MATERNAL LEARNING AND CREEP-FEEDING: TWO STRATEGIES DETERMINING THE POST-WEANING PERFORMANCE OF PIGLETS

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Als meus pares, el Josep Mª i la Francesca.

Per ser un exemple a seguir,
i pels valors que m'heu transmès.

COM EL BATEC DE L'AIGUA EN ELS NIELLS

M'ho deia la mare: tu i jo, un dia,
Pujarem a una petita barca i navegarem
mar endins; serà tan petita la barca que
només allargant una mica el braç
podrem acaronar l'aigua, només
allargant una mica el braç. Jo era petit,
tanmateix petit, i la mare semblava tenir
totes les forces per a ben menar la barca.

M'ho va dir el vent, molt més tard: no sempre
trobaràs un port o un amic; ni tan sols
totes les promeses es poden complir.
Jo havia crescut, de sobte, i els rems
ja doblegaven les tremoloses mans de la mare.
Sol i amb el llast de la urgència
vaig fer-me a la mar. Pirata, un temps, vaig ser,
em bategava el cor com l'aigua en els niells
sempre a proa i amb vents favorables
un tresor d'algues em floria a cada cala.

Mal oratge, altre temps, s'esdevingué, i no tenia
ni sabia llibants on amarrar la barca, nàufrag
de mi mateix a la deriva fressava mars feréstegues
sense ports ni albades. D'una bonança
vaig aprendre que tot tal com arriba passa
i ara m'he tornat pescador, pescador del temps
que em resta i treballo ferm totes les jornades
i cada vesprada, a atzur morent, deleixo sempre més
aquella petita barca, una mà acaronant l'aigua, l'altra
encaixada a la de la mare.

Josep Civit i Mateu
Montblanc, 2006

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Glossary

CF	Creep-Feed
CS	Conditioned Stimulus
CTA	Conditioned Taste Aversion
ETEC	Enterotoxigenic *Escherichia coli*
G	Gestation
GIT	Gastrointestinal Rract
L	Lactation
SDBM	Spray-Dried Blood Meal
SDP	Srpay-Dried Plasma
TUTA	Triple-U-Testing Arena
UAB	Universitat Autònoma de Barcelona
US	Unconditioned Stimulus
W	Weaning

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SUMMARY / RESUMEN / RESUMÉ

In the commercial pig industry piglets are abruptly weaned at 21 or 28 days of age becoming the most critical period for pigs. During this period piglets suffer stress, neophobia to new food and anorexia in response to the sudden separation of piglets from their mothers, mixing with others litters, reallocation to a different environment and pass from a liquid diet (milk) to a solid food. There are some strategies to reduce the negative impact of weaning: (i) promoting the early creep-feed intake and number of eaters, (ii) utilising good quality, high digestibility and palatable feedstuff, (iii) including flavours and taste enhancers in the diet, but not always increases fed intake. Recent studies support the use of flavours in the sow and piglets diets as a means of promoting maternal learning. It has been suggested that flavour cues from the maternal diet can reach the foetus through the amniotic fluid and/or the placental blood stream. The early exposure of foetus to certain volatiles may result in a further preference for these compounds later in life and consequently can positively affect the acceptance of food containing a similar flavour before and after weaning.

The present study is based on maternal learning to increase feed intake and performance at weaning. The flavour studied is Fluidarom 1003®, a commercial flavour based on >25 % of anethole and cinnamaldehyde and more than 10 % of eugenol as major volatile compounds. The study is divided in three trials, the first one previously carried on at SNiBA (Servei de Nutrició i Benestar Animal), what it was the basis for the hypothesis and objectives of our second and third trials. In this work we describe all the three trials to easily understand the global results.

1-<u>First trial:</u> the aim of this trial was to determine the effects on piglet performance of Fluidarom 1003® supplementation in the sows diets, during late gestation (28 days before farrowing) and lactation, and in the weaning diets. We measured sow's performance and piglet's productive parameters during pre-starter and starter phase (0-35 d). There was not difference in sow's performance related to flavour. Higher feed intake and growth were observed along the pre-starter and starter phase (0 to 35 days post-weaning) mainly due to the maternal exposure to the flavour. Inclusion of Fluidarom 1003® in the sows diets was linked with the presence of flavour in the creep-feed, so piglets probably consumed a higher quantity of creep-feed (uncertain). However, we did not register creep-feed intake so were not able to distinguish the maternal effect from creep-feed effect.

2-Consequently, the <u>second trial</u> was based on the uncertain of the first. We aimed again to find out at what extent Fluidarom 1003® affect sow's and piglet's productive and performance parameters, including the determination of creep-feed consumption and evaluating the effect of maternal learning. In order to evaluate the effect of maternal learning on Day 2 after weaning we conducted a double choice test (DCHT) for thirty minutes between two diets supplemented either with Fluidarom 1003® or Lacto-Vanilla (negative control) based on >25 % vanillin and more than 10% of butyric acid and diacetyl. Sow productive parameters did not show difference between treatments. Piglets consumed more unflavoured creep-feed, and those piglets perinatally exposed to flavour with unflavoured creep-feed showed the highest ADFI and BW. DCHT in control animals (without any previous exposure to flavours) showed a high preference for the Lacto-Vanilla. Those animals previously exposed to flavour by maternal diet and creep feed showed no preference for any aroma. But those piglets exposed perinatally to Fluidarom 1003® but not in the creep-feed showed a high preference for the Fluidarom 1003®.

3-In the third trial we go into detail about if each one, pre-natal (foetal environment) and post-natal (maternal milk) contact by their own is able to contribute to reinforce the positive reward and preference in piglets after weaning. We performed an experimental design to discern the independent role of Fluidarom 1003® inclusion (with or without) in late gestation, lactation and in creep-feed. We performed the double choice test (DCHT) like in the Trial 2. The results showed again that control pigs preferred the Lacto-Vanilla flavour. The other treatments did not prefer any flavour. The piglets prenatally exposed to the aroma but with unflavoured creep-feed consumed more feed with Fluidarom 1003® than Lacto-Vanilla, however it was not statistically significant.

It is concluded that the inclusion of Fluidarom 1003® in the sow diets for late gestation and lactation improved piglets' appetite when the same flavour is included at weaning. The positive reward associated to Fluidarom 1003® included in the sow diet is stronger if piglets are offered a non-flavoured creep feed. It was also observed that only exposure to the end of gestation would be enough to condition the piglets.

Key words: creep-feed, maternal learning, flavour, piglet, preferences

El destete de los lechones a edades tempranas (21-28 días) es la etapa más crítica en la producción intensiva de ganado porcino. Durante este periodo es frecuente que los lechones experimenten estrés, neofobia al pienso y anorexia provocada por la separación de la madre, el cambio de lugar y la dieta (de leche a pienso sólido). Para suavizar el destete se utilizan dietas palatables con alimentos frescos y de alta calidad, incluyendo aromasy potenciadores del sabor, que no siempre funcionan. También se da el pienso "creep-feed" durante la lactación para que los lechones se acostumbren al pienso sólido. Recientemente se ha vuelto a poner sobre la mesa la posibilidad de utilizar estrategias de aprendizaje temprano basado en la incorporación de aromas en las dietas maternas. Las claves aromáticas llegan hasta el feto mediante el líquido amniótico y/o el flujo sanguíneo placental. Esta previa exposición puede resultar en una preferencia por este aroma que permanece en el tiempo y en consecuencia, podría facilitar la aceptación del alimento con un aroma similar antes y después del destete.

Este estudio se basa en el aprendizaje materno para mejorar la adaptación del lechón durante el destete. El aroma estudiado es el Fluidarom 1003®, un aroma comercial basado en >25 % de anetol y cinamaldehido y más de un 10 % de eugenol, como compuestos principales. El estudio está dividido en tres ensayos, el primero realizado previamente en el SNiBA (Servei de Nutrició i Benestar Animal) ha servido de punto de inicio para el planteamiento de las hipótesis y objetivos de nuestro segundo y tercer ensayo. En la presente memoria describiremos los 3 ensayos para que el lector pueda comprender mejor el conjunto de resultados.

1-En el primer ensayo el objetivo era determinar las consecuencias productivas de añadir Fluidarom 1003® en la dieta de las cerdas (final gestación y lactación), así como en la ración que se administraba a los lechones durante el destete. Se registraron los parámetros productivos de las cerdas y los rendimientos de los lechones durante la transición (0-35 d). No se observó ninguna diferencia en los rendimientos de las cerdas asociada a la administración del aroma. Se observó durante toda la transición (0-35 d) un incremento de la ingestión y del peso vivo de los lechones expuestos perinatalmente al aroma. De estos resultados planteamos la incertidumbre de si el aroma en la dieta de las cerdas y en el pienso "creep-feed" se daba conjuntamente, es posible que el efecto se deba en parte a que los lechones consumieron más cantidad de pienso "creep-feed". Sin embargo, no se registró la ingestión de pienso "creep-feed" y no se pudo discernir el efecto maternal (gestación y lactación) del efecto "creep-feeding".

2-Partiendo de esta incertidumbre se realizó el segundo ensayo, donde esta vez se volvió a evaluar los efectos de la inclusión de Fluidarom 1003® pero teniendo en cuenta el factor "creep-feeding". Se registraron los parámetros productivos de las cerdas y los rendimientos de los lechones durante la transición (0-14 días), así como el consumo del pienso "creep-feed". Para poder evaluar el efecto del aprendizaje materno se realizó un test de preferencia doble (DCHT) durante treinta minutos a los 2 días post-destete entre el Fluidarom 1003® y el aroma Lácteo-Vainilla (control negativo) basado en más de un 25 % de vanilina y más de un 10 % de ácido butírico y diacetilo. No se observaron diferencias en los parámetros productivos de las cerdas. Los lechones, consumieron más pienso "creep-feed" sin aroma; y en la transición el mayor consumo y crecimiento se registró en los animales perinatalmente expuestos al Fluidarom 1003® pero sin aroma en el pienso "creep-feeding". En el DCHT los animales control (los que no recibieron nunca aroma) mostraron una alta preferencia hacia el Lácteo-Vainilla. Sin embargo, los animales previamente expuestos al aroma mediante la dieta materna no mostraron preferencia por ningún aroma. Pero aquellos lechones expuestos perinatalmente al aroma pero no en el pienso "creep-feed" mostraron una mayor preferencia por el aroma.

3- En el tercer ensayo se quiso profundizar más en conocer la etapa que resulta más importante para configurar el aprendizaje del lechón por el nuevo flavour. Se realizó un diseño experimental que nos permitiera discernir el papel independiente que ejerce la inclusión de Fluidarom 1003® (con o sin) en la dieta materna de final de gestación, de la lactación, y en el pienso "creep-feed" que se administraba a los lechones durante la lactación. Para ello se realizó un DCHT en las mismas condiciones que en el segundo ensayo. Los resultados volvieron a mostrar que los lechones control, preferían el aroma Lácteo-Vainilla. El resto de tratamientos no presentaron diferencias significativas en la preferencia entre aromas. Los lechones pre-natalmente expuestos al aroma pero con un pienso creep-feed sin aroma añadido consumieron más pienso con Fluidarom 1003® que de Lácteo-Vainilla, pero sin ser estadísticamente significativo.

En conclusión, la incorporación de Fluidarom 1003® en la dieta de las cerdas (final de gestación y lactación) y la administración de un pienso "creep-feed" sin aroma es suficiente para crear en los lechones una mayor apetencia al consumo de pienso postdestete con el aroma incluido. También se aprecia que dar el aroma solamente al final de gestación sería suficiente para condicionar a los lechones al aroma.

Palabras clave: "creep-feed", aprendizaje materno, aroma, lechón, preferencia

Le sevrage des porcelets à un âge précoce (21-28 jours) est l'étape la plus critique dans la production porcine intensive. Pendant cette période, les porcelets souvent expriment le stress, la néophobie alimentaire et l'anorexie causée par: la séparation de la mère, le changement d'emplacement et de l'alimentation (lait par l'aliment solide). Pour faciliter le sevrage des aliments appétissants sont utilisés avec des produits frais et de haute qualité, y compris les arômes, qui ne fonctionnent pas toujours. On peut également donner du "creep-feed" au cours de l'allaitement, afin que les porcelets s'habituent aux aliments solides. Récemment, on est revenu sur la possibilité d'utiliser des stratégies d'apprentissage précoce basé sur l'incorporation d'arômes dans l'alimentation maternelle. Des touches aromatiques atteignent le liquide amniotique et le flux de sanguin fœtal et / ou placentaire. Cette exposition prénatal précoce du fœtus aux touches aromatiques peut entraîner une préférence pour cette arôme qui reste au fil du temps et peut donc faciliter l'acceptation de la nourriture avec un arôme similaire avant et après le sevrage.

Cette étude est basée sur l'apprentissage maternel pour améliorer l'adaptation du porcelet pendant le sevrage. L'arôme étudiée est Fluidarom 1003®, une arôme commerciale basée en >25 % de l'anéthole et de l'aldéhyde cinnamique et plus de 10 % d'eugénol. L'étude est divisée en trois essais, le premier réalisé précédemment dans SNiBA (Servei de Nutició i Benestar Animal) a servi de point de départ pour planifier les hypothèses et les objectifs de notre deuxième et troisième essais. Dans cette mémoire, nous décrivons les 3 essais pour que le lecteur puisse mieux comprendre l'ensemble de résultats.

1-Dans le premier essai L'objectif était de déterminer les conséquences productives de l'ajout de Fluidarom 1003® dans l'alimentation des truies (en fin de gestation et de l'allaitement) et aussi dans la ration qui a été administré aux porcelets durant le sevrage. Les paramètres productifs des truies et les rendements des porcelets ont été enregistrés au cours de la transition (0-35 d). Il n'y avait aucune différence dans les performances de truies associées à l'administration de l'arôme. On a observée tout au long de la transition (0-35 d) une augmentation de la consommation et le poids vif des porcelets exposés pendant la période périnatale à l'arôme. A partir de ceci se pose l'incertitude de savoir si l'arôme dans l'alimentation des truies et l'aliment "creep-feed" a été donné en même temps, il est possible que l'effet est dû en partie au faite que les porcelets consommaient plus de quantité de l'aliment "creep-feed". Cependant, on n'a pas enregistré une consommation de l'aliment "creep-feed" et on ne pouvait pas distinguer entre l'effet de la mère (gestation et allaitement) et l'effet "creep-feeding".

2-Sur la base de cette incertitude on a réalisé le deuxième essai, où cette fois on a essayez d'évaluer les effets de l'inclusion du Fluidarom 1003® mais en tenant compte le facteur "creep-feeding". Également on a enregistré de nouveau les paramètres productives et rendements des porcelets au cours de la transition (0-14 jours), en plus de la consommation de l'aliment "creep-feed". Afin d'évaluer l'effet de l'apprentissage maternel on a effectué un test de préférence double (DCHT) pendant trente minutes aux cours du 2e jour après le sevrage entre Fluidarom 1003® et l'arôme Lait-Vanille (contrôle négatif) basé sur plus de 25 % de la vanilline et plus de 10% de l'acide butyrique et le diacétyle. Les résultats ont montré qu'il n'y a pas de différence dans les performances des truies. Les porcelets ils ont consommé plus "creep-feed" sans arôme, au cours de la transition la plus grande consommation et croissance se sont produites chez les animaux exposés pendant la période périnatale à Fluidarom 1003® mais sans arôme dans "creep-feed". Au cours du 'DCHT' les animaux témoins a montré une forte préférence pour le Lait-Vanille. Toutefois, les porcelets exposés pendant la période périnatale n'ont montré aucune préférence pour arôme. Mais les porcelets exposés pendant la période périnatale à l'arôme mais pas dans "creep-feed" ont montré une plus grande préférence pour le Fluidarom 1003®.

3-La troisième essai était destiné à connaitre l'étape la plus importante pour configuré l'apprentissage du porcelet pour la nouvelle arôme. On a réalisé un modèle expérimental qui a permis de discerner le rôle indépendant que exerce la inclusion de Fluidarom 1003® (avec ou sans) dans le régime alimentaire de la mère de la fin de gestation, de lactation et dans "creep-feed" qui a été administré aux porcelets pendant la lactation. Pour cela un 'DCHT' a été réalisé dans les mêmes conditions que dans la 2e essai. Les résultats ont montré que les des animaux témoins, préféraient l'arôme Lait-Vanille. Le reste des traitements ne présentent pas de différence significative pour la préférence entre les arômes. Les porcelets pré-natalement exposé à l'arôme mais avec "creep-feed" sans arôme ajoutée ont consommé plus d'aliments avec Fluidarom 1003® que du Lait-Vanille, mais sans être statistiquement significative.

