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Prenatal flavour exposure through maternal diets influences flavour preference in piglets before and after weaning

J. Figueroa, D. Solà-Oriol, L. Vinokurovas, X. Manteca, J.F. Pérez*

Grup de Recerca en Nutrició, Maneig i Benestar Animal, Department de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

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ABSTRACT

Flavour cues present in amniotic fluid are used in mammals to find their mother early, but they are also useful for having contact with environmental flavours before birth. Three experiments have been performed to evaluate if piglets, during lactation and post-weaning, have the ability to prefer natural or artificial cues previously added to the gestating diets. For 7 min, pigs, in pairs, were offered a triple-choice stimulus through a Triple-U-Testing Arena among maternal amniotic fluid, alien amniotic fluid or water (Experiment 1) or among a flavour added to the late-gestation diet, a control flavour and water (Experiment 2). The same prenatal strategy was used to study the piglet's preference for flavoured or unflavoured creep feed during the suckling period (Experiment 3). Suckling piglets preferred amniotic-fluid flavours from their own mother over an alien amniotic fluid and they also preferred specific flavour cues given to the sows during late gestation (anise and milky-cheese). However, prenatal flavour exposure did not improve the preference of piglets for a flavoured compared to an unflavoured creep feed diet. Pre-natal exposure to flavours via maternal diet influences the piglet's preferences for new flavours, probably through a positive association between flavours and the hedonic reward of the uterine experience and a familiarity effect. Nevertheless, our results do not exclude alternative routes of exposure of the newborn piglets to sow feed odours. Preferences acquired before birth seem to be long-lasting. This may be an important factor to reduce neophobia for specific flavours in young pigs.

1. Introduction

Exposure to novel feed ingredients or flavours may cause negative emotions of fear and anxiety in the animals, which generally sample small amounts of feed in a phenomenon known as feed neophobia (Miller and Holzman, 1981). After weaning, piglets face a large list of stressful challenges, also including the need to eat new feed. During this period, piglets respond with variable periods of underfeeding and anorexia that make them vulnerable to diarrhoea, weight loss and an increase in the mortality rate (Madec et al., 1998). Solid feed included during lactation, or "creep feed", is frequently used to habituate animals to solid-feed intake. These diets may help to improve the development and function of the gastrointestinal tract and have been positively related to performance in the immediate post-weaning period (Bruininx et al., 2002). However, only few piglets tend to eat creep feed during the suckling period (Pluske et al., 2007).

Feeding behaviour in mammals is based upon genetic components, but also on learning; a process which is established along the one's entire life to create temporal or permanent preferences for some ingredients or flavours of the diet. Animals

* Corresponding author. Tel.: +34 93 581 1504; fax: +34 93 581 1494.
E-mail addresses: josefrancisco.perez@uab.cat, josefrancisco.perez@uab.es (J.F. Pérez).

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can learn feeding behaviour from their mothers (either at the prenatal or postnatal stage) (Schaal et al., 2000; Mennella et al., 2001; Nicolaïdis, 2008), from social interactions with con-specifics (Galef and Whiskin, 1998, 2000) or by trial and error tests (Ackroff et al., 2001; Sclafani, 2004; Dwyer et al., 2009).

Newborn mammals have the ability to approach their own mother after birth by the detection of the natural flavours of amniotic fluid (Hepper, 1987; Morrow-Tesch and McGlone, 1990; Soussignan et al., 1997) which are considered a useful tool for the animal's orientation in the nest area and the initiation of suckling. Moreover, a calming effect on newborn subjects could be driven by those sensorial cues released from the amniotic fluid, which is closely in contact with the newborn during the delivery process and just after birth (Varendi et al., 1998). Amniotic fluid also plays a fundamental role in prenatal learning and provides a first opportunity for the foetus to learn about safe and available feedstuffs present in the environment (Schaal et al., 2004; Hepper and Wells, 2006). Some flavours are known to be transferred in small amounts from the maternal diet to the amniotic fluid and milk (Nolte et al., 1992; Mennella et al., 1995; Hausner et al., 2008). Thus, Campbell (1976) reported that prenatal exposure to flavours through amniotic fluid may be an effective method to facilitate feed detection and reduce the time to the first feed contact in weaned piglets. However, Langendijk et al. (2007) failed to repeat those results, and neophobia after weaning was still affecting feeding behaviour. Oostindjer et al. (2010) did not find any effect of prenatal flavour exposure on the future preferences in weaned piglets, but they reported an increase in weight gain after weaning when animals were offered the flavoured-conditioned diet, likely associated with reduced stress of the animals.

