).

The fact that limonene was also present (though in much lower concentrations) in the colostrum and milk of sows not supplemented in limonene can be explained by the fact that this molecule is ubiquitous, meaning that it can be found in various biological environments or matrices, and notably in the main ingredients of the sows' diet such as wheat and barley ([Bianchi et al., 2007](#); [Niu et al., 2016](#)). A contamination of the different feeds or animals via indirect contact (via animal caretakers or air) might also explain why carvone and anethole were also found in the colostrum and milk of sows that did not receive these molecules in their respective diets. It is important to notice that, despite this possible contamination, control piglets/pigs had a lower feed intake and growth. Further studies aimed at investigating the impact of different doses of additives in the feed are required.

Literature on the compounds composing FA2 is scarcer, but there is interesting evidence showing behavioral and metabolic effects of menthol, anethole, and carvone. Transfer of anethole to the amniotic fluid was already demonstrated in sows ([Blavi et al., 2016](#)), but the same authors failed to demonstrate a transfer to milk. In human mothers, the ingestion of capsules containing menthol, anethole, and carvone induced a peak of anethole and carvone in the maternal milk 2 h after intake ([Hausner et al., 2008](#)). Such a transfer in colostrum and milk is clearly confirmed for anethole and carvone in our study, but is also highly probable for menthol, which was detected at PND14 and PND28 in FA2 sows' milk. Menthol, which induces cold sensation, can increase the activity of endogenous signaling lipids and heat production ([Ehrlich et al., 2016](#)) or improve physical performance in hot environments ([Tran Trong et al., 2015](#)). Topical application of L-menthol can also

reduce pain intensity, mechanical and heat hyperalgesia, and neurogenic inflammation induced by the administration of a hot compound (Andersen et al., 2016). Anethole can have anti-inflammatory, immunomodulatory, and neuroprotective effects (Aprotosoie et al., 2016). Interestingly, Hatano et al. (2012) showed an anxiolytic effect of carvone in rats subjected to the elevated T-maze test. However, a phytogetic additive characterized by menthol and anethole only had a tendency towards improved zootechnical performance and apparent ileal absorption of phosphorus in broilers, whereas encapsulated essential oils of carvacol, thymol, and limonene significantly improved performance and digestibility (Hafeez et al., 2016). Interestingly, Blavi et al. (2016) showed that a feed additive containing anethole and provided to sows during gestation and lactation caused piglets to consume more feed and gain more weight.

Convergent data are still lacking to illustrate the impact of these phytogetic compounds on eating behavior and body weight, but the effects observed on performance in our study are more likely related to early programming mechanisms rather than appetite facilitation through sensory habituation processes, because the group with the best outcomes was that with maternal exposure only. Previous studies already showed an impact of biologically active compounds such as seaweed or ginger extracts supplemented in the sow's diet on the progeny's body weight, performance, and immunity, without direct exposure of the piglets (Leonard, 2010 #115; Lee, 2013 #116). Our own results even suggest that exposure to the additives after weaning had rather negative consequences or no consequence at all. As previously stated, this is in contradiction with some studies in pigs and humans showing in younglings a better acceptability of a flavor that was previously incorporated in the maternal diet (Oostindjer et al., 2009; Nicklaus, 2016b). Even though there was no aversion to the sensory additives included in the piglets' feed, since feed consumption and performance did not differ from the control group, we failed at demonstrating a positive impact of the additives incorporated to the weaned piglets' feed.

