Universitat Autònoma de Barcelona

Facultad de Veterinaria

Departament de Ciència Animal i dels Aliments

Learning strategies to increase piglets feed intake after weaning

PhD. Thesis

Jaime Figueroa Hamed

Bellaterra, 2012

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Supervisors:

Dr. José Francisco Pérez **Dr. Xavier Manteca i Vilanova**

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CERTIFICAN

Que la memoria titulada "**Learning strategies to increase piglets feed intake after weaning"** presentada por Jaime Figueroa Hamed para optar al grado de Doctor en Producción Animal, ha estado realizada bajo nuestra dirección y considerándola acabada autorizamos su presentación para que sea juzgada por la comisión correspondiente.

Y para que conste a efectos oportunos, firman el presente certificado en Bellaterra, a
5 de Noviembre de 2012.

Dr. José Francisco Pérez **Dr. Xavier Manteca i Vilanova**

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¿Quién no echa una mirada al sol cuando atardece?
¿Quién quita sus ojos del cometa cuando estalla?
¿Quién no presta oídos a una campana cuando por algún hecho tañe?
¿Quién puede desoír esa campana cuya música lo traslada fuera de este mundo?

Ningún hombre es una isla entera por sí mismo.
Cada hombre es una pieza del continente, una parte del todo.
Si el mar se lleva una porción de tierra, toda Europa queda disminuida, como si fuera un promontorio, o la casa de uno de tus amigos, o la tuya propia.
Ninguna persona es una isla; la muerte de cualquiera me afecta, porque me encuentro unido a toda la humanidad; por eso, nunca preguntes por quién doblan las campanas; doblan por ti.

John Donne, Londres (1572-1631)

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"La sabiduria no consiste en contar cuantas gotas de agua hay en una cascada. La sabiduria consiste en saber por que el agua busca la tierra"

Glossary

ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AMY	Amygdala
AW	After Weaning
BW	Body Weight
CNS	Central Nervous System
CP	Crude Protein
CR	Conditioned Response
CS	Conditioned Stimuli
CS-	Conditioned Stimuli Associated with Neutral or Negative Consequences
CS+	Conditioned Stimuli Associated with Positive Consequences
D1	Dopamine like-receptor SCH23390
D2	Dopamine like-receptor Raclopride
DA	Dopamine
DCHT	Double-choice test
DE	Digestible Energy
DM	Dry Matter
FC	First Contact Measure
FGR	Feed:Gain Ratio
G1	Group one
G2	Group two
GHSR	Growth hormone secretagogue receptor
GLP-1	Glucagon-like peptide-1
IG	Intra-gastric
KO	Knock Out
LH	Hypothalamic Nuclei
LiCl	Lithium Chloride
mGluR4	Specific taste receptor mediate the savoury taste umami
mPFC	Medial Prefrontal Cortex ()
MSG	Monosodic Glutamate
NAc	Nucleus Accumbens

NaCl	Sodium Chloride
NTS	Nucleus Tractus solitarius
PDP	Porcine Digestible Peptides
PVH	Paraventricular Hypothalamus
PW	Post-Weaning
SPC	Soybean Meal Concentrate
T1R1/T1R3	Umami Taste Heterodimer Receptor
T1R2	Taste Receptor Family1 Member 1
T1R2/T1R3	Sweet Taste Heterodimer Receptor
T1R3	Taste Receptor Family1 Member 2
T2Rs	Taste Receptor of Family 2
TR	Taste Reactivity
TUTA	Triple-U-Testing Arena
UAB	Universitat Autònoma de Barcelona
US	Unconditioned Stimuli
VTA	Ventral Tegmental Area

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Summary

Sudden changes inside intensive pigs' production cause adaptation problems to new environmental conditions that reduce feed intake and welfare specially during weaning. Pigs have a strongly developed oro-nasal sensory system highly use to recognize and prefer feeds cues previously learned as a result of positive experiences. The present work studies different learning strategies to increase the preference for flavours cues and ingredients during lactation and after weaning. We propose that pigs may learn flavour preferences from their mother (Chapter 1 and 5), from trial and error tasks (Chapter 2 and 3) or from social interactions with conspecifics (Chapter 4) and that this learning may improve intake of new diets.

Chapter 1 evaluate if piglets have the ability to discriminate and prefer natural and artificial cues of maternal amniotic fluid after birth. During 7 minutes piglets couples were tested to prefer between maternal amniotic fluid, alien amniotic fluid and water (Experiment 1) or between a flavour added into late gestation maternal diet, a control flavour and water (Experiment 2). The same prenatal strategy was used to study piglet's preferences for flavoured or unflavoured creep feed during the suckling period (Experiment 3). Suckling piglets preferred amniotic fluid flavours from their own mother over an alien amniotic fluid and also they preferred flavours cues given to the sows during the gestation period. However, prenatal flavour exposure only attenuated intake differences with the preferred unflavoured creep feed diets.

Chapter 2 study if piglets could learn by trial and error to prefer a new flavour cue. In this way flavour preferences were conditioned by a protein source in weaned pigs. Animals were trained to drink (30 min/day) one flavour (CS+) mixed into a 2% protein solution (Soybean Protein Concentrate or Porcine Digestible Peptides) and another flavour (CS-) mixed into water during 6 alternate sessions. Animals preferred the CS+ over the CS- at all test days. No differences were observed between the conditioning effects of the two proteins. In a second experiment a conditioned flavour preference also enhanced the attraction to a protein (PDP) when the flavour and protein were combined showing a synergy effect between the CS+ and US attraction.

Chapter 3 also study how pigs could learn a new flavour preference due to experience. Unlike Chapter 2, flavour preferences were conditioned only by the post-ingestive effect of nutrients in pigs. Pigs were trained during 8 d with one flavour (CS+) into a protein or sucrose solutions on odd days and another flavour (CS-) into 100 mM of monosodium glutamate (MSG) or 1% sucrose + 0.08% saccharine solutions on even days (5 L-bottle for 24 h). Piglets showed higher intakes for CS+ than CS- acquiring flavour preferences through associative learning between flavours and post-ingestive effects of nutrients.

Chapter 4 describes a specific way of social learning of feeding behaviour in pigs in which a naive animal could learn specific flavour preferences by a close and brief contact with a conspecific. Those social interactions may help animals to learn new features of their environment without a trial and error process. Piglets showed a preference for flavoured feed following brief social interactions (30 minutes) with an experienced demonstrator. However, higher intakes of demonstrators consumed flavoured feed were observed only when demonstrators and observers were familiar.

Chapter 5 study the importance of milky flavour continuity in the post-weaning period and explore the influence of pre and postnatal exposure to porcine digestive peptides via maternal diet on the productive performance of post-weaned piglets fed a diet containing the same protein. No differences were found between the productive parameters of animals fed with dairy or PDP diets. However, animals showed a higher preference and acceptance for diets containing dairy products than for PDP diets. When the PDP was added to sows diets, piglets coming from those sows tended to show higher ADFI and ADG than control piglets (15-33d after weaning). Pre and postnatal exposure to PDP via maternal diet may influence piglet performance because of maternal learning in the presence of the same cue.

It is concluded that pigs may learn to prefer neutral flavours or ingredient cues by a previous associative learning with a positive consequence. The three ways of feeding behaviour learning observed in this thesis demonstrate that pigs have several cognition abilities to acquire new patterns of alimentary behaviours. Flavour preferences learned through maternal transference, associative learning with nutrients and social interactions could increase pigs options to fit against new environment feed cues. These three ways of learning could act also together increasing the adaptive value of the learned feed.

General Introduction

Weaning, a challenge of how to adapt without time for learning

Weaning is probably the most problematic period that pigs have to face along their productive life. The problem mainly arise in the intensive pig industry, where piglets have to be separated from their mother at a very early age (3-4 weeks of life in the intensive production systems) to increase sows productivity (Orgeur et al. 2002) but also because nutritional requirements' of these highly selected pigs increase and cannot be fully satisfied by their mother's milk (Counsilman & Lim 1985). Environment conditions (temperature, housing, social and feeding) are artificially changed in few hours (Worobec et al. 1999) and piglets have to cope with and learn from these changes under fear and stressful factors. All these conditions may directly affect learning performance and decrease motivation to eat.

A direct consequence of these changes is that almost 45% of weaned animals don't have contact with solid feed during the first 15h and still 10% remain with this problem for more than 40h after weaning (Bruininx et al. 2001). Stress and anorexia may also affect intestinal integrity and nutrient absorption, promoting pathogens proliferation diarrhea, and growing and behavioural problems that occasionally result in the death of the animal (Pluske et al. 1997). Therefore, feed intake after weaning have a direct effect in the short-term performance, but also have an important impact in the subsequent future performance of productive pigs.

After weaning, piglets need several days to reach to the necessary feed intake levels to meet energy requirements for maintenance, relying strongly on body energy reserves (Cera et al. 1988; Dividich & Sève 2001). This transitory anorexia can be explained due to weaning stress but also to the natural reject of new dietary components; also known as "neophobia". In contrast, wild pigs are exposed to a long and progressive weaning process that occurs between the 9th and 22th week of age, allowing animals to learn and adapt to the new conditions. They learn about feed and water sources without any interruption of their milk intake. Also social interactions with experienced conspecifics and with their mother smooth the transmission of feeding behaviour patterns necessary for the weaning adaptation (Graves 1984).

This abrupt weaning situation, limited also because of housing restrictions, may affect the expression of natural behaviours, could create stress responses and productivity losses until the new behaviour is created (Held et al. 2002). To affront weaning in the intensive pig industry with appropriate tools, piglets might start to learn new future conditions during lactation or even before, and maternal transferences of knowledge but also environment trial and error learning are needed. (Wechsler & Lea 2007).

Social interactions are restricted during lactation only to penmates (most of them sib brothers) and to their own mother. This situation is different in wild pigs were sows tend to move in groups of 2 or 3 mothers and animals have the opportunity to increase their social communication and also to interact with animals of different ages (Graves 1984). Because of this situation wild pigs have less fight or stress problems after weaning due to this richer previous social experiences. D'Eath (2005) showed that mixing litters in productive systems during lactation may increase social interactions and prevent social problems after weaning.

To improve feeding behaviour, solid feed "creep feed" may be offered during lactation to habituate animals to future feed. These diets may help to improve the development of the gastrointestinal tract structure and their enzymatic activity. However, only few piglets tend to eat creep feed during the suckling period. This low intake and variability may be explain in part by body weight differences between littermates. Strongest (higher body weight) piglets successfully fight to get the best position during lactation leaving to lighter animals, ironically, the opportunity to have more experience with solid feed. Bruininx et al. (2001) showed that these lighter animals present more daily visits to feed after weaning and tended to present a lower interval between weaning and the first intake. However a higher body weight is correlated, as it was said before, with higher learning abilities and a more development of the gastrointestinal system.

In conclusion, weaning is still a major challenge for the pig industry which it is largely unsolved. Alternative strategies through learning which allow a likely reduction in the stress of the animals and ways to encourage an early feed intake are required. Some of these strategies are explored in the present manuscript.

Literature Review

From the innate to the acquired preferences

Evolutionary imperative of survival allow mammals to replenish energy. Pleasure or hedonism (from the ancient Greek word hedone, from the sweet taste of honey, hedus) and reward play a central role in the control of food intake. The mechanisms mostly depend on all five primary sensory systems as well as the visceral sensory system and gut-brain interactions (Kringelbach et al. 2012). Sensorial evaluation of feed has evolved in the animals to provide information about its reward value. However, other rewards as sex and conspecifics compete for time and resources and therefore typically follow a cyclical time course. On the other hand, some authors also suggest that feed stuffs in the environment have also evolved to express sensorial attractive cues (i.e. Sweet; Apple, Umami; Tomato) to stimulate animal's consumption in their optimal pruning state (when seeds are ready to germine i.e) (Goff & Klee 2006). However, there are mechanisms that prevent an uncontrolled food intake. Mammals present cyclical time course of eating with distinct phases related to expectation, intake and satiety. In this way, satiation (process that terminates eating) and satiety (feeling of fullness) help animals to control their energy intake (Kringelbach et al. 2012). Even decerebrated rats are able to stop feed consumption, suggesting that the caudal brainstem act as an important factor (Grill and Norgren, 1978).

There is evidence that some mammals present unlearned positive reactions or innate preferences to only a very few taste. Pigs developed over 9000 years of domestication different sensorial abilities that put their internal state in contact with external cues. It has been observed that pigs can learn olfactory cues for faster discrimination than visual discrimination tasks (Croney et al. 2003). This situation may be explained because senses of smell and hearing are quite well developed in pigs and they rely more on these than on sight. In this way pigs shows problems to discriminate between green and red colours or even green or red versus colours like grey (Eguchi et al. 1997). They have a sense of smell 2000 times higher than human (Jones et al. 2000) that allows them to easily detect volatile compounds coming from feed specially low molecular weight lipofilics compounds (Pichersky et al. 2006). Moreover it can also affect on food seeking and selection as well on their feed preferences (Roura et al. 2008). Pigs like other species tend to prefer sweet (energy), umami (protein) and low salty (minerals and electrolytes) flavours, and reject acid (non-mature) and bitter (poisoning) flavours (Hellekant & Daniliva 1999; Hudson & Distel 1999). Solà-Oriol et al. recently evaluated feed ingredients preferences in piglet's diets and showed that the nature of the ingredients included in their diets (kind of cereal base or proteins) affect directly voluntary feed intake (Figure 1 and 2). Each ingredient appears to be linked to their own organoleptic proprieties, which could be related at some extent to their nutritive value.

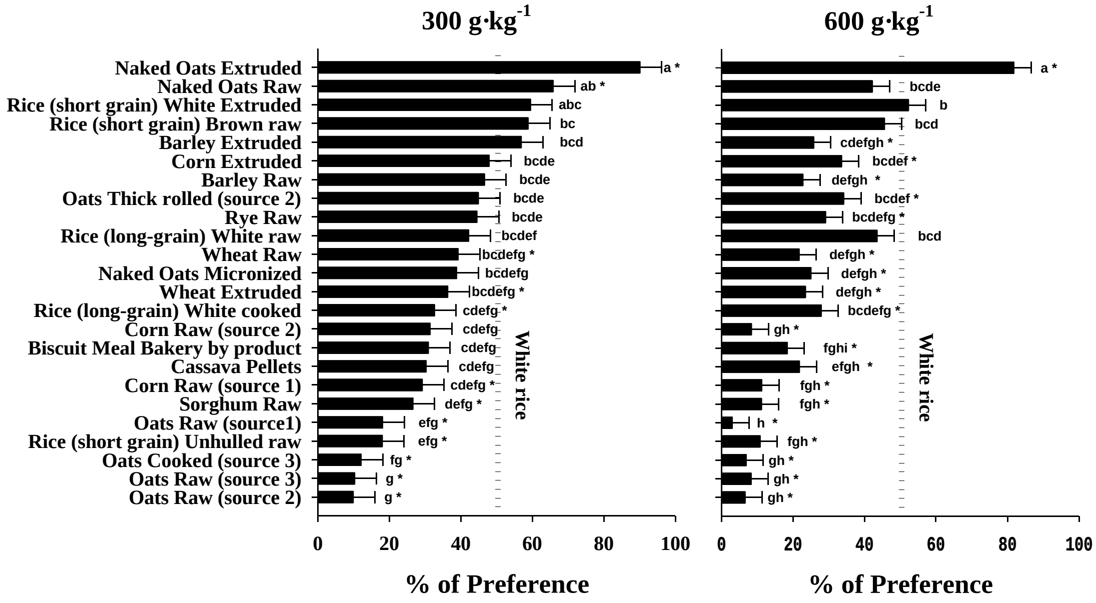

Figure 1. Cereal preferences during a 4-day choice test in relation with a reference diet (White rice). Preferences values are different if they differ from the neutral value (50%). Adapted from Solà-Oriol et al., (2009).

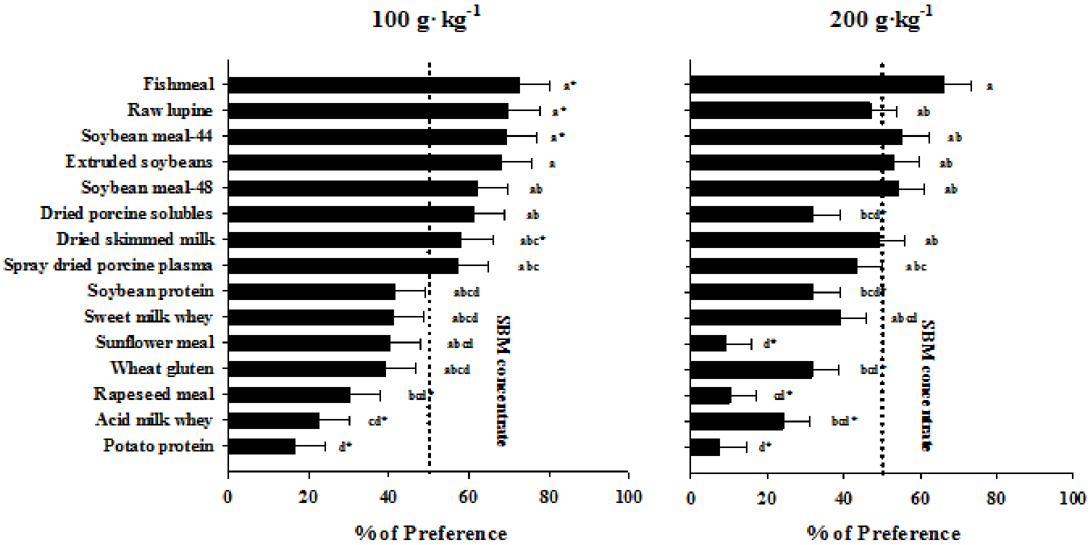

Figure 2. Protein preferences during a 4-day choice test in relation with a reference diet (SoyBean Meal Concentrate). Preferences values are different if they differ from the neutral value (50%). Adapted from Solà-Oriol et al. (2011).

Literature describes five tastes in mammals: sweet, umami, bitter, sour and salty. However there are evidences that this number may increase. (Liang et al. 2012; Sclafani 2004b). There is evidence for example that rodents can detect the taste of polysaccharides and find their taste attractive (Sclafani 1987; Dwyer 2008).

Sweet

Sweet tastes are highly preferred in pigs as well in other mammals because of their hedonic and post-ingestive effects. It is suggested that sweetness of an ingredient maybe correlated by the animal with the energy that this compound possesses. Sweet taste is detected as well as umami and bitter by TR proteins discovered in the early 2000s (Mombaerts 2000; Dulac 2000). The specific heteromeric receptor for sweet components is the T1R2-T1R3 that has been described in several tissues of mammals (oronasal, stomach, etc.) (Hoon et al. 1999; Nelson et al. 2001: Bachmanov & Beauchamp 2007). The total absence of this receptor (double knockout animals) eliminates behavioural responses in front of sweet stimulus (Li et al. 2002) but not the partial absence (T1R2 or T1R3 KO animals) (Zhao et al. 2003). Intakes of sweet solutions present an inverted u curve where medium concentrations showed the highest intakes. The inclusion of sucrose (3-5%) in pigs diets increased total feed intake (Legagneur & Fevrier 1956). A threshold for sucrose ranging from 5 to 10mM is highly preferred as well as glucose (10-30mM) or even artificial compounds like saccharin (5-10mM) compared to water. However sodium saccharin at elevated concentrations is rejected and the preferences at low concentration never increase the 90% as the other sweet solutions due to its bitter components (Kennedy & Baldwin 1972). Glaser et al. (2000) showed that sucrose present the highest sweet intensity of all carbohydrates and he described other sweet preferences in pigs (Table 1).

Umami

Umami presents also hedonic proprieties in pigs. Some compounds, such as monosodic glutamate (MSG) or L-amino acids are commonly used in their diets. This taste stimulates test specific heterodimer receptor T1R1 T1R3. (Danilova et al. 1999; Glaser et al. 2000). Some authors showed that the responses for this taste are abolished or reduced in T1R1 and T1R3 KO mice (Damak et al. 2003; Zhao et al. 2003). This taste is mainly related to protein, peptides and L-amino acids (especially L-glutamate) and it is present in several foods (Nelson et al. 2002; Tedo et al. 2011).

Table 1. Comparison (on a molar basis relative to 2% sucrose) between the sweetness potencies and preferences to various natural and artificial compounds known to be sweet in humans and pigs. Adapted from Glaser et al. 2000.

Carbohydrates	Potencies in humans	Preferences in pigs
Sucrose	1	1
d-Fructose	0.5	0.5
Lactose	0.33	0.146
Maltose	0.33	0.146
d/l-Glucose	0.25	0.125
Non-Carbohydrates		
Sucralose	1160	47
Saccharin	215	3.34
Aspartame	155	-

Bitter

The bitter taste is controlled by T2Rs receptors family in mammals (Mombaerts 2000; Matsunami & Amrein 2003; Shi & Zhang 2006). These compounds are rejected by pigs (Nelson & Sanregret 1997), and associated with anti-nutritional factors, poison drugs or toxics. When bitter tastes are added to the feed, animals show aversion behaviour and feed intake decrease dramatically. Comparisons between water and bitter compound like caffeine and quinine showed strong aversions for these bitter compounds (Nelson & Sanregret 1997).

Sour

Sour and salty taste activates taste cells through ion channels in the apical cell membrane (Roura et al. 2008). Its response is proportional to the proton concentration. Pigs present a special sensitivity for these compounds. Danilova et al. (1999) showed that citric and ascorbic acid are able to create electrophysiological responses. Pigs prefer (two feeder test) not-acidified than acidified diets (organic acids). However, they did not presented differences in an appetence test of one feeder test (Ettle et al. 2004). Acidifiers are commonly used in weaned diets to improve intestinal health due to pH environment change that prevent pathogens proliferation (Partanen 2001).

Salty

Animals present a specific appetite for sodium chloride (NaCl) or natriophilia. Salt appetite leads to a strong drive to seek and consume salt from a variety of sources and allows animals to maintain their ion and water homeostasis. Sodium appetite increases in response to hypovolemic and hypoosmotic deficits and can be altered even before birth because of gravidic dehydration (vomit i.e.) that created hypernatriophilia in rats (Nicolaïdis 2008) and humans (Crystal & Bernstein 1995, 1998). Pigs respond to salty products as chloride salts (Danilova et al. 1999) and sodium chloride use specially after weaning (Mahan et al. 1996).

Polysaccharides (Starch)

Polysaccharide taste may represent a sixth taste quality that certain nonhuman species use to detect starch-rich foods. Rats, unlike humans, are very attracted to the taste of polysaccharides derived from starch (also known as glucose polymers, maltodextrins, maltooligosaccharides (Sclafani, 1987). Polycose and sucrose stimulate different taste qualities. That is, learned aversions to Polycose and sucrose are only weakly cross-generalized (Nissenbaum & Sclafani 1987; Ramirez 1991; Sako et al. 1994). The two saccharides produce also different neural response profiles in the nucleus of the solitary tract (Giza et al. 1991). The hypothesized polysaccharide taste receptor(s) in rats, mice and other species remains to be identified (Sclafani 2004b).