The hypothesis tested in the present study is that flavour choices in piglets during the suckling period may be influenced by the prenatal experience with those flavours. The objectives are: (1) to determine whether suckling piglets show a preference for either the odour of maternal amniotic fluid or the odour of alien amniotic fluid (Experiment 1) and (2) to evaluate if pre-natal flavour exposure via maternal diet modifies flavour and creep-feed preferences in suckling and weaning piglets (Experiments 2 and 3, respectively).

2. Materials and methods

Three experiments were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (UAB). Experiment 1 was designed to study whether piglets prefer their own mother's amniotic fluid, when compared to the amniotic fluid of another sow; Experiments 2 and 3 were designed to evaluate whether prenatal flavour exposure through the maternal gestation diet can create flavour (Experiment 2) and feed preferences (Experiment 3) in suckling and post-weaning piglets. Experimental procedures were approved by the Ethical Committee on Animal Experimentation of the Universitat Autònoma de Barcelona.

2.1. Experimental design and diets

A total of 82 sows and their litters ([Large White × Landrace] × Pietrain) were used (10 litters in Experiment 1, 20 litters in Experiment 2, and 52 litters in Experiment 3). In Experiment 1, forty piglets (mixed sexes) coming from the 10 litters (four piglets/litter) were used to test their attraction for three olfactory stimuli (own mother's vs. alien mother's amniotic fluid vs. water) on days 4, 14 and 21 of life. In Experiment 2, a total of 20 sows (Large White × Landrace) were assigned to one of three diets for the last two weeks of pregnancy; two flavoured diets (anise flavour, active principle concentration 261 g/kg dosed at 0.75 g/kg or milky-cheese flavour, containing 200 g/kg of active principle dosed at 1.5 g/kg; Lucta SA; Montornès del Vallès, Spain; $n = 10$) and an unflavoured diet ($n = 10$). After farrowing, each sow ate a commercial, unflavoured lactation diet. Eighty male/female piglets (mixed sexes) coming from these 20 sows (four piglets/litter) were used to test their attraction for three olfactory stimuli (anise flavour vs. milky-cheese flavour vs. water in a triple-choice test arena, TUTA, Fig. 1) at days 14, 21 and 26 after birth (2nd post-weaning). An extended period of tests was performed to evaluate the persistence of choice differences between treatments before and after weaning. In Experiment 3, a total of 52 litters (10–12 piglets/sow) were used to evaluate the preference for flavoured creep diets, as compared to an unflavoured diet, if previously conditioned or not through prenatal exposure. Choice tests were performed from days 22 to 28 of lactation. Animals were separated into 3 treatments; Thirteen litters came from sows that, during late-gestation (two weeks before parturition), were offered a garlic flavoured diet (active principle concentration 94 g/kg dosed at 0.75 g/kg), other thirteen litters came from sows that, during late-gestation were offered an anise flavoured diet (active principle concentration 261 g/kg dosed at 0.75 g/kg; Lucta SA; Montornès del Vallès, Spain, Flavoured group), while the rest of the litters (26) came from sows that were fed an unflavoured diet (control group).

The piglets of each experiment were individually identified at birth by using a plastic ear tag, and they remained with their mother and littermates inside the farrowing crates during the entire suckling period (28 days), meeting the welfare standards in the European Union. The farrowing room was provided with a controlled temperature (22.4 ± 2.05 °C sow environment and 28.3 ± 2.70 °C piglet environment (HOBO U10, data logger; MA, USA) and automatic ventilation. Inside each crate, piglets had access to a heated area to provide a warm resting space, which was also enriched with shavings, sawdust and dry material (Biosuper CONFORT + Gratecap Services, La Rochelle, France). During the lactation period, following a farm's usual feeding routine, sows were fed twice a day, increasing the daily amount of feed offered according to litter size and the sow's body condition until *ad libitum* was reached after 1 week of lactation. Sows and piglets had *ad libitum* access to water. An unflavoured creep-feed diet was offered *ad libitum* from day 10 after birth onwards to litters in Experiments 1 and 2 by using a pan feeder. No solid feed was offered to piglets of Experiment 3 until the beginning of the choice tests.

