# *Long-term exposure to sensory feed additives during the gestational and postnatal periods impacts sows' colostrum and milk sensory profiles, piglets' growth and feed intake*

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Running head: Sensory feed additives in sows and piglets

# Long-term exposure to sensory feed additives during the gestational and postnatal periods impacts sows' colostrum and milk sensory profiles, piglets' growth and feed intake[1]

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## ABSTRACT

This study investigated the effect of feed supplementation in sows and/or their progeny with two 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 day 1, 14, and 28 of lactation for gas chromatography-mass spectrometry (GC-MS) 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 three 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.

**Keywords:** feed additives, feed transition, colostrum and milk sensory properties, performance, sensory conditioning, nutritional programming, *Sus scrofa*

## INTRODUCTION

In pig production, sensory feed additives are commonly used in an attempt to improve feed palatability and zootechnical performance (Franz et al., 2010; Jacela et al., 2010; Windisch et al., 2008), but discrepancies between studies are frequent (Clouard et al., 2012; Clouard and Val-Laillet, 2014; Jugl-Chizzola et al., 2006; Michiels et al., 2012; Seabolt et al., 2010; 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' (Figueroa et al., 2013; Mennella et al., 2001; Oostindjer et al., 2010; Wells and Hepper, 2006).

The aim of our study was to validate and compare the use of two different feed additives (FA) combining different phytogenic molecules, known to have behavioral and neurophysiological effects, to compare the impact of perinatal and/or post-weaning exposure to the feed additives (compared one to another and to a control feed). In mammals, flavors from the maternal diet can reach the fetus before birth through the amniotic fluid (El-Haddad et al., 2005; Mennella, 1995; Mennella et al., 1995). 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 (N°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 three consecutive batches (N=14 in January 2016, N=13 in February 2016, and N=13 in March 2016) with homogenous body weight and parity amongst treatments and batches, were used for this study and reared at the experimental center of INRA (St 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 6-8 individuals of same perinatal exposure (**Fig. 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 (**Fig. 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 two 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 divulgated. 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 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 lactation (28 days after farrowing), because it is commonly accepted that mammal fetuses are able to perceive flavors during the last third of gestation (Lecanuet and Schaal, 1996; Nicklaus, 2016a; Oostindjer et al., 2010; Schaal et al., 2000; Smotherman et al., 1991). During 10 days after weaning, the piglets received a pre-starter feed and then a starter feed until PND70. A three-day transition period was organized to familiarize piglets to the starter feed at the end of the pre-starter period. After PND70, the animals received a growth diet until slaughter at PND160 (**Table 1**). Dietary treatments per group are summarized in **Fig. 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 pre-starter, 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 pre-starter, starter, and growth feeds supplemented with 0.015% of sweeter and 0.1% of feed additive 1 or 2. Except for one 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 to the control group with sweetener alone, was aimed at discussing the specific impact of the sweetener, independently from the other additives. The experimental diets were produced at the feed mill of the INRA St Gilles experimental facilities.

***Colostrum and Milk Sampling and Analysis***

Colostrum or milk samples (at least 60 mL) were collected from all sows on the morning of PND1 (PND0 corresponding to farrowing), PND14, and PND28, after an intramuscular injection of oxytocin (1-2 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 St 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 mg/L to 20 mg/L) of menthol, limonene, cinnamaldehyde, carvone, and anethole were prepared in triacetin. A 20-mg/L solution of 246-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 mg/L, 0.1 mg/L, 0.25 mg/L, 1 mg/L, 2.5 mg/L, 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 was stored were then shaken manually for 10 seconds 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. In order to prepare a calibration curve for quantification of the compounds of interest, 50 μL of each standard solution were 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 four samples were analyzed from each diet (Control, FA1, FA2) at three collection points (Day 1, Day 14, and Day 28) from each of 3 batches (1, 2, and 3), *i.e.* a total of 79 samples.

*Solid-phase Microextraction*

Automated solid-phase microextraction (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 seconds on and 2 seconds off. After incubation, the headspace above the sample was extracted for 60 minutes at 60 °C using an SPME syringe containing a 1-cm Stable-flex fiber coated with 50/30 µm DVB/Carboxen on 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 (GC-MS)*