En conclusion, l'incorporation de Fluidarom 1003® dans l'alimentation des truies (en fin de la gestation et de la lactation) et de "creep-feed" sans arôme suffit pour les porcelets à créer un appétit d'ingestion d'aliments de post sevrage avec l'arôme inclus. Il a été montré que donner l'arôme seulement à la fin de gestation serait suffisante pour crée une préférence pour l'arôme.

Mots clés: "creep-feed", apprentissage de la mère, arôme, porcelet, préférence

1. INTRODUCTION

THE WEANING

Commercial weaning is a sudden change in piglet life during which piglets have to be adapted to eat a novel food (usually solid diet) after the separation from their mother. In natural conditions weaning is a gradual process that piglets perform during a long lactation and may be not completed until 17 weeks of age (Jensen, 1988). However, in the modern pig industry it is commonly used the "early weaning" with the objective of reducing and improving sow productive cycle. It means that weaning often occurs abruptly at 21-28 days of age and becomes a stressful process.

Weaning for piglets represents a period of adaptation and stress in response to the simultaneous stressors imposed on pigs at weaning. The sudden separation of piglets from their mothers, mixing with others litters and reallocation to a different environment drive piglets to exhibit aggressive behaviour while leading to the formation of a new social group (Varley and Wiseman, 2001). Moreover, piglets at weaning are switched from highly-digestible milk to a less-digestible more-complex solid feed, composed by cereals (starch source) and vegetal proteins (Pluske et al., 2003) and often supplemented with artificial flavours and savouries.

As a consequence piglets usually suffer a post-weaning "growth check" which last 7-14 days (Pluske et al., 2003). Piglets respond with low and variable feed intake or directly by stop eating, poor and variable growth rate and increased susceptibility to enteric pathogens. Anorexia brings about gastrointestinal disturbances; with alterations in small intestine architecture and enzyme activities, transiently-increased mucosal permeability, disturbed absorptive-secretory electrolyte balance and altered local inflammatory cytokine patterns (Lallès et al., 2007). The major diseases are diarrhoea, particularly from enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* (Pluske et al., 2003; Lallès et al., 2007).

For this reason main strategies to reduce the negative impact of weaning focus into the objective of reducing stress and anorexia (Pluske et al., 1997; Varley and Wiseman, 2001); such as by weaning pigs at older ages, creating eaters of creep-feeding, avoiding environmental challenges, and utilising good quality, high digestibility and palatable feedstuff. Recent studies support the use of flavours in the sow and piglets diets as a means of promoting maternal learning.

In conclusion, weaning is a major challenge in commercial pig husbandry, which requires an effort to promote an early feed intake of the animals. The aim of this work was to explore whether providing strategies of maternal learning may increase the opportunities of piglets to increase food intake before and after weaning. This work is based on the results of a previous study performed in our group.

2. THE PREVIOUS STUDY

EFFECT OF DIETARY (GESTATION & LACTATION) FLUIDAROM 1003® INCLUSION ON SOW, LITTER AND POSTWEANING PIGLET PERFORMANCE

Our background is based on the results obtained after sows and piglets diet supplementation with Fluidarom 1003® (Norel, S.A Spain) that is a commercial flavour based on more than 25% of anethol and cinnamaldehyde and more than 10% of eugenol as major volatile compounds.
The hypothesis proposed that dietary Fluidarom 1003® supplementation in gestation and lactation diets could represent a familiar cue for piglets that may facilitate the initiation to feed intake at weaning. The aim of this trial was to determine the effects on piglet performance of Fluidarom 1003® supplementation in the sows diets, during late gestation (28 days before farrowing) and lactation, and in the weaning diets. A total of 80 primiparous and multiparous sows were included in the study and, at weaning 480 piglets were moved to the weanling unit and split into four experimental treatments following a 2 x 2 factorial arrangement (Table 1). The main factors were the maternal contact with Fluidarom 1003® and the Fluidarom 1003® inclusion in weaning diets (pre-starter and starter) until 35d post-weaning.

Table 1. The experimental treatments.

Treatment Sow (G+L+CF)	Fluidarom 1003®	Treatment After weaning	Fluidarom 1003®
T-1	- (CG)	T-1 T-3	- +
T-2	+ (TG)	T-2 T-4	- +

+: inclusion of Fluidarom 1003® (375g/Tm) **-**: No inclusion of Fluidarom 1003®

Throughout the study, sow's body weight, body condition scores, backfat thickness and feed intake were measured. Sow's productive parameters per litter, number of total piglets born, born alive and stillbirth, weight of piglets born alive and stillbirth, number of weaned piglets, and pre-weaning mortality were measured by sow. Piglets were weighted at farrowing and when litter size was standardized after cross-fostering, and at weaning. Feed disappearance was recorded on days 7, 14, 21, 28 and 35 post-weaning. Average daily feed intake (ADFI), average daily gain (ADG) and feed: gain ratio (FGR) was calculated.

The relevant results of this study were that inclusion of Fluidarom 1003® in sow's diet and simultaneously in creep-feed:

1) Produced an increment in the litter body weight at weaning (73.5 versus 66.7 kg, P=0.04).

2) Significantly raised the feed intake of weaning phase and the body weight of piglets on days 7, 14, 21, 28 and 35. The body weight of piglets at Day 35 was 20.06 versus 19.35 kg (P = 0.048) in piglets from sows fed diets with or without Fluidarom 1003® supplementation, respectively.

3) The treatment without Fluidarom 1003® in the sow diet and in weaning diet showed the lowest consumption and body weight at the end of transition.

4) The inclusion of Fluidarom 1003® in weanling diets also showed a significant increases in feed intake (P = 0.010).

With these results it can be concluded that the inclusion of 375 g/Tm of Fluidarom 1003® in the sow diets for late gestation and lactation improved weight gain and feed intake of weanling piglets throughout the pre-starter and starter phase. However:

These results show a higher effect associated to the incorporation of the flavour to the sow's diet. If we take into account that inclusion of Fluidarom 1003® in the sows diets was linked with the presence of flavour in the creep-feed, we could suggest that piglets probably consumed a higher quantity of creep-feed. However, we did not registered creep-feed intake so we were not able to distinguish the maternal effect from creep-feed effect.

Consequently, to deal with this uncertain, in this study we aim to differentiate the likely role of creep-feed consumption on the observed Fluidarom 1003® effects.

3. LITERATURE REVIEW

3.1 PIGLET AT WEANING: the low feed intake

Weaning in natural conditions represent a gradual transition from a liquid diet to a solid diet, over a period of several weeks. This gradual transition (exposure to solid feed) leads to piglet to stimulate enzyme system and develop gastrointestinal tract (GIT) (Gestin et al., 1997; Makkink et al., 1993) and also provides time for changes in the ecology of the GIT (microbial population adapts to the new feed inputs) (Buddington, 1998). On the other hand the weaning on commercial pig industry occurs abruptly 21 to 28 days of age, and pass from a liquid diet of 20% DM to a compound diet with 85% DM (Varley and Wiseman, 2001). The nutritional, psychological and environmental changes produces stress response, and anorexia on the first days after weaning (Pluske et al., 2007); even some piglets abstain from eating for over 50 hours (Bruininx et al., 2002).

There is positive relationship between feed intake and villous height or villus/crypt ratio (Pluske et al,. 1996), so it seems that low feeding intake immediately after weaning is responsible for some changes in gut morphology. In the review of Dong and Pluske (2007) demonstrated that level of feed intake was the most important determinant of mucosal function and integrity. Therefore, anorexia produces a reduction of gut functions: a reduced enzyme activity and absorption (Kenworthy and Allen, 1966). In addition in a study of Pluske et al. (1996) suggested that the villous atrophy could be avoided if post-weaning feed consumption is maintained.

Moreover, weaning piglets show a reduced low acid secretion (lack of lactose substrate) and consumption of large meals at infrequent intervals results in elevated pH (Kidder and Manners, 1978). At this higher pH, the gastric conditions could allow pathogens to survive and is a greater opportunity to colonise the digestive tract (Yen, 2001).

Weaning piglets are less mature of digestive system; they have limited capacity of digestion and absorption of feeds, mainly vegetal proteins (Souza et al., 2012). In addition, the high feed intake after the underfeeding period may saturate the absorptive capacity, which causes undigested material beginning undesirable microbial activity (Makkink et al., 1993).

Another factor which influences feed consumption at weaning is neophobia. It is known as rejection or aversion to the new ingredients, flavours or scents. It happens when an animal is exposed to novel food and is reinforced by the associated novel environment (Hursti and Sjödén, 1997).

Neophobia can be adaptive under natural conditions as it prevents toxicosis while animals are learning from the post-ingestive consequences of eating a novel and potentially toxic feed (Rozin and Vollmecke, 1986), so is a precaution mechanism. It is characterized by a period of low feed intake, followed by increased consumption leading to a relatively stable level of intake (Figure 1). If the consumption of the new feed does not result in a gastro-intestinal disease, then the animals increase the new feed consumption. On the other hand, if eating the new feed results in illness, the animal forms a dislike for the feed called a conditioned taste aversion (CTA) (Launchbaugh, 1995).

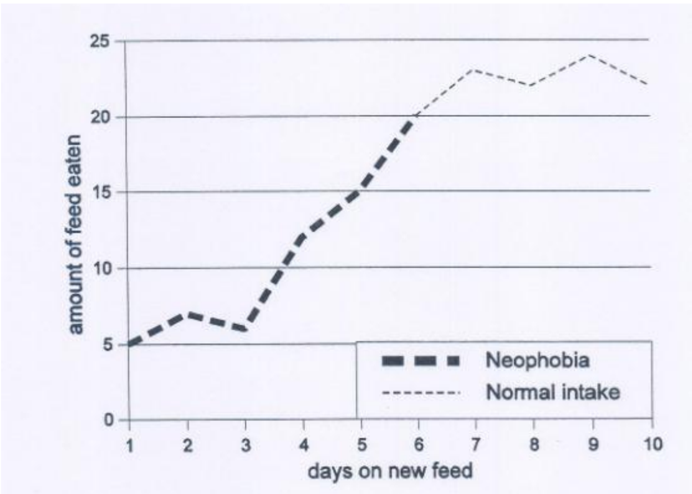

Figure 1. Intake pattern typical of animals expressing neophobia adapted from (Launchbaugh, 1995).

However, neophobia can be reduced through a learning process before or after birth involving contact with flavour cues and associations between those components and positive consequences that induces a food recognition and then its higher consume (Mennella et al., 2001; Wells and Hepper, 2006; Oostindjer et al., 2010; Figueroa et al., 2013a).

<u>Methods to increase feed consumption at weaning:</u>

a. Weaning pigs according to their size and ability to cope (including the possibility of weaning different pigs in a single litter at different times).
b. Accustoming pigs to the consumption of viable quantities of solid feed before they are weaned (creep-feeding). These diets should be highly digestible and include ingredients with high palatability (Solà-Oriol et al., 2012). Creep-feed may also help to

satisfy requirements of piglets to achieve a good growth potential and weaning weight, in addition to prepare the digestive system of the sucking pigs to cereal-based solid diets after weaning (Pluske et al., 2003). This item will be explained more extensively below.

c. Avoiding environmental (especially temperature) challenges and competitive stress.
d. Good quality and fresh ingredients: weaned pig is very sensitive to dietary mold growth and rancidity, so addition of for example proper amount of mold inhibitors and antioxidants to the manufactured diet is very important to prevent reduction in palatability during storage.
e. Optimum balance among nutrients in the diet: provide adequate amounts of vitamins, trace minerals and limiting amino acids in the diet.
f. Using weaning diets that are highly digestible: the digestibility of diets has a positive relationship with the feed intake. Whittemore and Kyriazakis (2006) developed an equation to show this relationship:

   Maximum voluntary feed intake (kg/d) = 0.013BW (1-DM digestibility coefficient)

   Where BW is Body Weight (kg) and DM is Dry Matter.
g. Palatable feedstuff with palatable ingredients: in the revision of Dong and Pluske (2007) showed that lactose-containing products, spray-dried plasma (SDP), spray-dried blood meal (SDBM), and high quality fish meal are palatable ingredients for the newly-weaned pigs. In addition, the inclusion of whey or lactose in the starter diet ensures continuation of bacterial fermentation and some, though reduced, lactic acid production (Kidder and Manners, 1978). However, improving the palatability of feed offered has variable and mostly only minor effects (Appleby et al., 1991; Pajor et al., 2002).
h. In relation with the previous point, inclusion of flavours and taste enhancers in the weaning diets may increase fed intake, but not always (Dong and Pluske, 2007).
i. Promoting ways of a maternal learning based on learning from flavours added in the maternal diet. It has been suggested that flavour cues from the maternal diet in uterus can reach the foetus through the amniotic fluid and/or the placental blood stream. This early exposure of foetus to certain cues generally may result in a preference for these flavours later in life and consequently can positively affect the acceptance of food with a similar flavour before and after weaning (Hepper, 1988; Mennella et al., 2001). This flavour preference can be more important when the flavour is also present

in maternal milk (Galef and Henderson, 1972). It has been demonstrated that maternal learning improves feed intake, maladaptive behaviour, gastro-intestinal problems and poor growth (Figure 2) (Oostindjer et al., 2010). This item will also be explained more extensively below, so is the central hypothesis of our study.

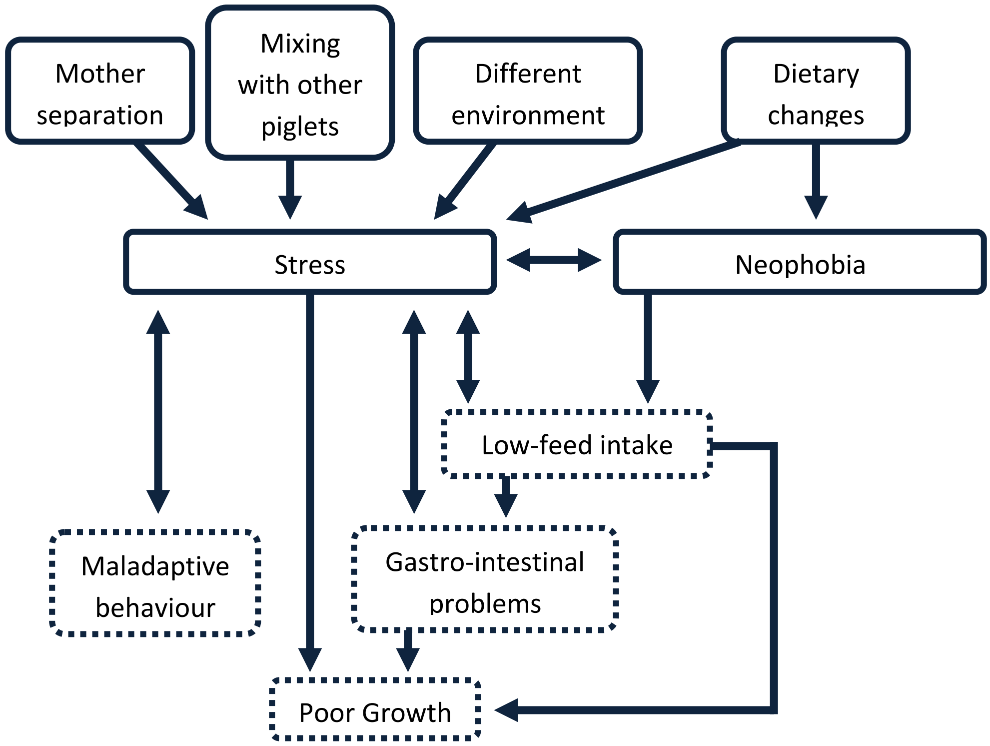

Figure 2. Schematic overview showing hypothetical links between neophobia, stress and performance at weaning. At weaning piglets suffered dietary and other changes like mother separation, mixing with other piglets in a different environment, thus create to piglets neophobia and stress and in consequence may negatively affect feed intake, gastrointestinal health, growth rate and behaviour. Dotted lines indicate variables which were found to be affected by prentatal or pre- and postnatal flavour learning. Adapted from (Oostindjer et al., 2010).

Among the previous strategies, we will focus those that involve an early learning of the piglet to the new food. Piglets need to establish a connection of continuity between the suckling period and post-weaning, to reduce the low feed intake and thus the “growth-check”. Therefore, piglets should learn what to eat and how to forage before weaning. The following sections of this bibliographic revision will focus on creep-feeding and maternal learning as likely strategies to smooth the weaning.