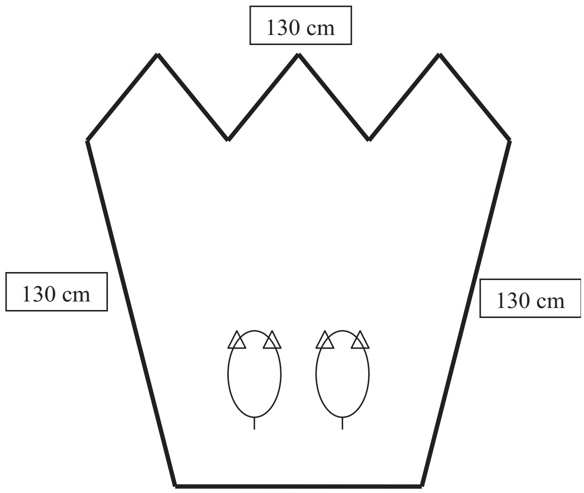

Fig. 1. Triple-U-Testing Arena (TUTA). Two sibling littermates were randomly selected and moved to the TUTA to conduct the triple-choice test. The saw-tooth area at the top of the figure corresponds to the triple inverted U area in which the corresponding cotton strips (impregnated in amniotic fluid, flavour or water) were hanged during the test.

2.2. Choice test procedures

Triple-choice tests were performed in Experiments 1 and 2 using the "Triple-U-Testing Arena" module (TUTA, Fig. 1), located in an isolated room close to the farrowing area. In Experiment 1, olfactory cues were tested by using adsorbent strips impregnated with amniotic fluid collected from their own mother (maternal amniotic fluid), alien amniotic fluid (mix of amniotic fluid from three alien sows), and water in the middle of the TUTA as a negative control. The placenta from each sow was collected and processed just after farrowing. Amniotic fluid was obtained by placenta compression with cheesecloth, which is a loose-woven gauze-like cotton cloth used primarily in cheese-making and cooking. Then, this fluid was centrifuged (3420 × *g*, 4 °C, 15 min) and filtered (0.22 μm Millipore filters). The liquid obtained was stored in 15 ml tubes and kept frozen (−20 °C) until test days. At the beginning of each test (9.00 am), tubes with maternal amniotic fluid and alien amniotic fluid contents were unfrozen by using warm water until reaching room temperature in order to have the samples ready to perform the test. During the test-period, TUTA's soil was completely covered with sawdust in order to simulate farrowing-pen conditions. Cotton strips were impregnated with maternal amniotic fluid, alien amniotic fluid or water and placed into the TUTA just before each test. The piglets selected for the test were temporarily removed from their mothers and placed in the middle of the TUTA in pairs of littermates in order to avoid fear and distractive behaviours during the test. The same pair of piglets was tested on the successive test days (days 4, 14 and 21 of life). Each test lasted 7 min, during which the time spent by the piglets in nasal contact with each strip was measured by direct observation by a previously trained observer. Strips were changed and the positions of maternal amniotic fluid and alien amniotic fluid were rotated for each litter-pair tested.

In Experiment 2, olfactory cues tested included strips impregnated with an anise flavour solution (0.375 g/L), milky-cheese solution (0.75 g/L), or tap water used as negative control at days 14, 21 and 26 after birth (2nd post-weaning). The flavours were identified as "maternal flavours" or "alien flavours", depending on the flavour included in the sow gestational diet (pre-exposed piglet) of the pair of piglets in each test. For control piglets (without previous exposure to the flavours), both flavours were considered to be alien. Flavour solutions were prepared with warm water and the choice test conditions were the same as in Experiment 1. The positions of maternal and alien flavours were also rotated between tests.