After extraction, the fiber was desorbed in the injection port of the gas chromatograph at 250 °C for 20 minutes onto a 30 m × 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/min 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: one quantifying ion (shown in bold) and three qualifiers. Each ion was monitored for 50 ms. All six compounds measured were well separated by GC, so six separate SIM windows could be prepared, one for each compound. The ions measured in Window 1 (start time 0 min) were 68, 67, 121, **136** (limonene); Window 2 (20 min) were **121**, 120, 126, 79 (TMP); Window 3 (30 min) were **138**, 81, 71, 95 (menthol); Window 4 (33 min) were **82**, 150, 54, 108 (carvone); Window 5 (35.5 min) were **148**, 147, 117, 133 (anethole); and Window 6 (40 min) were 131, **132**, 103, 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, in order to calculate the concentrations of the compounds of interest in samples. Because some samples were used for method development, they went missing 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 two weeks until slaughter. The average daily weight gain (ADG g/d) was calculated for the suckling period (PND1 to PND28), for the post-weaning 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 post-weaning 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, two-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 two-way ANOVA with repeated measures, with maternal

diet and batch as main factors, and parity as a cofactor. Piglets' body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (growth:feed ratio G:F) were analyzed with different complementary statistical procedures depending on the question/objective:

- Body weight was analyzed with a two-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 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).
- Body weight was analyzed with 2 three-way ANOVA with repeated measures (before weaning and after weaning) on two different data subsets, *i.e.* FA1 or C sows x FA1S or S piglets, as well as FA2 or C sows x FA2S or S piglets (3 factors and 4 groups in each three-way ANOVA), with maternal diet, progeny's diet and batch as main factors, and sow/litter as 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.
- Body weight at PND1 (birth), PND28 (weaning), PND70 (transfer to another building) and PDN160 (slaughter), as well as ADG, ADFI and G:F were analyzed for each period of interest with a two-way ANOVA on the whole dataset, with maternal diet and batch as main factors (three groups compared: C, FA1, FA2).
- Body weight at PND1, PND28, PND70 and PDN160, as well as ADG, ADFI and G:F were analyzed for each period of interest with a two-way ANOVA on the whole

dataset, with progeny's diet and batch as main factors (four groups compared: C, S, FA1S, FA2S).

- Body weight at PND1, PND28, PND70 and PDN160, as well as ADG, ADFI and G:F were analyzed for each period of interest with a three-way ANOVA on two different data subsets, *i.e.* FA1 or C sows x FA1S or S piglets, as well as FA2 or C sows x FA2S or S piglets (3 factors and 4 groups in each three-way ANOVA), with maternal diet, progeny's diet and batch as main factors, and sow/litter as 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).
- Body weight at PND1, PND28, PND70 and PDN160, as well as ADG, ADFI and G:F were analyzed for each period of interest with a two-way ANOVA on the whole dataset, with treatment and batch as main factors (4 groups: "No FA", "Addition", "Removal", "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 ± 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**, **Fig. 2**. Four of the five 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 two 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 four compounds, compounds present between 0.02 and 0.05 ppm were labeled trace while 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, while 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 three compounds increasing with time (between D1, D14, and D28 of lactation) in the colostrum/milk of sows that respectively received 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 ($P = 0.0332$), while 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

298 significantly affected by the maternal diets; menthol did not show a significant effect (only a
299 trend $P = 0.058$). Time of collection effect was only significant for limonene ($P = 0.038$),
300 while only carvone showed a batch effect ($P = 0.0178$).

301 Limonene, carvone, and anethole were all significantly higher in the milk of sows receiving
302 the diets to which they were added (FA1 for limonene, FA2 for carvone and anethole).

303 ***Zootechnical Parameters***

304 There was no difference between sows' groups in terms of parity ($4 \pm 0.3$; $P = 0.915$) and
305 body weight (at the onset of dietary treatment: $255 \pm 5$ kg, $P = 0.776$; early lactation:
306 $279 \pm 5$ kg, $P = 0.752$; end of lactation: $250 \pm 5$ kg, $P = 0.546$). There was an interaction
307 between parity and batch on body weight ($P = 0.035$), but no significant effect of batch
308 ($P = 0.099$) and no interaction with dietary treatment. There was no effect of group, batch, or
309 parity, and no interaction between factors on litter size at farrowing ($16.0 \pm 0.5$ piglets,
310 $P > 0.1$), but there was an interaction between group and batch for the piglets' survival at
311 weaning ($12.2 \pm 0.5$ piglets, $P = 0.038$), with no remaining difference after pairwise
312 comparisons. Over the 638 piglets that were born from the 40 sows of this study, there were
313 26 stillbirths and 64 additional piglets that died the day of farrowing. There was no difference
314 between groups in terms of sows' feed consumption during lactation ($216 \pm 4$ kg, $P = 0.447$).
315 Sows' back fat deposition did not differ between groups before farrowing ($16 \pm 1$ mm,
316 $P = 0.843$) and at the end of lactation ($13 \pm 1$ mm, $P = 0.680$). There was a significant
317 decrease of fat deposition for all groups between the end of gestation and the end of lactation
318 ($P < 0.0001$), as well as a batch effect ($P < 0.0001$), but no group effect ($P = 0.610$) and no
319 interaction between factors.