3.2 CREEP-FEEDING: effects of pre-weaning experience

Creep-feeding is defined as the milk replacer solid diet provided to the suckling litter during lactation in order to establish an early contact with solid feed and to make easier the transition from milk to solid feed at weaning. Creep-feed formulation may be very complex and varies from one feed mill to another; but milk by-products are always included as a source of lactose. Milk replacers commonly contain cereals (oats, corn, barley…), protein sources of animal origin (milk whey and whey powders, egg meal; animal plasma, fishmeal), protein sources of vegetal origin (soybean meal, soybean meal concentrate, wheat gluten, pea gluten, potato protein…) and lactose as main ingredients. For this period, technological treatments of the feed ingredients are also a common practice so that it is very common the use of heat processed cereal and vegetal origin protein sources (cooked, extruded, thick rolled), decorticated, micronized with the aim to obtain highly digestible ingredients and highly digestible diets. In commercial practice, the creep-feed is introduced from Day 7 to 10 of life, because piglet attraction for dry feed during the first week of life is very low (Pluske et al., 2003).

The main reasons that are suggested to supply solid food (creep-feed) during lactation are:

1- First, creep-feed will familiarize piglets feeding behaviour and intestinal physiology to the next changes caused by weaning (Pluske et al., 2003). Physiologically, creep-feed stimulates the gastrointestinal tract to produce amylases and proteases enzymes (Aumaitre, 1972) for digestion of complex carbohydrates and proteins. Also introduction of creep-feeding can reduce the enteropathologic changes, as Makinde et al. (1997) observed that only piglets without creep-feed had diarrhoea compared to piglets with creep-feed. According to feeding behaviour, early creep-feed consumption may allow the weaned piglets to focus more on feed intake and less on exploratory behaviour (Bruininx et al., 2002; Sulabo et al., 2010d).

2- Second, creep-feed will allow higher daily weight gain (270 g/day) (NRC, 2012). A higher piglet weight at the end of lactation is possible because creep-feed may compensate the decreasing milk production throughout lactation (Klindt, 2003; Bruininx et al., 2004). Whittemore and Morgan (1990) describe the lactation curve for a sow and daily milk yield. It can be observed that at day 21 with 12 piglets per litter, the piglets demand >3 kg/d of milk than the sow production (Figure 3). This result confirms that piglets require extra sources of energy and nutrients. It has been also described that creep-feed consumption occurs at a higher extent in smaller piglets within a litter (Sulabo et al., 2010b) and if only these piglets have the necessity to consume creep-feed (Solà-Oriol, 2013).

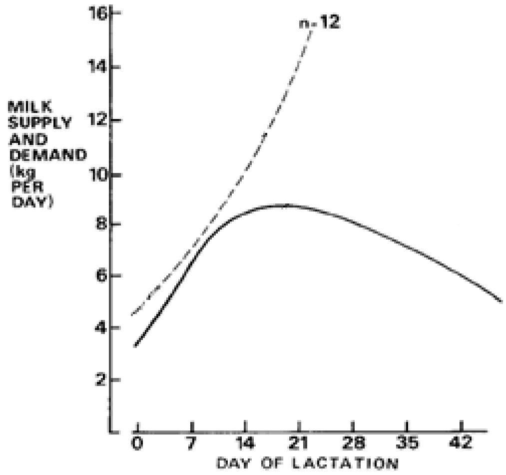

Figure 3. Supply of milk from the sow (solid line) and demand for milk by the litter (broken line), *n* is the litter size, adapted from (Whittemore and Morgan, 1990).

3- Finally, and probably as a consequence of the two previous reasons, creep-feed stimulates feed intake after weaning (Bruininx et al., 2002; Sulabo et al., 2010d).

In summary, it is accepted that creep-feed helps to smooth the adaptation of piglets after weaning**.**

However, it has been also suggested that the use in creep-feed of vegetal origin feed ingredients with antinutritional factors or antigenic compounds, like glycinin and β-conglycinin from soybean products, could sensitize piglets to antigens. Exposure of sensitized pigs to an increased intake of the same dietary antigens after weaning may rise to a hypersensitivity response (Pluske et al., 2003). Although, Friesen et al. (2010) observed that all pigs (previously fed to soybean and not) developed a tolerance to soy proteins within 2 weeks post-weaning.

3.2.1 Creep-feed intake

Creep-feed consumption is highly variable between litters, within litters (Barnett et al., 1989; Pluske et al., 1995; Bruininx et al., 2002; Sulabo et al., 2010d), and among studies (Table 1). Creep-feed contribution to the total daily energy intake before weaning at 21 to 35 d of age ranged from 1.2 to 17.4% (Pluske et al., 1995).

Table 1. Creep-feed intake per litter, per piglet and the duration of creep-feeding in different studies.

Study	Creep-feed Intake per litter	Creep-feed Intake per piglet	duration of Creep-feed (d)
Barnett et al., 1989	107-1550g	13-194	18
Pajor et al., 1991	2-205 g/day	27±16.8 g/day	18
Delumeau and Meuniersalaun, 1995	0-2382g	0-674g	14
Bruininx et al., 2002	445-7840g	377±200 g	17
Bruininx et al., 2004	-----	301±151 g	17
Pluske et al., 2007	380±332.5 g/day	37±32.7 g/day	19
Sulabo et al. 2010b	263-2349 g	------	18
Sulabo et al. 2010d	350-1034g	------	13

Factors that influence the large variation in creep-feed intake are difficult to study. However, it should be noted that most studies considered feed disappearance as feed intake and wastage may have a high impact as a source of variation. There are different theories about causes of the variable creep-feed intake. One is that some piglets may eat more creep-feed because of a more mature gastro-intestinal tract, which allows them to utilize nutrients from solid feed at an earlier age (Aumaitre, 1972). Another theory was that smaller piglets eat more creep-feed to compensate inadequate milk consumption (Algers et al., 1990), but Bruininx et al. (2004) did not observed a relationship between creep-feed consumption and body weight. Finally Sulabo et al. (2010d) speculated that creep diet composition, creep access, and feeder design may strongly contribute to this variability.

3.2.2 Creep-feeding behaviour

In order to account for this variability, it is important to remark that not all piglets consume creep-feed within a litter; approximately only 60% of pigs are creep-feed eaters (Sulabo et al., 2010d; Barnett et al., 1989).

For this reason, some authors (Barnett et al., 1989; Bruininx et al., 2002; Kuller et al., 2007; Sulabo et al., 2010b) have tried to discern which piglet eats creep-feed inside a litter, using a dye. Most of them use 1% of chromic oxide. However, Barnett et al. (1989) and Bruininx et al. (2002) reported that a considerable proportion of piglets (33%) were not be categorized with chromic oxide due to indistinguishable colours of the faeces. Pluske et al. (2007) used indigo carmine (5g/Kg) to classify 100% of piglets, because this dye clearly stains faeces (cyan).

Females show higher creep-feed intake than males (Bruininx et al., 2001; Kuller et al., 2007) and are more implicated in the creep-feeding activity than castrated males (Delumeau and Meuniersalaun, 1995). As a consequence females with higher activity in creep-feeding may become familiarized with the solid diet and they start early to consume after weaning. Then, gilts refrain less from eating after weaning than boars (Bruininx et al., 2002) and shows a greater ADFI and ADG in the first days of post-weaning period (Delumeau and Meuniersalaun, 1995; Bruininx et al., 2001). However, the same author in a posterior study did not observe a gender effect neither on the latency time or post-weaning ADFI and ADG (Bruininx et al., 2004).

The position of piglets to suckle (teat order) and their BW may also determine creep-feed consumption. Piglets that nurse from posterior teats that produce less milk are smaller than those nursing from anterior teats with greater milk production (Table 2) (Gill and Thomson, 1956; Fraser and Jones, 1975; Solà-Oriol, 2013).

Table 2. Teat Order of suckling period and its relationship with BW (from Solà-Oriol, 2013).

Position held on the udder	Front	Middle	Rear
Body Weight (kg)	1.72	1.65	1.48

Small piglets have less ability to compete at the udder and extract milk (Sulabo et al., 2010d) and they suckled teats that were less productive (Solà-Oriol, 2013). This could predispose small piglets to consume more creep-feed (Table 3). In contrast, heavier piglets sucking from anterior teats consume more milk during lactation so grew faster and were heavier at weaning (remaining 1.1kg heavier (Pluske et al., 2007) compared to their counterparts sucking from the posterior teats). Heaviest piglets may not find interesting to consume creep-feed, and as a consequence reduces post-weaning feed intake (Pluske et al., 2007). Nevertheless, Bruininx et al. (2004) failed to find a relationship between body weight and the pre-weaning consumption of creep-feed; and Pajor et al. (1991), Delumeau and Meuniersalaun (1995) and Pluske et al. (2007) did not observed any relationship between the teat order and the creep-feeding activity.

Table 3. Percentage of piglets that were consumers of creep-feed (offered from the 10th day of age) at 18, 21 and 25 days of age according to the position held on the udder during the lactation (Solà-Oriol, 2013).

	Position held on the udder		
Days of age	Front	Middle	Rear
18	7 %	5 %	12 %
21	17 %	13 %	21 %
25	41 %	35 %	42 %

3.2.3 When is creep-feed consumed?

Previously it was believed that creep-feed consumption increases linearly throughout lactation (Barnett et al., 1989) but more recently it has been described that a 60 to 80% of total creep-feed intake is consumed in the last week before weaning, either if pigs are weaned at 3 week (Sulabo et al., 2010d) or 4 weeks of age (Pajor et al., 1991; Fraser et al., 1994; Bruininx et al., 2002; Pluske et al., 2007).

Thus, Sulabo et al. (2010b) scored that daily creep-feed intake of litters increased quadratically (R^2 =0.22) from day 3 to weaning:

$$\text{Litter creep-feed intake, g/d} = (0.8965 \times \text{Age,d}^2) - (10.607 \times \text{Age,d}) + 37.683$$

It is clear that piglets consume more creep-feed as weaning date approaches, but why at the end of lactation? Sulabo et al. (2010c) introduced creep-feed 3 days before weaning and observed that this short period was enough to create 70% of eaters. Hence it could be concluded that individual creep-feed consumption is more related to the age of piglets rather than how many days creep-feed is offered to the litter (Kuller et al., 2007; Sulabo et al., 2010c). Moreover, piglets at the end of lactation have more digestive maturity and thus increase the opportunity to consume more creep-feed (Barnett et al., 1989; Pajor et al., 1991). However, in another study the same authors observed that as longer is the duration of creep-feed offer (13 d vs. 2 d) higher is the proportion of piglets that consume creep-feed but, without any effect on the pre-weaning performance (Sulabo et al., 2010d). Therefore, there are still contradictions about if it is better to introduce creep-feed early at the lactation or just few days before weaning.

Total creep-feed consumption increases according to litter size (6 to 13 pigs per litter) so larger litters have higher feed consumption despite of less individual creep-feed intake and lower daily gains during lactation (Barnett et al., 1989). In contrast, Klindt (2003) introduced creep-feed in litters with more than 8 pigs and observed an increased lactation ADG and weaning weight. It could be suggested that creep-feed could be beneficial for the piglets especially for those sows with a large litter. Pajor et al. (2002) observed that creep-feeding reduced lactation BW loss and weaning-to estrus interval; but Sulabo et al. (2010b) did not observed any effect, and estimated that creep-feeding provided energy equivalent to only 1.27 kg of sow BW loss. The difference in these results may be due to the different length in the lactation period (28d in the first study and 21d in the second). It is known that during the last period of lactation (21 to 28 days) milk production decrease a 12,5% (Whittemore and Morgan, 1990) and ME intake (kcal/day) of piglets increases 422 kcal/day (NRC, 2012) to pass 6.9 kg (medium weight at 21 days) to 8.8 kg (medium weight at 28 days).

3.2.4 Does Creep-feed affects body weight at weaning?

There are some studies that observed a positive effect of creep-feeding on the weaning weights (Pajor et al., 1991; Fraser et al., 1994; Klindt, 2003) while others did not show any effect (Barnett et al., 1989; Bruininx et al., 2002; Sulabo et al., 2010b,c,d).

Pajor et al. (1991) observed that the intake of creep-feed accounted for the 37% of the variation in weight gain in the week before weaning but only a 7% of the BW gain variation from day 10 to weaning.

These differences in pre-weaning response, as we have described above, may be due to the extent of creep-feed intake, type of creep-feeder, creep diet composition, duration of creep-feed, piglets genetic and may also be due to the smaller piglets are eaters, and thus implies that eaters of creep-feed have the lighter BW at weaning.

3.2.5 Post-weaning performance related to creep-feed

Creep-feed effects on post-weaning performance have been inconsistent across studies. Fraser et al. (1994) only noticed that 1-4% of the variation in post-weaning gain (0-14 days) is caused by creep-feeding. Barnett et al. (1989), Pajor et al. (1991) and Delumeau and Meuniersalaun (1995) did not find any relation between pre-weaning feed consumption on post-weaning gain. The lack of results could be due that in Barnett et al. (1989) piglets (eaters and non -eaters) were mixed at weaning and feed intake was measured on a pen level; and in Pajor et al. (1991) and Delumeau and Meuniersalaun (1995) did not difference between eaters and non-eaters.

On the other hand, consumption of creep-feed during lactation stimulated food intake and growth after weaning (higher ADFI and ADG) during first days of post-weaning (Bruininx et al., 2002, 2004; Cartensen et al., 2005; Sulabo et al., 2010d) and increases total BW gain (Kuller et al., 2007; Sulabo et al., 2010b) in eaters piglets.

Hence, if the response is focused in eaters, it can be observed that eaters have a higher post-weaning feed intake, greater ADG and total BW gains than non-eaters and no-creep pigs. Thus the differences between publications may fall on to the fact that they take into account or not the differences among eaters and non-eaters.

For example, Table 4 describes studies when eaters and non-eaters are identified. The highest post-weaning ADFI was for eaters, which demonstrates that exist a positive relationship between creep-feed intake and post-weaning feed intake.

Table 4. Creep-feed intake and post-weaning ADFI, differentiating between eaters and non-eaters.

Study	Creep-feed Intake		Post-weaning ADFI g/d/piglet	Period post-weaning ADFI
Bruininx et al., (2002)	445-7840 g/litter 377 g/piglet±200.5g	Eaters Non Eaters	202 160	0-8 days
Bruininx et al., (2004)	301 g/piglet ±151g	Eaters Non Eaters	154 105	0-4 days
Sulabo et al., (2010d)	350-1034 g/litter	Eaters Non Eaters	160 138	0-7 days

ADFI: Average Daily Feed Intake.

Kuller et al. (2007) described a relationship between creep-feed intake during lactation ($ADFI_{lact}$) and feed intake in the first week after weaning ($ADFI_{aw}$):

$$ADFI_{aw}=136+0.26*ADFI_{lact}\ (R^2=0.69).$$

Moreover, creep-feed decreases latency time (the time between weaning and first food intake). While fifty per cent of the "eaters" piglets during the suckling period started eating within 4h after weaning, the "non-eaters" and "no-feed" piglets needed about 6.7 and 6.9 h, respectively (Bruininx et al., 2002).

3.2.6 Strategies to increase creep-feed consumption

Several strategies have been described to increase the percentage of eaters within the litter and also the consumption of creep-feed:

1-Creep-feed composition: in a study of Okai et al. (1976) observed the effects of three types of creep-feed: simple, semi-complex and complex. The complex creep-feed was characterized by a lower presence of cereals and vegetable proteins (soybean) and an increased dextrose, sucrose, milk proteins and the percentage lysine. They observed better performance after weaning when complex creep-feed was offered to the animals. Also Fraser et al. (1994) compared low-complex (LC) creep-feed diet (based on corn, barley and soybean meal) to high-complex (HC) diet formulated using the ideal protein concept and without soybean meal. They observed that piglets fed the HC diet consumed more creep-feed, tended to gain more during the week before weaning, converted feed more efficiently and gained more weight in the 2 weeks after weaning. The study seems to show that using a HC formulate (without vegetal proteins) improves creep-feed intake. In the same line Pajor et al. (2002) observed that piglets consumed 50% more complex creep-feed (richer in fat and protein) than piglets provided the standard creep-feed. These results could suggest that during the first weeks of life, the gastrointestinal tract of piglets is better adapted to digest lactose, fat and milk proteins, casein and whey (Pluske et al., 2003). Cabrera et al. (2013) observed improved feed conversion when supplemented a creep-feed and pre-starter diet with L-glutamine and AminoGut (containing L-glutamine and glutamate).