In Experiment 3, creep-feed choice tests were performed on each litter to test the preference for flavoured diets if previously conditioned (26 sows) or not (26 sows) through prenatal exposure by performing a six-day choice test at the end of the lactation period. The control diet (Table 1) included corn, barley and wheat (547 g/kg), sweet whey (141.2 g/kg), soybean protein concentrate (166 g/kg), spray-dried animal plasma (50 g/kg) and a vitamin and mineral premix (10 g/kg) without artificial flavours. The modified diet included one flavour (garlic or anise flavours; 0.75 g/kg, Lucta SA; Montornès del Vallès, Spain). Thirteen sows received an anise flavoured gestation diet, and their litters a choice between anise-creep feed and the control diet, and 13 sows received a garlic-flavoured gestation diet, and their litters a choice between garlic creep-feed and the control diet. In the control group (26 sows), flavours (garlic or anise) were incorporated into the creep feed during the choice test (13 groups were offered a choice between anise creep-feed and the control diet, and 13 groups a choice between garlic creep-feed and the control diet). Choice feeding tests were conducted in the farrowing pens by using the entire litter as the experimental unit, and two pan-feeders containing the two diets *ad libitum* in a mash form (side by side). Feeder positions (left/right; flavoured/unflavoured) were alternated between litters. At the end of the choice test (D6) the total litter intake of each feeder was calculated.

Table 1
Ingredient composition and calculated nutrient content (g/kg diet as fed basis) of the experimental diets of sows (gestation and lactation) and piglets (creep feed; Experiment 3).

	Diets		
	Gestation	Lactation	Creep feed
Ingredients			
Barley	272	220	220
Wheat	200	200	175
Corn	100	250	151
Wheat bran	160	50	
Soybean hulls	24		
Sugar beet pulp	75		
Gluten feed	50	25	
Canola meal	45	25	
Soybean meal 440 g CP/kg	30	174	25.4
Full fat soybean meal			165
Whey			141
Animal plasma 800 g CP/kg			50
Soybean oil			38
Lard	13.3	22.9	
Limestone	15.3	13.7	9.4
Monocalcium phosphate	5.2	7.9	8.5
Sodium bicarbonate	2.0	3.0	
Salt	3.0	3.0	2.7
L-Lysine HCL	1.1	1.3	4.8
DL-Methionine			2.4
L-Threonine			2.0
L-Tryptophan			0.5
Vit–min premix	4.0[a]	4.0[a]	4.0[b]
Flavours			0.75[c]
Calculated content[d]			
NE (MJ/kg of feed)	9.30	9.63	10.96
CP	135.0	167.8	204.4
Lysine	6.28	8.93	15.52
Methionine	2.19	2.66	5.01
Methionine + cystine	5.08	6.01	9.05
Threonine	4.88	6.11	10.38
Tryptophan	4.98	6.69	3.21
Ca	9.50	8.50	7.20
P total	5.67	6.10	6.07
P digestible	3.00	3.50	3.86

[a] Sows gestation and lactation diets Premix Supplied (g/kg): 12,500 IU of vitamin A, 2000 IU of vitamin D3, 20 mg of vitamin E, 2 mg of vitamin K3, 4 mg of vitamin B1, 5 mg of vitamin B2, 25 mg of vitamin B3, 2.6 mg of vitamin B6, 0.02 mg of vitamin B12, 12 mg of calcium pantothenate, 25 mg of nicotinic acid, 0.100 mg of biotin, 300 mg of choline-Cl, 100 mg of Fe, 10 mg of Cu, 0.5 mg of Co, 100 mg of Zn, 80 mg of Mn, 0.5 mg of I, and 0.22 mg of Se.

[b] Creep feed diet Premix Supplied (g/kg): 7000 IU of vitamin A (acetate); 500 IU of vitamin D3 (cholecalciferol); 250 IU of vitamin D (25-hydroxicholecalciferol); 45 mg of vitamin E; 1 mg of vitamin K3; 1.5 mg of vitamin B1; 3.5 mg of vitamin B2; 1.75 mg of vitamin B6; 0.03 mg of vitamin B12; 8.5 mg of D-pantothenic acid; 22.5 mg of niacin; 0.1 mg of biotin; 0.75 mg of folacin; 20 mg of Fe (chelate of amino acids); 2.5 mg of Cu (sulphate); 7.5 mg of Cu (chelate of glycine); 0.05 mg of Co (sulphate); 40 mg of Zn (oxide); 12.5 mg Zn (chelate of amino acids); 12.5 mg of Mn (oxide); 7.5 of Mn (chelate of glycine); 0.35 mg of I, 0.5 of Se (organic), and 0.1 mg of Se (sodium).

[c] Garlic or Anise flavours added to flavoured creep feed diets (Lucta SA, Montornès del Vallès).