320 The two-way ANOVAs with repeated measures performed on the whole dataset revealed an
321 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 three-way ANOVAs with repeated measures performed on the two 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 ($P = 0.186$) (**Fig. 3A**). These differences disappeared at weaning (9.26 ± 0.09 kg; $P = 0.623$). The ratio between piglets' birth weight 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 to 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) (**Fig. 3A**). The three-way ANOVAs performed on the two 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

347 birth ($P$ = 0.016), PND70 ($P$ = 0.020) and at slaughter (PND160, $P$ = 0.022), but only a trend
348 at weaning (PND28, $P$ = 0.088).

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

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

367 Overall, there was an effect of the maternal diet and transition condition between the pre- and
368 post-weaning periods on ADG and ADFI, but not on G:F, whereas no effect of the piglets'
369 diet was observed on these variables (**Table 3**). The cofactor 'mother identity' had no
370 significant effect on these variables ($P$ > 0.15 for all). A significant effect of maternal diet for
371 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 (**Fig. 3BC**). 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 post-weaning exposure, had higher body weight at birth and PND160 than pigs with no exposure at all (**Fig. 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 (**Fig. 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) (**Fig. 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 post-weaning periods is likely due to the pre-weaning than the post-weaning exposure to FA. Moreover, the group 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.* three 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, this had no major 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 (Blavi et al., 2016; Langendijk et al., 2007; Oostindjer et al., 2011; Oostindjer et al., 2009; Oostindjer et al., 2010) demonstrating that prenatal exposure to some flavors affects eating behavior and growth of piglets and growing pigs. Similarly to Oostindjer et al. (Oostindjer et al., 2011; Oostindjer et al., 2009; Oostindjer et al., 2010), 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., 2011; Oostindjer et al., 2009; Oostindjer et al., 2010). On the contrary, the beneficial effects observed in our piglets exposed to FA during gestation and lactation were independent to 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, they 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 (Chang and Shen, 2011; Goes et al., 2012; Jafarzadeh et al., 2013; Lehrner et al., 2000), as well as anxiety symptoms (Faturi et al., 2010; Goes et al., 2012; Morrone et al., 2007; Saiyudthong and Marsden, 2011) 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 serotoninergic 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 (Clouard and Val-Laillet, 2014; Simitzis et al., 2008), 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 a feed additive containing cinnamaldehyde and provided to sows during gestation and lactation made piglets to consume 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 two hours 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, as well as neurogenic inflammation induced by the administration of a hot compound (Andersen et al., 2016). Anethole can have anti-inflammatory, immunomodulatory, and neuroprotective effects (Aprotosoaie 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 phytogenic 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 caravacol, 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 phytogenic 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 (Nicklaus, 2016b; Oostindjer et al., 2009). 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 programing, both in humans and pigs (Nicklaus, 2016a, b; Roura et al., 2016).

Further studies are needed to investigate the impact of early exposure to phytogenic products, and especially during gestation and lactation, on brain development and plasticity, as well as nutritional and behavioral programming. For example, Todrank et al. (2011) showed the effects of *in utero* odorant exposure on neuroanatomical 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 phytogenic 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 post-weaning 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 from 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.

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678 **Table 1.** Composition of the animal feeds used in the study. The gestation and lactation feeds
679 were provided to the gestating or lactating sows. The pre-starter, starter, and growth feeds
680 were provided to the piglets. ++ and + symbols indicate very small and infinitesimal
681 quantities of compounds added in the diet.
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| | 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 |
| Mono-calcic phosphate | | | 0.8 | 0.97 | |
| Bi-calcic 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 | | + | ++ | ++ | + |
| Méthionine | | + | ++ | ++ | + |
| Thréonine | | + | ++ | ++ | + |
| Tryptophane | | | + | + | |
| 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 fibre | 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 |

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**Table 2.** Concentrations (ppm) of four target compounds in sows' colostrum/milk. Samples with values lower than 0.05 ppm were labeled trace, while 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 ± SE.