Two hypotheses can be proposed to explain these results. First, the additives concentration or inclusion rate used for sows might not be adapted to piglets. Previous studies showed that the concentration of the additive is very important for perception and hedonic processes, especially in young animals (Clouard et al., 2012; Clouard and Val-Laillet, 2014; Val-Laillet et al., 2016). A dose-effect study is consequently needed to identify the

optimal concentration for acceptance and palatability of the additives in piglets. Second, it is possible that the beneficial effects of the additives are related to a particular developmental stage, during which specific events/exposures can shape further metabolic and behavioral processes. Perinatal exposure is determinant for the development of flavor preferences, appetite regulation, and nutritional programming, both in humans and pigs (Nicklaus, 2016a, 2016b; Roura et al., 2016). Further studies are needed to investigate the impact of early exposure to phytogetic products, and especially during gestation and lactation, on brain development and plasticity, and nutritional and behavioral programming. For example, Todrank et al. (2011) showed the effects of in utero odorant exposure on neuro-anatomical development of the olfactory bulb and odor preferences, describing larger tagged glomeruli in mice exposed to these activating odorants in amniotic fluid and later in mother's milk, as well as significant preferences for the activating odor.

In conclusion, our study demonstrated that phytogetic additives in the maternal diet during gestation and lactation could modulate the sensory and biochemical profiles of maternal colostrum and milk, as well as the progeny's growth and performance even in the absence of postweaning exposure to these additives. Notably, the transfer of limonene, carvone, anethole, and probably menthol from the maternal feed to sows' colostrum and milk was demonstrated, which was unprecedented. No beneficial effect was observed when the additives were supplemented in the piglets' solid feed after weaning, with or without early exposure. These results highlight the importance of the exposure to bioactive sensory compounds during the perinatal period for nutritional programming and/or sensory conditioning and further performance, and suggest that the effects observed after weaning were independent of a familiarization process to the organoleptic and sensory properties of the additives. The potential mechanisms underlying this programming/conditioning phenomenon need further investigation to validate the putative action modes of the additives.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Journal of Animal Science* online.

ACKNOWLEDGMENTS

We gratefully acknowledge the efforts and cooperation of INRA technical staff, and especially Sylvie Guérin, Isabelle Nogret, Régis Janvier,

Daniel Boutin, Josselin Delamarre, Hervé Demay, Bruno Duteil, Fabien Guérin, Georges Guillemois, Patrice Roger, Jean-François Rouaud, Patrick Touanel, and Yannick Surel for participating in this study and taking care of the animals.