Sensory specific satiety

When an animal eat the same diet for a long period their sensory system start to get tired and this may cause a decrease in the consumption rate (Rolls et al. 1981). This fatigue can be produced during the feeding process (even if the animal is still hungry) or could start after several days of consumption. Animals react decreasing the pleasantness and palatability value of the specific feed. However, when you give animals the opportunity to change the diet (changing sensorial cues), they start to increase their intake again. This phenomenon is known as sensory specific satiety.

Wild animals have the opportunity to select a range of available feeds in the nature, increasing the variability of their diets to obtain an adequate amount of nutrients depending on their needs (Goff & Klee 2006). However, pigs in productive systems don't have the opportunity to choose what to eat, and components of their diets are mixed in different proportions depending on the productive phase. The mixed diet in term of sensorial proprieties may act as a single unit, or white flavour, even if it has several ingredients inside (Snitz et al. 2011). This situation could increase the sensory specific satiety problem.

The concept of palatability

Maybe the simpler description of palatability is "the pleasure or hedonism that an animal experience with a particular food or fluid". In this way, palatability depends on diet characteristics (flavours, taste, viscosity, density, texture particle size and water compounds released) that animals are able to detect through oro-nasal sensing mechanisms (generally related with feed quality), but also on the physiological (hunger or satiety i.e.) and psychological state of animals. Palatability has been directly related with the energy density of a compound like sucrose (Davis & Smith 1992; Spector et al. 1998). However, preferences or total intake during a meal are not totally related with energy density and animals shows an inverted U-shaped function between concentration and total consumption, with the highest levels of consumption at intermediate levels of concentration (Figure 3). This demonstrates that palatability is independent of consumption level.

Several studies in productive systems have tried to estimate palatability mistakenly by comparing ad-lib intakes of different diets or fluids (one feeder or bottle test) or by choice or preference tests assuming that preferences are correlated with palatability. However, as we have said, consumption and palatability may present different intake functions. Moreover, choice tests allows animals to have experience with the two options at the same time (actually rats can mix fluids in their mouth) changing the perception of palatability. In this way, if a feed or fluid is preferred or more consumed it doesn't mean necessary that is more palatable. Thus, preference or even total intake test in animals are not a way to measure palatability and most of farm studies tend to confound concepts.

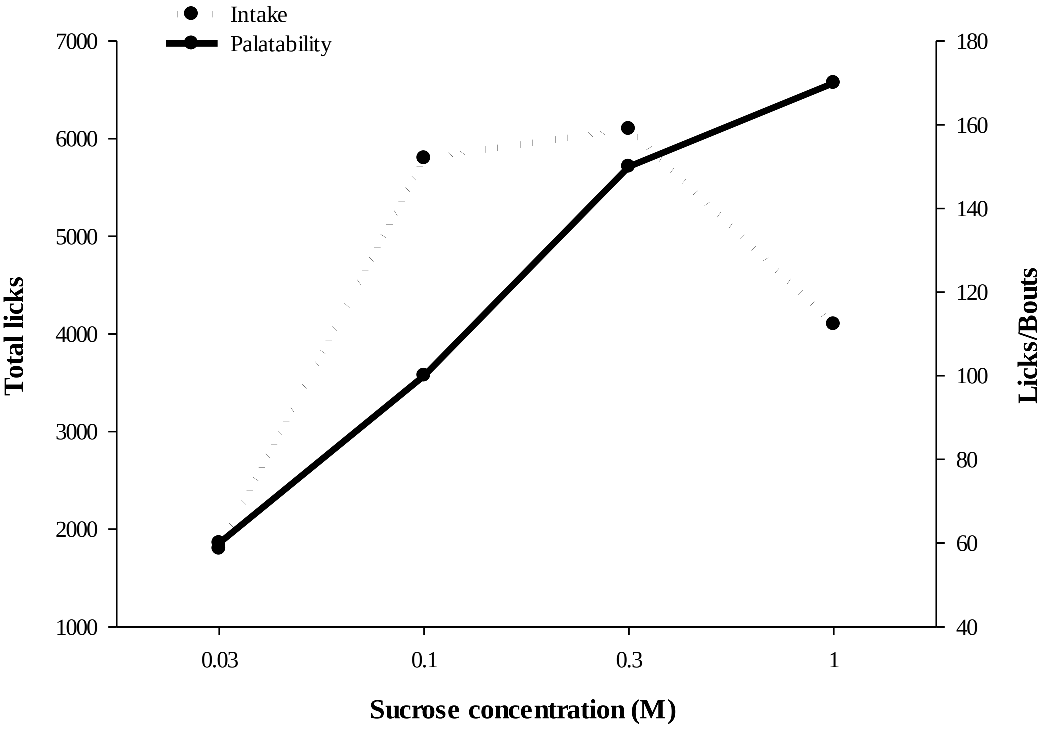

Figure 3. Rats intake (Total licks) and palatability (Licks/bouts) in relation with different sucrose concentrations. Adapted from Davis & Smith (1992).

Palatability is known to affect meal patterns. The rate of eating was described in pigs by Forbes (2007) as the amount of feed eaten per unit of time during the feeding behaviour. Auffray & Marcilloux (1983) discovered that unpalatable feeds presented a different pattern of consumption (slower), but no studies exist until now that associate these intake patterns with palatability in pigs. Indirect and wrong measures as total feed intake or preferences are usually applied to measure it in pig's studies. There are three different accepted techniques to measure palatability in animals; Reactive Tests, Cluster size tests and short time one bottle (feeder) test;

Taste reactivity tests (orofacial reactions)

Taste reactivity (TR) test has been the most commonly used method for investigating palatability changes. Several studies in mammals used this test to quantify the hedonic value of diets. Reactivity tests consist in the measure of facial “negative” or “positive” expressions patterns during consumption (Mennella et al. 2004). When an animal (rats, humans i.e.) drink or eat a specific hedonic or palatable cue they present a typical pattern of facial expressions measurable by videotapes. There are described also other facial expressions for not-palatable compounds (Figure 4). Figueroa et al. 2011 (unpublished data) observed that pigs also tend to change also their facial expressions when a more palatable diet is eaten (Head movements, lick patterns etc). However, reactivity test technique does have a number of pragmatic problems like the introduction of a context change if training is not done by passive infusion or behaviour samples are too small (Dwyer et al. 2009).

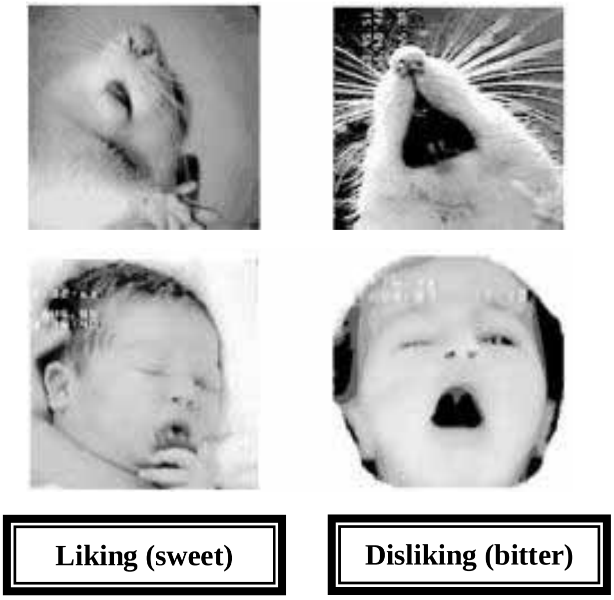

Figure 4. Oro-facial reactions (taste reactivity test) of rats and human to sweet and bitter components. Adapted from Kringelbach et al. 2012.

Cluster size test

Microstructural analysis of ingestive behaviour has been used to study palatability of different compounds in rats. Analyzing the microstructure of licking behaviour during consumption provides an alternative and less complicated way to study palatability of solutions. Rats present rapid and rhythmic licks "clusters" each time that they approach to solutions separated by pauses of varying lengths (Dwyer 2008). The mean number of licks in a cluster presents a direct monotonic relationship to the concentration of palatable fluids like sucrose. In this way, cluster size can be used as an index of stimulus palatability (Davis & Perez 1993; Davis & Smith 1992; Spector et al. 1998). When an aversive taste is tested, cluster size decreases monotonically with increasing concentrations of unpalatable solutions (Hsiao & Fan 1993). Cluster size (palatability) is not directly related to the amount of solution consumed, since animals as it was described before typically exhibit an inverted U-shaped function between concentration and total consumption of palatable fluids as sucrose (Dwyer et al. 2008). The idea that cluster size should be considered to directly reflect palatability receives further support from the finding that pharmacological treatments thought to influence palatability also have a direct effect on cluster size (Asin et al. 1992; Higgs & Cooper 1998).

Short term intake test

Palatability is important in the beginning of the intake behaviour. More palatable compound tends to present also more detectable cues to be attractive to animals. One simple way to measure palatability is to measure intakes during the first 30-40 seconds of consumption. In a short time one bottle test (<30 sec), palatability have a good correlation with total intake and may be easily used to study this factor in productive facilities.

To increase the feed intake of new diets nutritionists try to increase their palatability or hedonism. In this way, ingredients like animal proteins, dairy products and artificial or natural flavours are included in the first solid diets. However, palatability as other consumption factors may be increased also by changing animal's perception of the feed due to a learning process (Dwyer et al. 2008; Myers & Sclafani 2001b). Conditioned preferences are associated with increases in lick cluster size; conditioned flavours aversions also produce a change (decreases) in lick cluster size as well as other hedonic measures. In this way, a flavour preference or aversion by associative learning produce changes in flavours palatability. However, these changes on palatability tend to extinguish or disappears during just a few tests and before the reduction in consumption and then they do not explain the long persistence of conditioned preferences (Dwyer et al. 2009).

In contrast to innate preference for some tastes, animal tends to reject the intake of new ingredients or tastes. Neophobia “reject of the new” is a natural survival mechanism in front of new feed components of the diet to prevent possible toxic effects. Mammals tend to prefer and eat known feeds over unknown feeds. However the positive effects of consumption may change a neophobic reaction into a preference, or at least may change feed perception, making it able for future consumption (Provenza & Balph 1987). Neophobic reactions also can be modified by the repeated exposure to new diet compounds (Birch et al. 1998). In this way, innate preferences and learning processes through development set the different feed stuffs that an animal will eat during their life.

Because the first meal could be also the last one, neophobic mechanisms are of essential importance during the weaning period. Therefore, maternal and social transference of feed information (learning) become essential to increase the number of feedstuffs accepted in the early life period, and mechanisms that increase this kind of transference should be promoted.

Feeding behaviour learning

From the first days of life animals have to get environment cues to adapt themselves to new environmental conditions in order to fit with their current sexual and feeding requirements. This adaptation involves in many species to have substantial cognitive abilities that facilitate learning and memory processes of these external cues (Broom & Zanella 2004).

Learning plays a major role in food seeking and selection, and feed preference for omnivorous animals, even before birth. Nutrients that cover animal’s needs are transmitted in a passive way before birth together with some volatile compounds that pass from maternal diet to amniotic fluid (Schaal et al. 2000; Simitzis et al. 2008). Even if foetus are not active by searching for feed strategies inside maternal womb, the positive value of the amniotic fluid may active the opioid system specially during the last weeks of gestation. It has been suggested that these feed cues create an associative learning with the positive value given by the opioid system (Arias & Chotro 2007) which helps newborn animals to know what kind of secure feed are available in the external environment (by searching their volatile compounds).

Later, after birth, mammals start the intake of their own nutrients through milk, which early find following maternal odours at the moment of birth. After ingestion the positive value of milk (hedonic and post-ingestive) will reinforce the preference of the young animals for this solution. In fact, flavours that arrive to amniotic fluid from maternal diet during gestation are similar to flavours that may arrive to milk during lactation if the mother does not dramatically change the feed ingredients of their diet. In this way milk may be also preferred because of the flavour continuity between the internal and external environment as it was demonstrated in humans (Mennella & Beauchamp 1999).

After weaning, young animals are challenged to select the feed to eat. If the contact with the mother remains, these still become a first direct example of how to select nutritive components and their offspring start to learn the save feed locations and compounds by imitation of maternal behaviours (Hoppitt & Laland 2008; Oostindjer et al. 2011). Young animals can also copy older and more experienced conspecifics feeding behaviour patterns (Galef & Giraldeau 2001; Held et al. 2000). Thus, it has been suggested that copy models allow mammals, that share a common genetic and physiological background, to respond similarly to different feed types (Provenza & Balph 1987) enhancing the imitation of the behaviour. Prenatal, milk and observational learning of feed cues prevent that young eat toxic or low nutritive feeds, help to reduce the first contact “neophobia” and also decreases searching time of environment nutrients.

Despite this, environment may change and not all strategies learned from the mother or conspecifics could be adaptive in the new situation. In addition to mother or conspecific cues, trial and error learning of the feeding behaviour is also essential for the animals to get a direct relation of the actual benefits of a feed. It has been show that the variability of the environment affects the way that animal prefers to learn (socially or by trial and error) (Galef & Whiskin 2004). The first trial and error learning of the feeding behaviour may occur at the same time that animals are learning by social interaction if they participate in an active way (eating the feed source) during the process (Oostindjer et al. 2011). When a new feed is consumed, the consequences of that consumption, positive feedback as post-ingestive or hedonic effects, or negative as toxic effects, will be associated with the feed and all the volatile cues implicit in the feed. In the next opportunity, the animal will have to consume the feed, accepting or rejecting it, respectively (Sclafani 1997).

There are other several positive or negative effects that can also act to create associative learning during consumption with feed cues. The familiarity with a feed stuff (several exposures) also increases the acceptance of that feed. However, the effects promoted by familiarity over preferences and appetence are lower than the effects of associative learning (Myers & Sclafani 2006).

Associative learning during feeding behaviour

Animals can learn from the components of a novel feed and the consequences of its ingestion. Thus, during feed consumption, the animals associate their flavours and other cues with its positive or negative effects during (hedonic) and after (post-ingestive) intake. In this way, variety of procedures can be used to counteract neophobia and establish a preference for a novel flavour. Pairing the novel flavour with recovery from illness (Green & Garcia 1971), with the positive effects of nutrients such as carbohydrates (Sclafani & Nissenbaum 1988) or fats (Lucas & Sclafani 1989), with the positive value of social interactions (Galef & Whiskin 2001) with maternal fluids (Arias & Chotro 2007) or with a particularly palatable taste that does not contain nutrients such as saccharin (Holman 1975) can be used as strategies to increase the acceptance of the new flavours cues. These conditioned preferences can be remarkably persistent even when the positive consequences on which the preference was originally based are no longer present (Capaldi et al. 1983; Harris et al. 2004).

Conditioned flavour aversions

If the food has toxic consequences and produces gastrointestinal distress, animals rapidly learn to avoid its flavour, these kind of learning process may occur in just a few training sessions and it is well documented in literature (Braveman & Bronstein 1985). When the negative consequence is detected by the organism, animals could stay weeks, months or even all their life rejecting the feed and all the cues associated with it. Food conditioned aversions can be easily differentiable from food conditioned satiety because involves a reduction in the intake of the flavour cue from the start of the meal; whereas conditioned satiety involves a reduction in the intake of the flavour cue towards the end of the meal (Booth 1985). The most common associative cues that are used in studies to create conditioned aversions are quinine (unpalatable taste) and Lithium chloride (LiCl; Toxic effects).

Conditioned flavour preference

Flavours that are associated with post-ingestive nutritional or positive hedonic effects tend to be preferred in future exposures. Conditioned preferences can convert a neutral or even a normally avoided flavour to a preferred flavour. It has been showed for example that rats trained with a bitter solution paired with IG nutrient infusions subsequently preferred the bitter solution to plain water (Drucker et al. 1994). Several experiments in this way have studied the conditioned preferences in rats using different nutrients *(*glucose, polycose, casein, corn oil, ethanol, monosodium glutamate i.e.), different animal internal states (deprived or nondeprived) and training sessions times with solutions (10–30 min or 20–23 hr) (Sclafani 1997). This trial and error positive learning innate and social acquired feed cues will constitute the feeding preferences patterns of an animal during its life.

Therefore, the learning processes for a feeding behaviour can be explained by Pavlov conditioning paradigm or “classical conditioning” where a neutral cue or conditioned stimuli (CS) change its perception value because of repeated exposures with intrinsic positive or negative unconditioned stimuli (US) (Sclafani 1997). In this way, conditioning depends on the formation of an association between the representation of the cue and a representation of the reinforce such that sensation of the cue alone will activate the representation of the reinforcer, thus leading to conditioned responding (Dwyer 2005).

This response or learning outcome may be measured by giving to the animals the choice between the flavour associated with positive consequences (CS+) and a neutral or negative flavour cue (CS-) (Two bottle test; rats, Double choice test; pigs) or by measuring the total intake of the learned cue (one-bottle or feeder test). A flavour have to be offered the same number of times during conditioning to eliminate exposure effects over preferences; and is also suggested to equilibrate their intakes (Sclafani 2004a)

Tests can be performed with non-extinguished flavours cues (with the US present) or with extinguished flavours cues (without the US). The increase of absolute intake (acceptance) of learned solutions not always occur even if animals show an elevated preference. That is, whereas conditioned rats consume more CS+ than CS- in a preference test, they may consume comparable amounts CS solutions in an acceptance test (one bottle or feeder). However, the idea that flavour preferences are based on a Pavlovian process might be questioned by the fact that they have been shown to be resistant to extinction (Capaldi et al. 1983; Harris et al. 2004), which suggests that other processes might be involved (Dwyer 2005).

Conditioned trials in pigs started long time ago. These procedures were used in the early 1900s (Yerkes & Coburn 1915). Pigs may learn easily classical and operant conditioning tasks using an aversive or appetitive US. (Baldwin 1969; Baldwin & Stephens 1973; Kratzer 1971). During 1945 Moore & Marcuse looked for a salivary conditioned response (CR) after a training period with the association of one tone (CS) and the presence of feed (US) in an experimental setting similar to that used by Pavlov to prove positive associative learning. Noble & Adams (1963) studied the effects of associative aversive learning by using an increase in light intensity (CS) and electric shocks (US). After just a few sessions, pigs showed escape behaviours (CR) in front of light stimulus. However just a few experiments continued with this research line probably because classical conditioning experiments are of limited interest to cognitive researchers, and other animals models like rodents gave more experimental options.

The reward quality of food (that can be used as a US) is determined by both, its oro-sensory properties and its post-oral nutritive consequences (Sclafani 1997). Post-ingestive and hedonic effects of food over flavour conditioned preferences play different roles and have to be separated. The failure to differentiate taste preferences may be partially explained by the fact that many nutrients also have palatable tastes and thus both processes may be confounded (Dwyer 2005).

There are three components that explain rewards: a hedonic or ‘‘liking’’ component, a motivational or ‘‘wanting’’ or incentive component, and a learning component (Fig 5). The motivational value of food reward may be increased by the nutritive effects of food. The hedonic value also is increased by associative learning using palatable cues. Thus, the glucose infusions did not appear to enhance the rats’ ‘‘liking’’ of bitter or sour CS+ although it did increase the animals’ ‘‘wanting’’ of the CS+ solution as evidenced by the conditioned increase in CS+ intake (Sclafani 2004a). To separate both processes intragastric infusions of the US is recommended to assess post-ingestive learning and a positive non-nutritive US like an artificial sweetener of sham procedures are recommended to separate and study the hedonic effects. There is also another easier way to separate both learning process that consist in previously match the post-ingestive effects of 2 different hedonic cues (to study the hedonic learning) and to match the hedonic effects of two compounds that differ in the nutritive value (to study to post-ingestive learning) (Warwick and Weingarten 1994).

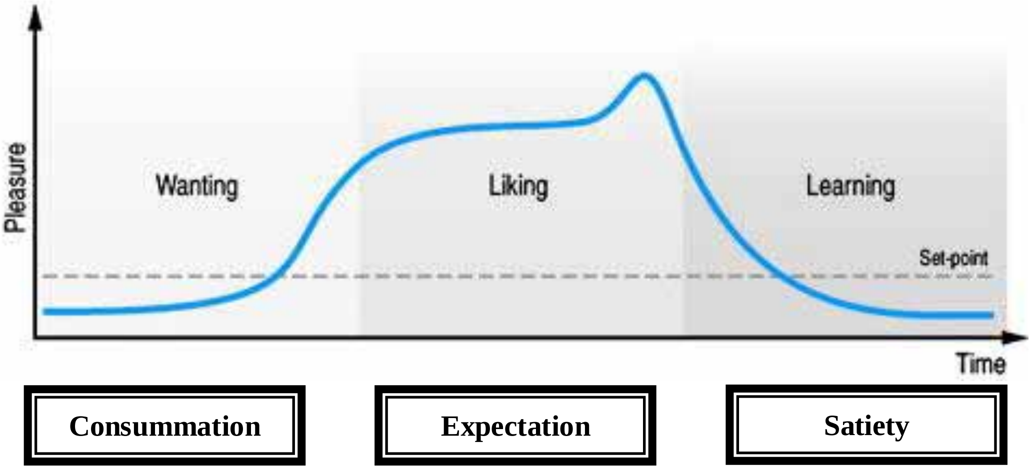

Figure 5. Food pleasure cycle. Rewarding phases of expectation or wanting, consummation or liking and satiety or learning. Adapted from Kringelbach et al. 2012.

Postingestive learning

Nutrient intra-gastric (IG) infusions are very potent in conditioning flavour preferences. This is demonstrated in numerous experiments in which the consumption of one novel flavour (conditioned stimulus; CS+) is paired with a nutrient infusion (e.g., glucose), while another flavour (CS-) is paired with water or saline infusions. In subsequent two-bottle choice tests, rats typically display strong and persistent preferences for the CS+ flavour over CS- flavour. IG nutrient infusions can also greatly stimulate the acceptance of an initially non-preferred flavour, and condition a strong preference for that flavour relative to the CS- flavour, plain water and even a normally preferred saccharin solution (Ackroff & Sclafani 2011; Myers et al. 2005; Sclafani 2001, 2004a). However, post-oral nutrient stimuli by themselves have minimal reward effects as indicated by the relative ineffectiveness of IG nutrient infusions to support operant responses in the absence of oral stimulation (Holman 1968).

Hedonic learning

Sweet and fatty flavours can reinforce preferences for arbitrary cue flavours when they are mixed together during conditioning sessions (Elizalde & Sclafani 1990; Holman 1975). These hedonic measures include measures of short-term intake and licking, sham-feeding intakes, taste reactivity responses, and operant performance (Sclafani 1987).

Palatability after conditioning

As it was explained before, flavours conditioned by the positive value of food not only may change their relative preferences but also their palatability (Myers & Sclafani 2001b). Harris et al. 2004 suggested that consuming a CS with sucrose served to pair the CS with the hedonic reaction elicited by the sucrose, which, in turn, resulted in the CS's having the same hedonic properties as sucrose. However, nutrients and palatability effects differ in their susceptibility to extinction. Thus, changes in the hedonic response to the conditioned flavours cannot explain the resistance to the extinction of learned flavour preferences (Dwyer et al. 2009).