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

**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). 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.

| | 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 | .63 ± .02 | .58 ± .02 | .58 ± .01 |
| **C/S** | 296 ± 7 | 511 ± 17 | 893 ± 24 | 774 ± 18 | 691 ± 15 | 807 ± 7 | 1557 ± 18 | 1330 ± 12 | .63 ± .02 | .57 ± .02 | .58 ± .01 |
| **C/FA1S** | 298 ± 9 | 512 ± 24 | 932 ± 22 | 801 ± 20 | 713 ± 17 | 861 ± 27 | 1594 ± 36 | 1373 ± 32 | .60 ± .03 | .59 ± .02 | .59 ± .02 |
| **C/FA2S** | 292 ± 7 | 474 ± 31 | 937 ± 28 | 793 ± 26 | 705 ± 21 | 716 ± 11 | 1591 ± 20 | 1325 ± 17 | .66 ± .04 | .59 ± .02 | .60 ± .02 |
| **FA1/S** | 305 ± 10 | 529 ± 23 | 983 ± 18 | 842 ± 15 | 748 ± 13 | 855 ± 22 | 1760 ± 66 | 1485 ± 49 | .62 ± .02 | .57 ± .02 | .58 ± .02 |
| **FA1/FA1S** | 309 ± 12 | 485 ± 26 | 936 ± 19 | 796 ± 17 | 711 ± 15 | 782 ± 26 | 1593 ± 74 | 1348 ± 58 | .62 ± .03 | .62 ± .04 | .61 ± .03 |
| **FA2/S** | 304 ± 9 | 516 ± 32 | 957 ± 19 | 820 ± 22 | 730 ± 18 | 799 ± 28 | 1636 ± 57 | 1382 ± 38 | .64 ± .03 | .60 ± .03 | .61 ± .03 |
| **FA2/FA2S** | 300 ± 9 | 531 ± 24 | 956 ± 18 | 823 ± 17 | 732 ± 15 | 844 ± 34 | 1700 ± 75 | 1485 ± 36 | .63 ± .02 | .58 ± .02 | .56 ± .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 | .63 ± .01 | .58 ± .01 | .59 ± .01 |
| **FA1 progeny** | 307 ± 8 | 508 ± 17 | 960 ± 13 | 819 ± 12 | 730 ± 10 | 820 ± 18 | 1678 ± 50 | 1418 ± 39 | .62 ± .02 | .59 ± .02 | .59 ± .02 |
| **FA2 progeny** | 302 ± 6 | 524 ± 20 | 956 ± 13 | 822 ± 14 | 731 ± 12 | 822 ± 22 | 1669 ± 47 | 1435 ± 27 | .64 ± .02 | .59 ± .02 | .58 ± .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 | .63 ± .02 | .58 ± .01 | .58 ± .01 |
| **Addition** | 295 ± 6 | 493 ± 19 | 934 ± 18 | 797 ± 16 | 709 ± 13 | 789 ± 18 | 1592 ± 20 | 1349 ± 18 | .63 ± .02 | .59 ± .01 | .58 ± .01 |
| **Removal** | 304 ± 7 | 522 ± 19 | 970 ± 13 | 832 ± 13 | 739 ± 11 | 828 ± 18 | 1699 ± 44 | 1435 ± 32 | .63 ± .02 | .59 ± .02 | .59 ± .02 |
| **Continuity** | 304 ± 8 | 508 ± 18 | 946 ± 13 | 810 ± 12 | 722 ± 10 | 814 ± 22 | 1648 ± 53 | 1418 ± 35 | .63 ± .02 | .60 ± .02 | .58 ± .02 |