2-Creep-feed presentation: Stimulating the exploratory behaviour may be a way to increase creep-feed intake. At this respect, presenting the new solid diet in a more variable form, using newness to incite exploration, can increase exploration activity. Some examples to stimulate creep-feed intake in young piglets are: offering freshness feed and small amounts of feed (Pajor et al., 1991), increasing feeding space (Appleby et al., 1992; Delumeau and Meuniersalaun, 1995), increasing accessibility (Wattanakul et al., 2005), daily replacement of the feed (Appleby et al., 1991). This frequent arrival of fresh feed stimulates the inherent curiosity of the piglets and its exploratory behaviour. Moreover it is important the type of feeder. For example creep-feeder with hopper create more eaters with less feed wastage (Sulabo et al. 2010c), while a playfeeder (an open trough with 3 protusions) stimulated to explore this feeder and thereby getting accustomed to the creep-feed (Kuller et al., 2010). Thus, exploratory investigation and social activity seems to be a key to start creep-feeding (Pajor et al., 1991; Delumeau and Meuniersalaun, 1995; Kuller et al., 2010).

Liquid creep-feed diets (water or milk + creep-feed) have been also proposed as a way to allow an enhanced creep-feed intake, pre-weaning growth, and performance after weaning (Pluske et al., 2003).

3-Intermittent suckling (IS): It is promoted when piglets are separated from the sow during 12 hours a day last week before weaning, and it has been related with an increased creep-feed intake (Kuller et al., 2007). Nevertheless, intermittent suckling did not increase the percentage of eaters within a litter, suggesting that intermittent suckling increased creep-feed intake of piglets that were already eating before the period of separation.

4-Flavours in the creep-feed: Studies on rats have shown that they ate more when fed different flavoured feeds (Shafat et al., 2009; Myers and Sclafani, 2006). However, the incorporation of flavour to the creep-feed diet did not increase creep-feed consumption nor increased the proportion of piglets consuming creep-feed (Sulabo et al., 2010a). Moreover, Figueroa et al., (2013a) observed that piglets preferred an unflavoured creep diet to a flavoured diet. The results suggest that the addition of new flavours or ingredients may affect the palatability of piglet's creep-feed diets. Piglets showed a preference for simple diets and showed neophobia or innate aversion to new flavours.

3.3 LEARNING STRATEGIES: how to increase feed consumption

For mammals, the selection and ingestion of an appropriate diet is a complex challenge that needs the incorporation of information from a wide variety of sources. It has been observed that mammals are able to have an innate recognition and preference for high energy (sweet savours), proteins (umami savours) and even electrolytes (savoury savours) feed (Pérez et al., 1995; Wald and Leshem, 2003; Rolls, 2009,). The rest of flavours are identified like a challenge. When the animal learn to discern if post-eating consequence of these feeds are positive or negative, then the animal acquire an associative learning that relates the sensory cues of food with the benefit (hedonic and/or post-ingestive) or disadvantage. Then, learning is considered important for developing feed preferences.

Learning of the feeding behaviours appears as an evolutionary mechanism that has been established to facilitate the search of food, making it more efficient and adaptive. Three types of learning have been described: trial and error (or direct experience with food and its consequence) (Myers and Sclafani, 2006; Dwyer et al., 2009), social learning acquired with direct or indirect contact with others animals (Galef and Whiskin, 2004) and maternal learning, which is established during gestation or lactation (Mennella et al., 2001).

3.3.1 Trial-and-error learning

Trial-and-error learning is defined as the ability of animals to change their consumption or preferences after experiencing food consumption, due to its sensorial experience or associated to nutrient absorption or digestive response (Solà-Oriol et al., 2012). By this way they learn to distinguish safe and nutritious food items from toxic ones (Galef, 1996a). One of items that pigs are based on sampling food is flavour. It is detected by three different chemosensory systems: olfaction, taste and trigeminal-mediated sensation (Bolhuis et al., 2009).

Sweet and umami taste are high preference compounds and they are used to increase palatability and intake of diets. However, they also can create associative learning in neutral clues (like flavour) as they have hedonism and post-ingestive effects. The associative learning of a flavour can be explained in terms of Pavlovian conditioning, where a new flavour serves as a conditioned stimulus (CS) associated to a biological consequence of feed intake acting as unconditioned stimulus (US). The US may be either aversive (like an unpalatable taste or toxicosis) or reinforcing (palatable taste or post-oral nutrient actions) (Myers and Sclafani, 2006). For example the sweet taste of an apple can serve as US (for hedonic and nutritive power) and during its consumption is associated to its flavour. When pigs consume some apples, they will be able to either know the benefit of eating an apple and also to prefer the characteristic flavour of this fruit. The power of conditioning is for its hedonism (sweet taste) or its post-ingestive effect (energy) and many times it happens together (Solà-Oriol et al., 2012). Furthermore preference for nutritional conditioning would be more resistant to extinction and more significant palatable (Dwyer et al., 2009). Weaned piglets are able to acquire preference for cue flavours added to sweet compounds like sucrose (Figueroa et al., 2012b) and recently our group has also demonstrated flavour condition when associated to protein products as porcine digestible peptides (PDP) and soybean protein concentrate (SPC) (Figure 4) (Figueroa et al., 2012a).

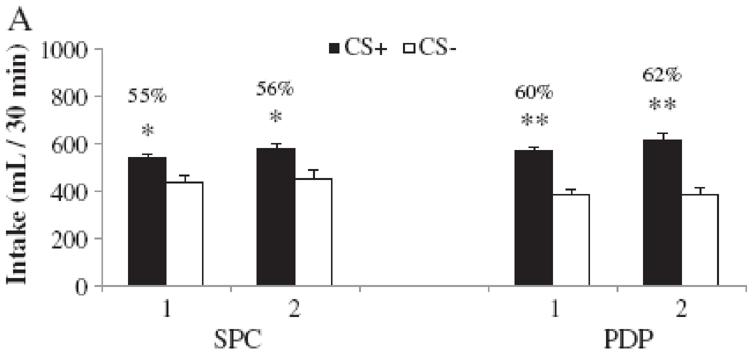

Figure 4. CS+ and CS- consumption during 30 minutes. In two preference test (Test 1 and test 2), when the flavours are dissolved in drinking water after being conditioned CS+ or non CS- previously by a soybean protein concentrate (SPC) or by porcine digestible peptides (PDP). Numbers in the top of bars indicate the average value of the corresponding percentage of preference. Asterisks indicate that CS+ intake is significantly different than CS- intake (*P<0.05; **P<0.001) (Figueroa et al., 2012a).

3.3.2 Social learning

Sometimes development of appropriate responses to food through their own experience in inexperienced animals can be a risk. Consequently, it appears safer and more efficient to benefit from the expertise of other animals (their mother, their littermates…), in relation about how, where, when and what to eat (Galef, 1996b). It has been reported that food information can be transferred from one individual to another through social learning (Oostindjer et al., 2011a). In addition, it is known that pigs are highly social animals and begin to form social relationships with littermates within hours of birth (Graves, 1984).

Social learning can be defined like that conduct that leads to transmit specific behavioural guidelines from one animal to the other animal. Through social learning it is not necessary to search a variety of range or sample potentially toxic foods (Galef, 1996b).

In our group (Figueroa et al., 2013b), observed that flavoured feed intake was greater in observer piglets when they interacted with familiar demonstrator than unfamiliar, flavours were previously eaten by familiar and unfamiliar piglet demonstrators (Figure 5). This demonstrates that pigs are able to learn a preference for flavoured feed following social interactions with a conspecific.

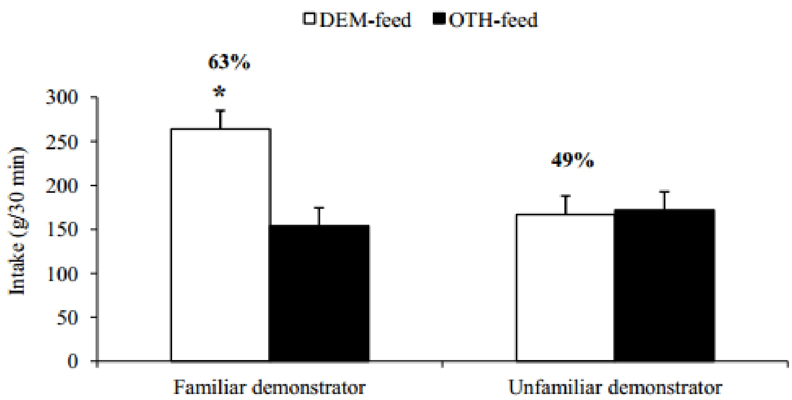

Figure 5. Feed intake by observers of flavoured feed consumed by demonstrators (DEM-feed) and other flavoured feed (OTH-feed) after 30 min choice tests when they previously interacted with unfamiliar or familiar demonstrators. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the flavoured feed previously consumed by demonstrators. Asterisks indicate that intake different between feeds within each group (*P<0.001) (Figueroa et al., 2013b).

Piglets can also learn by observing their mother. This is called vertical social learning (Oostindjer et al., 2011a). Learning from mother will be the best model for social information transfer and also could be more powerful than learning from other experienced animals (Thorhallsdottir et al., 1990). Furthermore, mother and its offspring are genetically related, so they will respond similarly to the food (Laland et al., 1993).

In pigs there are few studies about vertical transfer in pigs. In (Nicol and Pope, 1994) concluded that it would be better to learn from the mother than from piglets of the same age. However, in modern pig husbandry is difficult that piglets could observe and learn the feeding behaviour of their mother, as sows are confined in crates during lactation being the troughs inaccessible for them.

3.3.3 Maternal learning

It is known that pigs have a well-developed sense of smell (Morrow-Tesch and McGlone, 1990) and piglets can discriminate among auditory, olfactory, visual and tactile stimuli immediately after birth (Parfet and Gonyou, 1991). Furthermore it has been observed that pigs can learn olfactory cues for faster discrimination than visual discrimination tasks (Croney et al., 2003). It has been hypothesized that piglets have a well-developed olfactory system at birth so neonatal piglets were able to recognize their mother's faecal and skin odours (Morrow-Tesch and McGlone, 1990). Figueroa et al. (2013a) realized a triple-choice stimulus among maternal amniotic fluid, alien amniotic fluid or water and observed that piglets preferred their own maternal amniotic fluid; but this preference disappeared with age (Figure 6). This also happens in other species (mammals, birds and amphibians) (Bolhuis et al., 2009). For example, Hepper (1987) demonstrated that rat pups, either natural born or caesarean, recognized the flavour previously offered to the mother, indicating that the preference is acquired prenatally. So when a mammal is born they have a rudimentary idea of who are their parents/family. This leads to animals a smoothly transition from the prenatal environment to postnatal life, a maternal recognition, nipple localisation and initiation of suckling (in mammals) (Hepper, 1987).

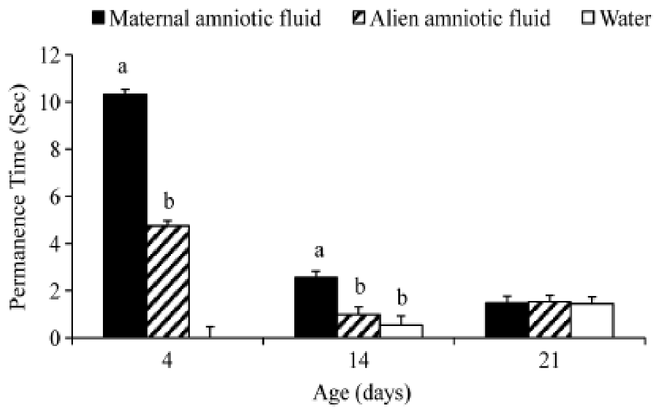

Figure 6. Piglet's permanence time in contact with strips impregnated with maternal amniotic fluid, alien amniotic fluid and water for a 7-min preference test during lactation. Means with different subscripts are different (P<0.05) (Figueroa et al., 2013a).

If mothers know what kind of food is good to eat and what kind of food is present to their environment, maternal learning would allow offspring to prefer to eat the same. Then, maternal learning starts before farrowing, when the sow eats some compounds of feed that pass to foetus.

It has been described the volatile compounds may cross the placental barrier and enter the foetal blood stream, consequently diffuse out of the nasal blood capillaries and come into contact with foetal olfactory receptors. In addition, flavours come into the amniotic fluid; the foetus inhales or swallows it and then stimulates olfactory receptors or taste buds (Hepper, 1988). As a result this exposure to cues lead to create preference for this flavours later in life and also can positively modulate the acceptance of food with a similar flavour before and after weaning (Mennella et al., 2001). After birth, maternal learning continues through milk, the hedonism and lactic post-ingestive effect with the pleasure of nursering may create an associative learning with cues in milk (Hepper and Wells, 2006). Thus, this flavour preference can be strengthened more when the flavour is also present in the maternal milk (Mennella et al., 2001; Oostindjer et al., 2009). However only milk exposure does not increase flavour preference in all species (Hepper and Wells, 2006). Although in rabbit pups the ingestion of mother's milk (Bilkó et al., 1994) was sufficient to influence the dietary preference; in piglets postnatal exposure alone did not reduce weaning-associated problems (Oostindjer et al., 2010).

What kinds of flavours can cross the placental barrier? Table 5, shows some of the flavours that have been described to create postnatal effects. Most of them increases post-natal olfactory preference for these flavours or reduces aversion. Some of them only focussed on the short-term recall by neonates and the others observed that flavour preferences may be long-lasting and persist until weaning and beyond.

For example (Bilkó et al., 1994) observed that the ingestion of juniper berries on the day of weaning was five times higher in rabbit pups from does fed juniper berries during gestation compared to control ones. Another example is in humans, where (Mennella et al., 2001) also observed an increased acceptance, enjoyment and intake of a carrot-flavoured cereal meal in infants of 6 month-old from mothers who had a regular consumption of carrot juice during pregnant and lactation (Figure 7).

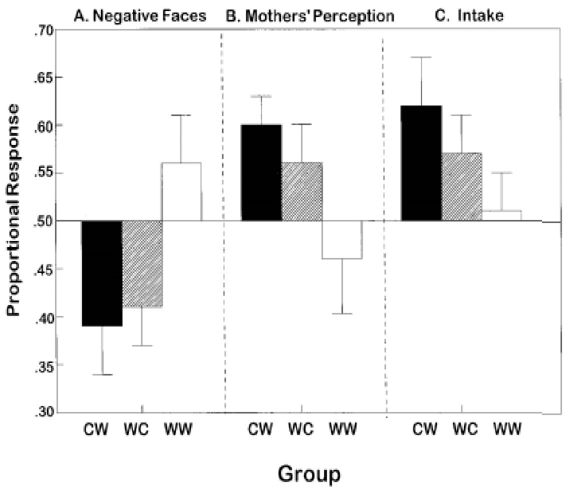

Figure 7. The infants' relative acceptance of the carrot-flavoured cereal as indicated by display of negative facial expressions (A), mothers' rating of their infants' enjoyments (B) and intake (C). Proportional responses were calculated by dividing each infant's response to the carrot-flavoured cereal by his or her response to the carrot cereal plus plain cereal (carrot/[carrot+plain]). CW group indicates that mothers drank carrot juice during pregnancy and water during lactation; WC did the opposite and finally WW drank water during both exposure periods (Mennella et al., 2001).

Table 5. Overview of experiments demonstrating that prenatal exposure to flavours from the maternal diets affects postnatal preferences. Species, type of flavour, postnatal effects and references are indicated, adapted from (Bolhuis et al., 2009).

Species	Flavour[1]	Postnatal effects[5]	References
Rats	Garlic	↑ olfactory preference d12	Hepper, 1988
Rabbits	Juniper[2]	↑ intake juniper at weaning d28	Bilkó et al., 1994
	Cumin	↑ olfactory preference d0	Coureaud et al., 2002
Sheeps	Citral[2]	↑ olfactory preference d0	Schaal et al., 1995
	Oregano[3]	↑ intake oregano-flavoured feed at 3,4.5,6,7.5 mo	Simitzis et al., 2008
Dogs	Anise (trans-anethole)	↑olfactory preference d0	Wells and Hepper, 2006
		↑ intake anise-flavoured treats w10, only when exposed through milk also	Hepper and Wells, 2006
Pig	Anise[2]	↑ intake the same flavour on diet, ↑ body weight first days post-weaning and ↓ diarrhoea	Oostindjer et al., 2009
		Positive effects on behaviour	Oostindjer et al., 2009
		↑olfactory preference d14, 21 and 26	Figueroa et al., 2013a
	Milky-cheese[2]	↑olfactory preference d14, 21 and 26	Figueroa et al., 2013a
Humans	Anise[2]	↑ olfactory preference d0 and d4	Schaal et al., 2000
	Garlic	↑ olfactory preference d0	Hepper, 1995
	Carrot[4]	↓ negative facial responses while eating carrot-flavoured cereal at 6 month	Mennella et al., 2001
		↑ enjoyment perceived by mother while eating carrot-flavoured cereal	Mennella et al., 2001

[1]Provided in the maternal diet throughout gestation unless indicated otherwise.
[2]Last two gestational weeks.
[3]Day 50-130 of gestation.
[4]Last three gestational weeks.
[5]Relative to (non-exposed) control groups. d=day; w=weeks; mo=months.