[d] Nutrient contents were calculated based on feed evaluation values described by Fundación Española para el Desarrollo de la Nutrición Animal (Fedna, 2010).

2.3. Statistical analyses

Mean permanence times for each strip were compared by General Linear Models using the GEN-MOD procedure of SAS® (SAS Inst., Inc., Cary NC). In Experiment 1, the mathematical model used was, $Y_{ijk} = \mu + \alpha_i + \beta_j + \chi_k + \alpha\beta_{ij} + \alpha\chi_{ik} + \beta\chi_{jk} + \alpha\beta\chi_{ijk} + \varepsilon_{ijk}$, where Y_{ijk} is the time for the observations of each experimental treatment; μ is the general mean of all observations; α_i is the effect of treatment (maternal amniotic fluid, alien amniotic fluid and water); β_j is the effect of the age of piglets at the moment of the test (4, 14 or 21 days old); χ_k is the effect of the of the position of the treatments at the moment of the test (right or left hand respect to water); $\alpha\beta_{ij}$, $\alpha\chi_{ik}$, $\beta\chi_{jk}$ and $\alpha\beta\chi_{ijk}$ are the interactions between and among the experimental treatment, the age of piglets and the position of the treatments at the moment of the test; and ε_{ijk} is the unexplained random error.

In Experiment 2, the mathematical model used was, $Y_{ijk} = \mu + \alpha_i + \beta_j + \chi_k + \alpha\beta_{ij} + \alpha\chi_{ik} + \beta\chi_{jk} + \alpha\beta\chi_{ijk} + \varepsilon_{ijk}$, where Y_{ijk} is the time spend for the observations of each experimental treatment; μ is the general mean of all observations; α_i is the effect of treatment (maternal flavour, alien flavour and water); β_j is the effect of the age of piglets at the moment of the test (14, 21 and 26 days old); χ_k is the prenatal exposure [piglets coming from sows that ate (pre-exposed) or not (control) flavours during

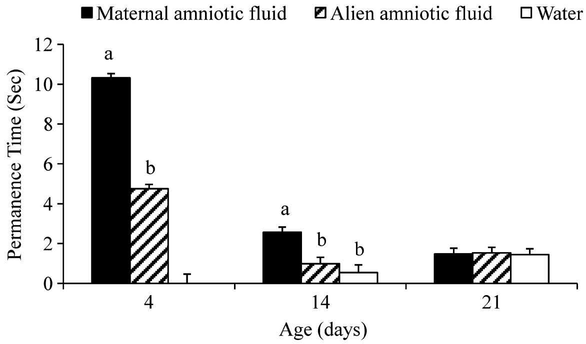

Fig. 2. Piglet's permanence time in contact with strips impregnated with maternal amniotic fluid, alien amniotic fluid and water for a 7-min preference test during lactation. Means with different subscripts are different (P<0.05). Bar size indicates SEM (Experiment 1).

late gestation]; $\alpha\beta_{ij}$, $\alpha\chi_{ik}$, $\beta\chi_{jk}$ and $\alpha\beta\chi_{ijk}$ are the interaction between and among the experimental treatments, age of the animals and the prenatal exposure; and ε_{ijk} is the unexplained random error. The maternal flavour treatment considered in the model included the two flavours counterbalanced gave them to the sows during the gestation (milky-cheese and anise). Prior to segregate the two different flavoured groups in one (maternal flavour) it was observed in a previous analysis that no effects were observed for the flavoured diets used prenatally (milky-cheese or anise) over piglet's preferences after birth. However, a significant interaction was observed between prenatal flavour exposure and the maternal flavour preference (either milky-cheese of anise).

Litters feed consumption during the choice test in Experiment 3 was analyzed with ANOVA by using mixed linear models with the MIXED procedure of the statistical package SAS® (SAS Inst., Inc., Cary NC). The following mathematical model used was: $Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij}$, where Y_{ijk} is the feed consumption for the observations of each flavour used; μ is the general mean of all observations; α_i is the effect of the flavour used (anise or garlic); β_j is the effect of litter origin (treated or control sows); $\alpha\beta_{ij}$ is the interaction between the flavour used and litter origin; and ε_{ijk} is the unexplained random error. Pen during the choice test was considered a random effect specifying the covariance structure of the residual matrix as completely general (unstructured). The mean values are presented as LSMeans. To test the hypotheses, P<0.05 adjusted by Tukey was considered significant.