**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.

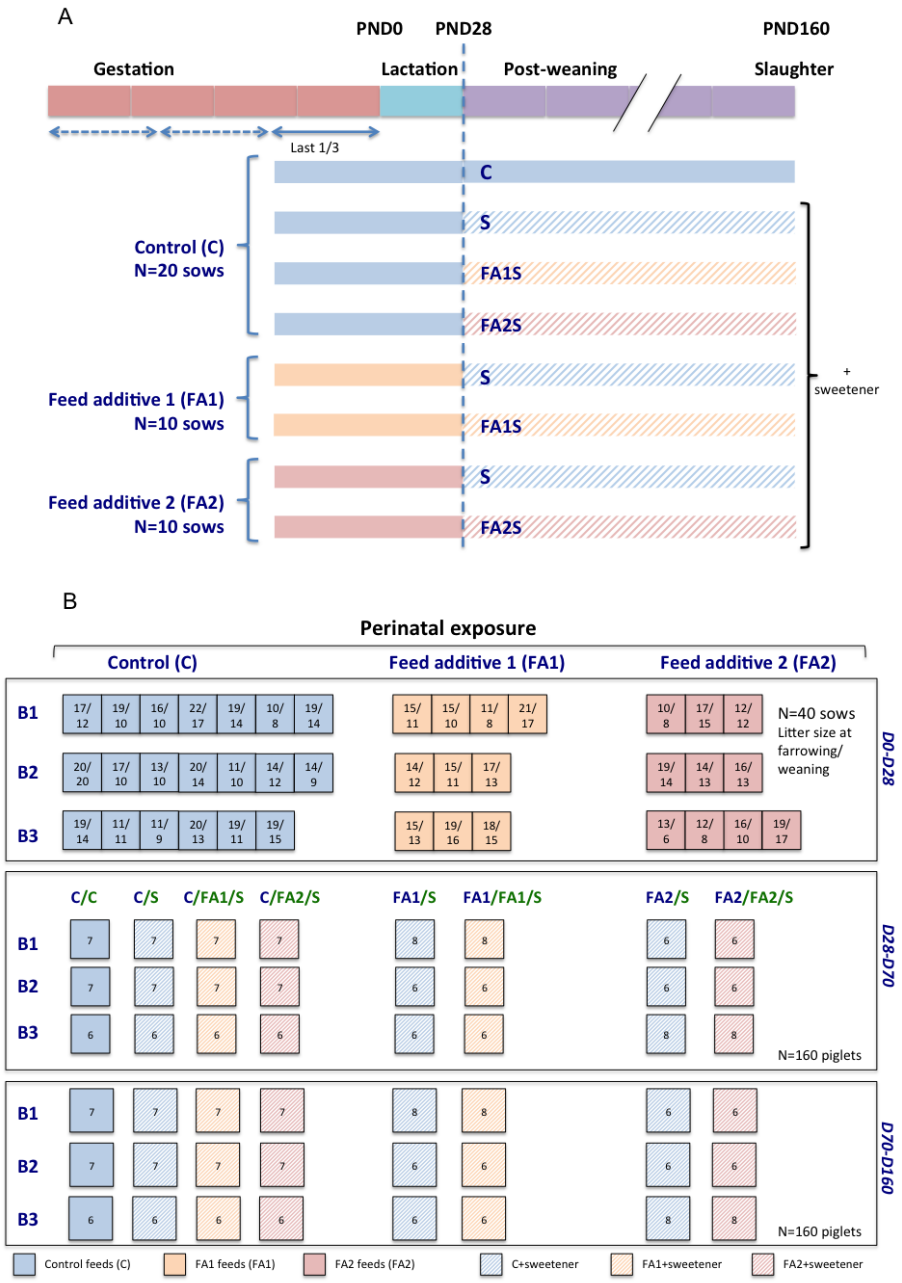


**Figure 2.** Concentrations of four target compounds in the colostrum/milk of sows fed a control (N=9), FA1 (N=10), or FA2 (N=10) diet. Limonene (A) and cinnamaldehyde were added to the FA1 diet, whereas menthol (B), carvone (C), and anethole (D) were added to the FA2 diet. Cinnamaldehyde was always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