LITERATURE CITED

- Andersen, H. H., P. Gazerani, and L. Arendt-Nielsen. 2016. High-concentration L-menthol exhibits counter-irritancy to neurogenic inflammation, thermal and mechanical hyperalgesia caused by trans-cinnamaldehyde. *J. Pain*. 17:919–929. doi:10.1016/j.jpain.2016.05.004
- Aprotosoaie, A. C., I. I. Costache, and A. Miron. 2016. Anethole and its role in chronic diseases. *Adv. Exp. Med. Biol.* 929:247–267. doi:10.1007/978-3-319-41342-6_11
- Bianchi, F., M. Careri, C. Conti, M. Musci, and R. Vreuls. 2007. Comparison of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry and gas chromatography-mass spectrometry for the qualitative characterisation of roasted barley by solid-phase microextraction. *J. Sep. Sci.* 30:527–533. doi:10.1002/jssc.200600380
- Blavi, L., D. Solà-Oriol, J. J. Mallo, and J. F. Pérez. 2016. Anethol, cinnamaldehyde, and eugenol inclusion in feed affects postweaning performance and feeding behavior of piglets. *J. Anim. Sci.* 94:5262–5271. doi:10.2527/jas.2016-0760
- Camacho, S., S. Michlig, C. de Senarclens-Bezençon, J. Meylan, J. Meystre, M. Pezzoli, H. Markram, and J. le Coutre. 2015. Anti-obesity and anti-hyperglycemic effects of cinnamaldehyde via altered ghrelin secretion and functional impact on food intake and gastric emptying. *Sci. Rep.* 5:7919. doi:10.1038/srep07919
- Chang, K. M., and C. W. Shen. 2011. Aromatherapy benefits autonomic nervous system regulation for elementary school faculty in Taiwan. *Evid. Based. Complement. Alternat. Med.* 2011:946537. doi:10.1155/2011/946537
- Clouard, C., M-C. Meunier-Salaün, and D. Val-Laillet. 2012. The effects of sensory functional ingredients on food preferences, intake and weight gain in juvenile pigs. *Appl. Anim. Behav. Sci.* 138:36–46. doi:10.1016/j.applanim.2012.01.016
- Clouard, C., and D. Val-Laillet. 2014. Impact of sensory feed additives on feed intake, feed preferences, and growth of female piglets during the early postweaning period. *J. Anim. Sci.* 92:2133–2140. doi:10.2527/jas.2013-6809
- Ehrlich, M., M. Ivask, A. Raasmaja, and S. Koks. 2016. Analysis of metabolic effects of menthol on WFS1-deficient mice. *Physiol. Rep.* 4. pii: e12660. doi:10.14814/phy2.1266
- El-Haddad, M. A., Y. Jia, and M. G. Ross. 2005. Persistent sucrose stimulation of ovine fetal ingestion: lack of adaptation responses. *J. Matern. Fetal. Neonatal Med.* 18:123–127. doi:10.1080/14767050500233860
- Faturi, C. B., J. R. Leite, P. B. Alves, A. C. Canton, and F. Teixeira-Silva. 2010. Anxiolytic-like effect of sweet orange aroma in Wistar rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 34:605–609. doi:10.1016/j.pnpbp.2010.02.020
- Figueroa, J., D. Solà-Oriol, L. Vinokurovas, X. Manteca, and J. F. Pérez. 2013. Prenatal flavour exposure through maternal diets influences flavour preference in piglets before and after weaning. *Anim. Feed Sci. Technol.* 183:60–167. doi:10.1016/j.anifeedsci.2013.04.023
- Franz, C., K. H. C. Baser, and W. Windisch. 2010. Essential oils and aromatic plants in animal feeding—a European perspective. A review. *Flavour Fragr. J.* 25:327–340. doi:10.1002/ffj.1967