In pigs the first description of maternal learning was from (Campbell, 1976), who demonstrated that piglets are able to choose diets related on their mothers consumption during lactation. They observed a 12% increase on feed intake of weaning diets with the same flavour that was added in sow’s diet and gained 24% more weight.

However, it has been long later when new studies on maternal learning in piglets have been published. Oostindjer et al. (2009, 2010) observed positive effects of perinatal flavour learning on feed intake, growth and behaviour. They saw that during first days of weaning the piglets previously exposed to flavour increased feed intake of the same flavour in the diet and growth (Figure 8). Piglets showed also fewer vocalizations, escape, fighting and aggression, but no increased preference for the flavour. In our department Figueroa et al. (2013a) found that prenatal flavour exposure via maternal diet influenced a piglet's preferences of the pure flavours during lactation and post-weaning (Day 26).

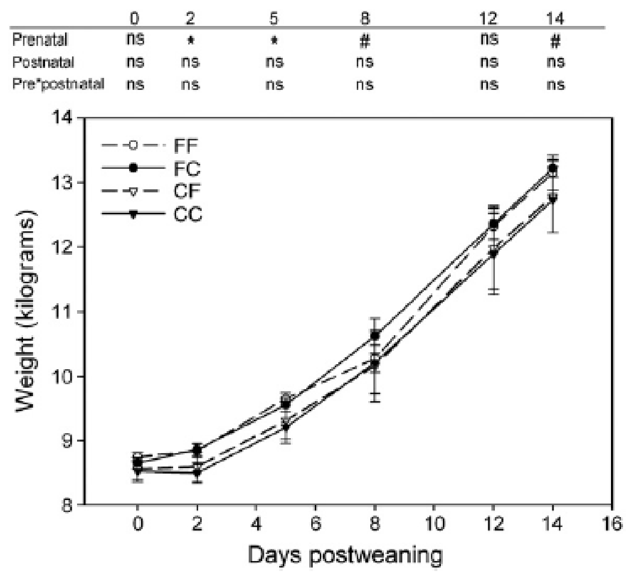

Figure 8. Body weight during the first 14 days post-weaning for animals that had been exposed to anise both pre- and post-natally (FF), prenatally (FC), postnatally (CF), or never (CC) *P<0.05, # P<0.1, ns not significant (Oostindjer et al., 2010).

In another study, Oostindjer et al. (2011b) did not observe that pre-weaning (anethol-flavoured diet during gestation and lactation) or post-weaning (anethol either in food or in air) treatment affected food intake, preference or growth. But flavour piglets played more and showed less damaging behaviours than control piglets, indicating a reduced stress around weaning when the familiar flavour is present.

So it can be concluded that early experience with flavours (prenatal learning), plays a role in postnatal feeding behaviour and development of dietary habits, increasing later acceptance and improving adaptation to weaning.

Focusing to post-natal learning, volatiles can be also transferred to the mammary glands via vascular routes from the digestive or respiratory system (Dougherty et al., 1962). It has been observed that pre-weaning exposure to feed flavour (during lactation) from the maternal diet can reduce food neophobia and promote the intake of an otherwise unfamiliar feed after weaning (Bolhuis et al., 2009). For example in Table 6, the studies used two- or three-way food choice test to assess the influence of the maternal diet on feed preferences of the offspring in a wide range of species.

Table 6. Overview of experiments demonstrating that exposure to flavours from the diet of the lactating mother affects post-weaning preferences. Species, type of flavour, post-weaning and references are indicated. Adapted from Bolhuis et al., (2009).

Species	Flavour[1]	Testing Age[8]	Post-weaning effects[9]	References
Rats	Lab chow[2]	d21 (w)	↑ intake lab chow maternal diet	Galef and Sherry, 1973
		d 19-23 (w)	↑ intake lab chow maternal diet	Galef and Henderson, 1972
	Lab chow[3]	d23 (w)	↑ intake in 30-min test if weanling diet matched with maternal diet	Bornstein et al., 1975
	Garlic[4]	d29-40	↑ intake of garlic-flavoured water	Capretta and Rawls, 1974
	Onion	d 21, d23 (w) d25	↑ intake of onion-flavoured diet	Wuensch, 1978
Mice	Fennel	d22 (w)	↑ intake of fennel	Mainardi et al., 1989
Rabbits	Juniper	d28 (w)	↑ intake of juniper	Bilkó et al., 1994
Goats	Rice straw[5]	6 mo	↓ latency to first ingestion ↑ intake of rice straw	Van Tien, 2002
Dogs	Anise[6]	10 w	↑ intake anise-flavoured treats, only when exposed prenatally also	Hepper and Wells, 2006
Pigs	Firanor nº3	3 w	↑ intake firanor nº3 and ↑growth rate	Campbell, 1976
Humans	Carrot[7]	6 mo (w)	↓ negative facial responses while eating carrot-flavoured cereal	Mennella et al., 2001
			↓ intake of carrot-flavoured cereal shortly after exposure to carrot-flavoured mother's milk	Mennella and Beauchamp, 1999

[1]Provided in the maternal diet throughout the lactation period unless indicated otherwise.
[2]Day 5-weaning.
[3]Day 2-weaning.
[4]Three days before parturition-weaning at day 22.
[5]Last three weeks before weaning at 3 months.
[6]First 20 days of lactation
[7]First two months of lactation, for four days per week.
[8](w) indicates that animals were tested at weaning or were not fully weaned at the time of testing. d=days; w=weeks; mo=months.
[9]Relative to (non-exposed) control groups.

In pig, Campbell (1976) demonstrated that flavour can be transferred by mother's milk and affect flavour preferences after weaning, but Oostindjer et al. (2010) was not able to demonstrate it.

Moreover, we have to keep in mind that during lactation, piglets have also contact with the smell of the mother's body, breath, feed particles clinging to her skin, fur or teeth or faecal droppings (Bolhuis et al., 2009). Thus, there are reinforcing conditions like nursing which may further strengthen their preference and acceptance of these flavours.

As a result, the postnatal learning can also facilitate the acceptance of a similarly flavoured feed at weaning and thus increases the consumption after weaning. In other words, breast milk may bridge the experiences of flavours *in utero* to those in solid foods.

As seen in this review many studies have studied maternal learning in different species. However, there is little practical knowledge in the pig industry about the mechanisms underlying flavour learning and how perinatal flavour learning can increase acceptance of diets after weaning.

4. HYPOTESIS AND OBJECTIVES

The hypothesis of this study is that maternal learning, adding Fluidarom 1003® on sow diets, may create a flavour habit on the piglets, either in utero or via the milk suckling. Fluidarom 1003® could represent a familiar volatile cue for piglets that may facilitate the initiation to feed intake and improve the post-weaning performance when the same flavour is reintroduced in weanling diets. The reason could be that: 1.- the flavour in creep-feed will stimulate the consumption, creating more creep-feed eaters and/or 2.- reintroducing the same flavour in weaning diets will reduce feed neophobia and therefore piglets will consume earlier the solid diet.

To confirm or deny these hypotheses, we performed the following trials with the following corresponding objectives:

1) First trial: Productive and Performance Outcome

The objective was to determine the sow's and piglet's productive and performance parameters associated to the incorporation of Fluidarom 1003® in the sow (late gestation and lactation) and weaning diets.

2) Second trial: Study of the Preference for Fluidarom 1003® in Suckling and Weaned piglets.

In the second trial according to the literature it was hypothesised that the positive results previously observed in Trial 1, highest ADFI and BW for piglets perinatally exposed to flavour, could be mainly explained by an early creep-feed contact and intake following the familiar link with volatile compounds exposed via amniotic fluid and/or milk. Thus, the main objective was to determine the preference for Fluidarom 1003® throughout a test of preference during lactation and at weaning.

The trial also aimed to find out at what extent Fluidarom 1003® affect sow's and piglet's productive and performance parameters, including the determination of creep-feed consumption.

3) Third trial: is the Fluidarom 1003® Habit mainly formed in Uterus or via the Milk Suckling?

The results of the first and second trial indicated that diet supplementation with Fluidarom 1003® throughout gestation and lactation has a positive impact on growth performance when the same flavour is included in weanling diets. However, we do not know if each one, pre-natal (foetal environment) and post-natal (maternal milk) contact by their own are able to contribute to reinforce the positive reward and preference in piglets after weaning.

5. MATERIAL AND METHODS

The trials were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (UAB). Experimental procedures were approved by the Ethical Committee on Animal Experimentation of the UAB (CEAAH 1406).

5.1 PRODUCTIVE AND PERFORMANCE OUTCOME (First Trial)

5.1.1 Pre-weaning performance

A total of 80 (Landrace x Large White) sows were selected, and distributed in two experimental groups according to parity number, body condition score, and body weight at Day 42 before farrowing (late gestation). Sows were either exposed to a flavoured (TG, n=40) or control (CG, n=40) feed from Day 73 to farrowing and during 28 days of lactation. The composition and estimated nutrient content for the gestation and lactation diets are presented in Tables 1 and 2. Diets were offered twice a day at 8.00 and at 16:00 pm in mash form. Lactating diets were offered *ad libitum* (*ad libitum* controlled situation) from farrowing onwards. During lactation piglets from control group (CG) were offered a non-flavoured creep-feed and those piglets corresponding to the treatment group (TG) were offered the same creep-feed supplemented with 375g/Tm of Fluidarom 1003®, from Day 10 of live onwards. All diets were formulated to meet or exceed the requirements for each period (NRC, 2012).

Body weight, body condition score and backfat thickness (measured by ultrasound system) of sows was measured at Days 73 and 110 of gestation and at weaning. Feed intake was registered between Day 73 of gestation until farrowing and from Day 1 after farrowing to the weaning day (taking weaning day as the day of lactation of the last farrowed sow (in this case 23d). Litter size was standardized immediately after birth. Sow's productive parameters per litter, number of total piglets born, number of piglets born alive, number of stillbirth piglets, weight of piglets born alive and stillbirth, number of weaned piglets and pre-weaning mortality were measured by sow. Piglets born alive and stillbirth were weighted at farrowing. Standardized litters were weighted after cross-fostering and at weaning. All piglets were processed with the same protocol than in commercial farms (24 hours after birth) and were individually identified by using plastic ear tags.

Table 1. Composition of the gestation, lactation and creep-feed diets (%).

Ingredient	Gestation	Lactation	Creep-Feed
Barley	27.3	22.0	22.0
Wheat	20.0	20.0	17.5
Wheat bran	16.0	5.0	-
Maize	10.0	25.0	-
Sweet beet pulp	7.5	-	-
Corn	-	-	15.1
Gluten feed	5.0	2.5	-
Canola meal	4.5	2.5	-
Soybean meal 44%CP	3.0	17.5	-
Soybean meal concentrate	-	-	2.5
Soybean hulls	2.4	-	-
Full fat soybean meal	-	-	16.5
Sweet Milk Whey	-	-	14.1
Animal plasma 80%CP	-	-	5.0
Soybean oil	-	-	3.8
Lard	1.33	2.29	-
Calcium carbonate	1.53	1.37	0.94
Mono-calcium phosphate	0.52	0.79	0.85
Salt	0.30	0.30	0.27
Sodium bicarbonate	0.20	0.30	-
L-Lysine-HCl	0.11	0.13	0.48
DL-Methionine	-	-	0.24
L-Threonine	-	-	0.20
L-Tryptophan	-	-	0.50
Vit-Min complex (*)	0.40[a]	0.40[a]	0.40[b]

[a] Sow gestation and lactation diets Premix Supplied (g/kg): 12500 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), 250IU 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 glycine), 0.05 mg of Co (sulphate), 40 mg of Zn (chelate of amino acids), 12.5 mg of Mn (oxide), 7.5 mg of Mn (chelate of glycine), 0.35 mg of I, 0.5 mg of Se (organic) and 0.1 mg of Se (sodium).

Table 2. Estimated nutrient content (% as feed basis) for gestation, lactation and creep-feed diet.

Nutrients	Gestation	Lactation	Creep-Feed
Moisture	10.99	10.92	8.5
Net Energy (kcal/kg)	2125	2305	2618
Crude Protein	13.50	16.78	20.44
Fat	3.68	4.95	8.41
FND	21.43	14.75	8.96
Lysine	0.628	0.892	0.155
Methionine	0.218	0.266	0.500
Met + Cys	0.506	0.600	0.904
Threonine	0.488	0.611	0.103
Tryptophan	0.163	0.202	0.320
Isoleucine	0.497	0.668	0.813
Valine	0.660	0.807	1.015
Calcium	0.950	0.850	0.720
Total P	0.566	0.610	0.606
Dig. P	0.300	0.350	0.386
Sodium	0.201	0.213	0.368

5.1.2 Post-weaning performance

A total of 480 weanling piglets [Pietrain x (Landrace x Large White)] were selected at weaning (26 days old; average BW= 7.4 SD=1.28kg). Piglets were distributed into 48 pens (10 pigs/pen). Two rooms with 24 pens each. Rooms were equipped with central heating, automatic, forced ventilation and a complete slatted floor. Each pen (3.2 m^2 in floor area) had a lidded feeder with 3 feeding spaces and an independent water supply. The animals had *ad libitum* access to feed and drinking water. Piglets were distributed by initial body into three blocks (body weight category; heavy=8.97 kg; medium=7.65 kg and light=5.77 kg) and allotted into four experimental treatments following a 2 x 2 factorial arrangement (Table 3) where the main factors were the maternal contact with Fluidarom 1003® and the Fluidarom 1003® inclusion in weanling diets (pre-starter and starter) until Day 35 post-weaning.

Table 3. Experimental treatments of Trial 1.

Treatment Sow (G+L+CF)	Fluidarom 1003®	Treatment Post-weaning	Fluidarom 1003®
CG	-	T-1	-
		T-3	+
TG	+	T-2	-
		T-4	+

+: inclusion of Fluidarom 1003® (375g/Tm); **-**: No inclusion of Fluidarom 1003®
CG: Control Group; TG: Treatment Group

Pre-starter diets were offered *ad libitum* for fourteen consecutive days and starter diets were offered from Day 14 to 35 post-weaning. All diets were presented in mash form. Zinc oxide (at 2500 ppm (2000 ppm of Zinc)), Colistin sulphate (120 ppm) and Amoxicillin (300 ppm) were used as common antimicrobials for weaning diets. The composition and the estimated nutrient content are presented in Tables 4 and 5.

Body weight and Feed disappearance was weekly monitored on Days 0, 7, 14, 21, 28 and 35 post-weaning. Piglets were weighted by group. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) was calculated by pen.

Table 4. Composition (%) of the experimental diets during the pre-starter and starter phase (0 to 14 and 14 to 35 days post-weaning).

Ingredients (*)	**Pre-starter**	**Starter**
Maize	28	35
Barley	15	17.07
Wheat	15	18
Sweet Milk Whey	10	-
Extruded Soybeans	9.44	11.4
Soybean meal 44%CP	5	6
Plasma animal 80% PB	5	-
Whey powder (50% Fat)	5	2.5
Fishmeal LT	2.5	5
Soybean oil	1.85	-
Calcium carbonate	1.13	0.25
Monocalcium Phosphate	0.84	2.84
Salt	0.08	0.45
L-Lisina HCL	0.47	0.63
Vit-Min Premix	0.4[a]	0.4[a]

(*) Antibiotics in feed: Amoxicilin: 300 ppm; Colistin sulphate: 120ppm; zinc oxide: 2500ppm.

[b] Pre-starter and starter diet Premix Supplied (g/kg): 7000 IU of vitamin A (acetate), 500 IU of vitamin D3 (cholecalciferol), 250IU 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 glycine), 0.05 mg of Co (sulphate), 40 mg of Zn (chelate of amino acids), 12.5 mg of Mn (oxide), 7.5 mg of Mn (chelate of glycine), 0.35 mg of I, 0.5 mg of Se (organic) and 0.1 mg of Se (sodium).

Table 5. Estimated nutrient content of the basal experimental diets during the pre-starter phase (0 to 14 days post-weaning (%).