Neuronal control of flavours learned preferences

Eating decisions are controlled by neuronal processing in higher-order cognition brain regions (Figure 6). Physiological cues, previous experience and other multiple factors may change the rewarding value of a food and as consequence animal's preference and acceptance of specific diets (Olszewski et al. 2011). Primary sensory areas for taste and smell are not modulated by motivational state, and that hedonic processing occurs in higher-order, multi-modal areas such as the orbitofrontal cortex and regions of mid-insular cortex. In the orbitofrontal cortex, activity is elicited by auditory (Frey et al. 2003), gustatory (Small et al. 1999), olfactory (Zatorre et al. 1992), somatosensory (Rolls et al. 2003) and visual (Aharon et al. 2001) inputs, as well as information from the visceral sensory system (Critchley et al. 2002). Primate orbitofrontal cortex receives input from all of the five senses.

Cues associated with rewarding foods can act as incentive motivators (i.e., wanting) for food independent of basic homeostatic system (Dickinson & Balleine 1994). Learned flavour preferences in this way, may change the food reward value (Myers & Sclafani 2001a; Sclafani & Ackroff 2006) and could modify the neurochemical response to food in mammals. However, both kind of flavour conditioned preferences (Flavour-flavour, hedonic; and flavour-nutrient, post-ingestive) appear to be mediated to some extent by separate neural mechanisms (Touzani et al. 2010).

The brain mesolimbic dopamine (DA) system regulates neuronal processing of natural rewards such as feeding and sex (Berridge & Robinson 1998). Dopamine is a critical player in the neurochemical controls of incentive motivation like the appetite created by Pavlovian conditioning (Di Ciano et al. 2001) and act in the "wanting" step of the feeding behaviour. In this system, DA neurons located in the midbrain (ventral tegmental area (VTA)) project to cortical and limbic structures including the nucleus accumbens (NAc), amygdala (AMY) and the medial prefrontal cortex (mPFC) (Swanson, 1982).

Acquisition of sweet taste-conditioned flavour preferences depends upon both D1 (SCH23390) and D2 (raclopride) like-receptor signalling. The full expression of a previously learned CS+ preference also requires D1 and to a lesser degree D2 signalling (Touzani et al. 2010). However, unlike flavour-flavour learning, flavour-nutrient learning with sugars is critically dependent only on D1-like receptor signalling. The systemic administration of antagonism of D1-like, but not D2-like receptors blocked flavour preferences conditioning by IG sugars. D2 antagonist raclopride (200 or 400 nmol/kg) throughout training or before the choice do not alter flavour conditioning. However rats showed a decrease in their CS+intake but they continued to drink more CS+ than CS− (Sclafani et al. 2011).

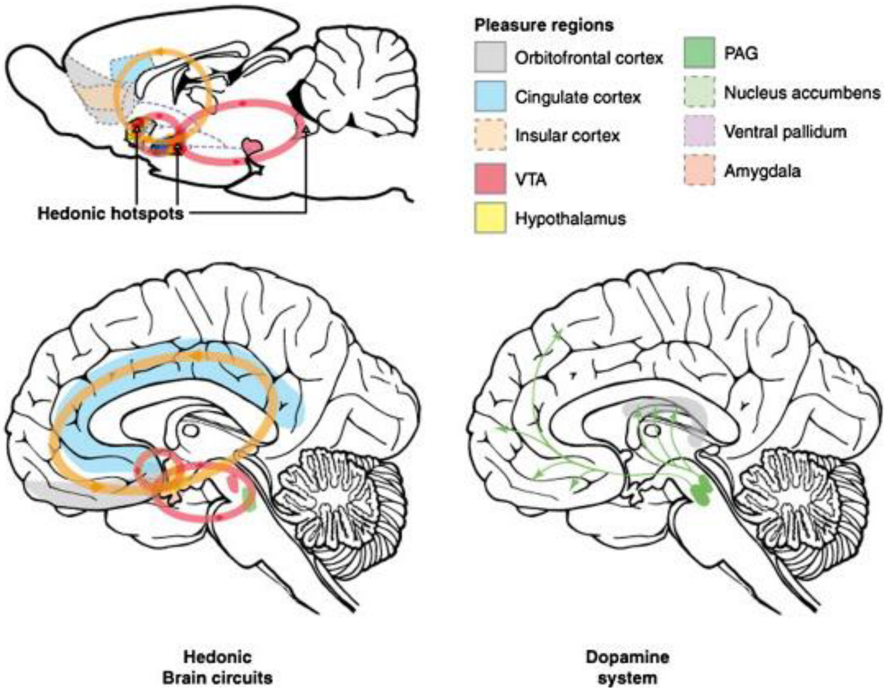

Figure 6. Pleasure networks in the mammalian brain. Pleasure regions in the adult rat (upper) and human (lower) brains. The hedonic circuitries have been revealed using behavioral and subjective measures of pleasure to food stimuli. The pleasure networks (in the middle panel) include the orbitofrontal cortex (gray), the cingulate cortex (light blue), ventral tegmental area in the brainstem (light red), hypothalamus (yellow), periventricular gray/periacqueductal gray (PVG/PAG, green), nucleus accumbens (light green), ventral pallidum (light purple), amygdala (light red) and the insular cortices (not shown). The right-most panel shows the dopaminergic system in the human brain. (Kringelbach et al. 2012).

D1-like receptor signalling contributes to the acquisition of a flavour-nutrient preference, but not the expression of previously learned preference. Touzani et al (2010), explained that when rats are treated with SCH23390 (200 nmol/kg) during a training session of flavour learning, failed to prefer the CS+ to the CS− but the expression of CS+ preference was not blocked when D1-like receptor was administrated before the choice test. As systemic injections, specifics microinjections of D1-like receptor SCH23390 in the NAc, AMY, mPFC, or LH are able to block or significantly attenuate the acquisition but not the expression of flavour conditioned preferences learned by the post-ingestive effects of sugars (Touzani et al. 2008; Touzani et al. 2009). A network is essential for post-oral flavour conditioning by sugars infusions. Is the system fail DA reward system could find food more rewarding (hypersensitive) and animals could suffer overeating and obesity There is also an opposing view that reduced food reward may, paradoxically, stimulate overeating in some individuals.

On the other hand, hedonic or “liking” values of food cues are mediated by opioid peptide signalling in a distributed Central Nervous System (CNS) network including hindbrain, midbrain, and forebrain regions, such as the nucleus tractus solitarius (NTS) (Giraudo et al. 1998) the nucleus accumbens (NAc) (Kelley et al. 2002; Zhang & Kelley 2002; Zhang et al. 1998), the amygdala (Stanley et al. 1998; Glass et al. 2000), the ventral tegmental area (VTA) (Kim et al. 2009), hypothalamic nuclei (LH) and paraventricular hypothalamus (PVH) (Stanley et al. 1998; Glass et al. 2000). The opioid effects over feeding are larger with preferred palatable foods and solutions (Naleid et al. 2007; Woolley et al. 2007; Woolley et al. 2006). It is well established that general and selective opioid receptor antagonists as naloxone and naltrexone (longer duration of pharmacodynamic action than naloxone) may suppress food and fluid intake in a variety of situations (Bodnar 2004; Levine 2006). Naloxone reduced the intake and preferences of natural and artificial sweet solutions more than that of plain water (Sclafani et al. 1982; Cooper 1983; Le Magnen et al. 1980), reduced sugar solution intakes in sham-feeding tests (Kirkham & Cooper 1988; Rockwood & Reid 1982), and suppressed hedonic taste reactivity responses to intraoral sugar infusions (Parker et al. 1992). However, endogenous opioids are not intimately involved in flavour preference conditioning by the sweet taste of sugar. Although naltrexone treatment reduced training intakes of the CS solutions (0.1– 10 mg/kg), it did not prevent the acquisition (animals injected during training) or expression (animals injected before choice tests) of flavour conditioning preference (Touzani et al. 2010).

As DA and opioid systems regulate food intake, hormones like leptin or ghrelin also influence appetitive behaviour depending on previous learned relationships between internal context cues and food access. The hippocampus participate in the control of food intake and contains receptors for these hormones (Scott et al. 2009; Zigman et al. 2005; Guan et al. 1997), as well as for GLP-1 (Merchenthaler et al. 1998), and insulin (Zahniser et al. 2006; Pratchayasakul et al. 2011) that controlled the anorectic/inhibitory control. Also hormones as GHSR in the hippocampus may modulate feeding by increasing how effectively environmental cues trigger food-related memories and stimulate meal initiation. Disruption of hippocampal inhibitory control over behaviours directed at obtaining food can yield further overconsumption of the same foods that contributed to hippocampal dysfunction in the first place, a “vicious circle” model of energy deregulation (Davidson et al. 2007; Davidson et al. 2005; Davidson et al. 2008; Kanoski et al. 2007; Kanoski & Davidson. 2011).

Glutamate is also critical for reward related learning in part by interacting with DA systems in these areas (Kelley 2004) as well as cannabinoids that interact with opioid and DA systems to promote intake of palatable food and food reward (Cooper 2004; Cota et al. 2006; Gardner 2005).

Social influence over feeding behaviour

Social learning refers to those instances in which the acquisition of behaviour is influenced by observation of or interaction with another animal or its products (Heyes 1994). There are several evidences in pigs and other species that the feeding behaviour of a conspecific may influence the future feeding behaviour of a naive animal (Gieling et al. 2011; Held et al. 2000; Laland 2004). By the observation of the feeding place of conspecifics and the attraction to that feeding site (Nicol & Pope 1994; Held et al. 2000), stimulation of feed intake when an inexperienced piglet is housed with an experienced conspecific (Morgan et al. 2001) or even by brief interactions where olfactory cues are transmitted (Galef & Whiskin 1997, 2003), observer animals may copy a new feeding pattern that may or may not be adaptive. If this social transmitted behaviour of feed preferences is stable because adaptive characteristics, it might persist in animal’s populations through weeks, years and also generations. Social learning acts primarily to introduce a new feeding behaviour into an individual’s repertoire; while the experience of their consequences determine the persistence of that behaviour (Galef & Whiskin 2001)

The possibility that non-human animals living in natural environments have ‘culture’ has been of great interest. Several years ago Dawkins in “*The Selfish Gene”*, based in the genes term, created the denomination of memes to talk about the culture units that pass through generations. As well as genes, memes could have a transmission pattern and more adaptive behaviours may be “selected” and be easier to replicate and maintain. If selection acts directly on the phenotypic expression of memes, rather than on memes themselves, then socially transmitted behaviour should be even more locally adaptive than behaviour learned by individual trial and error (Galef 1995). In this way, feed memes in animals may be necessary, as well as genes, to develop an optimal feeding behaviour in wild and productive species.

If a behaviour is observed in many members of a social group and in no members of another, and there are no obvious differences in the ecology in which those groups live and no reason to believe that the groups differ genetically, then the behaviour is provisionally accepted as traditional (Whiten et al. 1999). However, “traditions” studies in laboratory animals have been successfully replicated only few times under natural conditions (Galef 2010; Müller & Cant 2010; Galef & Allen 1995).

The maintenance and propagation of traditions of food preference depend on several factors rather than the conspecific contact taking place within complex environmental and social situations. The time that an animal have to explore the different options of their environment after a social transference of a feeding behaviour affect directly the longevity of that behaviour. Studies performed with rats (Galef & Whiskin 1997) have demonstrated that colonies that had food available 2 h/day present more longevity of the learned feed behaviour than colonies that had food available 24 h/day. Opportunities to learn a-socially to eat a food other than that preferred reduced the stability of a food preference especially if the previous learned preference is no reinforced anymore or is less rewarded (maladaptive preference) than the other foods. Also when animals eat for a short period of time (hrs/day), as it usually occurs in natural conditions, opportunities to maintain traditional pattern of food preferences increase because of the simultaneously consumption of the group of animals. As well as the amount of time that animals spent in individual learning, population replacement rate may influence if a feeding behaviour stays or not inside the population (Laland 2004).

However, social influence on feed choice is transitory when animals find better options inside their environment. In terms of memes, feeding behavioural traditions in animals will spread if they have an adequate fidelity, longevity and fecundity (Dawkins 1989). With optimal conditions, a food preference tradition in laboratory colonies of Norway rats can survive for several generations (Galef & Allen 1995). However, Galef & Whiskin, (1995) showed that maladaptive preference for a feed lacking sufficient protein to support normal growth learned in a social way was soon reversed in the absence of continuing social influence. Individual learning in this way change the already learned behaviour when is maladaptive or present less reward value than the alternative behaviours in the order of days or weeks rather than generations. Therefore, individual learning and social influence are not independent processes (Galef & Allen 1995), with the exception of learning by imitation, social learning is described as social biasing of individual learning, and animals need to get rewarded to maintain a behaviour (Heyes 1993). Pig's productive environments create difficult conditions for the transmission of feeding behaviours through generations and even inside the same productive period. All in-all out sanitary management do not allow to the different generations of pigs to have any kind of contact. Each generation have to spend more time exploring the environment to know the consequences of the feeding stuffs than in natural condition. In this way, with this minimal social learning facilitation, maternal transmission of behaviours and trial and error experiences become the only way to modify the feeding behaviour of an animal. However, housing and feeding managements may also make difficult this natural transmission of feeding cues.

The importance that the social learning of a feeding behaviour may have in intensive pigs conditions is to introduce new behaviours into an individual's repertoire reducing in some way the neophobic factor that appear when the diets are changed. It also may delay trial-and-error learning that can lead individuals to acquire patterns of behaviour different from those shown by others of its social group. This situation could create more homogeny preferences in piglets and by the maintaining of these traditions through their productive life, memes could also be transferred between generations if we create adequate management conditions reducing in this way the learning cost of future animals. This culture of feed preferences could be possible also because the minimal variability of the productive conditions and the unique diet that pigs ate eliminates the trial and error learning of more rewarded feed options.

Objectives

Objective 1

To estimate if sucking piglets are able to discriminate between their own mother's amniotic fluids versus an alien one at different ages and to confirm the influence of pre-natal flavour exposure via maternal diet in flavour and flavoured creep feed preferences on suckling and weanling piglets.

Objective 2

To determine if newly weaned pigs will learn to prefer a flavour (CS+) that is paired with a protein source (US) and to determine if a conditionally preferred flavour would enhance the attraction to that protein source when the flavour (CS+) and protein (US) are combined.

Objective 3

To evaluate if the post-ingestive effects of protein and sucrose (unconditioned stimulus; US) are sufficient to establish flavour preferences in pigs because a previous associative learning between these nutrients and a neutral flavour stimulus (conditioned stimulus; CS).

Objective 4

To study if pigs may learn to prefer a certain flavoured feed following brief social interactions with their conspecifics during the post-weaning period, and to study if this learning is affected by the familiarity of the demonstrator or the nature of the alternative choice.

Objective 5

To study if the incorporation of Porcine Digestive Peptides in post-weaning diets, free of dairy products and lactose, may keep the productive performance of weaned piglets as compared to those animals fed on diets containing significant amounts of whey and lactose in the pre-starter and starter diet; and to explore if a pre- and postnatal exposure to PDP via the sow diet may improve feed intake and productive performance of weaned piglets fed on a diet containing PDP.

Chapter 1

Flavour preferences in piglets conditioned by prenatal flavour exposure through the maternal gestation diet.

Abstract

Flavour cues present in the amniotic fluid are commonly used in mammals to early recognize their mother but also to have contact with environment flavours cues before birth. Three experiments were performed to evaluate if piglets have the ability to discriminate and prefer natural and artificial cues of maternal amniotic fluid after birth (during lactation and post-weaning). During 7 minutes piglets couples were used to choose inside a Triple-U-Testing Arena between maternal amniotic fluid, alien amniotic fluid or water (Experiment 1) or between a flavour added into late gestation maternal diet, a control flavour and water (Experiment 2). The same prenatal strategy was used to study piglet's preferences for flavoured or unflavoured creep feed during the suckling period (Experiment 3). Suckling piglets preferred amniotic fluid flavours from their own mother over an alien amniotic fluid and also they preferred flavours cues given to the sows during the late gestation period. However, prenatal flavour exposure only attenuated intake differences with the preferred unflavoured creep feed diets. Pre-natal exposure to flavours via maternal diet influences piglet's preferences in front of new flavours, probably through a positive association between flavours and the hedonic reward of the uterine experience and a familiarity effect. However, volatile components of some ingredients in the sow diet may also create this association and it is hard to increase preferences or intake over a simple unflavoured diet. Preferences acquired before birth seems to be highly resistant to extinction. This may be an important factor to reduce neophobia, such as during sucking or weaning period occurs.

Introduction

Exposure to novel feed ingredients or flavours may cause negative emotions of fear and anxiety to the animals, which generally sample little amounts of feed in a phenomenon known as feed neophobia (Miller and Holzman, 1981). Piglets after weaning face a large list of stressful challenges, including also the need of eating a new feed. During this period, piglets respond with variable periods of underfeeding and anorexia that make piglets vulnerable to diarrhea, weight losses and increase in mortality rate (Madec et al., 1998). Solid feed included during lactation or "creep feed" is frequently used to habituate animals to the solid feed intake. These diets may help to improve the development of gastrointestinal structure tract and enzymatic activity. However, only few piglets tend to eat creep feed during the suckling period and only a few volatile compounds can generate an attractive stimulus (Pluske et al., 2007).

Feeding behaviour in mammals is based on genetic components, but also on learning; a process which it is established along the whole life to create temporal or permanent preferences for some ingredients or flavours. In this way, animals can learn feeding behaviour from their mothers (either at the prenatal or postnatal stage) (Schaal et al., 2000; Mennella et al., 2001; Nicolaïdis, 2008), from social interactions with conspecifics (Galef and Whiskin, 1998, 2000) or by trial and error tests (Ackroff et al., 2001; Sclafani, 2004; Dwyer et al., 2009).

Natural flavours of amniotic fluid are used by newborn mammals to recognize their own mother after birth (Hepper, 1987; Morrow-Tesch and Mcglone, 1990; Soussignan et al., 1997). Amniotic fluid cues are considered a useful tool for the animal orientation in the nest area and the initiation of sucking. Moreover a calming effect on newborn subjects could be driven by those sensorial cues released from the amniotic fluid which it is closely in contact to the newborn during the delivery process and just after birth (Varendi et al., 1998).

Amniotic fluid also plays a fundamental role over maternal learning of the feeding behaviour and provides a first opportunity for the foetus to learn about safe and available feeds sources present in the environment (Schaal et al., 2004; Hepper and

Wells, 2006). Some volatile compounds are known to be transferred in small amounts from maternal diet to the amniotic fluid and milk (Nolte et al., 1992; Mennella et al., 2001; Hausner et al., 2008). Thus, this adaptive mechanism may create a conditioning effect to the foetus that affects the feeding preference and acceptance of the newborn animals (Mennella et al., 1995). In 1976, Campbell reported that prenatal exposure to flavours through amniotic fluid may be an effective method to facilitate feed detection and reduce the time to first feed contact in weaned piglets. However, Langendijk et al. (2007) failed to repeat those results and neophobia after weaning was still affecting the feeding behaviour. Oostindjer et al. (2010) did not find any effect of the prenatal flavour exposure over the future preferences in weaned piglets, but refereed an increase on the weight gain after weaning when animals were offered the flavoured conditioned diet, likely associated to a reduced stress of the animals.

The hypothesis tested in the present study was that piglets flavour choices during the suckling period are largely influenced by the prenatal period. The objectives were to determine whether sucking piglets are able to discriminate between their own mother’s amniotic fluids versus an alien one at different ages (Experiment 1) and whether a pre-natal flavour exposure via maternal diet modify flavour and creep feed preferences on suckling and weanling piglets (Experiment 2 and 3, respectively).

Materials and methods

Three Experiments were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (UAB). Experiment 1 was designed to study if piglets recognise their own mother amniotic fluid over an alien mother amniotic fluid; Experiment 2 and 3 were designed to evaluate if a prenatal flavour exposure through the maternal gestation diet can create flavour (Experiment 2) and feed preferences (Experiment 3) in sucking and post-weaning piglets. Experimental procedures were approved by the Ethical Committee on Animal Experimentation of the Universitat Autònoma de Barcelona.

Experimental design and diets

A total of 111 sows and their litters ([Large White x Landrace] x Pietrain) were used (10 litters in Experiment 1, 20 litters in Experiment 2, and 81 litters in Experiment 3). In Experiment 1, forty male/female piglets coming from the 10 litters (4 piglets/litter) were used to test their attraction for 3 olfactory stimuli (own mother vs alien mother amniotic fluid vs water) on day 4, 14 and 21 of life. In Experiment 2, a total of 20 sows (Large White x Landrace) were assigned to one of two diets during the last 2 weeks of pregnancy; a flavoured diet (Aniseed, 0.75 $g \cdot kg^{-1}$ or Vanilla, 1.5 $g \cdot kg^{-1}$; Lucta SA, Montornès del Vallès, Spain; n=10) and an unflavoured diet (n=10). After farrowing each sow ate a commercial unflavoured lactation diet. Eighty male/female piglets (mixed sexes) coming from these 20 sows (4 piglets/litter) were used to test their attraction for 3 olfactory stimuli (aniseed vs vanilla vs water in a triple-choice test) at day 14, 21 and 26 after birth (2d post-weaning). In Experiment 3, a total of 81 litters (10-12 piglets/sow) were used to test the choice feeding preference for flavoured creep feed diets as compared to a simple diet during a 6 day choice test at the end of the lactation period (22 to 28d-old). Twenty nine litters were used to evaluate the innate feed preferences for simple or commercial diets; and the rest 52 litters to test the preferences for flavoured diets if previously conditioned or not through a prenatal exposure. In the latest, half of the litters (26) came from sows that during late gestation (2 weeks before parturition) were offered flavoured diets (garlic or aniseed, Flavoured group) while the rest of the litters (26) came from sows that were fed with an unflavoured diet (Control group).

Experimental piglets were individually identified at birth by using a plastic ear tag and they rested with their mother and littermates inside the farrowing crates (0.5 m wide, 2.0 m large and 1.03 m high) and their corresponding area for piglets (total available area 4.63 m^2; 4.15 m^2 of complete slated floor and 0.48m^2 of concrete heat area) during the entire suckling period (28d). The farrowing room was provided with controlled temperature (22.4±2.05°C sow environment and 28.3±2.70°C piglet environment, (HOBO U10, data logger, MA, USA) and automatic ventilation. Inside each crate, piglets had access to a heated area to provide a warm resting space, which was also enriched with shaving, sawdust and drying material (Biosuper CONFORT +, Gratecap Services, La Rochelle, France). Free access to water was provided to sows (by using a commercial drinking nipple and an extra source of water provided at feeding time) and piglets (stainless steel nipple) from the birth. Sows were fed twice a day 8.00am and 16.00 pm. Feed was offered to the sows by hand following and controlled ad-libitum situation according to the lactating yield curve. An unflavoured creep feed diet was offered ad-libitum from day 10 of birth onwards to litters in Experiment 1 and 2 by using a pan feeder. No solid feed was offered to piglets of experiment 3 until the beginning of the choice tests.