3. Results

3.1. Experiment 1

The time spent by piglets in nasal contact with the different strips is shown in Fig. 2. Latency time to oro-nasal contact accounted for more than 6 min of the 7-min test. Older piglets became increasingly agitated, looking for alternative routes to escape, and they also had more playing and exploring behaviours in the TUTA. Piglets showed preferential responses towards maternal amniotic fluid (P<0.001), but these preferences disappeared with age (solution approached × piglet age, P<0.001). Compared to alien amniotic fluid and water, higher oro-nasal contact for maternal amniotic fluid was observed at days 4 and 14 (P<0.05). No differences were observed at day 21. On the first test day (D4), piglets not only showed more nose approach to strips impregnated with maternal amniotic fluid (P<0.01), but they also spent more time near this area. Between days 4 and 14, piglets showed a dramatic decrease in their attraction for placenta solutions; despite this, they again showed longer residence time near maternal amniotic fluid than alien amniotic fluid on day 14 (P<0.05).

3.2. Experiment 2

Prenatal flavour exposure via maternal diet significantly influenced a piglet's preferences during lactation (P<0.001, Fig. 3). Piglets born from flavour-treated sows showed preferential responses towards maternal flavours on D14 and D21 (during lactation) and D26 (after weaning) of age, as compared to alien flavours and water. No different preferences were observed between flavours for control piglets. In addition, piglets born from sows fed flavour-supplemented diets during late gestation also had a higher number of oro-nasal contacts with the corresponding flavour than did control piglets during preference tests on days 14 (P=0.08), 21 (P<0.05) and 26 (P<0.05). These preferences acquired before birth for the prenatally exposed flavour were long-lasting, as they were still observed two days after weaning (D26). However, latency time in the post-weaning test to oro-nasal contact also accounted for more than 6 min of the 7-min test. Post-weaning piglets (D26) showed less time spent by piglets in nasal contact with the different strips than did lactating piglets (P<0.05), expressing more exploration and playing behaviours in the TUTA module. No different preferences were observed between flavours used as prenatal cues (anise or milky-cheese). Both flavours showed high preferences on all testing days in the pre-exposed group (P<0.05).

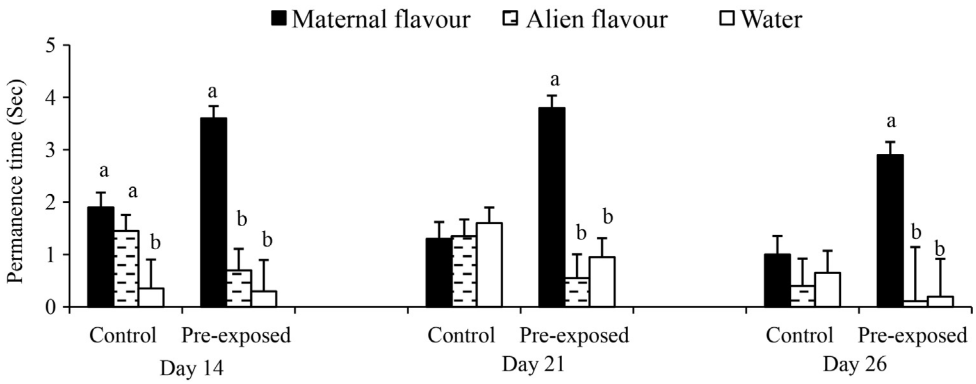

Fig. 3. Piglet's permanence time in contact with strips soaked with different fluids (maternal flavour, alien flavour and water) during a 7 min. preference test on days 14, 21 and 26 of lactation. Piglets were organized as control, piglets coming from control sows, or pre-exposed, piglets coming from sows that ate flavours during late gestation (anise 0.75 g/kg or milky-cheese 1.5 g/kg). Means in the same group and day with different subscripts are different (P<0.05). Bar size indicates SEM. (Experiment 2).

3.3. *Experiment 3*

When the litters had the opportunity to choose between the unflavoured and flavoured creep-feed, control animals as well as piglets coming from sows that ate the flavoured diet during late-gestation preferred the unflavoured diet (P<0.001, Fig. 4). No differences on creep-feed intake were observed between flavour treatments (garlic vs. anise flavour; P>0.05).