Nutrients	Pre-starter	Starter
Moisture	10.2	11.2
Net Energy (kcal/kg)	2584.2	2447.4
Ash	5.46	6.22
Etheric Extract	8.18	5.95
Linoleic Acid	1.93	2.20
Crude Fibre	2.62	3.15
Neutro Detergent Fibre	8.14	9.74
Starch	34.24	41.50
Sugars	10.79	3.12
Crude Protein	19.00	18.00
Lysine	1.496	1.417
Dig. Lysine	1.355	1.292
Methionine	0.446	0.504
Dig. Methionine	0.412	0.468
Met+Cys	0.823	0.779
Dig Met+Cys	0.741	0.703
Threonine	0.897	0.850
Dig. Threonine	0.722	0.648
Tryptophan	0.269	0.255
Dig. Tryptophan	0.231	0.221
Valine	0.943	0.810
Dig. Valine	0.813	0.690
Isoleucine	0.741	0.693
Dig. Isoleucine	0.644	0.601
Calcium	0.830	0.830
Total Phosphorous	0.622	1.008
Dig. Phosphorous	0.400	0.660

Magnesium (0.12 and 0.13 %), Sodium (0.31 and 0.24 %), Calcium (0.49 and 0.54 %), Potassium (0.72 and 0.63 %), Copper (6.16 and 6.36 %), Iron (91.50 and 176.61 %), Vitamin E (15.7 and 19.3 %), Biotin (0.2 and 0.1 %), Coline (1153.6 and 1233.3 %), Sulphur (0.2 and 0.2 %), Zinc (28.5 and 34.5 %) and Manganese (10.9 and 12.6 %) for pre-starter and starter diets, respectively.

5.2 STUDY OF THE PREFERENCE FOR FLUIDAROM 1003® IN SUCKLING AND WEANED PIGLETS (Second Trial)

5.2.1 Pre-weaning performance and creep-feed intake

A total of 32 (Landrace x Large White) sows were used. An overview of all experimental treatments and procedures is given in Table 6 and Figure 1. Four groups of sows were arranged according to parity, expected farrowing date and body condition scores achieved 28 days before farrowing and allotted to four experimental treatments (Table 6). Control treatment (T4) was a commercial gestation, lactation, creep-feed and pre-starter diets, T1 consisted on the same diets than T4 but containing Fluidarom 1003® (375g/Tm), T2 the same that T1 but with a non-flavoured pre-starter diet and finally T3 was the same that T1 but with a non-flavoured creep-feed. Sows were exposed to a flavoured feed from Day 87 to farrowing and during 28 days of lactation. Composition and the estimated nutrient content were the same as trial 1 and are presented in Tables 1, 2, 4 and 5. Feeding programme was the same than for the first trial.

Table 6. Experimental treatments of Trial 2.

Experimental treatment	Gestation	Lactation	Creep-Feed	Pre-starter
T1	+	+	+	+
T2	+	+	+	-
T3	+	+	-	+
T4 (control)	-	-	-	-

+: inclusion of Fluidarom 1003® (375g/Tm) **-**: No inclusion of Fluidarom 1003®

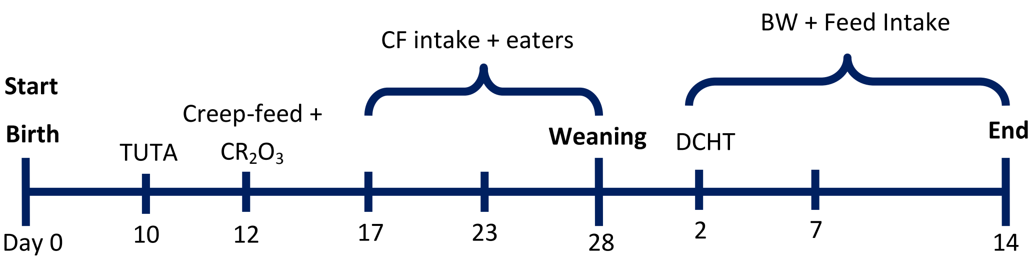

Figure 1. Overview of all controls made in second trial.

Sow's body weight was measured at Days 110 of gestation and at weaning; body condition scores was measured at Days 87 and 110 of gestation and at weaning; and feed intake was registered along the entire experimental period. After birth, the number of piglets (born alive and stillbirth), weight of piglets (born alive and stillbirth) and number of weaned piglets were registered. Litters were standardized by cross-fostering after birth. Piglets were weighed after cross-fostering and at weaning following the same procedures as those described in the first trial.

On Day 10 after birth a preference test was performed following a Tripe-U-Testing Arena (TUTA) previously used by (Figueroa et al., 2013a). The preference test was conducted among three different diets: a control diet (non-flavoured) and two flavoured diets in order to study a possible effect of flavour pre-exposure on the maternal diet on piglet preference during lactation. Flavoured diets were obtained by supplementing the control diet either with Fluidarom 1003® (375 g/Tm) or Lacto-Vanilla (500 g/Tm; a commercial flavoured feed additive based on >25 % of vanillin, >10 % of butyric acid and >10 % of diacetyl; Norel S.A., Spain). The cumulated time in contact with each diet by pair of littermates was measured for 5 minutes.

From Day 12 of age until weaning (Day 28), all litters were offered free access to creep-feed (Tables 1 and 2). Creep-feed was presented in mash form containing 1 % of chromium III oxide (Cr_2O_3) to determinate the number of piglets consuming creep-feed (eaters). Creep-feed was provided in commercial pan-feeders with hopper in order to ensure *ad libitum* access to feed. Creep-feed consumption was estimated by weighing the disappearance of creep-feed from the pan-feeder in three different periods (Days 17, 23 and 28). Marker appearance in faeces (Cr_2O_3) was individually monitored on days 17, 23 and 28 by sampling faeces with swaps, a green colour of the faeces was registered as a creep-feed eater according to the method previously described by (Bruininx et al., 2002).

5.2.2 Post-weaning performance and feed preference

A total of 24 complete litters (252 male and female, 10 SD=2 piglets/litter) [Pietrain x (Landrace x Large White)] were weaned at an average of 26 SD=2.5 days old. Piglets were weaned with an average BW of 7.3 SD=1.81 kg (selected by age, number of piglets/litter and litter weight at weaning) and moved to the weaning unit according to the previous exposure to Fluidarom 1003®. Entire litters were weaned and moved at the same pen (10 SD=2 pigs/pen) during 14 days period. Initial body weight was not balanced at weaning in order to keep the body weight differences achieved during the early pre-exposure of piglets to Fluidarom 1003®. The animals had *ad-libitum* access to feed and drinking water, except 1h before the preference test when piglets had no access to the commercial feeders during the test.

Pre-starter diets were offered in mash form for fourteen consecutive days post-weaning. Feed disappearance was recorded on Days 2, 7 and 14 post-weaning. Average daily gain (ADG) was calculated from 0-2 days, 0-7 days and 0-14 days. Moreover, on Day 2 after weaning a preference test was conducted following a Double choice test (DCHT) protocol between two diets supplemented either with Fluidarom 1003® (375g/Tm) or Lacto-Vanilla (500g/Tm) with 4 animals of each pen during 30 minutes. A total of 24 piglets per treatment were used (n=6 replicates per treatment).

5.3 IS FLUIDAROM 1003® HABIT MAINLY FORMED IN UTERUS OR VIA THE MILK SUCKLING? (Third Trial)

A total of 24 (Landrace x Large White) sows were used to study which is the main pre-exposure way before weaning for what piglets are familiarized with a flavour supplemented in sow diet and of its reinforcement via creep-feed. An overview of all experimental treatments and procedures is presented in Table 7 and Figure 2. Sows were selected and distributed at Day 86 of gestation into six experimental groups following a 3 x 2 factorial arrangement, taking into account the inclusion of Fluidarom 1003® in sow diets (without (Control), with during late gestation (Gestation) and with during lactation (Lactation)) and Fluidarom 1003® inclusion in the creep-feed (with or without) as main factors. Sow parity number and expected farrowing date were taken into account for the initial distribution. Dietary composition and the estimated nutrient content were the same as trial 1, and are presented in Tables 1, 2, 4 and 5. Diets were offered following the procedures described in the first trial.

Table 7. Experimental treatments of trial 3.

Treatment		Gestation	Lactation	Creep-feed	Post-weaning
Control	Ø -	-	-	-	-
	Ø +	-	-	+	-
Gestation	G -	+	-	-	-
	G +	+	-	+	-
Lactation	L -	-	+	-	-
	L +	-	+	+	-

-: Fluidarom 1003® not added; **+**: Fluidarom 1003® added.

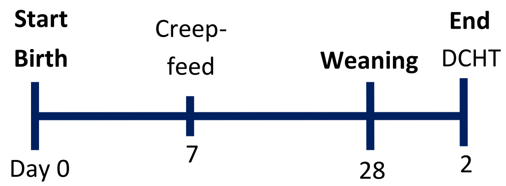

Figure 2. Overview of all controls made in third trial.

Again, litter size was standardized immediately after birth by cross-fostering within the same experimental treatment. All piglets were weighted at Day 7 and at weaning (Day 28) following the same procedures described for the first trial.

From Day 7 of age until weaning (Day 28) all litters were offered free access to a creep-feed diet. Half of the litters received a flavoured creep-feed (Ø +, G + and L +) and the other half received a non-flavoured creep-feed (Ø -, G - and L -).

5.3.1 Post-weaning feed preference

A total of 96 piglets [Pietrain x (Landrace x Large White)] were weaned at an average of 28 SD=1.5 days old and with an average BW of 8.35 SD=1.69 kg. Piglets were moved to the weanling unit taking into account the previous exposure to Fluidarom 1003® and distributed into 12 pens (2 pens per treatment, 8 pigs per pen). The weaning unit and pens have the same conditions as first trial. The animals had *ad-libitum* access to feed and drinking water.

After weaning, the animals received a pre-starter specification and feed was offered in mash form. Two days after weaning, piglets were individually weighed and a preference test was conducted with 2 animals (Double choice test (DCHT); n=8 per treatment) after 1h of fasting. Two diets differing in the flavour supplementation, either with or without Fluidarom 1003® (375g/Tm) or Lacto-Vanilla (500g/Tm), were provided at the same time and their intake evaluated after 30 minutes.

5.4 STATISTICAL ANALYSIS

5.4.1 Performance parameters

Parameters for sow and litter performance were analysed with ANOVA by using the GLM procedure of the statistical package SAS® (version 9.2, SAS Institute; Cary, USA). The statistical unit was the sow for all individual measurements and litter for piglet performance.

The following mathematical model was used:

$Yij = \mu + \alpha i + \beta j + \alpha\beta ij + \varepsilon ij$,

Where Yij were the general observations by sow category (i) and experimental treatment (j); μ was the general mean of all observations; α**i** was the effect of sow category; β**j** was the effect of experimental treatment; αβ**ij** was the interaction between treatment and effect of farrowing cycle and $\boldsymbol{\varepsilon \sim N(0,\sigma^2\varepsilon)}$ was the unexplained random error. Sow was also included as a random effect.

In order to get a balanced distribution amongst treatments for the data analysis, the farrowing number of sows was classified as sow category: 0= Zero parities (nuliparous) and 1; 1= Sows of 2, 3 and 4 farrows; and 2= parity 5 y 6.

In first trial average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) was analysed with ANOVA following a 2 x 2 factorial arrangement where the two main factors were Fluidarom 1003® supplementation in sows (gestation, lactation and creep-feed) diet and after weaning (pre- and starter diets) by using the GLM procedure of the statistical package SAS®. The statistical unit was the pen of pigs for all production measurements.

In second trial, piglet performance parameters before and after weaning were analyzed with a generalized linear model by using the MIXED procedure of the statistical package SAS® taking into account the day as a repeated measure, the creep-feed intake at each period and the experimental treatment (T1 to T4) as main factors, and their interactions. The statistical unit was the individual piglet for all production measurements.

The following complete mathematical model was:

*Y*ijkl = μ + αi + βj + δk +γl + αβij + αδik + αγil +βδjk +βγjl + δγkl + αβδijk + αδγijl + βδγjkl+ αβγijl + αβδγijkl + εijkl,

From our perspective some interactions had no sense and/or we were not interested in. Then, we removed them from the model. So the final mathematical model used was:

*Y*ijkl = μ + αi + βj + δk +γl + αδik + αγil +δγkl +αδγikl + εijkl,

Where *Y*ijkl were the parameters for all observations at each day (i), according to creep-feed intake in the first period (Day 12 to 17 of age) (j), creep-feed intake during the second period (Day 17 to 22 of age) (k), and for the different experimental treatments (l); μ was the general mean of all observations; α**i** was the effect of the day; β**j** was the effect of creep-feed intake during Day 12 to 16 of age; δ**k** was the effect of creep-feed intake during Day 17 to 22 of age; γ**l** was the effect of the experimental treatment; αδ**ik** was the interaction between day and the creep-feed intake during Day 17 to 22 of age; αγ**jl** was the interaction between day and the experimental treatment; δγ**kl** was the interaction between creep-feed intake during Day 17 to 22 of age and the experimental treatment; αδγ**ikl** was the interaction between day, creep-feed intake during Day 17 to 22 of age and the experimental treatment and **$\varepsilon \sim N(0,\sigma^2\varepsilon)$** was the unexplained random error.

All of the results are presented as Least Square Means taking into account a Tukey adjust and the alpha level used for the determination of significance for all of the analysis was 0.05.

5.4.2 Preference test

Triple-U-Test-Arena was analysed with General Linear Models using the GENMOD procedure of SAS®. The mathematical model used was:

Yij = μ + αi + βj + αβij + εij,

Where Yij was the time spend for the observations of each experimental treatment; μ was the general mean of all observations; α**i** was the effect of treatment (feed with Fluidarom 1003®, Lacto-Vanilla and without flavour); β**j** was the prenatal exposure [piglets coming from sows that ate (pre and post-exposed) or not (control) flavours during late gestation and lactation]; αβ**ij** was the interaction between experimental treatments and the prenatal exposure; and $\boldsymbol{\varepsilon \sim N(0,\sigma^2\varepsilon)}$ was the unexplained random error.

Double Choice Test was analysed with ANOVA using the MIXED procedure of the statistical package SAS®. The statistical unit was the pen of two or four piglets.

The mathematical model used was:

$Yij = \mu + \alpha i + \varepsilon ijk$,

Where Yij is the consumption estimated of each flavour; μ is the general mean of all observations; α**i** is the effect of treatment; and $\boldsymbol{\varepsilon \sim N(0,\sigma^2\varepsilon)}$ was the unexplained random error.

All of the results are presented as Least Square Means taking into account a Tukey adjust and the alpha level used for the determination of significance for all of the analysis was 0.05.

Additionally, the preference values for the Fluidarom 1003® were compared to the neutral value of 50 % by using a Student's t-test procedure of the statistical package SAS®.

6. RESULTS

6.1 PRODUCTIVE AND PERFORMANCE OUTCOME (First Trial)

In the present trial a total of 80 sow and their litters were used. Therefore a total of 480 piglets were selected at weaning to evaluate post-weaning performance for the entire transition period (35d post-weaning). No abnormal or clinical signs were detected in all animals during the entire experimental period. However, two sows of the treatment group (TG) were removed from the analysis due to abnormal performance and high mortality of the piglets, not associated with the experimental treatment. The results are presented as two main groups, sow (farrowing and lactation) and post-weaning performance.

6.1.1 Sow and litter performance parameters

Farrowing performance and productive parameters for the sows and their litters are presented in Table 1 in Annex. No significant effects were observed neither for body weight, body condition score and back fat thickness due to the experimental diets offered (P >0.05) along the gestation and lactation nor for farrowing performance (P >0.10; Annex Table 1). However, lower average daily feed intake (2.77 vs. 2.98 kg; P=0.002) was observed during the late gestation for the animals fed the Fluidarom 1003® supplemented diet (TG). During lactation no different feed intake was observed between treatments (P>0.1).

Litter performance during lactation was not affected due to the Fluidarom 1003® supplementation. However, higher litter body weight at weaning was observed for the sows fed diets supplemented with Fluidarom 1003® during late gestation and lactation (73.5 vs. 66.7 kg/litter; P=0.043).

6.1.2 Piglets performance after weaning (0 to 35 days post-weaning)

Body weight (BW), Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) for the entire period are shown in Table 1.