Choice test procedures

Triple-choice tests were performed in Experiment 1 and 2 using the module "Triple-U-Testing Arena" (TUTA, Figure 1), which it was located in an isolated room close to the farrowing area. In Experiment 1, olfactory cues were tested by using adsorbent strips impregnated with amniotic fluid collected from their own mother (maternal amniotic fluid), alien amniotic fluid (mix of amniotic fluid from 3 alien sows), and water in the middle of the TUTA as a negative control. The placenta from each sow was collected just after farrowing and processed. Amniotic fluid was obtained by placenta compression with synthetic gauze. Then, this fluid was centrifuged (3420x*g*, 4Cº, 15 minutes) and filtrated (0.22 μm Millipore filters). The liquid obtained was stored in 15 ml tubes and kept frozen (-20ºC) until test days. At the beginning of each test (9.00 am), tubes with maternal amniotic fluid and alien amniotic fluid contents were unfrozen by using warm water until environment temperature in order to have the samples ready to perform the test. During the test period TUTA`s soil was completely covered with sawdust in order to simulate farrowing pen conditions. Cotton strips were impregnated with maternal amniotic fluid, alien amniotic fluid or water and placed into the TUTA just before each test. The piglets selected for the test were temporary removed from their mothers and placed in the middle of the TUTA in litter-pairs in order to avoid fear and distractive behaviours during the test. The same litter-pairs were tested in the consecutive test days (day 4, 14 and 21 of life). Each test lasted 7 minutes, during which, the time spent by piglets in nasal contact with each strip was measured by direct observation by a previously trained observer. Strips were changed and the position of maternal amniotic fluid and alien amniotic fluid were rotated for each litter-pair tested.

In Experiment 2, olfactory cues tested included strips impregnated with an aniseed solution (0.375 g·kg^{-1}), vanilla solution (0.75 g·kg^{-1}), or tap water used as negative control at day 14, 21 and 26 after birth (2d post-weaning). The flavours were identified as "maternal flavours" or "control flavours" depending on the flavour included in the sow gestational diet of the pair of piglets in each test. Flavour solutions were prepared with warm water and the choice test conditions were the same than in Experiment 1. The position of maternal and control flavours were also rotated between tests.

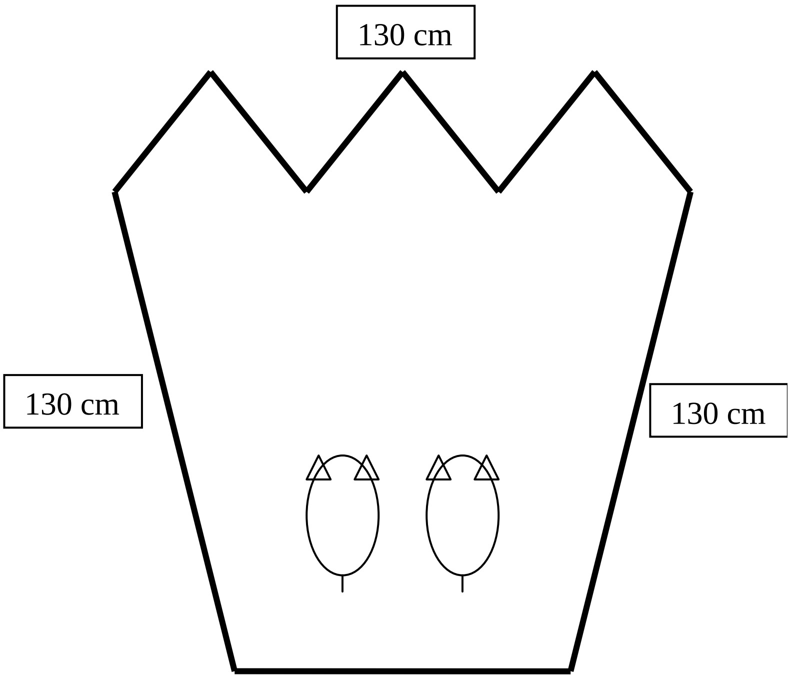

Figure 1. Triple-U-Testing Arena (TUTA).

In Experiment 3, creep feed choice tests were performed. The preference for 2 different modified creep feed diets was compared respect to a simple diet by performing a 6 day choice test at the end of the lactation period. The simple diet included corn, barley and wheat (547 g·kg^{-1}), sweet whey (141.2 g·kg^{-1}), soybean protein concentrate (166 g·kg^{-1}), spray dried animal plasma (50 g·kg^{-1}) and a vitamin and mineral premix (10 g·kg^{-1}) without artificial flavours. Modified diets included a commercial creep feed diet, commonly used in the farm (first test) or a modification of the simple diet (second test), based on the incorporation of one flavour (Garlic or Aniseed; 0.75 g·kg^{-1}, Lucta SA, Montornès del Vallès, Spain) (Table 1). The first test, performed to evaluate the innate feed preferences for simple or commercial diets used 29 litters; the second test was performed with 52 litters to test the preferences for flavoured diets if previously conditioned (26 sows) or not (26 sows) through a prenatal exposure.

Table 1. Ingredient composition and calculated nutrient content (g/kf diet as fed) of the experimental diets (Experiment 3).

Item	Simple diet	Commercial diet	Flavoured diet
Ingredient			
Barley	22.00	10.00	22.00
Wheat	17.70	17.50	17.50
Extruded soybean	16.50	15.00	16.50
Maize	15.00	15.00	15.20
Sweet milk whey	14.10	10.00	14.10
Animal Plasma 800 g CP/kg	5.00	2.50	5.00
Soybean oil	3.80	1.51	3.80
Soybean meal 440 g CP/kg	2.54		2.54
Fish meal		5.00	
Limestone	0.94	0.70	0.94
Monocalcium phosphate	0.85	0.75	0.85
L-Lysine HCL	0.48	0.39	0.48
Vit-Min Premix	0.40[1]	1.00[2]	0.40
Processed cereal blend[3]		20.00	
Salt	0.27	0.278	0.27
DL-Methionine	0.24	0.204	0.24
L-Threonine	0.20	0.153	0.20
L-Tryptophan	0.05	0.037	0.05
Flavours		*	0.075[5]
Calculated content			
NE, MJ/kg of feed	10.96	11.00	10.96
CP	204.44	202.30	204.44
Lysine	15.52	15.07	15.52
Methionine	5.01	5.55	5.01
Methionine +Cystine	9.05	9.15	9.05
Threonine	10.38	9.95	10.38
Tryptophan	3.21	2.89	3.21
Ca	7.20	8.00	7.20
P Total	6.07	6.45	6.07
P.dig	3.86	4.00	3.86

[1] Simple diet Premix Supplied ($g\cdot kg^{-1}$): 7,000 IU of vitamin A (acetate); 500 IU of vitamin D_3 (cholecalciferol); 250 IU of vitamin D (25-hydroxicholecalciferol); 45 mg of vitamin E; 1 mg of vitamin K_3; 1.5 mg of vitamin B_1; 3.5 mg of vitamin B_2; 1.75 mg of vitamin B_6; 0.03 mg of vitamin B_{12}; 8.5 mg of D-pantothenic acid; 22.5 mg of niacin; 0.1 mg of biotin; 0.75 mg of folacin; 20 mg of Fe (chelate of amino acids); 2.5 mg of Cu (sulphate); 7.5 mg of Cu (chelate of glycine); 0.05 mg of Co (sulphate); 40 mg of Zn

(oxide); 12.5 mg Zn (chelate of amino acids); 12.5 mg of Mn (oxide); 7.5 of Mn (chelate of glycine); 0.35 mg of I, 0.5 of Se (organic); 0.1 mg of Se (sodium).

[2] Commercial diet Premix Supplied (g·kg^{-1}): 7,500 IU of vitamin A (acetate); 750 IU of vitamin D_3 (cholecalciferol); 4500 mg of vitamin E; 380 mg of vitamin K_3; 150 mg of vitamin B_1; 400 mg of vitamin B_2; 380 mg of vitamin B_6; 38 mg of vitamin B_{12}; 1500 mg of niacin; 1100 mg of calcium pantothenate; 22500 mg of Choline chloride; 220 mg of folic acid; 150 mg of biotin; 7500 mg of Fe (chelate of amino acids); 14000 mg of Cu (sulphate); 7500 mg of Zn (oxide); 2250 mg of Mn (oxide); 150 mg of I, 220 mg of Se (sodium) and 230000 mg of a mixture of flavours*.

[3] Extruded cereals (wheat corn and rice; 410 g·kg^{-1}) and protein sources (Extruded Soybean and dairy products 400 g·kg^{-1}).

[5] Garlic or Anis flavours (Lucta SA, Montornès del Vallès).

Flavours used [garlic or aniseed; 0.75 g·kg^{-1}] were counterbalanced between animals. In this way, 13 sows received an aniseed flavoured gestation diet and their litters a choice between aniseed creep feed and the simple diet, and 13 sows received a garlic flavoured gestation diet and their litters a choice between garlic creep feed and the simple diet. In the control group flavours were contra-balanced to during the choice test. Choice feeding test were conducted in the farrowing pens in both tests by using two pan-feeders containing the two diets ad libitum in a mash form (side by side). Feeder's positions were side counterbalanced between litters. At the end of the choice test (6d) the total intake of each feeder was calculated.

Statistical analyses

Mean permanence time for each strip were compared by General Linear Models using the GENMOD procedure of SAS® (SAS Inst. Inc., Cary NC); taking into account the solution approached (Maternal amniotic fluid, Alien amniotic fluid and Water in Experiment 1; Maternal flavour, Control flavour and Water in Experiment 2), age of piglets at the test, Prenatal exposure [piglets coming from sows that ate (pre-exposed) or not (control) flavours during late gestation ; Experiment 2] and the position of solutions during the choice test as the main factors and their corresponding interaction. Feed consumption during the choice test in Experiment 3 was analysed with ANOVA by using mixed linear models with the MIXED procedure of the statistical package SAS® (SAS Inst. Inc., Cary NC); taking into account in Test 1 the effects of the creep feed consumed (simple diet vs commercial diet), and in Test 2 the effects of the creep feed consumed (simple diet vs. flavoured diet), flavour used (anis or garlic), litter origin (treated or control sows) and the interaction between litters origin and feed consumed as the main factors. Pen during the choice test was also included as a repeated measure specifing the covariance structure of the residual matrix as completely general (unstructured). All of the interactions that did not reach significance in a previous analysis were not considered in the final model. The mean values are presented as LSMeans. To test the hypotheses, P<0.05 adjusted by Tukey was considered significant.

Results

Experiment 1

The permanence time of piglets in contact with the different strips in are shown in Figure 2. Latency time accounted for more than 6 minutes of the 7 minute test. Older piglets became increasingly agitated, looking for alternative routes to escape and they also had more playing and exploring behaviours at the TUTA.

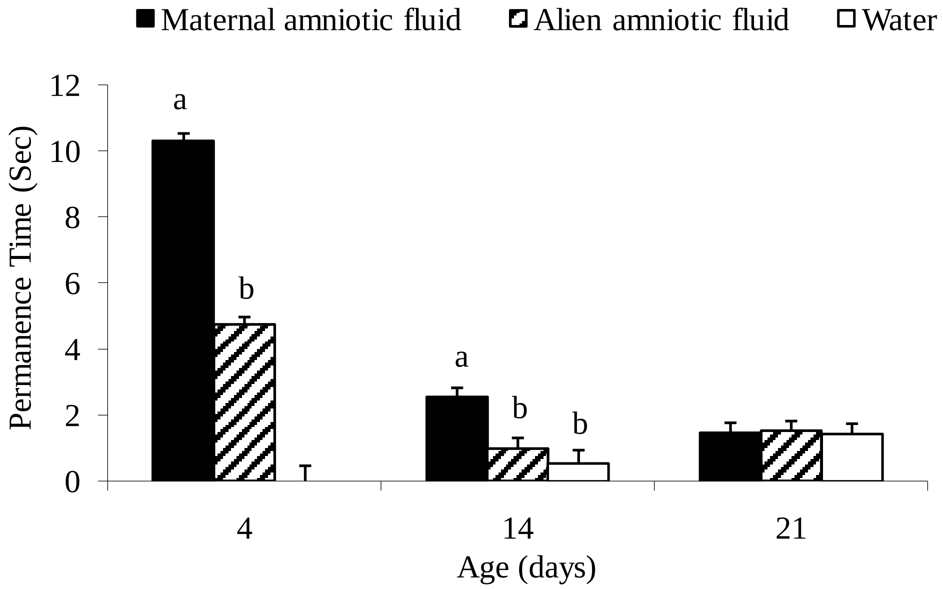

Figure 2. Piglet's permanence time in contact with strips (Maternal amniotic fluid; Alien amniotic fluid and Water) for a 7 minutes preference test during lactation. Means, by day with different subscripts are different (*p*<0.05). Flags: ±1 SEM. (Experiment 1).

Piglets showed preferential responses towards maternal amniotic fluid [*F*(2, 144) = 28.48, P < 0.0001] but these preferences disappeared with the age [Solution approached x piglets age; *F*(4, 144) = 12.6, P < 0.0001]. In this way, higher oro-nasal contact for maternal amniotic fluid was observed at day 4 and 14 compared to alien amniotic fluid and water. No differences were observed at day 21. In the first test day (4d-old) piglets not only showed more nose approach to impregnate strips with maternal amniotic fluid but also they spend more time near this area. Between day 4 and 14 piglets showed a dramatic decrease in their attraction for placenta solutions, despite this, they again showed longer residence time near to maternal amniotic fluid than alien amniotic fluid on day 14.

Experiment 2

Prenatal flavour exposure via maternal diet significantly influenced piglet's preferences during lactation [Prenatal exposure x Solution approached; $F(2, 161) = 10.72$, $P < 0.0001$, Figure 3]. Piglets born from flavour treated sows showed preferential responses towards maternal flavours on d 14 and 21(during lactation) and d 26 (after weaning) of age as compared to control flavours and water. No different preferences were observed between flavours for control piglets. In addition, piglets born from sows fed flavour supplemented diets during late gestation had also more occurrences of oro-nasal contact with the corresponding flavour than control piglets during preference tests at day 14 (NS $P = 0.08$), 21 ($P < 0.05$) and 26 ($P < 0.05$). These preferences acquired before birth for the prenatally exposed flavour were highly resistant to extinction as they were still observed at 2 days after weaning (26 old). However, latency time in the post-weaning test also accounted for more than 6 minutes of the 7 minute test. Post weaning piglets (26 old) showed a lower global permanence time than lactating piglets ($P < 0.05$), expressing more exploration and playing behaviour into the TUTA module. No different preferences were observed between flavours used as prenatal cues (Aniseed or Vanilla) into the power of piglet's preferences during testing days. Both flavours showed high preferences at all testing days.

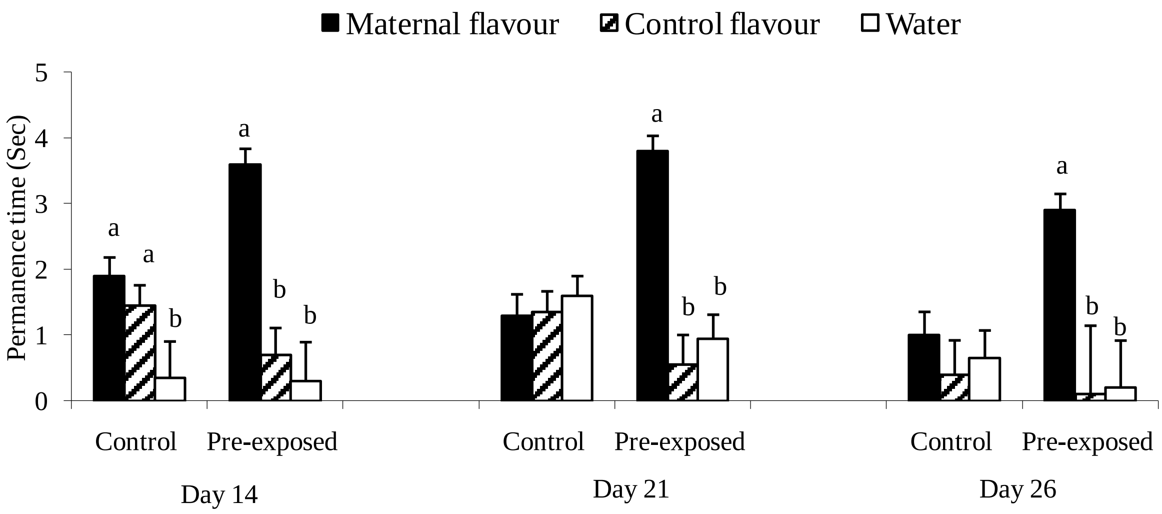

Figure 3. Piglet's permanence time in contact with strips soaked with different fluids (Maternal flavour, Control flavour and Water) during lactation as function of the prenatal exposure to flavours (Anis 0.75 $g{\cdot}kg^{-1}$ or Vanilla 1.5 $g{\cdot}kg^{-1}$) during late gestation (Control piglets coming from control sows or Pre-exposed piglets coming from sows that ate flavours during late gestation) during a 7 min. preference test. Means in the same group and day with different subscripts are different (p<0.05). Flags: ±1 SEM. (Experiment 2)

Experiment 3

Creep feed choice test showed that piglets preferred to eat the control unflavoured diet (Simple diet) over the commercial creep feed diet ($F(1, 28) = 35.40$, $P < 0.0001$, Figure 4). When piglets had the opportunity to choose between the simple diet and a flavoured creep feed, control animals as well as piglets coming from sows that ate the flavoured diet during late gestation preferred the simple unflavoured diet ($F(1, 25) = 42.04$, $P < 0.0001$, Figure 5). No differences were observed between feed intakes of flavours used inside the creep feed diets (garlic or aniseed; $P > 0.05$).

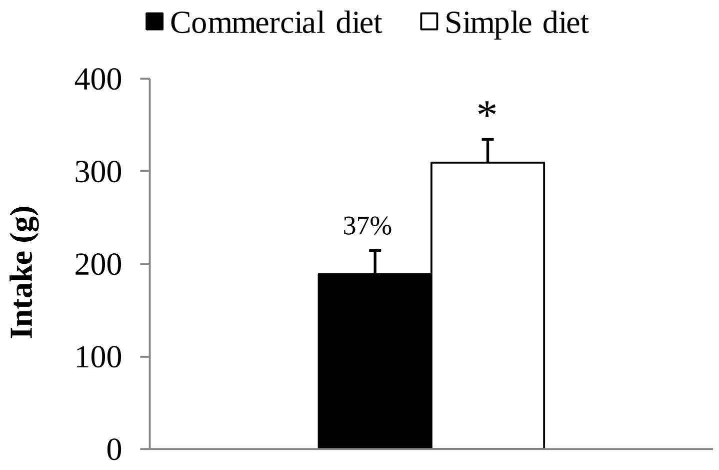

Figure 4. Creep feed intake of 3 weeks-old suckling piglets litters during a 6 day choice test between a commercial creep feed diet and a simple unflavoured diet. Numbers in the top of bars indicate the average percent preference for the flavoured feed. Asterisks indicate that control creep feed intake is significantly different than flavoured creep feed intake (*P<0.001). Flags: ±1 SEM. (Experiment 3).

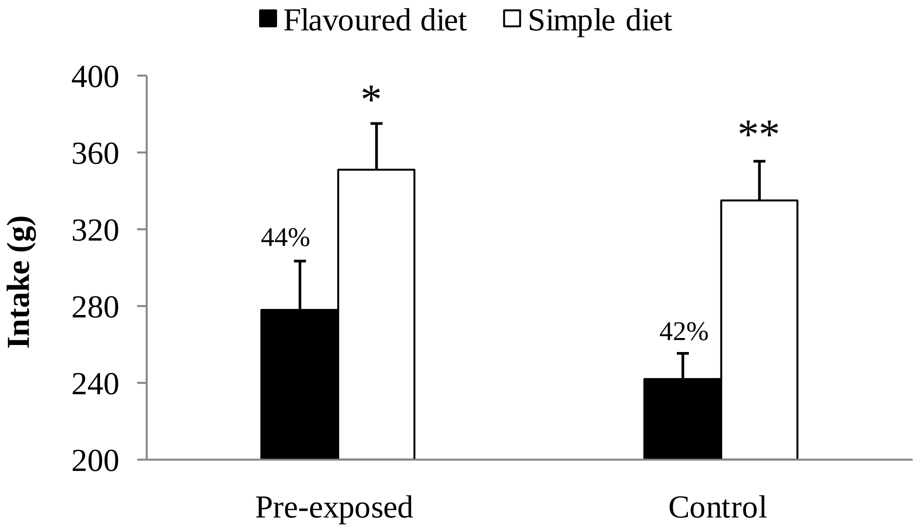

Figure 5. Creep feed intake during a 6 day choice test between a flavoured diet vs. a simple unflavoured diet in 3 weeks-old suckling piglets litters that received (Pre-exposed) or not received (Control) the flavour cues during late gestation (2 weeks) through their maternal diet. Numbers in the top of bars indicate the average percent preference for the flavoured feed. Asterisks indicate that simple diet creep feed intake is significantly different than flavoured creep feed intake (*P<0.005, **P<0.001). Flags: ±1 SEM. (Experiment 3)

Discussion

Wild life is plenty of examples where the newborns show an innate ability to find the protection of their mothers. This behaviour is also important, as piglets that quickly follow their mother have more chances to survive than piglets that stay behind (Graves, 1984). However, a question that arises is how newborn piglets know what kind of environment cues they have to follow without an apparent learning process? In the present study, piglets were able to prefer the contact with the amniotic fluid over water during the first days of life. This attraction was stronger early after born (4d-old). These results agree with previous results described in rats (Hepper, 1987), humans (Varendi et al., 1998) and rabbits (Coureaud et al., 2002). Thus, it can be suggested that piglets can be familiarized before birth with the oro-sensory cues of amniotic fluid. Such a prenatal learning could help the newborn to find its mother and teat seeking as it occurs in several species (Hepper, 1987; Morrow-Tesch and McGlone, 1990) by following impregnated previously born piglets or by following the amniotic fluid itself left by sows in their ventral zone.

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

Experiment 1 showed that 14 days old piglets still recognized and preferred the amniotic fluid odour of their own mother. This persistence proves that flavour recognition not only help them to look for mother´s teats (Morrow-Tesch and McGlone, 1990), own blood brothers (Hepper, 1986) or protection but also may act as an hedonic persistent cue. After 14 days, piglets still need mother nearness to satisfy their feeding requirements. However, from this time point, piglets also need to explore the environment and learn from it and milk rather than amniotic fluid could arise as a higher key role stimuli. In natural environments, piglets are exposed to a lot of new challenges, and trial and error learning is essential in natural life. Animals have to explore and learn more to know what are the new rules of life in a continuously changing environment (Galef, 1995), where challenges are gradual and distributed in time. However, productive challenges in intensive production conditions occur dramatically fast, and animals experience sudden changes with short time to choose or learn on the basis of trial and error.

One strategy to reduce this psychological impact in piglets is to give them links between one step and another that allow them to feel a familiar connection and reduce stress (Oostindjer et al., 2011). The common milk replacers, creep feed and weanling diets for young animals usually contain dairy products which help to smooth the transition between sucking and eating solid fed as well as to satisfy lactose requirements. Creep feed incorporation during the last lactation days is used as an strategy to reduce neophobia, however, just a few animals eat this feed at this period. In our second experiment we confirmed that young piglets are able to recognize flavours supplemented in the maternal gestational diet, showing that artificial cues can change amniotic fluid environment and consequently piglet’s behaviour and flavours preference. This flavour continuity, may act as a psychological link, bringing to piglets mind the hedonic remembrance of maternal environment during lactation or post weaning periods (Arias and Chotro, 2006). Several studies in other species have ratified our results showing that this prenatal experience can influence later recognition and preferences for flavours cues (Mennella et al., 2001; Hepper and Wells, 2006; Nicolaïdis, 2008; Simitzis et al., 2008). However, even if positive effects over pig stress and intake have been reported (Oostindjer et al., 2009), just a few piglets studies have been successful proved that preferences can be modified (Campbell, 1976).