- Goes, T. C., F. D. Antunes, P. B. Alves, and F. Teixeira-Silva. 2012. Effect of sweet orange aroma on experimental anxiety in humans. *J. Altern. Complement. Med.* 18:798–804. doi:10.1089/acm.2011.0551
- Hafeez, A., K. Männer, C. Schieder, and J. Zentek. 2016. Effect of supplementation of phytogenic feed additives (powdered vs. Encapsulated) on performance and nutrient digestibility in broiler chickens. *Poult. Sci.* 95:622–629. doi:10.3382/ps/pev368
- Hatano, V. Y., A. S. Torricelli, A. C. Giassi, L. A. Coslope, and M. B. Viana. 2012. Anxiolytic effects of repeated treatment with an essential oil from lippia alba and (R)-(-)-carvone in the elevated T-maze. *Braz. J. Med. Biol. Res.* 45:238–243.
- Hausner, H., W. L. Bredie, C. Mølgaard, M. A. Petersen, and P. Møller. 2008. Differential transfer of dietary flavour compounds into human breast milk. *Physiol. Behav.* 95:118–124. doi:10.1016/j.physbeh.2008.05.007
- Jacela, J. Y., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2010. Feed additives for swine: fact sheets—flavors and mold inhibitors, mycotoxin binders, and antioxidant. *J. Swine Health Prod.* 18:27–32.
- Jafarzadeh, M., S. Arman, and F. F. Pour. 2013. Effect of aromatherapy with orange essential oil on salivary cortisol and pulse rate in children during dental treatment: a randomized controlled clinical trial. *Adv. Biomed. Res.* 2:10. doi:10.4103/2277-9175.107968
- Jugl-Chizzola, M., E. Ungerhofer, C. Gabler, W. Hagmüller, R. Chizzola, K. Zitterl-Eglseer, and C. Franz. 2006. Testing of the palatability of thymus vulgaris L. And origanum vulgare L. As flavouring feed additive for weaner pigs on the basis of a choice experiment. *Berl. Munch. Tierarztl. Wochenschr.* 119:238–243.
- Khare, P., S. Jagtap, Y. Jain, R. K. Baboota, P. Mangal, R. K. Boparai, K. K. Bhutani, S. S. Sharma, L. S. Premkumar, K. K. Kondepudi, et al. 2016. Cinnamaldehyde supplementation prevents fasting-induced hyperphagia, lipid accumulation, and inflammation in high-fat diet-fed mice. *BioFactors* 42:201–211. doi:10.1002/biof.1265
- Komiya, M., T. Takeuchi, and E. Harada. 2006. Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. *Behav. Brain Res.* 172:240–249. doi:10.1016/j.bbr.2006.05.006
- Komori, T., R. Fujiwara, M. Tanida, J. Nomura, and M. M. Yokoyama. 1995. Effects of citrus fragrance on immune function and depressive states. *Neuroimmunomodulation* 2:174–180. doi:10.1159/000096889
- Langendijk, P., J. E. Bolhuis, and B. F. A. Laurensen. 2007. Effects of pre- and postnatal exposure to garlic and aniseed flavour on pre- and postweaning feed intake in pigs. *Livestock Sci.* 108:284–287. doi:10.1016/j.livsci.2007.01.083
- Lecanuet, J. P., and B. Schaal. 1996. Fetal sensory competencies. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 68:1–23. doi:10.1016/0301-2115(96)02509-2
- Lehrner, J., C. Eckersberger, P. Walla, G. Pötsch, and L. Deecke. 2000. Ambient odor of orange in a dental office reduces anxiety and improves mood in female patients. *Physiol. Behav.* 71:83–86. doi:10.1016/S0031-9384(00)00308-5