Average Daily Feed Intake was significantly affected by the dietary Fluidarom 1003® supplementation (P<0.01) to the sow (gestation + lactation) and by the Fluidarom 1003® supplementation after weaning (P=0.01) for the starter phase and entire period, but no interactions were observed between both main factors (P>0.10). Lower ADFI along the first week post-weaning (0 to 7 d; P<0.05), for the entire pre-starter phase (0 to 14 d post–weaning; P<0.05), for the starter phase (14 to 35 d post-weaning) and for the entire period (0 to 35d post-weaning) was observed in piglets from sows fed the control diet than those piglets from sows fed the diet containing the Fluidarom 1003®. On the other hand, higher ADFI was also observed for the animals that had been in contact with Fluidarom 1003® supplementation in all phases (pre-starter and starter phase).

Table 1. Effect of flavoured feed additive inclusion in late gestation and lactation diets and of flavour additive supplementation on weanling diets on body weight (BW), average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) during the pre-starter and starter phase (0 to 14 and 14 to 35 days post-weaning).

	Sow diet (Gestation –Lactation)					P-values		
	Control		Flavoured					
	Weanling diet		*Weanling diet*					
Item	Control	Flavoured	Control	Flavoured	Pooled SEM	Sow diet	Weaning diet	S diet x Wd
BW0, g	7415	7423	7426	7407	27.6	0.939	0.837	0.633
BW7, g	**8066** ^{b}	8319 ^{ab}	**8453** ^{a}	**8393** ^{a}	75.5	**0.004**	0.208	0.045
BW14, g	**9892** ^{b}	10219 ^{ab}	**10427** ^{a}	**10453** ^{a}	108.1	**0.001**	0.111	0.722
BW21, g	**11843** ^{b}	12317 ^{ab}	**12636** ^{a}	**12604** ^{a}	176.7	**0.004**	0.220	0.161
BW28, g	**15095** ^{b}	15845 ^{ab}	**16201** ^{a}	**16157** ^{a}	239.4	**0.005**	0.149	0.106
BW35, g	**18933** ^{b}	19774 ^{ab}	**20100** ^{a}	**20036** ^{a}	348.5	**0.048**	0.273	0.203
ADG07, g/d	92.1 ^{b}	128.1 ^{ab}	**146.7** ^{a}	**140.9** ^{a}	10.52	**0.003**	0.160	0.054
ADFI07, g/d	151.7 ^{b}	174.8 ^{ab}	**202.8** ^{a}	**196.3** ^{a}	7.98	**0.000**	0.307	0.072
FCR07	1.132	1.389	1.426	1.46	0.234	0.441	0.539	0.638
ADG014, g/d	174.4 ^{b}	199.8 ^{ab}	**212.0** ^{a}	**217.5** ^{a}	8.04	**0.002**	0.063	0.224
ADFI014, g/d	238.0 ^{b}	259.5 ^{ab}	**286.6** ^{a}	**297.0** ^{a}	8.72	**0.000**	0.076	0.525
FCR014	1.399	1.299	1.364	1.367	0.0339	0.326	0.162	0.136
ADG1435, g/d	360.9	393.3	407	402.5	28.28	0.335	0.625	0.519
ADFI1435, g/d	649.6 ^{b}	**721.5** ^{a}	**722.5** ^{a}	**741.5** ^{a}	16.65	**0.008**	**0.010**	0.121
FCR1435	1.864	1.917	1.852	1.912	0.1241	0.944	0.651	0.976
ADG035, g/d	286.3	315.9	328.9	328.5	16.73	0.107	0.390	0.375
ADFI035, g/d	484.9 ^{b}	**536.7** ^{a}	**548.1** ^{a}	**563.6** ^{a}	12.34	**0.001**	**0.010**	0.151
FCR035	1.678	1.67	1.656	1.694	0.0689	0.983	0.833	0.742

Although piglet BW category (block of BW) and their interaction were taken into account for the statistical analysis, table 1 only show the main factors (p-values for sow and weaning diet and their interaction) as the other factors did not affect the main results.

Piglet BW and ADG was significantly affected by the dietary Fluidarom 1003® supplementation of the sow diet ($P<0.05$) but not by the Fluidarom 1003® supplementation of the post-weaning diets ($P>0.10$) or their interaction ($P>0.10$).

Lower BW and ADG were observed for the control animals (never Fluidarom 1003® was included into diet). BW at 7, 14, 21, 28 and 35 days post-weaning ($P<0.05$) and ADG during the first week post-weaning (0 to 7d; P<0.003) and for the entire pre-starter phase (0 to 14d; P=0.002) showed in Table 1.

Feed conversion ratio was not affected by the main factors along the experimental phase (P>0.10).

6.2 STUDY OF THE PREFERENCE FOR FLUIDAROM 1003® IN SUCKLING AND WEANED PIGLETS (Second Trial)

Data from one sow of Treatment 1 was missed as the animal died before farrowing and another sow from the same treatment was removed from the analysis due to a delay in the farrowing day (because there was a larger deviation in age and BW than the rest of the litters within the same experimental treatment).

6.2.1 Sow and litter performance parameters

Farrowing performance and productive parameters of all sows and litters are presented in Table 2 in Annex. No significant differences were observed on the performance of sows and litters due to the experimental treatments (Fluidarom 1003® supplementation in the sows or piglets diets). However it is remarkable that the number of piglets in T-4 (control) tended to be lower than the number in others treatments as no cross-fostering was allowed between treatments.

Total creep-feed disappearance was measured from Day 10 after birth until weaning (Table 2). Piglets from treatments T3 and T4 (unflavoured creep-feed) showed higher creep-feed intake (Table 2) and higher body weight at weaning (7.6 kg) than animals with Fluidarom 1003® included in creep-feed (7.0 Kg; P<0.01)

No differences were observed on the average body weight (BW) after cross-fostering and at weaning (P>0.10; Table 3) neither for average daily gain (ADG) (P>0.1). However, a strong interaction (P=0.0004) on piglet body weight was observed between the experimental treatment and the day of BW measure (at cross-fostering or at weaning).

Table 2 Creep-feed intake (g/eater piglet/day) and percentage of eater piglets (in brackets) during the suckling period.

Item		Treatment (G+L+CF+W)				Pooled SEM	P-values
		T1	*T2*	*T3*	*T4*		Treatment
		+ + + +	+ + + -	+ + - +	- - - -		
Day 12-16 of live	**ADFI**	3.86[c]	5.98[b,c]	**11.02[a,b]**	**14.26[a]**	2.09	0.004
	% eaters	(42±24)	(60±25)	(81±19)	(67±17)		
Day 17-22 of live	**ADFI**	7.50[b]	11.29[b]	**19.36[a]**	**11.82[b]**	2.78	0.029
	% eaters	(72±14)	(81±16)	(91±13)	(84±18)		
Day 23-28 of live	**ADFI**	8.00	13.52	**18.24**	**22.86**	4.21	0.073
	% eaters 8	(96±6)	(96±6)	(97±7)	(94±12)		
Day 12-28 of live	**ADFI**	5.97[b]	11.11[a,b]	**14.73[a]**	**15.98[a]**	2.36	0.019
	% eaters	(70±27)	(79±18)	(90±8)	(82±14)		

+: Fluidarom 1003® added; **-**: Fluidarom 1003® not added.

Table 3. Body weight (BW) evolution during the lactating period (piglet performance).

Treatment	G	L	CF	W	Body Weight (g) Cross-Fostering	Body Weight (g) Weaning
T-1	+	+	+	+	1567	7090
T-2	+	+	+	-	1754	7214
T-3	+	+	-	+	1354	7140
T-4 Control	-	-	-	-	1513	**8361**
				Mean	1547	7451
				Pooled Standard Error	316.50	316.50
				Day (Pr>F)	<0.0001	
				Creep-feed intake (12-16d) effect (Pr>F)	0.772	
				Creep-feed intake (17-22d) effect (Pr>F)	0.117	
				Treatment effect (Pr>F)	0.214	
				Treat x Day effect (Pr>F)	**0.0004**	
				Treat x Creep-feed intake (17-22d) effect (Pr>F)	0.724	
				Day x Creep-feed intake (17-22d) effect (Pr>F)	0.056	
				Treat x Day x Creep-feed intake (17-22) effect (Pr>F)	0.281	

G: gestation; **L:** lactation; **CF:** creep-feed; **W:** weaning; **+**: Fluidarom 1003® added; **-**: Fluidarom 1003® not added
Creep-feed intake (12-16d) effect: creep-feed intake during the period of 12 to 16 days of age.
Creep-feed intake (17-22d) effect: creep-feed intake during the period of 17 to 22 days of age.

No differences were observed in the Triple-U-Testing Arena (TUTA) (P>0.1). Piglets became highly stressed, looking for alternative routes to escape without interest in solid feed (nor flavoured nor non-flavoured).

6.2.2 Performance parameters after weaning (0 to 14 days post-weaning)

Average daily feed intake (taking into account the initial body weight at weaning (BW0) as a covariate) was higher for T3 during the first week of pre-starter phase (P=0.016) and for the whole pre-starter phase 0 to 14 days post-weaning (P=0.010; Table 4).

Table 4. Average daily feed intake (g/d) during pre-starter phase. Using the initial body weight (BW0) as a covariate.

	Treatments (G+L+CF+W)				Pooled SEM	P-values	
	T1	*T2*	*T3*	*T4*			
Item	+ + + +	+ + + -	+ + - +	- - - -		**Treatment**	**BW0**
ADFI 0-2 days of live	112.74	95.23	151.68	119.88	15.83	0.102	0.057
ADFI 0-7 days of live	198.48[b]	179.13[b]	**248.21[a]**	197.53[b]	14.43	**0.016**	0.048
ADFI 7-14 days of live	333.87	314.94	393.12	342.97	21.94	0.096	0.377
ADFI 0-14 days of live	266.24[b,c]	247.09[b]	**320.28[a]**	287.49[a,c]	14.34	**0.010**	0.111

+: Fluidarom 1003® added; **-**: Fluidarom 1003® not added.

No differences in body weight were initially observed due to the experimental treatments and number of eaters classified at Day 17. However, a significant effect was observed on the interaction between the dietary treatment and day (higher BW was observed for T3 than T1, T2 and T4 on day 14 P<0.001; Table 5) and the number of eaters classified at Day 23 (higher BW was observed in non-eater than eater piglets, 9.5 vs. 8.4 Kg respectively; P<0.05). A triple interaction between the experimental treatment, day of measure and number of eaters after 11 days of exposure to creep-feed was also observed (P=0.048).

No different average daily gain (ADG) was observed between treatments for the first and second week post-weaning (0 to 7 or 7 to 14 days post-weaning; P>0.10) and for the entire pre-starter phase (0 to 14d post–weaning; P>0.10).

Table 5. Piglet body weight (BW, g) at weaning and on days 7 and 14 post-weaning.

Treatment	G	L	CF	W	Body Weight (g)		
					Day 0	Day 7	Day 14
T-1	+	+	+	+	7507	8480	10080
T-2	+	+	+	-	7630	8410	9970
T-3	+	+	-	+	7892	9308	**11099**
T-4 Control	-	-	-	-	7859	8786	10184
				Mean	7722	8746	10333
				Pooled Standard Error	531.66	531.69	531.72
				Day effect (Pr>F)		<0.0001	
				Creep-feed intake (12-16d) effect (Pr>F)		0.675	
				Creep-feed intake (17-22d) effect (Pr>F)		**0.014**	
				Treat effect (Pr>F)		0.673	
				Treat x Day effect (Pr>F)		**0.0006**	
				Treat x Creep-feed intake (17-22d) effect (Pr>F)		0.585	
				Day x Creep-feed intake (17-22d) effect (Pr>F)		0.241	
				Treat x Day x Creep-feed intake (17-22d) effect (Pr>F)		**0.048**	

G: gestation; **L:** lactation; **CF:** creep-feed; **W:** weaning; **+**: Fluidarom 1003® added; **-**: Fluidarom 1003® not added
Creep-feed intake (12-16d) effect: creep-feed intake during the period of 12 to 16 days of age.

Creep-feed intake (17-22d) effect: creep-feed intake during the period of 17 to 22 days of age

6.2.3 Feed preference between Fluidarom 1003® and a lacto-vanilla flavour 2 days after weaning

The preference for Fluidarom 1003® (375g/Tm of feed) over a lacto-vanilla (500g/Tm of feed) was directly compared. Results are presented as feed intake corrected by the number of piglets and BW. The percentage of preference is reported in Figure 1. Piglets from the control group (T4) showed a higher preference for lacto-vanilla (P<0.05) and no preferences for Fluidarom 1003® or lacto-vanilla were observed for piglets corresponding to treatments T1 and T2 (P>0.1). However, a strong preference (P<0.05) for Fluidarom 1003® was observed in T3 piglets (the ones that received an early contact with Fluidarom 1003® via the sow diet, but were offered an unflavoured creep-feed).

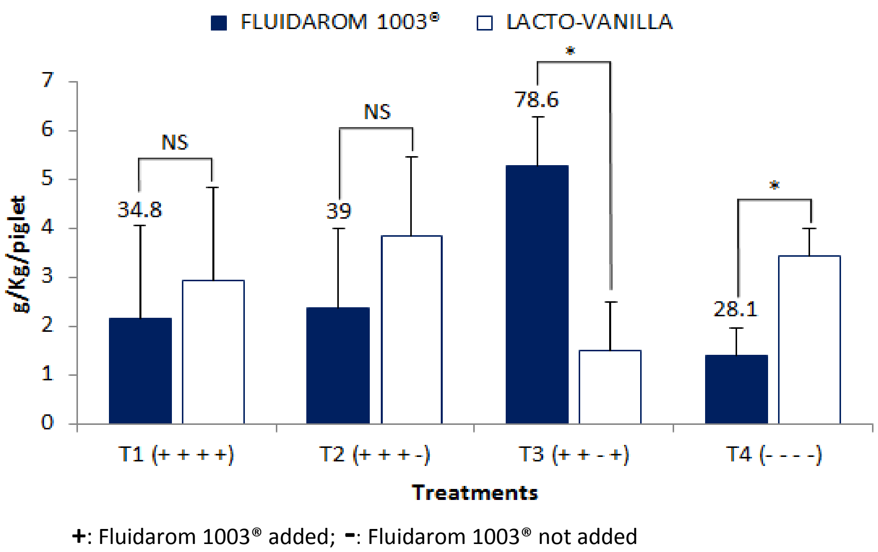

Figure 1. Feed consumption pondered by number and body weight of piglets and feed preference about Fluidarom 1003® as total consume percentage (number on top of the bars), statistical comparison against the preference neutral value (50%; indifference). NS: P>0.1; *: P<0.05 (Trial 2).

6.3 IS FLUIDAROM 1003® HABIT MAINLY FORMED IN UTERUS OR VIA THE MILK SUCKLING? (Third Trial)

6.3.1 Feed preference after weaning (Day 2 post-weaning) measured by using a Double Choice Test (DCHT)

The preference for Fluidarom 1003® over a lacto-vanilla (negative control) was directly compared. Results are presented as feed intake pondered by the number and BW of piglets in each pen. Additionally, the percentage of preference for Fluidarom 1003® compared to the negative control is also reported in Figure 2.

Piglets from the sows of the control group that were offered a creep-feed without Fluidarom 1003® inclusion (Ø-) showed higher preference for lacto-vanilla (P<0.05). In addition piglets previously exposed to Fluidarom 1003® during the pre-natal period (G+; Fluidarom 1003® supplemented on gestation diet and creep-feed) also tended to prefer Lacto-Vanilla (P<0.1).

No preferences for Fluidarom 1003® were observed two days after weaning for piglets corresponding to treatments Ø+, G-, L- and L+. However piglets pre-exposed during the pre-natal period (G-; dietary Fluidarom 1003® supplementation during late gestation but not on creep-feed) were those showing a quantitative preference (56.1%) for the Fluidarom 1003®

supplemented diet as compared to Lacto-vanilla (negative control) two days post-weaning (P=0.19).

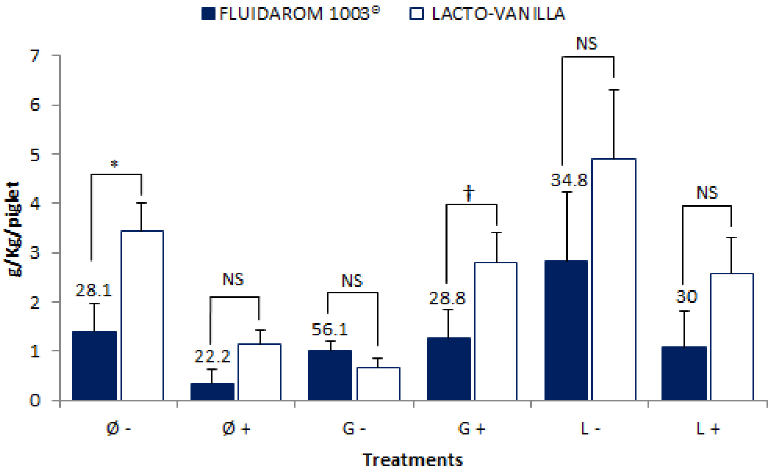

Figure 2. Feed consumption pondered by number and body weight of piglets and feed preference about Fluidarom 1003® as total consume percentage (number on top of the bars), statistical comparison against the preference neutral value (50%; indifference). NS: P>0.1; *: P<0.05; †: P<0.1 (Trial 3).