4. Discussion

In the wild there are plenty of examples where newborns show an innate ability to find maternal protection. Piglets that quickly follow their mother have more chances to survive than do piglets that stay behind (Graves, 1984). However, a question that arises is how newborn piglets know what kind of environmental cues they have to follow without undergoing an apparent learning process. In the present study, piglets were able to prefer the contact with amniotic fluid over water during the first days of life. This attraction was stronger in the first days after birth (D4). These results agree with previous results described in rats (Hepper, 1987), humans (Varendi et al., 1998) and rabbits (Coureaud et al., 2002). Thus, it seems that piglets may be familiarized before birth by oro-sensory cues from the amniotic fluid. This prenatal learning could help the newborn to find mother and teat, as occurs in several species (Hepper, 1987; Morrow-Tesch and McGlone, 1990) by following maternal olfactory cues impregnating other newborn piglets or the mother's skin.

This positive orientation towards maternal fluids once again suggests the existence of a transnatal olfactory continuity in mammals. Previously, Parfet and Gonyou (1991) showed that piglets can discriminate between and prefer maternal fluids from water. Our results also showed that piglets may discriminate between their mother's amniotic fluid and amniotic fluid from other sows during their first weeks of life (Morrow-Tesch and McGlone, 1990). Amniotic fluid may contain unique and specific cues that allow piglets to prefer their own mother's proximity. Amniotic fluid smell also can help the newborn to recognize its own siblings as related individuals, thus achieving benefits in their fitness by responding differentially to kin and non-kin (Hepper, 1986). Free fatty acids and proteins that transport hydrophobic components in maternal fluids could constitute a chemical signature that contributes to the successful transition between the pre-natal and post-natal environments (Guiraudie-Capraz et al., 2005).

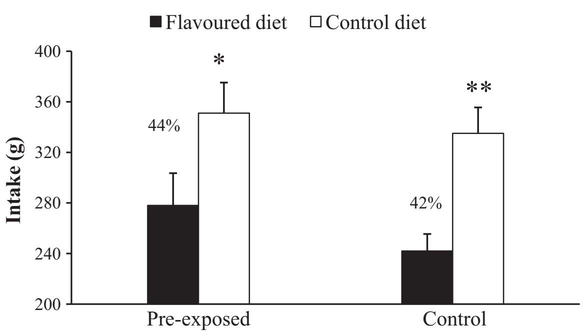

Fig. 4. Creep feed average intake of 3 weeks-old suckling piglets litters during a 6-day choice test between a flavoured diet vs. a control unflavoured diet. Piglet litters were classified as pre-exposed or control, depending of sows received or not received the flavour cues in feed during late gestation (2 weeks). Numbers in the top of bars indicate the average percent intake of flavoured feed as compared to total feed intake. Asterisks indicate that control diet creep feed intake is significantly different than flavoured creep feed intake (*P<0.005, **P<0.001). Bar size indicates SEM (Experiment 3).

Experiment 1 showed that 14-day-old piglets still preferred the amniotic fluid odour of their own mother. This persistence proves that maternal amniotic fluid odours not only could help them to look for their mother's teats (Morrow-Tesch and McGlone, 1990), own blood siblings (Hepper, 1986) or protection, but may also act as hedonic, long-lasting cues. After 14 days, piglets still need maternal contact to satisfy their feeding requirements. However, from this time-point on, piglets also need to explore the environment and learn from it, and milk, rather than amniotic fluid, could arise as a more important key stimuli. In natural environments, piglets are exposed to a lot of new challenges, and trial- and error-learning is essential in nature. Animals have to explore and learn more to know what the new rules of life are in a continuously changing environment (Galef, 1995), where challenges are gradual and distributed over time. However, in intensive pig production, animals experience sudden changes with little time to choose or learn on the basis of trial and error.