- Mennella, J. A. 1995. Mother's milk: a medium for early flavor experiences. *J. Hum. Lact.* 11:39–45. doi:10.1177/089033449501100122
- Mennella, J. A., C. P. Jagnow, and G. K. Beauchamp. 2001. Prenatal and postnatal flavor learning by human infants. *Pediatrics* 107:E88. doi:10.1542/peds.107.6.e88
- Mennella, J. A., A. Johnson, and G. K. Beauchamp. 1995. Garlic ingestion by pregnant women alters the odor of amniotic fluid. *Chem. Senses* 20:207–209.
- Michiels, J. et al. 2012. Effect of dose of thymol and supplemental flavours or camphor on palatability in a choice feeding study with piglets. *Czech J. Anim. Sci.* 57: 65–74.
- Morrone, L. A., L. Rombolà, C. Pelle, M. T. Corasaniti, S. Zappettini, P. Paudice, G. Bonanno, and G. Bagetta. 2007. The essential oil of bergamot enhances the levels of amino acid neurotransmitters in the hippocampus of rat: implication of monoterpene hydrocarbons. *Pharmacol. Res.* 55:255–262. doi:10.1016/j.phrs.2006.11.010
- Nicklaus, S. 2016a. Relationships between early flavor exposure, and food acceptability and neophobia. In: P. Etiévant, E. Guichard, C. Salles and A. Voilley, editors, *Flavor: from food to behaviors, Wellbeing and Health*. Sawston, UK, Cambridge, UK: Elsevier.
- Nicklaus, S. 2016b. The role of food experiences during early childhood in food pleasure learning. *Appetite* 104:3–9. doi:10.1016/j.appet.2015.08.022
- Niu, Y., L. Hua, G. Hardy, M. Agarwal, and Y. Ren. 2016. Analysis of volatiles from stored wheat and *Rhizopertha dominica* (F.) with solid phase microextraction-gas chromatography mass spectrometry. *J. Sci. Food Agric.* 96:1697–1703. doi:10.1002/jsfa.7274
- Oostindjer, M., J. E. Bolhuis, K. Simon, H. van den Brand, and B. Kemp. 2011. Perinatal flavour learning and adaptation to being weaned: all the pig needs is smell. *PLoS ONE* 6:e25318. doi:10.1371/journal.pone.0025318
- Oostindjer, M., J. E. Bolhuis, H. van den Brand, and B. Kemp. 2009. Prenatal flavor exposure affects flavor recognition and stress-related behavior of piglets. *Chem. Senses* 34:775–787. doi:10.1093/chemse/bjp063
- Oostindjer, M., J. E. Bolhuis, H. van den Brand, E. Roura, and B. Kemp. 2010. Prenatal flavor exposure affects growth, health and behavior of newly weaned piglets. *Physiol. Behav.* 99:579–586. doi:10.1016/j.physbeh.2010.01.031
- Roura, E., S. J. Koopmans, J. P. Lallès, I. Le Huerou-Luron, N. de Jager, T. Schuurman, and D. Val-Laillet. 2016. Critical review evaluating the pig as a model for human nutritional physiology. *Nutr. Res. Rev.* 29:60–90. doi:10.1017/S0954422416000020
- Saiyudthong, S., and C. A. Marsden. 2011. Acute effects of bergamot oil on anxiety-related behaviour and corticosterone level in rats. *Phytother. Res.* 25:858–862. doi:10.1002/ptr.3325
- Schaal, B., L. Marlier, and R. Soussignan. 2000. Human foetuses learn odours from their pregnant mother's diet. *Chem. Senses* 25:729–737. doi:10.1093/chemse/25.6.729
- Seabolt, B. S., E. van Heugten, S. W. Kim, K. D. Ange-van Heugten, and E. Roura. 2010. Feed preferences and performance of nursery pigs fed diets containing various inclusion amounts and qualities of distillers coproducts and flavor. *J. Anim. Sci.* 88:3725–3738. doi:10.2527/jas.2009-2640
- Simitzis, P. E., S. G. Deligeorgis, J. A. Bizelis, and K. Fegeros. 2008. Feeding preferences in lambs influenced by prenatal flavour exposure. *Physiol. Behav.* 93:529–536. doi:10.1016/j.physbeh.2007.10.013
- Smotherman, W. P., S. R. Robinson, A. E. Ronca, J. R. Alberts, and P. G. Hepper. 1991. Heart rate response of the rat fetus and neonate to a chemosensory stimulus. *Physiol. Behav.* 50:47–52. doi:10.1016/0031-9384(91)90496-B
- Todrank, J., G. Heth, and D. Restrepo. 2011. Effects of in utero odorant exposure on neuroanatomical development of the olfactory bulb and odour preferences. *Proc. R. Soc. Lond., B, Biol. Sci./The Royal Society.* 278:1949–1955.
- Tran Trong, T., F. Riera, K. Rinaldi, W. Briki, and O. Hue. 2015. Ingestion of a cold temperature/menthol beverage increases outdoor exercise performance in a hot, humid environment. *PLoS ONE* 10:e0123815. doi:10.1371/journal.pone.0123815
- Val-Laillet, D., S. Blat, I. Louveau, and C. H. Malbert. 2010. A computed tomography scan application to evaluate adiposity in a minipig model of human obesity. *Br. J. Nutr.* 104:1719–1728. doi:10.1017/S0007114510002667
- Val-Laillet, D., P. Meurice, and C. Clouard. 2016. Familiarity to a feed additive modulates its effects on brain responses in reward and memory regions in the pig model. *PLoS ONE* 11:e0162660. doi:10.1371/journal.pone.0162660
- Wells, D. L., and P. G. Hepper. 2006. Prenatal olfactory learning in the domestic dog. *Anim. Behav.* 72:681–686. doi:10.1016/j.anbehav.2005.12.008
- Windisch, W., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogetic products as feed additives for swine and poultry. *J. Anim. Sci.* 86(14 Suppl):E140–E148. doi:10.2527/jas.2007-0459
- Yang, W. Z., B. N. Ametaj, C. Benchaar, M. L. He, and K. A. Beauchemin. 2010. Cinnamaldehyde in feedlot cattle diets: intake, growth performance, carcass characteristics, and blood metabolites. *J. Anim. Sci.* 88:1082–1092. doi:10.2527/jas.2008-1608