7. DISCUSSION

The present work aimed to study the effects on the piglets behaviour of a pre- and postnatal exposure through the maternal diet to Fluidarom 1003®, a commercial flavour based on >25 % anethol, >25 % cinnamaldehyde and >10 % eugenol. Preference and acceptance of piglets towards the same flavoured feed at weaning were the main studied parameters, as well as the subsequent growth in the post-weaning period.

The first study revealed that pre- and postnatal exposure to Fluidarom 1003® throughout the maternal diet improved growing performance after weaning. Higher feed intake and growth were observed along the pre-starter and starter phase (0 to 35 days post-weaning) mainly due to the maternal exposure to the flavour. We suggest that Fluidarom 1003® supplementation in sow diet may promote an early food habit formation in the piglets that could affect the piglets feed intake and performance after-weaning. The basis of this effect could be related with the transfer of some compounds to the womb and/or the milk. It has been shown that anise (anethol) offered in swine diets (Langendijk et al., 2007) may be transferred to both amniotic fluid and milk (Hepper, 1988). Moreover, higher feed intake after weaning could also be related with a stress-reducing effect by piglet exposure with a familiar flavour acquired before weaning. This effect widely accepted in other mammals like humans (Varendi et al., 1998) and rats (Smotherman, 1982).

However, in our first trial we were not able to discriminate between the pre-natal (gestation) and post-natal (lactation + creep-feed) effects, because both were administered together. Then, piglet learning could have been achieved through one of these steps, or by their interconnection and positive reinforcement from pre-natal to weaning experience. To solve and fractionate these mechanisms, we hypothesized in the second trial that an early creep-feed contact and intake may play an important role on feed and growth performance after weaning. Therefore, the use of the same flavour in maternal diets and in the creep-feed may facilitate creep-feed intake and their further positive reward after weaning when the same flavour is used.

The consumption of creep feed and the influence of flavours in the creep feed diet

Unexpectedly, the highest growth of piglets during lactation in the second trial was observed in piglets without pre- and post-natal contact to Fluidarom 1003®. We related this effect to the consumption of unflavoured creep-feed.

Thus, in our second trial we observed that piglets preferred to consume non-flavoured than flavoured creep-feed. Both, litters corresponding to the control sows (non-flavoured diets) and litters corresponding to sows fed a flavoured diet showed higher intake of control creep-feed (non-flavoured) than the creep-feed containing the flavour (277 vs. 153 g/eater piglet, respectively). Therefore, we observed a negative impact of the flavour supplementation on piglet feed intake during lactation, which is different to the results described previously by Langendijk et al. (2007) who observed that creep-feed intake may be stimulated when specific flavours associated with the diet and the milk of the sow are offered.

No preference of the suckling piglets for a creep-feed supplemented with a flavour, even if previously was given to the sows, was also reported by (Figueroa et al., 2013). It appears that during the lactating period piglets preferred a simple unflavoured diet (cereal base, without added flavours). Moreover Sulabo et al. (2010a) reported that flavour supplementation to creep-feed diets did not improve daily creep-feed intake, average daily gain or feed efficiency. Our results and those described in the literature suggest that a non-flavoured creep-feed is more consumed than flavoured creep-feed.

However, we should remark that what we call "a non-artificial flavoured diet" is in fact a diet containing the intrinsic flavours of their own ingredients. The high content of the creep-feed in dairy by-products, like milk whey, lactose and even skimmed milk, may be one of the main reasons that explain that piglets preferred a plain creep feed over a flavoured modified diet. It could be speculated that some of these intrinsic flavours may be also found in the sow's diets and their fluids and secretions, favouring the attraction of piglets towards these diets. We could also suggest that the use of creep-feed diets with similar ingredients than those used in maternal diets should be taken into account for creep-feed formulation. To reduce feed neophobia in the piglets, a variety of ingredients should be included in the maternal diets. This idea has been exposed for humans, as higher is the variety of the ingredients consumed by the mother, larger is the number of new volatiles that the baby may be get used to while sucking (Mennella et al., 2001) and lower the negative impact on food acceptance when complex diets are offered to the babies later on.

Creep feed intake is one of the other controversial points because often it is difficult to standardise the different condition in which the studies has been performed, and therefore the results obtained in the literature are not comparable. Among the wide range of intakes reported in the literature, (Pluske et al., 2007) reported creep-feed consumption of 703 g per piglet (SD= 608) in 19 days of lactation, while (Pajor et al., 1991) found an average creep-feed consumption of 468 g (range 13-1385) per piglet in 18 days of lactation and (Bruininx et al., 2002) observed that total creep-feed disappearance was 377 g per piglet (SD=220.5) in 17 days of lactation. In our study we registered a creep feed consumption of 153 g per piglet (SD= 41) with flavoured creep-feed and 277 g per piglet (SD= 41) with non-flavoured creep-feed. The creep-feed consumption of the present study is lower than in others studies which could depend on the different composition of creep-feed, the animal's genetics, animals per litter and type of creep-feeding. The differences highlight the difficulty of comparing creep-feeding among different studies.

Among the main causes that affect creep-feed consumption, Sulabo et al. (2010b) remarked that only 60% of pigs were creep-feed eaters. In our second trial a higher amount of creep-feed eaters was observed for pigs fed non-flavoured (86%) than for pigs fed the flavoured diet (74.5%). Sulabo et al. (2010a) observed that addition of the feed flavour to creep-feed did not affect the proportion of creep-feed eaters. The difference between studies results might be due to the difficulty of marker detection (chromium III oxide (Cr_2O_3)) detection in faces. Bruininx et al. (2004) reported that a considerable proportion of piglets (33%) could not be designated into any categories due to "indistinguishable colours" of the faces with this dye. Contrarily, Pluske et al. (2007) reported that by using indigo carmine as indigestible marker almost up to 90% of piglets could be classified as eaters due to the presence of with creep feed as their faces staining faeces (cyan).

The influence of flavour in the sow diet to guide the post-weaning feed intake of piglets

In natural environments, piglets are exposed to a lot of new challenges, and train- and error-learning is essential in nature. 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., 2011b). We observed that pre-natal (gestation) and post-natal (lactation + creep-feed) flavour exposure via maternal diet may reduce the negative impact of weaning when the same flavour was offered in post weaning diet (**Trial 1**). Moreover, those piglets with pre-weaning flavour exposure via maternal diet and creep-feed, showed higher BW and ADG along the entire transition period than those with no previous exposure to flavoured diets. The similar results were obtained for those piglets without pre-weaning exposure to flavour but with post-weaning flavour exposure. This could be explained because the different experimental treatments were randomized within the same farrowing room. Then sows and their corresponding litters shared the same ambient and a cross-contamination of volatiles within the room could have been occurred. Therefore, we can't exclude that piglets did not have a previous familiarity with those volatile compounds.

In the **second trial (Trial 2**) we hypothesised that the positive results previously observed in Trial 1 could be explained by the early creep-feed contact and intake following the familiar link with volatile compounds exposed via amniotic fluid and/or milk.

However, and contrarily to our hypothesis, the incorporation of the flavour into the creep-feed diet did not improve feed intake during lactation and the piglet preference for Fluidarom 1003® at weaning. On the other hand, a higher effect of the maternal learning was observed when a non-flavoured creep-feed diet was administered to the piglets.

A high preference for Lacto-Vanilla, a commercial flavour based on butyric acid, vanillin and diacetyl was observed in those piglets without any previous exposure to flavours (control group). This might be explained because butyric acid is one of main fatty acids in milk; vanillin is the primary component of the extract of the vanilla bean and diacetyl is a natural by-product of fermentation that give butter its characteristic taste. It is possible that butyric acid and diacetyl, both found in sow's milk, may justify a high preference for this flavour.

Having rejected the hypothesis around the positive effect of flavouring creep-feed to bridge the connection between the prenatal learning and the post-weaning period, a **third experiment** was performed. This experiment aimed to study the most important maternal pathway that could explain a maternal flavour formation, either pre-natal or post-natal. Even though many studies examined perinatal flavour learning in a lot of species during four decades, there is still little information about the mechanisms affecting flavour learning and how perinatal flavour learning may improve feed acceptance and feed intake in piglets (Oostindjer et al., 2010). So, in our third trial we observed that piglets previously exposed to Fluidarom 1003® during the pre-natal period (only gestation) but not in the creep-feed were able to neutralize the high preference previously observed for lacto-vanilla. Those piglets offered a flavoured creep-feed preferred the Lacto-Vanilla flavour after-weaning. It seems again, that an aversive reaction to the flavour offered in the creep-feed diet turns down the preference for the pre-natal conditioned flavour. This result might be due to flavour supplemented in the creep-feed, and as we suggested previously, the use of a flavoured creep-feed induces to confusion while piglets are still sucking. Moreover, post-natal flavour exposure alone (milk pathway) did not exert any effect on the preference for the pre-weaning flavour exposure either with or without flavour in the creep-fed. As it was previously reported by Oostindjer et al. (2010) piglets only with post-natal flavour exposure (lactation) did not show any preference for the pre-exposed flavour, which suggests that only a pre-natal exposure is the most powerful pathway for maternal flavour conditioning of newborn piglets and it seems enough to create a post-weaning preference for a previously exposed flavour.

Figueroa et al. (2013a) also observed that prenatal flavour exposure (anise and milky-cheese) via maternal diet significantly influenced the piglet’s preferences during lactation and until 5 days after weaning (throughout a Triple-U-Testing Arena (TUTA)). However, a preference for Fluidarom 1003® was not observed during lactation in our trial by using the TUTA method in feed. This might be due to the use of solid feed instead of using adsorbent strips impregnated with a high concentration of flavour like the Figueroa et al. (2013a) study. We decided to offer directly a choice between three different diets in order to actually evaluate if piglets were able to discriminate between the two different volatile compounds (commercial flavours; Fluidarom 1003® and Lacto-Vanilla) supplemented on top of the creep-feed diets. Piglets are able to detect volatile compounds in feed at very low levels of inclusion, and then we tried to simulate as much as possible a practical situation. However, young animals did not pay much attention to the different diets likely because it was the first time to have contact with feed. On the other hand, piglets showed an escaping behaviour.

Then, we confirmed that young animals can learn about flavours from the maternal diet that appear in the amniotic fluid and also that these preferences acquired before birth for the prenatally exposed flavour were long-lasting. This flavour continuity, may act as a psychological link, remembering to piglets the hedonic remembrance of the maternal environment the post-weaning periods (Arias and Chotro, 2006).

8. CONCLUSIONS

1. Inclusion of 375g/Tm of Fluidarom 1003® in the sow diets for late gestation and lactation improves weight gain and feed intake of weaning piglets throughout all the phase (0 to 35 days).
2. Young animals can learn about flavours from the maternal diet (gestation and lactation), and this effect is reinforced using an unflavoured creep-feed. Adding flavour only at the end of gestation is enough to create preference for that flavour. These preferences are still persistent in post-weaning period (at least until 2 days post-weaning).
3. Flavour supplementation to creep-feed diets does not improve daily creep-feed intake.
4. A maternal learning strategy may be used in the pig industry to develop flavour preferences and to reduce flavour or feed neophobia in piglets during weaning (critical phase).

9. ANNEX

Table 1. Sow reproductive and productive parameters and litter performance due to Fluidarom 1003® supplementation in gestating and lactating diets (Trial 1).

	Experimental Treatments			P-values
	CG	*TG*	*Pooled SEM*	*Treatment*
n	40	38	-	
Body Weight (BW; kg)				
day 73 of gestation	246	248	4.22	0.782
day 110 of gestation	266	266	4.94	0.917
28 days after farrowing	216	215	6.09	0.911
Body Condition Scoring (BCS)				
day 73 of gestation	2.98	2.93	0.109	0.752
day 110 of gestation	3.10	3.09	0.107	0.945
28d after farrowing	2.67	2.58	0.112	0.541
Back Fat (BFP2; in mm)				
day 110 of gestation	15.6	15.5	0.83	0.926
28d after farrowing	12.1	12.6	0.83	0.688
Sow farrowing performance				
ADFI (late gestation; kg)	**2.98**	**2.77**	0.049	**0.002**
Total number of piglets born	16.8	15.9	0.82	0.410
Piglets born alive	13.8	13.3	0.67	0.536
Stillbirth	2.4	2.0	0.44	0.583
Mummies	0.65	0.63	0.251	0.941
BW of piglets born alive, kg	1.35	1.44	0.052	0.194
BW of stillbirth piglets, kg	1.17	1.16	0.087	0.934
Total litter weight at birth, kg	21.0	21.4	0.94	0.754
Sow and litter performance				
Initial number of piglets by litter (CF)	12.6	13.1	0.39	0.370
Final number of piglets by litter (23d)	11.0	11.6	0.28	0.100
Mortality rate (CF-23d; %)	11.9	10.6	2.91	0.730
ADFI (Cross-Fostering to 23d; kg)	5.43	5.72	0.210	0.319
TFI (Cross-Fostering to 23d; kg)	121.9	129.2	4.68	0.265
Litter weight at CF, kg	17.3	18.7	0.71	0.133
Litter weight at 23d, kg	**66.7**	**73.5**	2.48	**0.043**
Average initial BW (CF; kg)	1.38	1.44	0.060	0.452
Average final BW (23d; kg)	6.06	6.35	0.164	0.184
ADG (CF-23d; kg)	0.208	0.218	0.0065	0.256

The table 1 is simplified; only show the p-value of treatment. But the model included farrowing, batch and their interactions as factors to control the error experimental.
CG: Control group (No inclusion Fluidarom 1003® in diets); **TG**: Treatment group (inclusion Fluidarom 1003® in diets)

Table 2. Sow reproductive and productive parameters and litter performance due to Fluidarom 1003® supplementation in gestating and lactating diets (Trial 2).

	Experimental Treatments				*Pooled SEM*	**P-values**
	T-1 *G L CF W* *+ + + +*	***T-2*** *G L CF W* *+ + - -*	***T-3*** *G L CF W* *+ + - +*	***T-4*** *G L CF W* *- - - -*		***treatment***
n	7	8	9	9	-	
Body Weight (BW; kg)						
day 110 of gestation	262	259	250	258	9.56	0.820
28 days after farrowing	223	251	240	221	9.85	0.090
Body Condition Scoring (BCS)						
day 87 of gestation	3.72	3.38	3.50	3.75	0.22	0.450
day 110 of gestation	2.86	3.00	2.85	3.02	0.17	0.795
28d after farrowing	2.67	2.29	2.42	2.63	0.14	0.132
Sow farrowing performance						
ADFI (late gestation; kg)	3.11	3.15	3.10	3.24	0.09	0.637
Total number of piglets born	15.1	14	14.1	13.7	1.82	0.933
Piglets born alive	14.1	13.3	12.9	10.6	1.52	0.297
Stillbirth	0.7	0.6	1.1	2.3	0.60	0.123
Mummies	0.28	0.08	0.23	0.87	0.33	0.241
BW of piglets born alive, kg	1.544	1.518	1.256	1.568	0.10	0.118
Total litter weight at birth, kg	22.354	20.457	16.260	19.395	2.05	0.197
Sow and litter performance						
Initial number of piglets by litter (CF)	12	12.3	12.4	**10.2**	0.72	0.071
Final number of piglets by litter (28d)	10	11	11	10	0.75	0.624
Mortality rate (CF-28d; %)	13.8	11.6	13.7	1.7	0.05	0.211
ADFI (Cross-Fostering to 28d; kg)	4.66	4.76	4.83	4.90	0.11	0.435
TFI (Cross-Fostering to 28d; kg)	158.49	162.38	164.23	158.54	4.99	0.762
Litter weight at CF, kg	18.177	18.651	15.423	16.139	1.41	0.247
Litter weight at 28d, kg	71.503	76.923	77.042	78.884	7.10	0.851

The table 2 is simplified; only show the p-value of treatment. But the model included farrowing and their interactions as factors to control the error experimental.

G: Gestation; **L:** Lactation; **CF:** Creep-Feed; **W:** weaning.

+: inclusion of Fluidarom 1003® (375g/Tm) **-**: No inclusion of Fluidarom 1003®

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