One strategy to reduce this psychological impact in piglets is to give them links between one step and another that allow them to feel a familiar connection and reduce stress (Oostindjer et al., 2011). The common milk replacers, creep feed and weaning diets, for young animals usually contain dairy products which help to smooth the transition between suckling and eating solid feed as well as to satisfy lactose requirements. Offering creep feed during the last lactation days is used as a strategy to reduce neophobia, however, just a small percentage of piglets eat this feed at this period (Pluske et al., 2007). In our second experiment, we confirmed that young piglets were able to prefer anise and milky-cheese supplemented in the maternal gestating diet, showing that either some artificial cues can change the amniotic-fluid environment or piglets become exposed to flavours right after birth by direct contact to the sow or the sow's feed. This flavour continuity, may act as a psychological link, bringing to the piglets' mind the hedonic remembrance of the maternal environment during lactation or post-weaning periods (Arias and Chotro, 2006). Even if positive effects over pig stress and intake have been reported (Oostindjer et al., 2009), just a few piglets studies have been successful proving that preferences can be modified (Campbell, 1976). Several studies in other species have ratified our results by using other or the same flavours, showing that this prenatal or perinatal experience can influence later preferences for those specific flavour cues (Mennella et al., 2001; Hepper and Wells, 2006; Nicolaïdis, 2008; Simitzis et al., 2008). However, the positive effect of anise and milky-cheese during the second experiment does not necessary mean than all the flavours may be use as a pre-natal stimuli and the extrapolation of these findings to other flavours should be done with caution by looking for flavours with similar metabolic routes and stability.

Amniotic fluid may be a key factor in preferences after birth contributing to inhibit aversive responses before sensorial stimuli. This inhibition may be due to a familiar learning effect (repeated prenatal exposure) that would reduce neophobia, but it could also be due to an associative learning effect during pregnancy. The amniotic fluid and comfort of the womb could be associated with sensory stimuli and create a strong preference lasting long after birth. It has been reported that animals associate the flavours of amniotic fluid with the hedonic properties of this liquid (Arias and Chotro, 2007). However, piglets could also have experienced early contact with the diet of their mother, becoming indirectly familiarized with flavour cues contained in sows' diet. This could also be another way in which piglets could increase their range of familiar compounds, reducing the negative impact before new feeds at weaning. The long persistence of preferences in Experiment 2 shows us that we were probably faced with an associative learning process rather than with a familiarity or mere-exposure process. The positive hedonic uterine environment can act as unconditioned stimuli (US) and the amniotic fluid flavour as conditioned stimuli (CS) during late gestation. The association of US and CS can create a hedonic preference for the amniotic fluid flavour (Myers and Sclafani, 2001, 2006; Arias and Chotro, 2007). Despite this, we cannot rule out a synergy effect between the non-associative and associative components of learning.

The hypothesis in Experiment 3 was that a new flavour in the creep-feed diet previously added to the final gestation diet of sows could increase the intake instead of creating a neophobic behaviour in the lactating piglets. However, our results refuted the initial hypothesis and confirmed that aversion also appeared in piglets that came from sows that eat this flavour at gestation. A likely preference for the prenatal flavour in creep-feed diets was not found. This result is important because it shows that unflavoured creep feed is more preferred than is flavoured creep feed, likely because of neophobia before new cues. A possible reason could be that in these choice tests, the flavoured creep feed was compared versus a control unflavoured diet. Both diets contain dairy ingredients, but also some of the ingredients contained in the sow's diet (corn, barley, wheat or soybean protein concentrate), and likely dietary cues that piglets prefer because of their previous exposure to the sow (amniotic fluid, sow milk or fluids) or sow's diet.

Therefore, we can argue that a higher variety of ingredients in the sow's diets throughout gestation may help foetuses (unborn piglets) to be exposed to more flavoured cues during gestation, thus reducing the risk of future cases of neophobia (Mennella et al., 2001). Moreover, diet variety is also important to reduce sensorial specific satiety (Provenza, 1996) and to improve animal welfare, as it allows animals to satisfy their real individual nutritional requirements (Manteca et al., 2008). The prenatal learning process of feeding behaviour has no significant cost for maternal requirements and welfare. Animals would teach their offspring in a passive way, showing what kind of flavours they will face in the extra-uterine life just by eating the available and safe nutrients during gestation. This is probably the most elevated rate of cost/benefit in the feeding learning process (Galef et al., 2005).

5. Conclusions

Amniotic fluid becomes the first extra-uterine hedonic flavour in pigs, helping newborn animals to approach their own mother. Moreover, specific flavour components included in the sow diet during late gestation may change flavour preferences of piglets. These newly established preferences could be used to reduce flavour or feed neophobia in piglets during critical phases, such as at weaning.

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