Amniotic fluid is therefore a key factor in preferences acquisition contributing to inhibit aversive responses in front of sensorial stimulus. This inhibition may due to a familiar learning effect (repeated prenatal exposure) that would reduce neophobia, or also could be due to an associative learning effect during pregnancy, in which the amniotic fluid and comfort of the womb could be associated with the sensory stimulus and create a strong preference; in most cases, resistant to extinction. The flavours transferred may create a conditioning effect that not only affects preference but also the acceptance of the same cue (Myers and Sclafani, 2006). It has been reported that animals associate the flavours of amniotic fluid with the hedonic properties of this liquid (Arias and Chotro, 2007). In this way, activation of the opioid system plays an important role in the acquisition of flavour preferences during the last days of gestation (Kristal et al., 1990; Korthank and Robinson, 1998; Bonacchi et al., 2010). Thus, flavours uploaded and released during gestation may establish long-lasting preferences and a permanent influence on the individual behaviour. The long persistence of preferences in experiment 2 shows us that we probably were in front of an associative learning process rather than a familiarity or mere-exposure process (Myers and Sclafani, 2006). In this way, the positive hedonic uterine environment can act as unconditioned stimuli (US) and the amniotic fluid flavour as conditioned stimuli (CS) during late gestation. The association of US and CS can create a hedonic preference for the amniotic fluid flavour (Myers and Sclafani, 2001; Arias and Chotro, 2007). Despite this, we cannot rule out a synergy effect between the non-associative and associative components of learning.

The hypothesis in Experiment 3 was that a new flavour in the creep feed diet previously added in the final gestation diet of sows could increase appetite and voluntary creep feed intake instead of creates a neophobic behaviour in the lactating piglets. Our results showed that the lactating piglets preferred a simple unflavoured diet (cereal base, without added flavours) than the commercial creep feed currently used by the farm. This result is important because once again show that simple diets are more preferred than flavoured diets, likely because of neophobia in front of new cues. Main ingredients in the simple diet (corn, barley, wheat or soybean protein concentrate) were all contained in the sows diet. Then, it is likely that cereals and other feedstuffs ingredients may also have cues that piglets recognize from a previous experience with amniotic fluid or even the sow milk. Thus, even if commercial flavoured diets are offered intending to enhance feed intake in critical steps as at the end of lactation, our results suggest the inclusion of a new flavour may provoke neophobia in a 6 d double choice feeding study. Our results also denied the initial hypothesis and confirmed that aversion to a new flavour also appeared in piglets that came from sows that eat this flavour at gestation. A likely preference for the prenatal flavour in creep feed diets where not found. A possible reason could be that in these choice tests, we compared the flavoured creep feed versus a control unflavoured diet. It could be argued that this simple control diet contains whey but also the same ingredients that the sow's diet, and likely dietary cues that the piglets recognized because of their previous experience.

Considering the observed transference of flavour preferences towards flavours in the amniotic fluid, we can argue that a higher variety of ingredients in sow´s diets throughout gestation may help foetuses (unborn piglets) to be exposed to more flavoured cues or volatile components during gestation, reducing the risk of future cases of neophobia (Mennella et al., 2001). Moreover, diet variety is also important to reduce sensorial specific satiety (Provenza, 1996) and to improve animal welfare, as it allows animals to satisfy their real individual nutritional requirements (Manteca et al., 2008). Prenatal learning process of feeding behaviour has no significant cost for maternal requirements and welfare. Animals would teach their offspring in a passive way, showing what kind of cues they will affront in the extra-uterine life. This is probably the most elevate rate of benefit / cost in the feeding learning process (Galef et al., 2005). Animals can perceive one stimulus as pleasant or unpleasant according to their internal state (Cabanac, 1971). One flavour, in this way, will cause an increase in the intake of animals only if these flavours evoke positive hedonic or post-ingestive consequences in animal's memory or actual fitness.

Conclusion

Amniotic fluid becomes a first extra uterine hedonic flavour in pigs, helping newborn animals to recognize their own mother. Amniotic fluid flavours can be modulated by adding new flavour components to sows gestation diet during late gestation. Therefore, unborn piglets can learn cues before birth in an associative way that allows them to change their flavour preferences and maybe their feeding behaviour. These new established preferences could be used to reduce flavour or feed neophobia during common critical steps of pig's life such as weaning.

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Chapter 2

Flavour preferences conditioned by protein solutions in post-weaning pigs.

Abstract

Prior studies report in various mammalian species that a neutral flavour can become strongly preferred after being associated with a positive consequence of its consumption. Two experiments were performed to study flavour preferences conditioned by a protein source in weaned pigs. In experiment 1, pigs were trained to drink (30 min/day) one flavour (CS+) mixed into a 2% protein solution (Soybean Protein Concentrate; SPC or Porcine Digestible Peptides; PDP) and another flavour (CS-) mixed into water during 6 alternate sessions. The pigs in the SPC and PDP groups consumed more CS+ than CS- in the two-choice tests with both flavours presented in water (552 vs. 409 mL, 571 vs. 414 mL, respectively). In the last choice SPC and PDP animals preferred the CS+ over the CS- when both flavours were present in feed rather than water (650 vs. 536g and 678 vs. 513g, respectively). No differences were observed between the conditioning effects of the two proteins. In experiment 2, pigs were trained (30 min/day) with a garlic flavour (CS) mixed with 4% PDP in sessions 1, 3, 5 and 7 unflavoured tap water in sessions 2, 4, 6 and 8 (Conditioned group) or with garlic flavour in water in sessions 1, 3, 5 and 7 and 4 % PDP without added flavour in sessions 2, 4, 6 and 8 (control group). In subsequent choice tests conditioned pigs consumed more PDP+Garlic than PDP in Tests 1 (550mL vs. 372mL $P<0.05$) and 3 (763mL vs. 503mL, $P <0.05$). In addition, pigs in the Conditioned group made significantly more first contacts (FC, number of piglets at a pan during the first 15 sec) with the PDP+Garlic solution than PDP solution in Test 1 and 2 but not in Test 3. In contrast, the control group did not differ in their intakes of or first contacts to the two PDP solutions. The present results indicate that piglets can acquire preferences for a cue flavour added to protein products (PDP and SPC). The conditioned flavour preference also enhanced the attraction to the palatable protein (PDP) when the flavour and protein were combined.

Introduction

It is well known that mammals have innate attractions for only a few flavours [24], showing preferences for sweet (energy), umami (protein), moderately salty (electrolytes) and perhaps fatty acid (energy) tastes [30,33,50]. However, genetic predisposition interacts with learning to promote food preferences during the animal's life [5].

Mammals can learn food preferences from their mother (during pregnancy and lactation) [32,37,47,56], from social interactions with conspecifics [18,19] or by experience with new foods [1,16,57]. Flavours become increasingly acceptable with repeated exposure. This non-associative learning is the easiest way to decrease neophobic reactions because of familiarity [64]. However, while mere exposure learning may reduce neophobia, it commonly does not produce strong preferences. On the other hand, animals may learn to associate feed flavours with positive hedonic tastes (e.g., sweet), referred to as flavour-taste learning, or post-ingestive nutrient consequences, referred to as flavour-nutrient learning. Both kinds of conditioning normally act together during feeding behaviour [69,73]. However, there is evidence that flavours preferred through nutrient conditioning are highly resistant to extinction [6,13,70] and significantly more palatable [15,44]. Flavour learning is usually explained as a form of Pavlovian conditioning in which the flavour is the conditioned stimulus (CS) that becomes associated with the biologically significant consequences of food consumption, the unconditioned stimulus (US) [45]. Preference conditioning is commonly measured by subsequently offering subjects a choice between the flavour (CS+) previously paired with the US vs. a comparable flavour (CS-) not paired with the US.

Among mammals, pigs have substantial learning abilities [7] and evidence has accumulated about their ability to learn and be conditioned [23]. For example, pigs have been trained to find food in a test chamber or perform a response to avoid an electrical or temperature punishment. Previous studies showed that pigs can be trained to make a conditioned response to several kinds of environmental stimuli. However, olfactory cues appear to be the most effective, followed in order by auditory and visual cues. Also pigs have been trained to associate an auditory cue with food with the conditioned response being salivation as in the case of Pavlov’s dog [35]. Yet, pigs have been used only in a few classical conditioning studies during the last decades. Also, the nutritive or hedonic value of feed has been used more to create rewards for use in operant conditioning studies than to serve as a US in flavour conditioning studies [23]. A recent study reported a mild flavour conditioning effect in pigs trained with sucrose [10]. However, no references are available about the use of protein sources as reinforcers for pigs based on their preferred taste and post-ingestive effects.

Without human intervention, the pig becomes nutritionally independent of its dam at 10-17 weeks of age [34,46] having opportunities to show their learning abilities as they start the exploratory behaviour. However, the intensive industry imposes an abrupt weaning at 21-28 days of age, which offers few opportunities for the young piglet to learn the new feeds. Even if creep feed (feed available during the lactation period) is typically used as a practical strategy to habituate young animals to consume solid feed and new flavours [8], its consumption is variable and inconsistent, and learning strategies to enhance feed intake may be difficult to perform.

Therefore, early weaned piglets have to find their own food, which is more bulky and dry (usually >88%), less digestible than milk, and composed of ingredients that piglet has not previously encountered. In these conditions most weaned piglets are reluctant to eat the new solid feed (feed neophobia) because of uncertainty about the post-ingestive consequences of the new food [38,54,65]. Feed ingredients at this time have to be highly palatable and with a powerful post-ingestive effect, but these products are usually associated with a high cost [14] and even a highly palatable diet may not be sufficient to counteract under eating in the first days of the post-weaning period. Solà-Oriol (2011) conducted different double-choice feeding experiments to study the effect of selected protein sources on feed preference in pigs and it was reported that feeds with fish meal at 5 and 10%, porcine digestible peptides (PDP) at 5% and lupine, soybean meal with 44% CP, and dried skim milk at 10 % were preferred ($P < 0.05$) to the reference feed with soybean meal concentrate.

The aim of the present study was to first determine if newly weaned pigs will learn to prefer a flavour (CS+) that is paired with a protein source (US). The second aim was to determine if a conditionally preferred flavour would enhance the attraction to that protein source when the flavour (CS+) and protein (US) are combined.

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

Experiment 1

The first experiment investigated if piglets would acquire a protein-conditioned flavour preference during the post-weaning period. Two protein sources differing in their origin (animal or vegetable) were used as USs, and preference tests were conducted with the CS flavours presented in water or in solid feed.

Materials and methods

Subjects

A total of 480 entire male and female non-deprived weaned piglets ([Large White x Landrace] x Pietrain) served as subjects. During the lactation period these animals consumed a creep feed diet without supplemented flavours, i.e., only with the natural flavour of the feed ingredients contained in the diet. They were weaned at an average of 26 days old and weighing 8.0 ± 1.20 kg. At weaning they were distributed by initial body weight into three categories (Small: 6.7 ± 0.59 kg; Medium: 8.1 ± 0.32 kg; and Large: 9.3 ± 0.48 kg) and sex into 48 pens (10 pigs/pen) inside a room equipped with automatic, forced ventilation and a slatted floor. Each pen (3.2 m^2 in floor area) had a feeder with 3 feeding spaces and an independent water supply. The animals had ad-lib access to a commercial unflavoured feed (pre-starter; 0-14 d and starter diets 15-35 d after weaning) and drinking water except 1 h before and after experimental sessions which were conducted in the housing pens.

Procedure

During the first week after weaning (AW), animals were pre-trained by placing drinking water (1600 ml in 2 pans) in each pen from 09:00 to 11:00. They were then trained to drink during 6 days (alternate sessions) a flavoured solution (CS+P) that was mixed with a protein source (US) on odd days, and a different flavoured solution (CS-) mixed in water on even days. Animals were separated into two same body weight groups (24 pens/group). Pigs in group SPC were trained with 2% of Soybean Protein Concentrate (SPC; HP300®, Hamlet Protein, Horsens, Denmark) solution (1.12 g of CP, 0.31 kJ/mL) and pigs in group PDP were trained with 2% of Porcine Digestible Peptides (PDP; Palbio 62SP®, Bioibérica, Palafolls, Spain) solution (1.24 g of CP, 0.27 kJ/mL) as the US. Flavour products used as CSs (anise or garlic, 0.0375%; Lucta SA, Montornès del Vallès, Spain) were selected because of their similar preferences in naive animals and were counterbalanced across subjects to act as CS+ or CS- flavours. Training sessions were of short duration (30 min/day) and were performed with minimal (1 h) feed and water restriction. The animals received 2 pans with 1600mL (total amount) of the respective CS solution at 10 am each day. In this and all subsequent sessions, consumption was recorded by measuring the initial and final solution volumes.

After training sessions, flavour preferences were evaluated in two-choice tests with the CS+ and CS- flavours both presented in water (non-reinforced tests) on day 15 (Test 1) and 22 (Test 2) AW. In these tests two pans containing 800 mL of the CS+ and CS- flavour were placed in the front of each pen and consumption was measured after 30 minutes. To control for side preferences, the left/right positions of the CS+ and CS- pans were counterbalanced across subjects and between tests. A final choice test was conducted on day 29 (Test 3F) AW in which the CS+ and CS- flavours were presented in the unflavoured commercial feed (0.075%) and intakes were measured by weighting the initial and final feed amounts. At a first sight, spillage was not visually important and, as a consequence, was not accounted for when measuring feed and solution consumption. On the day prior to the first test session, the animals were given a two-choice test (30 min) with 2 pans of water to obtain a measure of baseline water intake.

Statistical analysis

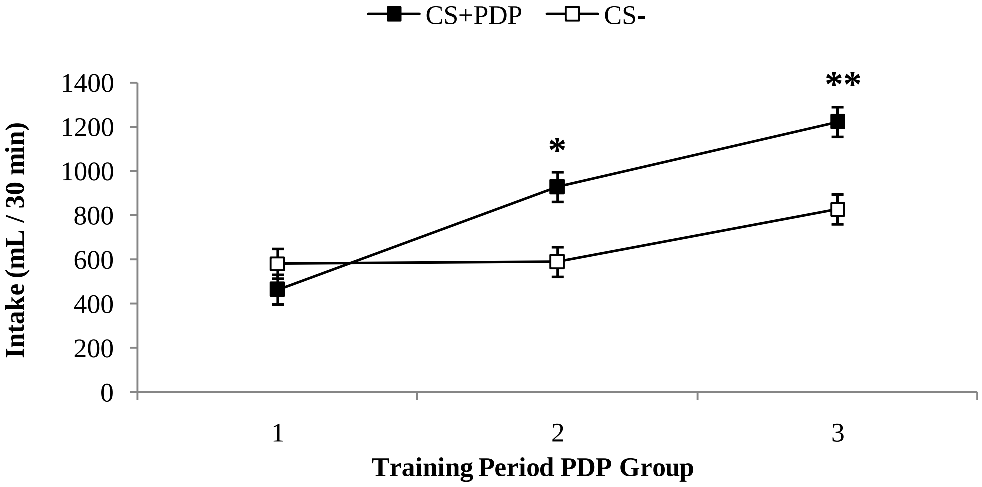
Mean pen solution intakes averaged across the training sessions and two-choice tests with the CS+ and CS- solutions were analyzed in separate tests. Consumption during the training period was analysed with ANOVA using the MIXED procedure of the statistical package SAS® with training session, animal size (large, medium or small), sex, and solution (CS+P or CS-) as the main factors for each group (PDP or SPC). All of the interactions that did not reach significance in a previous analysis were not considered in the final model. For the two-choice test the experimental unit (pen) was also included as a repeated measure specifying the covariance structure of the residual matrix as completely general (unstructured). The mean values are presented as LSMeans adjusted by Tukey and considering a significance level of 5%. Consumption during the choice tests was analyzed with the same procedure with tests (1 and 2), animal size (large, medium or small), sex, group (PDP and SPC) and solution (CS+ or CS-) as main factors. Analysis of the Test 3F was performed in the same way. Percent preference for the CS+ solution or feed was calculated as CS+ intake divided by total intake x 100. The alpha level used for the determination of significance for all the analyses was 0.05.

Results

Training sessions

Mean pen intakes of CS+P and CS- during the training sessions for SPC and PDP groups are summarized in **Figure 1**. The intake of CS+P (SPC or PDP) solution increased over training sessions. In the PDP group CS+P intake exceeded CS– intake in sessions 3-4 and 5-6 but not in the first 2 sessions [Day x CSs, *F*(2, 135)=9.29, *P*<0.001] , whereas in the SPC group CS+ intake was not significantly different from CS- intake except for a tendency (957 vs. 727mL; *P*=0.08) in sessions 5-6 [Day x CSs, *F*(2, 132)=4.29, *P*=0.016]. There was no significant effect of sex on training intakes although overall female pigs tended to drink more than male pigs [*F*(1, 276)=2.90, *P*=0.089]. There was also an interaction between size and solution consumed [*F*(2, 276)=5.54, *P*=0.004]. In this way, overall intake of CS+P (SPC or PDP) was higher than CS- intake in large but not medium or small animals.

1A)



1B)

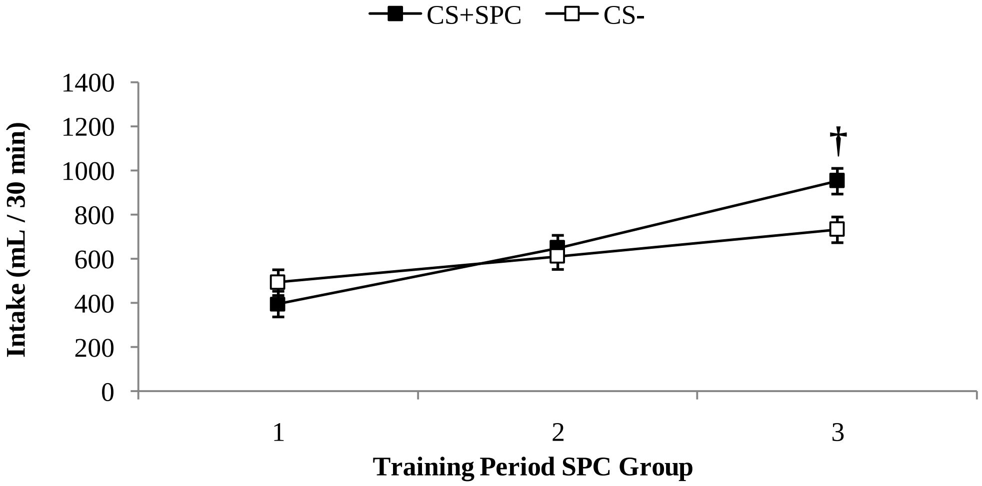


Figure 1, Experiment 1: Solution intake after 30 min. training session for CS+ and CS- in SPC (1A) and PDP (1B) groups. No feed or other fluids were available during training sessions. †P<0.1; *P<0.05; **P<0.01; indicate that solution intakes are significantly different. Flags: ±1 SEM.

Two-Choice Preference Tests

When pigs were given the opportunity to choose between CS+ and CS– solutions, overall they significantly preferred the CS+ to the CS- [*F*(1, 43)=40.09, *P*<0.001] in Test 1 and 2 (**Figure 2**). There were no differences between groups SPC and PDP. The CS+ intake during Test 1 was significantly greater than water intake on the previous day [*F*(1, 42)=17.74, *P*<0.001] in both groups. These differences persisted in Test 2 indicating that flavour conditioning not only produced a CS+ preference relative to the CS- but also stimulated consumption or "appetence" of the CS+ flavour relative to plain water. In addition there were size and sex effects: large animals consumed more than medium (553 vs. 479mL) and small ones (553 vs. 445mL) [*F*(2, 43)=7.64, *P*=0.001] and females a higher intake of CS- during sessions [443 vs. 380mL; *F*(1, 43)=4.13, *P*=0.048].

In Test 3F (15 days after the end of training) the animals in both groups showed preferences for the CS+ flavoured feed over the CS- flavoured feed [*F*(1, 42)=50.70, *P*<0.001] **(Figure 2).** There was an interaction between pig size and CS preference [*F*(2, 42)=4.31, *P*=0.019] with the larger animals showing higher intakes of CS+ over CS-. Large and medium pigs consumed more (P<0.001) CS+ feed than did small animals (674.3, 606.2, 525.6 g, respectively); intakes of CS- feed did not differ. As in the first two choice tests, overall total intakes were higher in female than male pigs (664.6 vs. 524.4; *F*(1, 42)=6.54, *P*=0.014). This difference was due specifically because female PDP pigs tend to consume more than male PDP pigs [sex x group; *F*(1, 42)=3.43, *P*=0.071]. Intakes did not differ for the SPC female and male pigs. There were no significant differences between the two-choice intakes of the SPC and PDP groups.

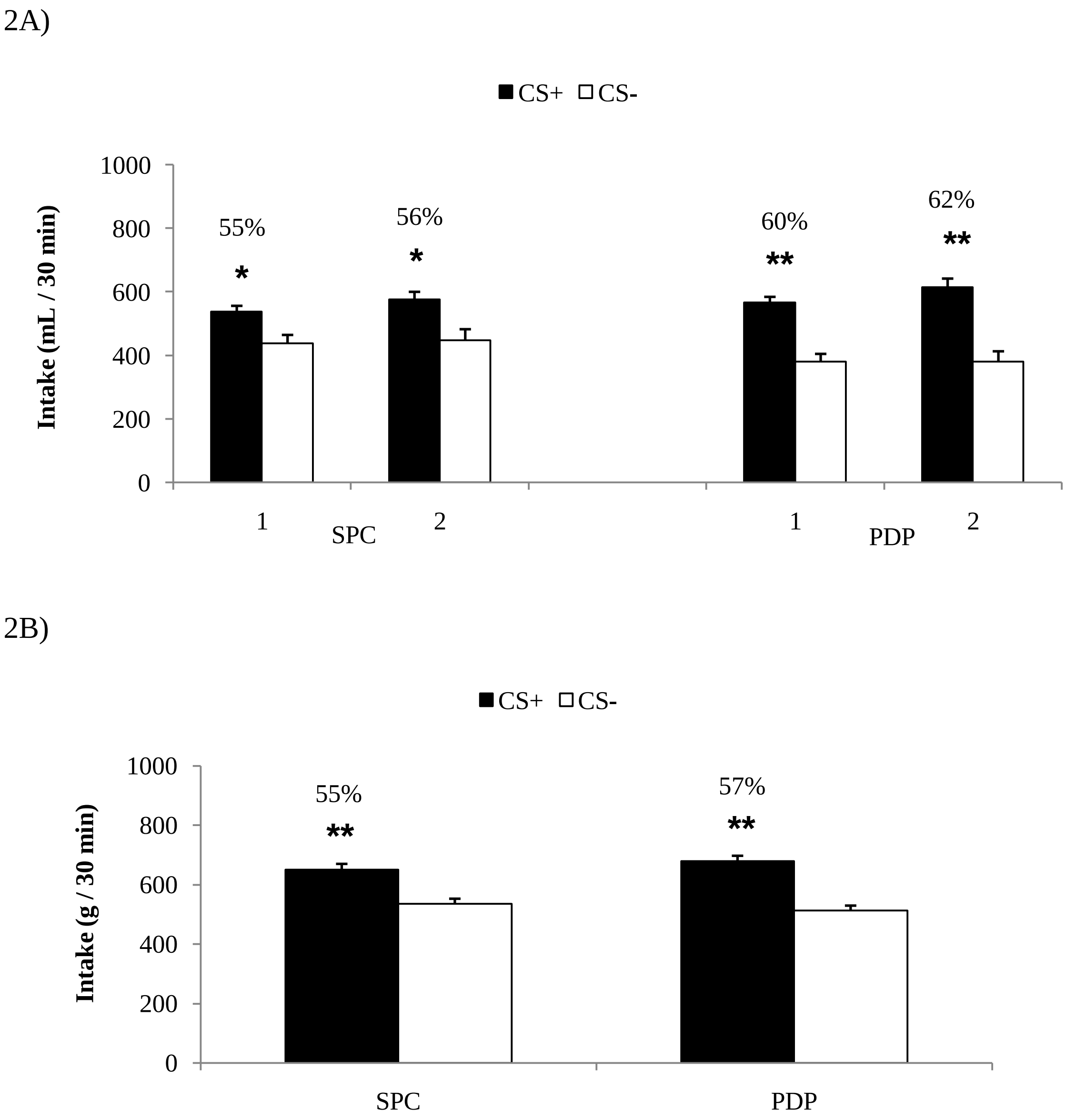


Figure 2, Experiment 1: Intake of CS+ and CS- solutions during 30 min. nonreinforced two-choice test (Test 1 and 2) when flavours were dissolved in drinking water (2A) or mixed into the regular feed on Test 3F (2B). No feed or other fluids were available during the test. Numbers in the top of bars indicate the average percent preference for CS+ solution. Asterisks indicate that CS+ intake is significantly different than CS- intake (*P<0.05; **P<0.001). Flags: ±1 SEM.