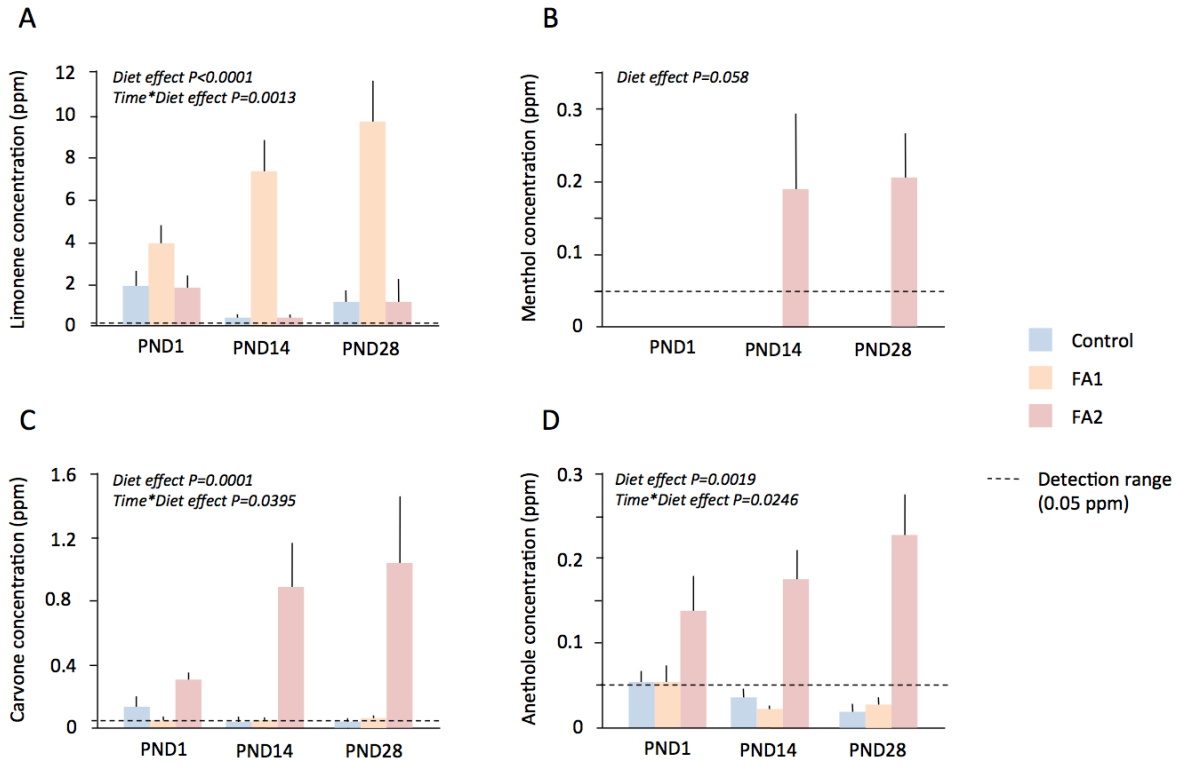


**Figure 3.** Impact of the maternal diet on the progeny's body weight (A), average daily gain (B), and average daily feed consumption (C) at different ages and periods from birth to slaughter (PND: postnatal day). C sows were subjected