Experiment 2

The previous experiment demonstrated that post-weaning piglets learned to prefer a flavour cue (CS+) that had been associated with the intake of a protein source (SPC or PDP). Experiment 2 tested if the conditionally preferred flavour would enhance the unconditioned preference for the protein US in post-weaning piglets, such that the animals preferred the CS+US mixture to the US alone [27].

Materials and methods

Subjects

A total of 240 entire male and female non-deprived post-weaning piglets ([Large White x Landrace] x Pietrain) were used. Animals were weaned at an average of 26 days old with an average BW at weaning of 8.0 ± 1.20 kg. During the lactation period the animals consumed a creep feed diet without supplemented flavours as in Experiment 1. At weaning they were distributed according to size (Small: 6.6 ± 0.60 kg; Medium: 8.0 ± 0.32 kg; and Large: 9.3 ± 0.48 kg) and sex into 24 pens (10 pigs / pen). The experimental procedures for the pre-training period during the first week after weaning (AW) were the same as the first experiment. The animals had free access to feed and fresh water except 1 hr before and after each training and test session. Training and tests were conducted in the housing pens.

Procedure

During the second week AW animals were given 8 training alternate sessions with a garlic flavour (0.0375%, Lucta SA, Montornès del Vallès, Spain) and the Porcine Digestible Peptides (4% PDP; Palbio 62SP®;Bioibérica SA, Palafolls, Spain) in water solutions. Four percent of PDP was used rather than 2% (Experiment 1) to increase the possible post-ingestive effects of the solution during training. Animals were separated into 2 groups with same body weight (12 pens/group). Conditioned group was trained with the garlic flavour (CS) mixed with PDP in sessions 1, 3, 5 and 7 and tap water in sessions 2, 4, 6 and 8. Control group was trained with garlic flavour in water in sessions

1, 3, 5 and 7 and 4% PDP in sessions 2, 4, 6 and 8. Therefore, both groups had experience with the PDP and garlic solutions, but in the conditioned group both products were mixed together in the same solution (associative learning) while in the control group they were offered separately (familiarity learning). Training sessions were of short duration (30 min/day) and were performed with minimal (1 h) feed and water restriction by giving 2 pans with 1600mL (total amount) of the corresponding solution at 10 am each day.

After training, flavour preferences were evaluated in two-choice tests between PDP+Garlic vs. PDP on days 16, 23, and 30 AW (Tests 1, 2 and 3). In these tests, two pans with 800mL of each solution were presented in the front of each pen. Solution intakes were measured during the 30-min tests. In addition, a first contact measure (FC, number of piglets at a pan during the first 15 sec) was recorded as a measure of the palatability of each solution. As in experiment 1, the order (left or right positions) of presentation of the two solutions was counterbalanced across subjects and across tests.

Statistical analysis

Consumption during the training period was analyzed as in experiment 1 for each group (conditioned and control). The mean values are presented as LSMeans considering a significance level of 5% adjusted by Tukey. Two-choice test consumption was analyzed with the same statistical procedure as experiment 1 with test (1, 2 and 3), animal size (large, medium or small), sex, group (conditioned and control) and solution (PDP or PDP+garlic) as main factors and the pen included as a repeated measure. First contact (FC) during choice tests as a measure of solution palatability was analyzed in the same way as consumption data.

Results

Training sessions

The training solution intakes across sessions are presented in **Figure 3.** The animals did not differ in their intakes of PDP+Garlic and water during the first sessions but they tended to consume more PDP+Garlic than water in the last two sessions (838 vs. 574mL; *P*=0.1). The control animals consumed similar amounts of PDP and Garlic water during the first six sessions but consumed considerably more PDP than Garlic in the last two sessions [1297 vs. 458mL *F*(3, 84)=24.54, *P*<0.001]. The control pigs showed an interaction between sex and solution during training [*F*(1, 84)=5.88, *P*=0.017]; females but not males consumed more PDP than garlic solution (686 vs. 361mL; P<0.001 and 570 vs. 450mL; P>0.5, respectively). The water and garlic intakes of the conditioned and control groups respectively were stable during training sessions whereas protein solution intakes (PDP+Garlic or PDP) increased over the last four sessions [*F*(3, 171)=20.88, *P*<0.001]. No differences between groups were observed in terms of protein consumption (PDP+Garlic or PDP) until the last training session where control group showed a higher intake of PDP than conditioned group of PDP+Garlic [Session x group x solution; *F*(7, 171)=4.68, *P*<0.001].

Two-Choice Tests

Figure 4 presents the FC (first contact) results for the three tests. Pigs in the Conditioned group made significantly more first contacts with the PDP+Garlic solution than PDP solution in Tests 1 and 2 but not in Test 3 [*F*(1, 19)=14.82, *P*=0.001]. No differences were observed in FC between PDP+Garlic and PDP for the control group. PDP+Garlic FC scores were higher in the Conditioned group than control group whereas the PDP FC scores were higher in the control group [*F*(1, 19)=14.82, *P*=0.001]. No sex or size effect was observed for the FC.

With respect to solution intake the previous treatment showed an effect on piglets choice (*F*(1, 19)=17.47, *P*<0.001) (Figure 5). Conditioned group consumed more PDP+Garlic than PDP in Tests 1 and 3. There were no sex or size effects on the consumption. No difference in solution intake was observed in the control group during tests 1-3. The preference for the PDP+Garlic displayed by the Conditioned group but not by the control group demonstrates that the addition of a conditionally preferred CS+ flavour (garlic) to the unconditioned stimulus (PDP) enhanced the preference and appetence for the US.

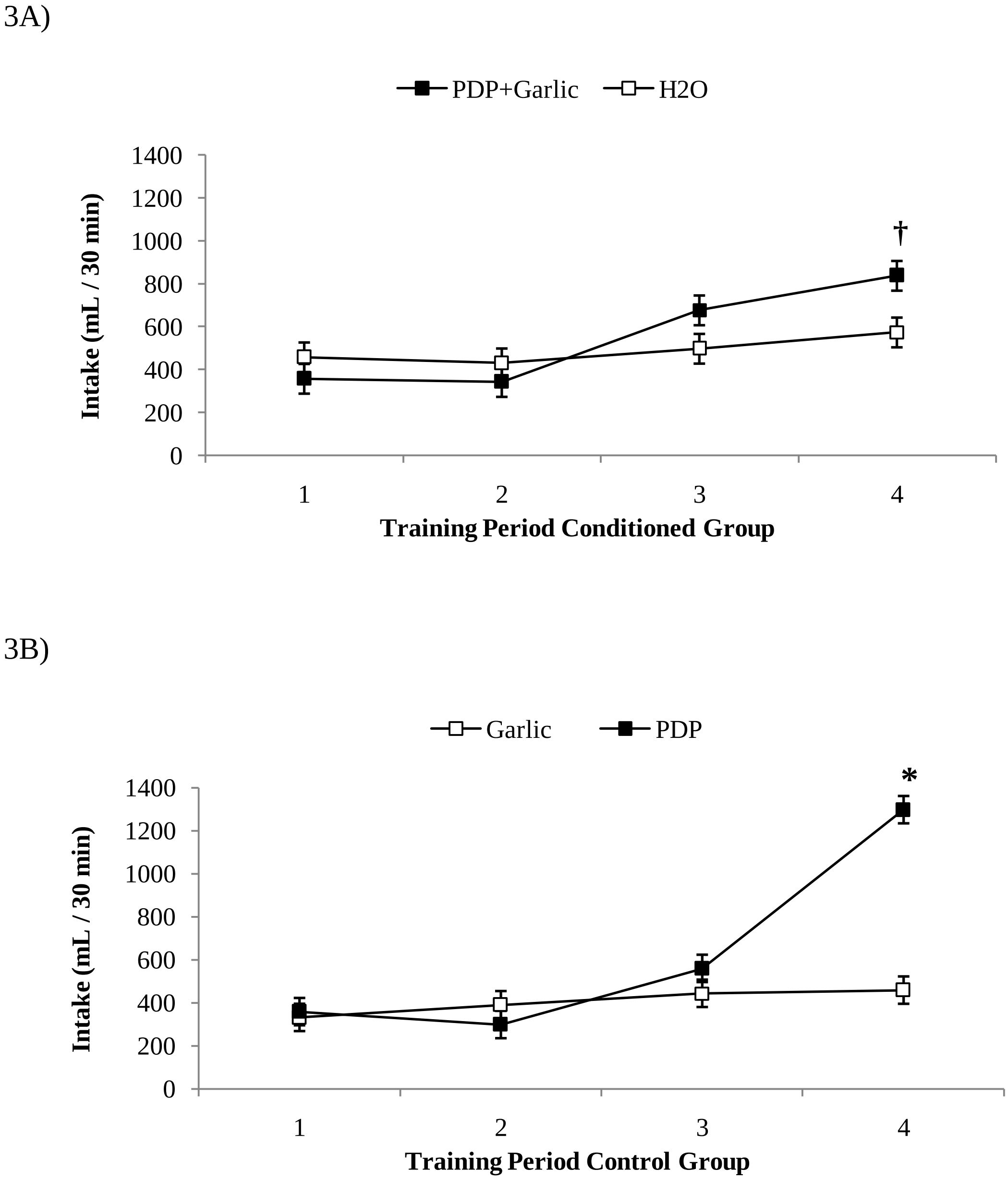
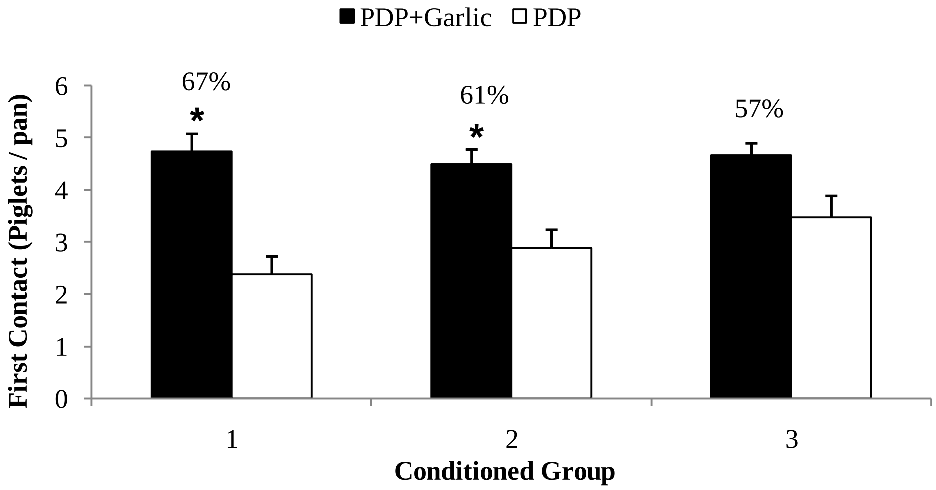


Figure 3, Experiment 2: Solution intake over 30 min. of training session of PDP+Garlic and water solutions in Conditioned group (3A) or Garlic and PDP solutions in Control group (3B). No feed or other fluids were available during training sessions. Asterisks indicate that solution intakes are significantly different (*P<0.05; †P=0.1). Flags: ±1 SEM.

4A)



4B)

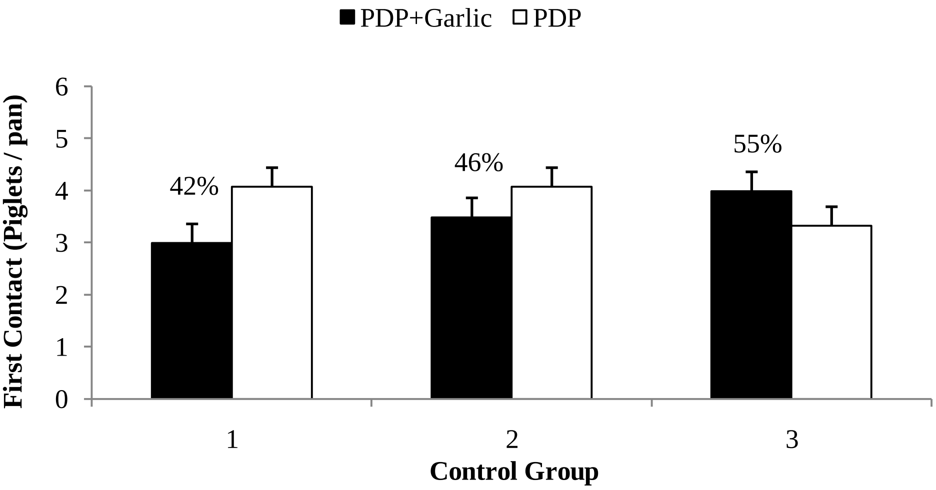
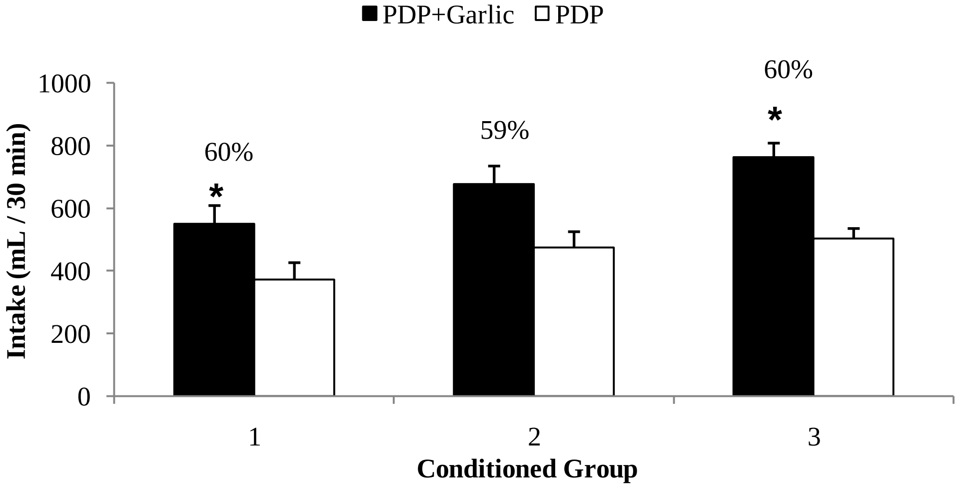


Figure 4, Experiment 2: First contact during 30 min. Two-choice test sessions of PDP+Garlic and PDP solutions (Test 1, 2 and 3) in Conditioned (4A) and Control group (4B). No feed or other fluids were available during the test. Numbers in the top of bars indicate the average percent of first contact for PDP+Garlic solution. Asterisks indicate that PDP+Garlic first contact is significantly different than PDP ($P<0.05$). Flags: ±1 SEM.

5A)



5B)

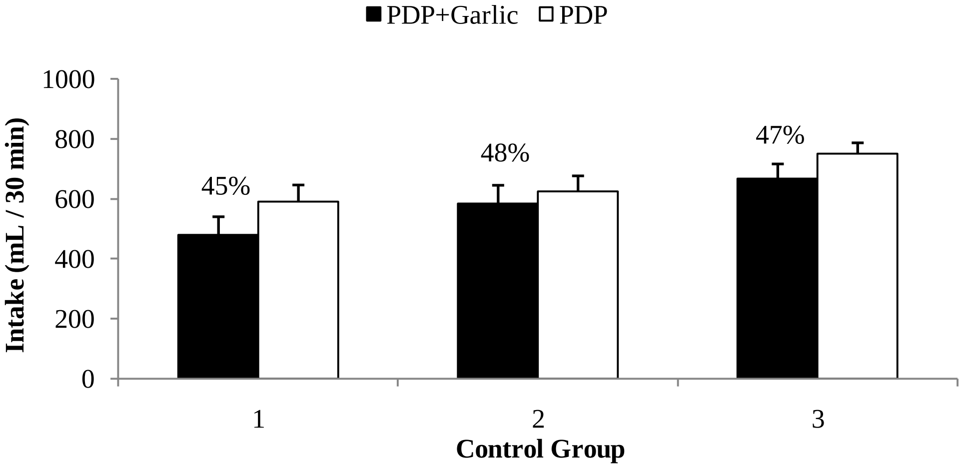


Figure 5, Experiment 2: Solutions intake during 30 min. Two-choice test sessions of PDP+Garlic and PDP solutions (Test 1, 2 and 3) in Conditioned (5A) and Control group (5B). No feed or other fluids were available during the test. Numbers in the top of bars indicate the average percent preference for PDP+Garlic solution. Asterisks indicate that PDP+Garlic intake is significantly different than PDP intake (P<0.05). Flags: ±1 SEM.

Discussion

The present study shows that a protein source can be used to condition flavour preferences in weaned pigs. It was already known that sugar, artificial sweeteners, and chocolate can be used for learning purposes because of their inherent palatability [3,10,39,41]. However to our knowledge the present findings provide the first report of protein-based flavour preference conditioning in pigs.

When post-weaned piglets were exposed to the new protein sources during the training phase of experiment 1 and 2, the animals required only a couple of sessions to show an elevated intake of the protein solutions (SPC or PDP). A feed neophobic response and/or maladaptive behaviours to the early weaning may have limited intakes during the initial exposure to the protein solutions. In later sessions, piglets tended to consume more of the protein sources, most likely because of the nutrient's attractive taste and post-ingestive effects. The choice conducted after the six training sessions in experiment 1 showed that piglets, like rats [51,52] learned to associate the flavour cue with the positive consequences of the protein source. This association created a persistent preference for the CS+ flavour as revealed in the choice tests with the CS flavours presented in water (Test 1 and 2) and feed (Test 3F). Preferences for the CS+ over the CS- (Exp.1) are considered learned preferences acquired through a Pavlovian conditioning process. Mere exposure learning can be ruled out because both flavours were presented the same number of times during the training period. The resistance to extinction the piglets showed for the CS+ preferences is typical of flavour-nutrient learning [45], and further illustrates the powerful effect of this kind of flavour conditioning. However, the higher intake of CS+Protein solutions in the last days of training increased the exposure to the CS+ relative to the CS-flavour, so that we cannot rule out a contribution of exposure learning to the acquired CS+ preference

The two protein sources did not differ in their ability to condition flavour preferences as revealed in the two-choice tests. However, PDP group consumed more of the of CS+ solution than did the SPC group which may be due to the more attractive taste of the PDP protein source and in a small difference in their CP content. The small intake differences observed in the training and choice tests are similar to the higher feed preference for PDP than other protein sources supplemented in nursery pig feed [61]. It is possible that the CS+ preference of SPC group was primarily due to the post-ingestive effects of the protein whereas in the PDP group both the palatable taste and post-ingestive effects contributed to the conditioned CS+ preference. However, the process by which the two protein sources reinforced the flavour preferences was not directly studied. Two ways of separating the taste and post-ingestive conditioning effects of the nutritive US in future studies could be to deliver the US directly into animal's digestive tract [53], or to match the hedonic taste value of two USs using flavour additives (e.g., saccharin or quinine) [69].

Experiment 2 showed that a protein-paired CS+ flavour can increase the preference and consumption of the preferred protein US when the CS+ is combined with the US. This synergy effect was not due to simple familiarity with the flavour because the control group failed to prefer the Garlic-PDP to the PDP solution. Thus, when the CS+ was associated with the US (PDP protein) during the training period, this experience created a positive value for the CS+ flavour that enhanced the attraction of the PDP protein. As previously reported with sweet USs, flavours paired with protein solutions may acquire a "protein" taste quality [58,63,70]. In this way, flavour of the CS+ may have enhanced the protein taste quality of the PDP US.

Some authors have suggested that the palatability of a CS flavour increases when flavour preferences are conditioned by a nutritional US [26, 44]. In experiments 1 and 2 the piglets, especially in the PDP group, tended to lick their mouth several times in the first minutes of drinking the protein solutions. Prior work in rats indicates that this licking pattern is correlated with the palatability of the solution or feed [12,62]. But palatability in pigs has not been systematically studied. Intakes during the first minutes of drinking are also thought to reflect a palatability response to a sapid solution [60]. In experiment 2, we attempted to measure palatability changes by measuring the first contacts (FC) with the solutions. The FC and intake preferences observed in the Conditioned group confirm that the association of a protein source (US) with a neutral flavour cue (CS) increased the attraction to the protein source. Conditioned changes in feeding behaviour, including changes in intake rate, bite rate and bite size, have been observed with other animals [66]. However, rat lick microstructure studies report that changes in CS palatability do not explain the resistance to extinction of the CS+

preference [16, 43]. These studies found that the CS+ preference measured in two-choice intake tests was more resistant to extinction than was the palatability change measured by lick microstructure analysis. Consistent with this finding, we observed in experiment 2 that the increase in CS+ FC disappeared by the last test while the increased CS+ intake in the choice test was still evident. Thus, in addition to acquiring the hedonic tone of the US, the CS+ acquires the incentive value of the US which can maintain elevated intake even in the absence of enhanced palatability [44].