to a control diet during the whole trial. FA1 and FA2 sows were 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 ± SE. Two different letters indicate a significant difference at $P < 0.05$.

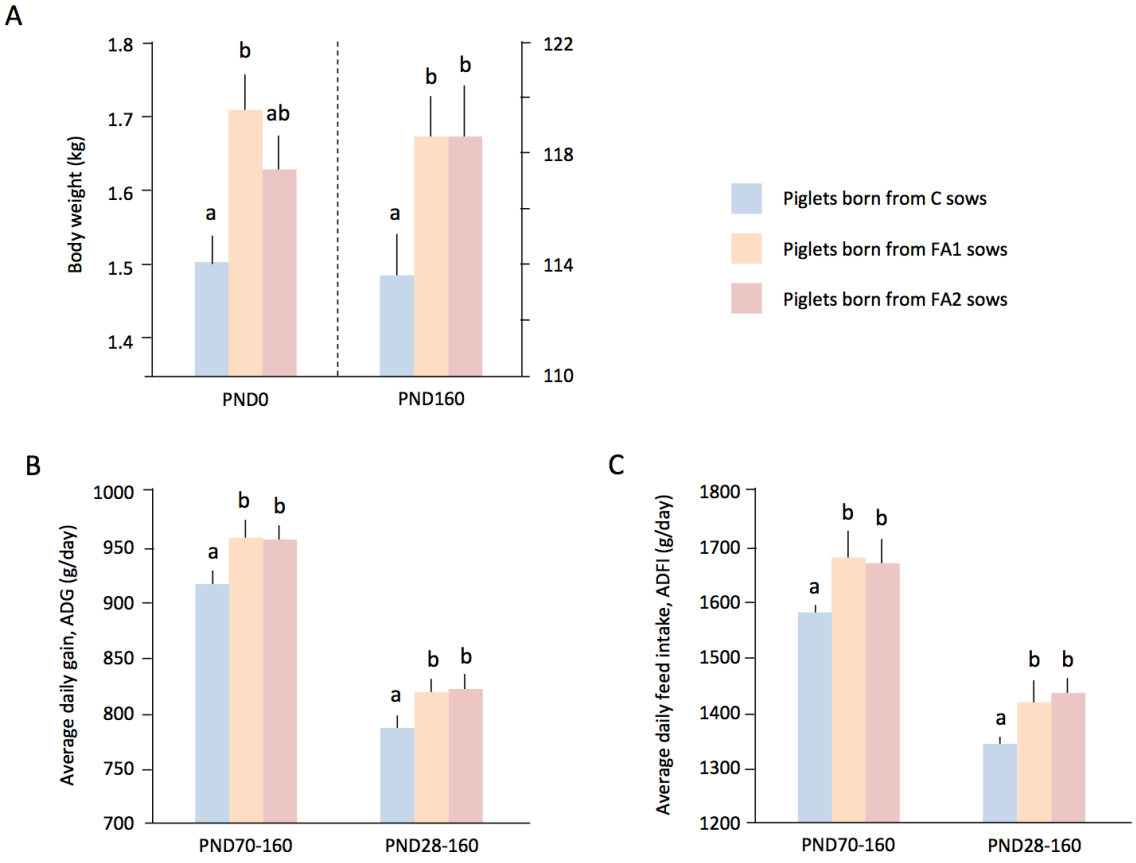
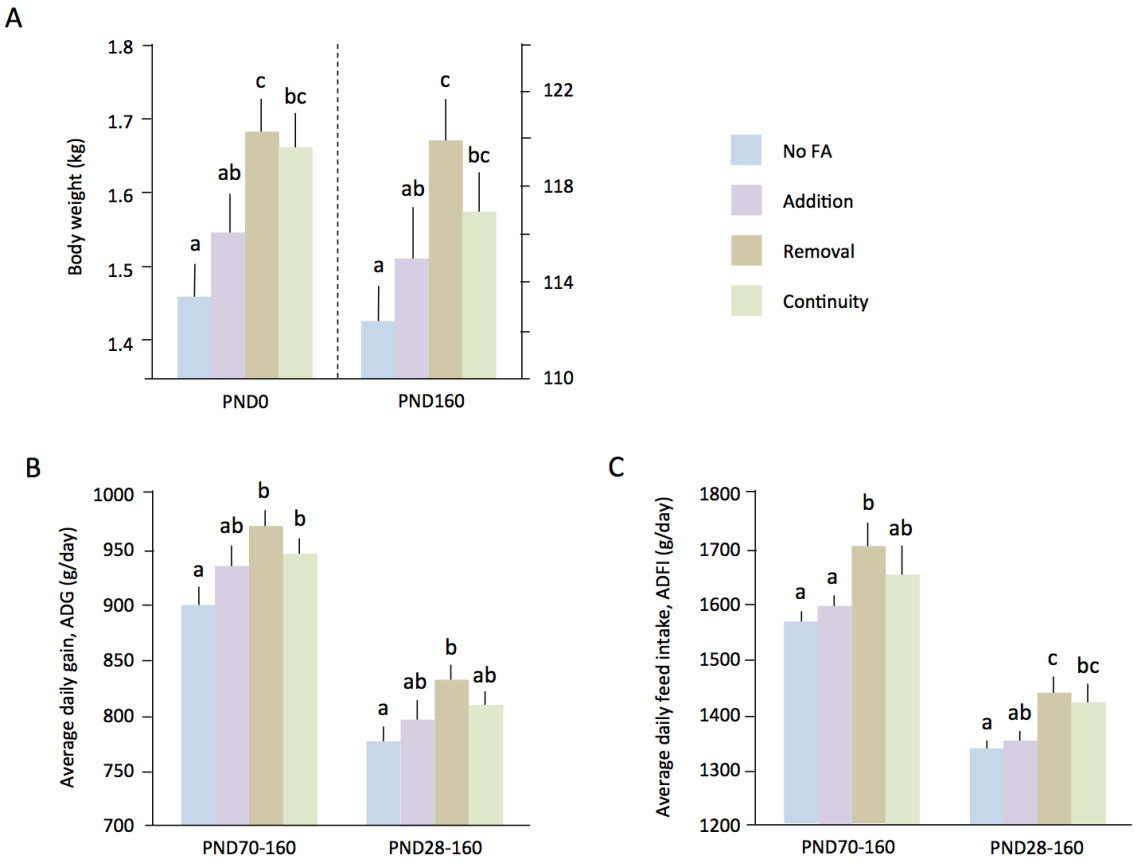


**Figure 4.** Impact of the transition type between the sows' diet and progeny's diet on the progeny's body weight (A), average daily gain (B), and average daily feed intake (C) at different ages and periods (PND postnatal day). The "No FA" condition corresponded to sows and their progeny subjected to 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 ± SE. Two different letters indicate a significant difference at $P < 0.05$.



Appendix 1: List and raw data (ppm) of the colostrum/milk samples analyzed at day 1, 14 and 28 of lactation for each of the four detected compounds.

| Batch | Animal | Diet | Limonene D1 | Limonene D14 | Limonene D28 | Menthol D1 | Menthol D14 | Menthol D28 | Carvone D1 | Carvone D14 | Carvone D28 | Anethole D1 | Anethole D14 | Anethole D28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 220965 | Control | 2.430 | 0.415 | 0.000 | 0.000 | 0.000 | 0.000 | 0.151 | 0.025 | 0.000 | 0.082 | 0.015 | 0.000 |
| 1 | 241978 | Control | 0.507 | 0.605 | 4.033 | 0.000 | 0.000 | 0.000 | 0.026 | 0.057 | 0.159 | 0.041 | 0.043 | 0.033 |
| 1 | 321402 | Control | 2.597 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.083 | 0.000 | 0.072 | 0.057 | 0.002 | 0.073 |
| 1 | 320424 | FA1 | 4.538 | 3.484 | 20.407 | 0.000 | 0.000 | 0.000 | 0.048 | 0.038 | 0.016 | 0.058 | 0.019 | 0.003 |
| 1 | 341560 | FA1 | 1.255 | 1.689 | 6.909 | 0.000 | 0.000 | 0.000 | 0.051 | 0.042 | 0.026 | 0.058 | 0.018 | 0.037 |
| 1 | 341566 | FA1 | 4.548 |  | 4.913 | 0.000 |  | 0.000 | 0.135 |  | 0.086 | 0.027 |  | 0.055 |
| 1 | 463860 | FA1 | 0.093 | 13.755 |  | 0.000 | 0.000 |  | 0.018 | 0.023 |  | 0.042 | 0.005 |  |
| 1 | 220966 | FA2 | 6.007 | 0.000 |  | 0.000 | 0.000 |  | 0.569 | 0.759 |  | 0.036 | 0.063 |  |
| 1 | 320423 | FA2 | 3.917 |  | 8.964 | 0.000 |  | 20.080 | 0.377 |  | 3.859 | 0.081 |  | 0.330 |
| 1 | 463856 | FA2 | 0.686 | 0.000 | 0.010 | 0.000 | 0.000 | 0.162 | 0.298 | 0.178 | 0.301 | 0.200 | 0.192 | 0.321 |
| 2 | 320839 | Control | 6.274 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.623 | 0.000 | 0.034 | 0.135 | 0.013 | 0.016 |
| 2 | 464887 | Control | 1.272 | 0.000 | 4.524 | 0.000 | 0.000 | 0.000 | 0.167 | 0.027 | 0.036 | 0.029 | 0.057 | 0.011 |
| 2 | 561152 | Control | 0.075 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.083 | 0.027 | 0.048 | 0.066 | 0.020 |
| 2 | 461869 | FA1 | 7.109 |  |  | 0.000 |  |  | 0.017 |  |  | 0.211 |  |  |
| 2 | 463862 | FA1 | 3.674 | 9.940 | 12.780 | 0.000 | 0.000 | 0.000 | 0.048 | 0.064 | 0.122 | 0.028 | 0.045 | 0.018 |
| 2 | 561621 | FA1 | 8.589 | 3.578 | 12.538 | 0.000 | 0.000 | 0.000 | 0.008 | 0.012 | 0.153 | 0.012 | 0.021 | 0.077 |
| 2 | 322770 | FA2 | 2.729 | 0.652 |  | 0.000 | 0.685 |  | 0.369 | 0.400 |  | 0.104 | 0.190 |  |
| 2 | 461871 | FA2 | 0.000 |  | 0.000 | 0.000 |  | 0.000 | 0.170 |  | 0.359 | 0.147 |  | 0.379 |
| 2 | 561619 | FA2 | 0.000 | 0.000 | 0.188 | 0.000 | 0.170 | 0.000 | 0.161 | 0.177 | 0.378 | 0.155 | 0.127 | 0.340 |
| 3 | 320834 | Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.020 | 0.000 | 0.025 | 0.008 | 0.009 |
| 3 | 320838 | Control | 3.021 | 1.475 | 0.972 | 0.000 | 0.000 | 0.000 | 0.095 | 0.070 | 0.000 | 0.047 | 0.011 | 0.007 |
| 3 | 464436 | Control | 0.000 | 0.334 | 0.000 | 0.000 | 0.000 | 0.000 | 0.012 | 0.020 | 0.000 | 0.018 | 0.105 | 0.004 |
| 3 | 230862 | FA1 | 2.039 | 6.819 | 7.341 | 0.000 | 0.000 | 0.000 | 0.021 | 0.023 | 0.014 | 0.047 | 0.010 | 0.022 |
| 3 | 321454 | FA1 | 4.463 | 9.241 | 9.075 | 0.000 | 0.000 | 0.000 | 0.108 | 0.113 | 0.018 | 0.060 | 0.030 | 0.017 |
| 3 | 462306 | FA1 | 2.101 | 9.978 | 2.662 | 0.000 | 0.000 | 0.000 | 0.082 | 0.022 | 0.010 | 0.000 | 0.026 | 0.002 |
| 3 | 320452 | FA2 | 0.182 | 0.809 | 0.000 | 0.000 | 0.000 | 0.242 | 0.137 | 1.529 | 0.786 | 0.037 | 0.270 | 0.075 |
| 3 | 460050 | FA2 | 3.123 | 0.000 | 0.000 | 0.000 | 0.000 | 0.191 | 0.447 | 1.066 | 0.729 | 0.115 | 0.080 | 0.047 |
| 3 | 460051 | FA2 | 0.289 | 0.000 | 0.000 | 0.000 | 0.000 | 0.480 | 0.134 | 2.196 | 1.055 | 0.049 | 0.140 | 0.051 |
| 3 | 460303 | FA2 | 0.549 | 0.870 | 0.000 | 0.000 | 0.665 | 0.351 | 0.353 | 0.785 | 0.809 | 0.457 | 0.355 | 0.280 |