The CS+ preference after associative learning reflect how attractive the nutrient US is to animals. Preferences may change, however, as the animal's nutrient needs change [68]. Cabanac [9] showed that animals can perceive flavours as pleasant or unpleasant according to their current physiological state. It has been reported that the post-ingestive actions of nutrients can condition flavour preferences in food satiated as well as hungry rats [72]. Thus, it is not necessary to use feed-restricted animals to produce learned associations between a CS and nutrient US although removal of feed during the training sessions presumably enhances the association between the CS and US. In the present study we obtained significant CS+ preferences in ad libitum fed piglets which minimize negative effects on their welfare and productivity. Nevertheless, the physiological state of the animal during two-choice tests may influence the magnitude of the CS+ preference expressed. Studies in rats and humans [4,22] indicate that a preference for a protein-paired CS+ flavour is reduced by a high-protein preload prior to the testing. Some previous experiments in rats also showed that calorically conditioned flavour preferences are expressed more strongly when animals were food deprived than non-deprived [17]. Nevertheless, in the present experiment with piglets under a constant growing condition, it was not necessarily to feed restrict the animals to obtain a significant CS+ preference. Piglets show rapid growth and lean tissue deposition which may account for their high protein appetite. In this way pigs may detect and prefer protein compounds to satisfy their internal needs and, because flavours conditioned by the positive effects of nutrients create a representation of the reinforcer, the sensation of the flavour cue alone will activate the representation of that reinforce and a consecutive preference too. Protein needs also may change with pigs size and this could explain the higher intakes of CS+P during the training or of CS+ during the feed choice test that larger animals presented in experiment 1.

It would be of interest in future experiments to determine if protein and carbohydrate sources differ in their ability to condition flavour preference in the weaning transition period. A recent study reported preference conditioning in juvenile pigs trained with a flavoured 10% sucrose solution [10]. However, in contrast with the results of experiment 1, the preference was displayed only in the first of two CS+ vs. CS- solution tests and was not displayed in flavoured feed tests. Juvenile pigs trained with a flavoured 2.25% maltodextrin solution failed to acquire a significant CS+ preference which contrasts with the effectiveness of the 2% protein solutions used in experiment 1. Taken together, these findings suggest that protein maybe more effective than carbohydrate in conditioning flavour preferences in pigs although procedural differences between the current and prior study may also be a factor. Note, however, that a recent rat study reported that flavour preferences were conditioned in rats by intragastric infusions of 60 mM (1%) monosodium glutamate but not 60 mM (1%) glucose [67].

The present results and along with prior finding [71] show that associative learning can produce stronger and more persistent flavour preferences than flavour exposure alone. However, the ability of flavour-nutrient learning to decrease post-weaning neophobia would be most useful if the conditioned preferences were established prior to weaning. During lactation, piglets mainly consume sow’s milk and eat only small amounts of solid feed making the introduction of external flavour stimuli to create new associations difficult. Nevertheless, one study demonstrated the ability of pre-weaning rats to acquire a flavour-nutrient preference that can be expressed after weaning [42]. Other feed learning strategies are possible such as prenatal learning of flavour cues [2,11,25,31,48], the learning of cues transmitted via milk from the maternal diet [28,36] or even the social learning with conspecifics [20,21,29]. In all these cases the US stimuli could create preferences for a neutral flavour by associative learning process. Note that a recent study reported that pre-natal or post-natal flavour exposure in pigs did not condition a flavour preference in piglets although it reduced weaning associated problems [49]. Perhaps a combination of these different feed learning procedures would be most effective in enhancing feed intake after weaning. Thus, new opportunities exist to apply psychological procedures to modify the feeding behaviour of pigs to improve their performance and welfare.

While the present findings document the attraction of piglets to protein solutions and their acquired preference for a protein-paired CS+ flavour, the molecular substrates mediating oral and post-ingestive protein detection remain uncertain. As recently reviewed in detail elsewhere [40,55,59], specific taste receptors including T1R1+T1R3 and mGluR4 mediate the savoury taste (umami) of glutamate and related amino acids in pigs and other mammals. However, whether umami taste represents a general “protein” taste that allows animals to inherently recognize the protein content of food remains is not established. The T1R1+T1R3 and mGlu4 umami taste receptors have also been identified in the gastrointestinal tract where they may, in theory, mediate the post-ingestive flavour conditioning response to glutamate and proteins although this requires further testing [59, 67]. The conditioning signal generated by amino acid detection in the gut appears to be transmitted to the brain via the vagus nerve which contrasts with a nonvagal pathway implicated in carbohydrate conditioning [59]. Several brain structures are implicated in post-ingestive carbohydrate conditioning but the specific brain mechanisms that mediate protein conditioning have yet to be investigated [59].

Conclusion

We conclude that post-weanling piglets can acquire a significant and highly resistant to extinction preference for a flavour paired with a protein source, which likely evokes positive hedonic or post-ingestive memories established by prior conditioning experience. This conditioned flavour preference may stimulate intake and may even enhance the attraction to the preferred protein source through a synergy mechanism. This may be a useful strategy to create long lasting feed preferences and stimulate feed intake in piglets during critical productive periods such as weaning.

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Chapter 3

Flavour preferences conditioned by post-ingestive effect of sucrose and porcine digestive peptides in post-weaning pigs.

Abstract

Pigs can learn to prefer a flavour if it has been associated previously to positive consequences. The aim of this experiment was to study flavour preferences conditioned by the post-ingestive effect of nutrients in pigs. A total of 240 weanling piglets were allocated in 24 pens (10 piglets/pen) and distributed to 2 groups of animals (12 pens per group). Pigs in the first group (G1) were trained during 8 d with one flavour (positive conditioned stimulus, CS+) into a protein solution (4% porcine digestible peptides, PDP) on odd days and another flavour (negative conditioned stimulus, CS-) into 100 mM of monosodium glutamate (MSG) solution on even days (5 L-bottle for 24 h). In the second group of pigs (G2), CS+ was mixed into a 4% sucrose solution in odd days and CS- into 1% sucrose + 0.08% saccharine on even days. Thus, treatments were defined as CS+, the flavour associated with PDP or sucrose on odd days, which were assumed to have a higher post-ingestive effect than MSG or saccharine + sucrose, the ingredients associated to CS-. Concentration of ingredients in the solutions were chosen to assure that hedonic attraction for PDP and MSG solutions, and for sucrose and saccharine + sucrose were similar (checked in previous double-choice studies). The amount of solution offered during training period was prepared to be totally consumed each day to equalize flavours intake. Flavours (anise or garlic, 0.0375%) were counterbalanced across replicates to act as CS+ or CS-. Double-choice test between flavours dissolved in water (CS+ and CS-) were performed by selecting 2 pigs/pen on d 1, 6 and 8 after the training period. Solution intake was measured after 30 min. Piglets showed higher intakes for CS+ than CS- in G2 (212 vs. 76 mL; 168 vs. 86 mL; $P < 0.05$ and 195 vs. 78 mL; $P = 0.15$) on d 1, 6 and 8, respectively. Differences between CS+ and CS- consumption were observed in G1 on d 8 (231 vs. 130 mL; $P < 0.05$). In conclusion, weanling pigs can acquire flavour preferences through associative learning between a flavour and post-ingestive effects of some nutrients.

Introduction

Pigs are frequently exposed to feed and environmental changes that have to face in a critical short time and without previous experience. Feed neophobia is a phenomenon commonly associated with those changes (Miller and Holzman, 1981) which may drive pigs to a variable period of under-feeding or anorexia. However, it is known that pigs have a high innate preference for sweet, umami and moderately salty compounds (Hellekant and Danilova, 1999). These compounds have been used to enhance feed palatability but also in other species to facilitate the intake of neutral flavours by an associative learning with their hedonic or post-ingestive effects (Ackroff and Sclafani, 2011). Both effects may play a different role on the learning process of flavour preferences and can be dissociated by using different techniques to assess their contribution. Warwick and Weingarten (1994) proved in rats that post-ingestive effects are strong enough by themselves to create flavour preferences by using the match of the hedonic effects of 2 components with different nutritive values. These effects are stronger than flavour-flavour associations; shows a higher resistance to extinction (Sclafani, 2004) and flavours become more palatable (Dwyer et al., 2009). In the present work it was hypothesized that this difference could be even higher in species with a high growing potential like pigs were nutrients may act as a powerful positive stimulus to create flavours preferences. The aim was to evaluate if the post-ingestive effects of protein and sucrose (unconditioned stimulus; US) are sufficient to establish flavour preferences in pigs because a previous associative learning between these nutrients and a neutral flavour stimulus (conditioned stimulus; CS).

Materials and methods

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

A total of 240 weaned piglets ([Large White x Landrace] x Pietrain) were weaned at an average of 26 d of age and allocated in 24 pens (10 piglets/pen) inside a room equipped with automatic, forced ventilation and completely slatted floor. During the first week after weaning, animals were pre-trained by offering plain water (1600 mL in 2 pans) in each pen from 09:00 to 11:00 h. All the animals had ad-libitum access to unflavoured feed (pre-starter; 0 to 14 d and starter diets 15 to 35 d after weaning). After the pre-training period pens were equally distributed into 2 groups (12 pens/group). Pens of Group 1 (G1) were trained to drink during 8 d (alternate sessions) a flavour as a positive conditioned stimulus (CS+) that was mixed with a protein solution [4% porcine digestible peptides (PDP); Palbio 62SP, Bioibérica, Palafolls, Spain] on odd days, and a different flavour (negative conditioned stimulus, CS-) into 100 mM of monosodium glutamate (MSG; Ajinomoto SAS, Paris, France) solution on even days. In pens of Group 2 (G2), CS+ was mixed into 4% sucrose solution in odd days and CS- into 1% sucrose + 0.08% saccharine (S1002, SIGMA, St. Louis, MO) on even days. It is assumed that MSG and 1% sucrose + 0.08% saccharine provide lower post-ingestive nutrients than 4%PDP and 4% sucrose (positive stimulus), respectively. On the other hand, we tried to match the hedonic attraction for PDP and MSG solutions, and for sucrose and saccharine + sucrose in a previous double-choice tests (30 min) by evaluating different concentrations of MSG vs. a fix 4%PDP and different concentrations of saccharine + 1% sucrose vs. 4% of sucrose. Thus, flavours CS+ were associated with a higher post-ingestive but the same hedonic value than flavours CS-. The total amount of solution offered during training session was prepared to be absolutely consumed in each day to equalize flavour intake (5L-bottle for 24h). Flavour products used as conditioned stimulus (anis or garlic, 0.0375%; Lucta SA, Montornès del Vallès, Spain) were counterbalanced in each group across replicates to act as CS+ or CS- (ie. half of the pens in G1 associated anise or garlic to PDP on odd days to act as CS+, and consequently garlic or anise as CS- to MSG on even days). After training sessions, flavour preferences were evaluated by using a double-choice test (DCHT) with the CS+ and CS- flavours both presented in water (unreinforced tests) and selecting 2 pigs/pen on d 1 (Test 1), 6 (Test 2), and 8 (Test 3) after the training period. In these tests 2 pans containing 800 mL of the CS+ and CS- flavours were placed in the front of each pen and consumption was measured after 30 min. Left/right positions of the CS+ and CS- pans were counterbalanced across subjects and tests days. Solution intakes during DCHT between CS+ and CS- was analyzed by group (1 and 2) and test day (1, 2 and 3) with ANOVA by using the GLM procedure of the statistical package SAS; taking into account the solution intake (CS+ or CS-) and the flavour (anise or garlic), and being the pen the experimental unit. The mean values are presented as LSMeans adjusted by Tukey. The alpha level used for the determination of significance was 0.05.

Results

Piglets coming from G1 showed a higher total solution intake during the choice tests than piglets of G2 (370 vs. 270 mL SE 30.8; $P < 0.05$). Although differences were not observed in G1 (Figure 1A) between CS+ and CS- at the first 2 tests, a higher intake of the CS+ solution was observed on d 8 for CS+ than CS- (231 vs. 130 mL SE 31.6; $P < 0.05$). On the other hand, piglets of G2 (Figure 1B) presented higher intakes for CS+ than CS- solutions on Test 1 (212 vs. 76 mL SE 42.6; $P < 0.05$) and 2 (168 vs. 86 mL SE 26.1; $P < 0.05$) but, no differences were observed in Test 3 (195 vs. 78 mL SE 55.5; $P = 0.15$).

Discussion

Our results confirm that piglets can acquire flavour preferences through an associative learning with the post-ingestive effects of sucrose. However, PDP failed to establish flavour preferences at the first 2 Tests. It is possible that post-ingestive effects of MSG could also have a positive association with the flavour (CS-), making differences between flavours less visible (Ackroff and Sclafani, 2011). Moreover, it could be speculated that the attraction for CS- flavour could have been enhanced by the hedonic attraction of MSG solution when a flavour is simultaneously added (Rolls, 2009). In this way the previous hedonic match between PDP and MSG could have been unbalanced. However, preferences conditioned by hedonic effects have a short extinction time because they evoke the palatability of the previous association (Dwyer et al., 2009). This could be the reason of finding a preference for the CS+ flavour at the last choice test when CS- attraction likely tended to be extinguished. In conclusion, pigs learn to select nutritious fluids by associating the flavour of consumed substances with their post-ingestive consequences. Learned flavour preferences may be used as a strategy to enhance voluntary intake and reduce neophobia during critical periods, such as weaning.

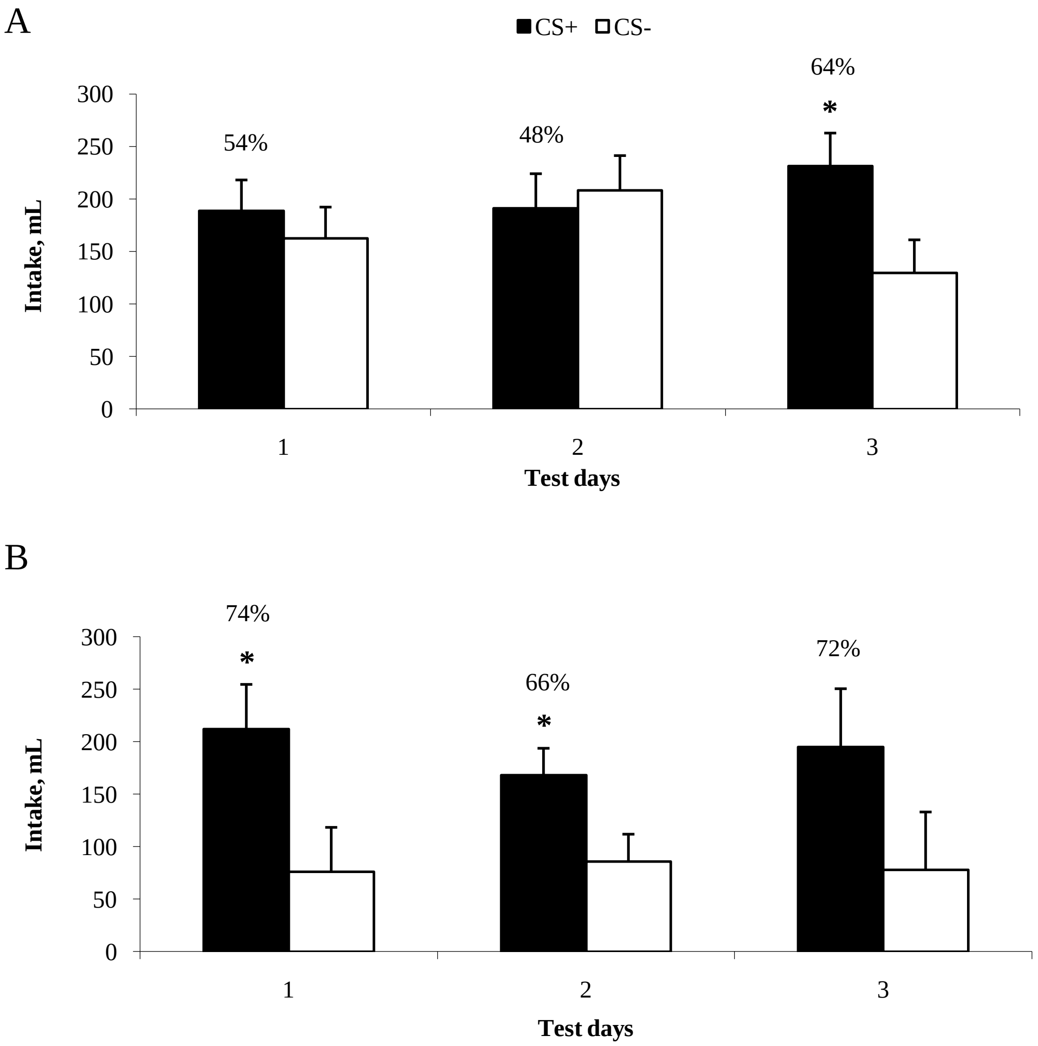

Figure 1: Intake of flavoured solutions (conditioned stimulus, CS+ and CS-) during the choice tests (Test 1,2 and 3, 30 min) of a group of pigs previously trained with a flavour (CS+) mixed with PDP (4%) and other flavour (CS-) mixed with MSG (100Mm) solutions (A) and of a second group of pigs (B) previously trained with a flavour (CS+) mixed with Sucrose (4%) and other flavour (CS-) mixed with Saccharine (0.08%) + sucrose (1%) solutions (B). Asterisks indicate that CS+ intake is different than CS- intake (**P* < 0.05). Flags: ±1 SEM.

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Chapter 4

Social learning of feeding behaviour in pigs: effects of neophobia and familiarity with the demonstrator conspecific.

Abstract

Social interactions help animals to learn new features of their environment without a trial and error process. It has been observed in other species that feed cues can be transferred from one model animal (demonstrator) to another (observer) due to a social learning process. Three experiments were performed to evaluate if weaned piglets showed a preference for a flavoured feed following brief social interactions (30 minutes) with an experienced demonstrator. After the social interaction between demonstrator and observer pigs, a 30 minute choice test between the flavoured feed previously eaten by demonstrators and a novel flavoured feed (Exp. 1 and 2) or a known unflavoured starter diet (Exp. 3) was performed with observer animals. Higher intakes of demonstrators consumed flavoured feed were observed when demonstrators and observers were from the same transition pen (Exp. 1) or from the same litter (Exp. 2), but not when observers and demonstrators were unfamiliar with each other. Observers also preferred flavours previously eaten by the conspecifics demonstrator over their unflavoured diet already known. Social interactions with a conspecific pig that had a recent experience with a flavoured feed enhanced the preference for that feed and could even override neophobia to new feed ingredients. The familiarity of conspecific demonstrators plays a key role in social learning probably due to selective exploration of new cues and therefore closer snout-to-snout contact.

Introduction

Feed preferences and aversions are strongly influenced by individual learning experience (Sclafani 2004; Sclafani & Zukerman 2009; Dwyer et al. 2009) but also, feed preferences are determined by social interactions (Galef & Whiskin 1997, 2001). In natural environments, social learning is a behaviour that increases the probability of survival especially in young or less experienced individuals that benefit from learning advantageous behaviour patterns from experienced group members (Galef & Giraldeau 2001; Thornton & Malapert 2009). It has been reported in different species that feed information can be transferred from one individual to another by a social learning process (Posadas-Andreus & Roper 1983, rats; Provenza & Balph 1987, ruminants; Yoerg 1991, hyenas; Oostindjer et al. 2011, pigs & sows). In rats, when a conspecific animal (demonstrator or model) tried a new feed or flavour, another rat (observer) could learn to prefer these components in a future exposure after a brief interaction with this experienced animal because of contact with the feed odours on the breath of the demonstrator rat. This learning was not affected by the age or familiarity of the demonstrator and even appeared when rats' experience indicated that such an act led to inferior outcomes (Galef 1996; Galef & Whiskin 2008a). However, a common observation in the social learning literature is that individuals often copy kin conspecifics with the expectation of obtaining the same rewards (Coussi-Korbel & Fragaszy, 1995; Laland 2004). Evolutionary theories explain that familiar animals also share a common environment and probably they are genetically related (Laland 2004; Valsecchi et al. 1996). Because of this, their physiology characteristics are similar and feed behavior is an expression of their similar needs making the new food behavior adaptive (Laland 2004).

Pigs are highly social animals and begin to form social relationships with littermates within hours of birth (Graves 1984). Nevertheless, there have been only a few studies of social learning of feeding behaviour in pigs. Nicol & Pope (1994) and Held et al. (2000) showed that an inexperienced pig could follow an experienced pig and learn about the location of food; and Morgan et al. (2001) also demonstrated that feed intake is enhanced when an inexperienced piglet is housed with an experienced conspecific. However, a “follow the leader” can be a local enhancement process in which the observer animal is attracted to the place that demonstrator visits. This can explain previous results and none of these 3 experiments demonstrated that pigs can learn specific feed cues from an experienced animal that allow them to change their feeding behaviour.

At weaning the piglet faces three main challenges in the intensive pig industry, including changes in the physical environment, the psychological stress that accompanies moving and mixing animals, and a major change in food composition. Early weaned piglets have to find their own food, which it is more bulky and dry (usually >88%), less digestible than milk, and composed of ingredients that the piglet has not previously encountered. In these conditions most weaned piglets are reluctant to eat the new solid feed, leading to anorexia on the first days after weaning (Fraser 1984; Pluske et al. 2007; Moeser et al. 2012). Neophobic avoidance of new feeds may seem a maladaptive process on some occasions for omnivorous species that need to consume a varied diet to obtain adequate nutrition and it becomes critical in pig production systems where new diets have to be incorporated during a short period of time. (Provenza & Balph 1988). However, neophobic reactions may be reduced with a learning process before or after birth by having contact with ingredients or flavour cues and associating those components with positive consequences (Hepper & Wells 2006; Mennella 2001; Ackroff & Sclafani 2010; Oostindjer et al. 2009, 2010).

Social interactions with experienced pigs could help naïve pigs to learn about conditions in a new environment without trial and error learning. By using social learning, individuals do not need to search their entire home range, or sample potentially toxic foods (Laland 2004). These interactions could help to override neophobia towards a particular ingredient or flavour after interacting with a conspecific that has eaten these components. In our first experiment we hypothesised that pigs can learn to prefer a certain flavoured feed following brief social interactions with their conspecifics during the post-weaning period, without participating or watching during the conspecific demonstrator intake. We also hypothesised that this learning could be affected by the familiarity of the demonstrator. In the following experiments we tried to establish whether these preferences were affected by the kind of familiarity between demonstrators and observers (penmates vs. littermates, Experiment 2) or may even increase feed preference over the previously known unflavoured feed (Experiment 3).

Experiments were conducted at the weanling unit of the pig facilities belonging to the Universitat Autònoma de Barcelona (UAB). Experimental procedures were approved by Ethical Committee on Animal Experimentation of the UAB. (CEAAH 1406).

Materials and methods

Subjects

A total of 448 entire male (224) and female (224) pigs ([Large White x Landrace] x Pietrain) of 49d of age (3 weeks after weaning) were used (256 in Trial 1, 64 in Trial 2 and 128 in Trial 3). Animals were individually identified at birth by using a plastic ear tag and they stayed with their mother and littermates inside the farrowing crates (standard farrowing create, 0.5 m wide, 2.0 m large and 1.03 m high) and their corresponding area for piglets (total available area 4.63 m^2; 4.15 m^2 of complete slatted floor and 0.48m^2 of concrete heat area) during the entire suckling period (28d). The farrowing room was provided with controlled temperature; 22.4±2.05°C sow environment and 28.3±2.70°C piglet environment (HOBO U10, data logger, MA, USA) and automatic ventilation. Inside each crate, piglets had access to a heated area to provide a warmed resting area, which was also enriched with wood shavings, sawdust and drying material (Biosuper CONFORT +, Gratecap Services, La Rochelle, France). An unflavoured creep feed diet was offered ad-libitum from day 10 of birth onwards to all litters by using a pan feeder. Piglets were weaned at an average of 26±2 days of age with a body weight of 7.3±1.1 kg. At weaning animals were moved to the weanling unit and distributed into weaning pens according to sex (8 piglets/pen). The room was provided with automatic, forced ventilation and completely slatted floors. Each pen (3.2 m^2 in floor area) had a feeder with 3 feeding spaces and an independent water supply next to the feeder. Animals had ad-lib access to unflavoured complete feed (pre-starter; 0-14d or starter; 15-35d post-weaning) except 1 hr before and after each test session. Free access to fresh water was provided to all of the animals for the entire experimental period and no environmental enrichments were applied during this period. At the second week after weaning animals were adapted to future experimental conditions by offering them two equidistant control dishes for 2 hours (each morning from 9-11am in each pen) with the same feed that they were eating before. During these 2hrs training periods the commercial feeder was removed from the pens. After the experiment, piglets continued with the normal process of commercial pig production in the same experimental unit of the UAB.

Procedure

On week 3 after weaning (49d-old), animals were divided into 2 groups differing in the familiarity of the demonstrators (experiment 1 and 2) or in the exposure or not to social contact (experiment 3). Some piglets in each pen participated as demonstrator in and the rest as observers (Figure 1). Demonstrator animals of each pen were temporary moved to an empty pen where 1000g of a flavoured starter feed was offered for 30 min inside 2 feeder pans. The flavoured feed was previously prepared by mixing the unflavoured starter commercial feed with 0.075% of garlic or aniseed (Lucta SA, Montornès del Vallès, Spain) by using a small experimental feed mixer during 10 minutes. Garlic and aniseed flavours were chosen because we previously confirmed similar preference between them when piglets were offered both flavoured feeds in a choice feeding test for the first time. Immediately after that, demonstrator piglets were mixed with the observer animals for 30 min. Flavours were counterbalanced across pens to act as the flavour consumed by demonstrators or novel flavour. After the interaction between demonstrators and observers, demonstrator animals were returned to the empty pen and a choice test between aniseed and garlic flavoured feeds was performed to the observers in two new dishes. Feed intake of the flavoured feed consumed by demonstrators and of both feeds during the choice test was measured after 30 minutes by weighting the feeders at the beginning and end of the test. At a first sight, spillage was not visually important and, as a consequence, was not accounted for when measuring feed consumption. The positions of the control and social flavours were counterbalance (left/right) across observer pens. No feed deprivation was applied to pigs in the experiment. However, feed was removed for 1 hour before the beginning of each test and it was returned to each pen at the end of the choice test.

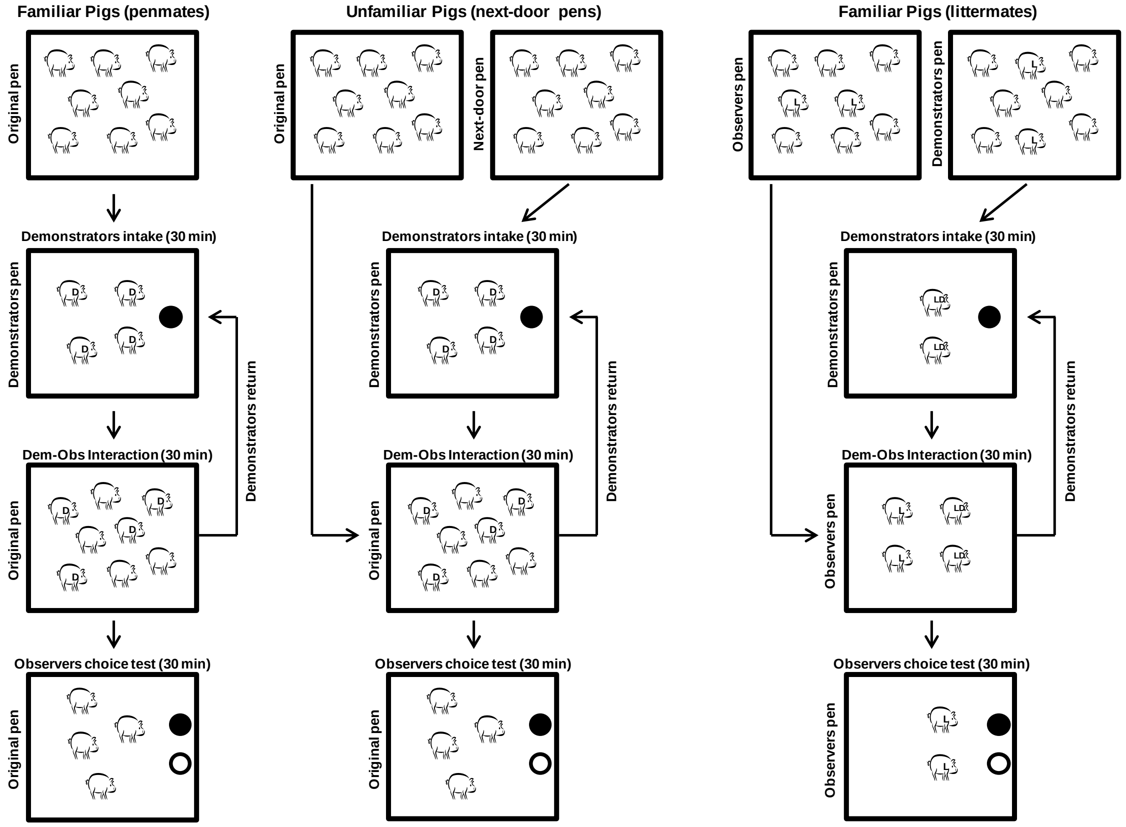

Figure 1. Experiments 1, 2 and 3. Layout of the social behaviour learning procedures between observers and demonstrators (D) post-weaning pigs under familiar [Experiment 1,2 (2 pigs) and 3] and unfamiliar (Experiment 1) interaction conditions. Control animals of experiment 3 were selected to act as observers without a previous social interaction and were not showed in this figure. Novel feed of experiment 3 had no added flavours. Close circles represent the flavoured feed eaten by demonstrator and open circles a novel flavoured feed (experiment 1 and 2) or an unflavoured known diet (experiment 3).

For Experiment 1 [effect of familiarity over social learned preferences (Familiar vs. Unfamiliar demonstrators)], 16 pens (8 for female and 8 for male, 8 pigs/pen) were selected for the familiar demonstrator group, and 16 pens for the unfamiliar demonstrator group. Four animals in each pen were randomly selected to act as observers and 4 as demonstrator or model. Penmate demonstrators were used in the familiar demonstrator group (16 pens) and next-door pen demonstrators were used in the unfamiliar demonstrator group (16 pens). For Experiment 2, [effect of littermates as familiar conspecifics over social learned preferences (Littermates vs. Penmate demonstrators)], 32 animals were used for the littermate demonstrator group (8 lactation littermates allocated in different weaning pens, 4 piglets/sow, 2 acting as demonstrators and 2 as observers) and 32 piglets in the penmate demonstrator group (8 pens, 2 pigs as demonstrator and 2 as observers). For Experiment 3, (preferences for a flavoured feed over their known unflavoured feed because of social interactions), we used 8 pens for the pre-exposed treatment (with penmate demonstrators as described in experiment 1) and 8 pens acted as a control group and no exposure to social interactions were done before the choice test (animals from each control pen were randomly selected at the moment of the choice test). In contrast to experiment 1 and 2 when animals were offered a choice between two flavoured feeds, in experiment 3, a choice test between the flavoured feed previously consumed by demonstrators and a control feed (known unflavoured starter diet) was performed on the observer animals.

Statistical analysis

Pen feed consumption during the observers choice test was analyzed with ANOVA by using mixed linear models with the MIXED procedure of the statistical package SAS® (SAS Inst. Inc., Cary NC); taking into account the effects of the group (familiar vs. unfamiliar in experiment 1; littermate vs penmate in experiment 2; Pre-exposed vs control in experiment 3), flavour eat by demonstrators (aniseed or garlic), sex (experiment 1), feed consumed (demonstrator consumed flavoured feed or novel flavoured feed) and the interaction between the group and feed consumed as the main factors. Pen during the choice test was also included as a repeated measure specifying the covariance structure of the residual matrix as completely general (unstructured). All of the interactions that did not reach significance in a previous analysis were not considered in the final model. Before ANOVA analysis normality and homoscedasticity of the dataset were analyzed by using the UNOVARIATE and GLM procedures with the Shapiro-Wilk and O'Brien's Test, respectively for each factor. As no significant p-values were obtained for any of the specific factors, the original hypothesis for normality and homogeneity of variance were accepted ($P>0.10$). The mean values are presented as LSMeans considering a significance level of 5% adjusted by Tukey.

Results

Familiar vs. unfamiliar demonstrators (Experiment 1)

The average flavoured feed (aniseed or garlic) intake by the demonstrator pens (4 pigs/pen) after 30 minutes of exposure before social contact was 324g/pen; SEM 34.5g (81g/pig). The intake of flavoured feeds by the observer's pens during the choice test is summarized in Figure 2. Observer animals exposed to social interactions with pen-mates showed a tendency to consume more feed than observers exposed to an unfamiliar conspecific during the choice test (*F* 1,28=3.13, *P*=0.087). The feed offered previously to demonstrator pigs had an effect over feed preferences of familiar observers (penmates) but not unfamiliar observers (next door pen observers) (GROUP x FEED CONSUMED; *F* 1,28=11.68, *P*=0.002). Flavoured feed intake was higher in observer pigs when those flavours were previously eaten by a familiar (transition pen-mate) rather than by an unfamiliar demonstrator (264g vs. 167g; SEM 27.9; *P*=0.002).

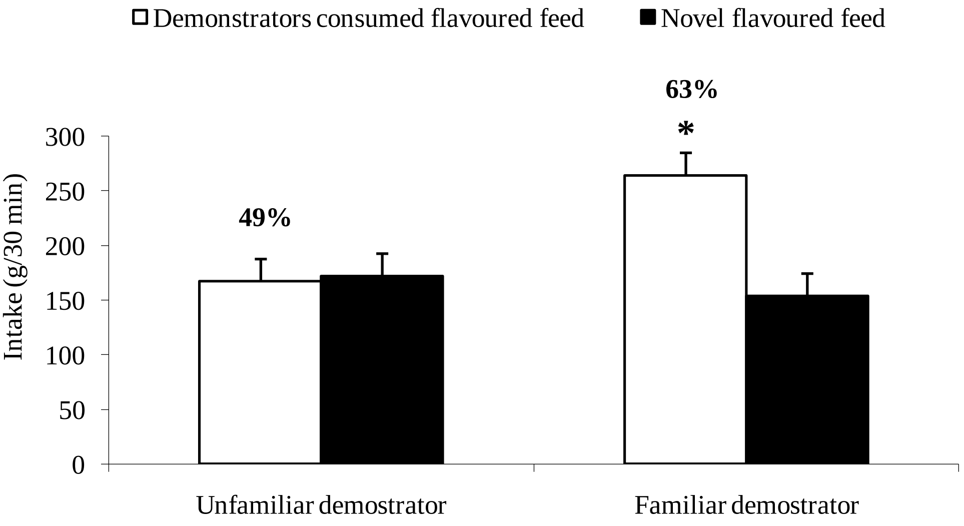

Figure 2. Experiment 1. Means (+SEM) observers feed intake of demonstrators consumed flavoured feed or novel flavoured feed after 30 min. choice test when they previously interact with unfamiliar or familiar demonstrators. Numbers in the top of bars indicate the average value of the corresponding percentage of preference for the flavoured feed previously consumed by demonstrators. Asterisks indicate that intake is significantly different between both feeds in each group (*P<0.001).

As an average, animals in the familiar (penmate) group consumed more demonstrators consumed flavoured feed than novel flavoured feed (264g vs. 154g; SEM 23.9g; *P*<0.001). Thus, 13 out of 16 familiar observer groups consumed more demonstrators consumed flavoured feed than novel flavoured feed. On the other hand, animals that interacted with unfamiliar conspecifics did not show different intakes of demonstrated versus novel flavoured feed. Observers feed intake did not differ significantly between the flavours used previously to fed demonstrators (188g garlic vs. 190g aniseed; SEM 22.3g; *F* 1,28=0.01, *P*=0.943) or sex of animals (222g females vs. 209g males; SEM 19.7g; *F* 1,32=0.15, *P*=0.697).

Littermates vs. Penmates demonstrators (Experiment 2)

It was observed in experiment 1 that pigs were able to develop a flavour preference after a brief contact with a familiar and experienced conspecific. In results of experiment 2, demonstrator animals ate an average of 227g/pen SEM 105g (113.5g/pig) of the flavoured feed during the 30 minutes of exposure before the social contact. Figure 3 shows the Demonstrators consumed flavoured feed and Novel flavoured feed intakes recorded during the choice session for the observer pigs, after an interaction with either penmate or littermate demonstrators. Observers pens exposed to social interactions with littermates showed a tendency to eat more (169g vs. 137g SEM 18.1g) during the choice test than observers pens exposed to penmate conspecifics (*F* 1,13=3.16, *P*=0.098). The feed offered previously to demonstrator pigs had an effect over feed preferences of both groups after the social contact. Thus, 6 out of 8 observer couples that interacted with penmates conspecifics consumed more the flavoured feed that demonstrators ate than novel flavoured feed. A similar situation was also observed for littermate demonstrators group where 7 out of 8 observer pairs consumed more feed containing the demonstrators consumed flavour than the novel flavour. As an average, animals in the penmate group consumed more demonstrators consumed flavoured feed than novel flavoured feed during the choice test (211 vs. 63g; SEM 24.1g; *P*=0.016). Similar results were observed for the littermate group (242 vs. 96g; SEM 24.1g; *P*=0.018) for demonstrators consumed and novel flavoured feed, respectively. No interaction was found between the feed consumed and groups (*F* 1,13=0.00236, *P*=0.962). As for the experiment 1, no significant effect of flavour used was observed in observer animals total intake (157g garlic vs. 149g aniseed SEM 18.1g; *F* 1,13=0.22, *P*=0.644).

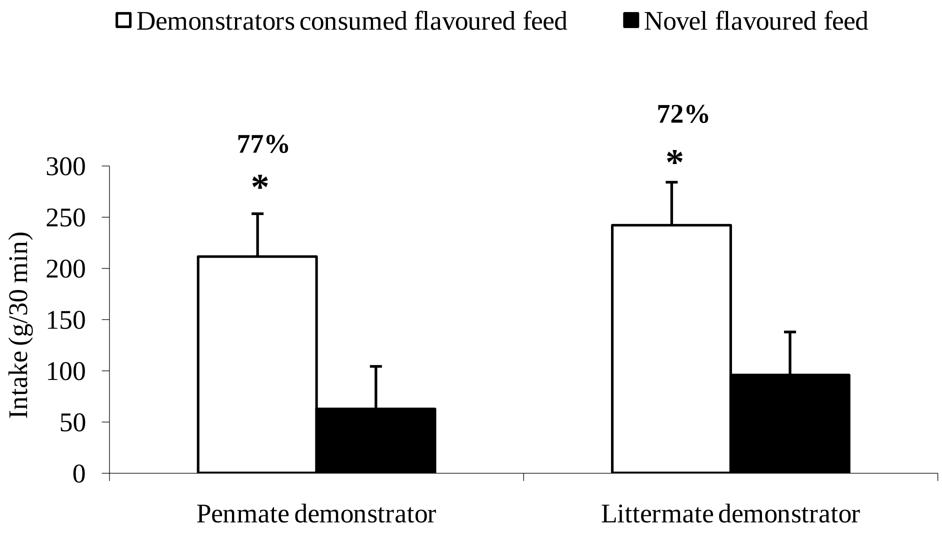

Figure 3. Experiment 2. Means (+SEM) observers feed intake of demonstrators consumed flavoured feed or novel flavoured feed after 30 min. choice test when they previously interact with pen-mates or littermates demonstrators. Numbers in the top of bars indicate the average percent preference for the flavoured feed previously consumed by demonstrators. Asterisks indicate that intake is significantly different between both feeds in each group (*P<0.05).

Flavoured feed vs. Unflavoured and known feed (Experiment 3)

Demonstrator animals in pre-exposed group consumed an average of 288g/pen, SEM 35.7g (72g/pig) of the flavoured feed (aniseed or garlic) during the 30 minutes of exposure before the social interaction. The intakes of the flavoured feed and the previously known control feed during the choice test session are shown in Figure 4 for animals that were either exposed or not exposed to social interactions with demonstrator conspecifics that previously consumed the flavoured feed. Pigs exposed to social interactions with conspecifics did not show a different total feed intake during the choice test from control animals (*F* 1,14=0.54, *P*=0.475). However, preferences were affected by the social exposure (GROUP X CHOICE; *F* 1,14=23.21, *P*<0.001). Pens exposed to social interaction showed a higher intake of the flavoured feed than control pens (389 vs. 283g; SE 42.3; *P*=0.026). As was the case in experiments 1 and 2, during the choice test, observers exposed to social interactions preferred the feed that their demonstrators had eaten before. In this way, higher intakes of flavoured feed compared to the already known control feed (389 vs. 264g; SE 24.4g; *P*<0.001) were consumed by the observer animals in the choice test. In this group, animals in 7 out of 8 observer pens ate more flavoured feed than control known feed. No difference in feed intake was observed in the control group between the two feeds (283 vs. 325g; SEM 24.4g; *P*=0.109, for flavoured and control feed). However, in 7 out of 8 pens of the control group animals ate more the control known feed than flavoured feed.

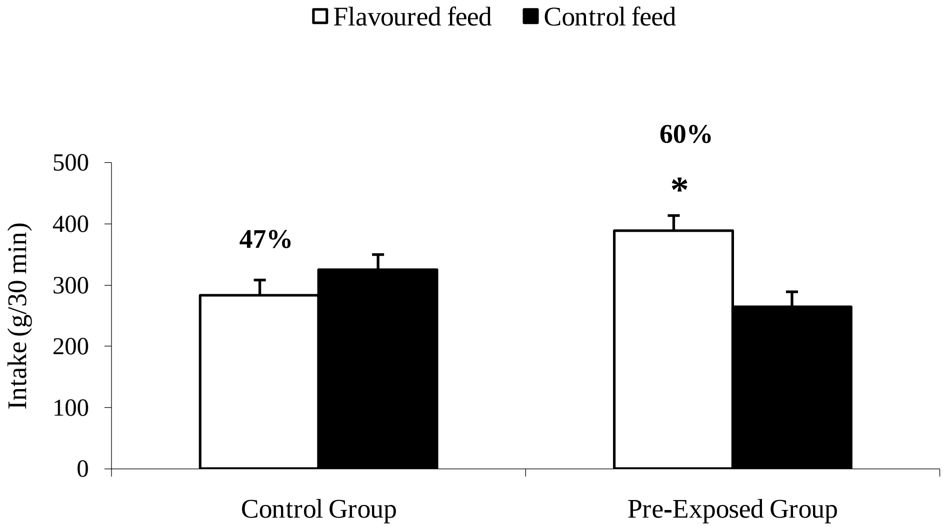

Figure 4. Experiment 3. Means (+SEM) feed intake of flavoured feed or a known control unflavoured feed after 30 min. choice test in animals previously exposed to a social interaction with familiar conspecifics that ate the flavoured feed or control animals (not exposed to social interactions). Numbers in the top of bars indicate the average percent preference for the flavoured feed. Asterisks indicate that flavoured feed intake is significantly different than control feed intake (*P<0.001).

Discussion

The present manuscript shows that pigs, like rats (Galef 1995) and non-human primates (Hopper et al. 2011), were able to learn a preference for flavoured feeds following social interactions with a conspecific animal. A brief contact with a demonstrator that recently ate a flavoured diet was enough to change the feeding behaviour of naive observers. This could have a great importance in some critical stages of pig development, reducing the possible costs associated with individual trial-and error learning and first feed contact time. However, social learning does not appear in all conditions, and demonstrator characteristics (age, sex, familiarity, illness i.e.) may affect the behaviour acquisition (Coussi-Korbel & Fragaszy 1995; Galef & Wigmore

1983; Galef et al. 1984, 1998). In experiment 1, it was observed that the familiarity of the demonstrator was important to establish these preferences in observer pigs. Interaction with familiar (same transition pen) but not with unfamiliar (next-door pen) pig demonstrators was sufficient to induce a preference for the feed that demonstrators had previously eaten. These results are in agreement with previous results of Valsecchi et al. (1996) where they reported, in a study with gerbils, a greater effectiveness of familiar animals to learn feed cues in a social way. Familiar animals in nature have a high probability to be kin and selection may have favoured learning between kin subjects. In this way, demonstrators and observers may experience the same environment from an early stage, could reproduce similar behaviour variants and get similar rewards experiences. Because kin share genes, individuals may have more to gain by providing reliable information to kin than to nonkin, making the probability of social learning being directly proportional to the coefficient of relatedness of observer and demonstrator (Laland 2004). Nevertheless, Galef & Whiskin (2008b) showed that in rats the familiarity of demonstrators is not important at all and social learning of the feeding behaviour may be even higher when the demonstrator is unknown because of the prolonged oro-nasal recognition contact with an unfamiliar animal. Saggerson (2006) support this view because of “attentional modulation” that made observer put less attention to a familiar conspecific.

The effect of the demonstrator familiarity on observer pig’s acquisition of feeding behaviours may be also explained by evasive behaviours between unfamiliar members when they meet for the first time. This may cause a reduction of pigs contact and a decrease in the flavour transference. Demonstrators’ familiarity could be also important because of the selective exploration that animals perform to the unknown cues when they interact with a conspecific. In this way, observer pigs probably spend more time interacting “snout-to-snout” with familiar than with unfamiliar demonstrators, reducing observer probability to achieve flavours cues coming from an unknown demonstrator’s subject even if they share the common environment or age. Theoretically, this social interaction “snout-to-snout” could also help to transfer flavour cues from sows to piglets during the lactation period. However, previous studies using the sow as the demonstrator failed to establish feed preferences in the observer groups (piglets) when animals interact after the meal without the possibility of seeing or participating in their mothers intake (Oostindjer et al. 2011). Probably the different response in the last experiment was due of the piglets age and conditions, because they tried to promote a social learning in lactating animals, when milk remains as the main reinforce for the young mammals. Kevin Myers et al. (2005) have also reported that it is easier to create flavour preferences by nutrient post-ingestive effects in post-weaning animals as compared to lactating young conspecifics.

In intensive pig production, familiarity between animals becomes a flexible concept as those animals are mixed on multiple occasions (cross fostering, weaning, pre-growing and even before slaughter). However, it has been shown that pigs are able to recognize littermates after several weeks (Ewbank & Meese 1974; Gieling et al. 2011). Studies in wild boars (Graves 1984) also demonstrated that early social interactions can be remembered until adulthood. In experiment 2 it was showed that piglets were able to recognize old litter members 3 weeks after weaning, and learn a feeding behaviour from them. The results obtained confirm that piglet’s interactions during lactation persist even 3 weeks after weaning and may be important to allow feed communication between conspecifics.

Social interactions are important not only to create preferences for a diet in observer naive animals but also to override neophobic reactions or unlearned aversions (Nicol 2004; Galef 1986). It has been shown that these kinds of interactions may also change previous preferences for a known or even highly preferred feed. In experiment 3 it was observed that social interaction with a conspecific piglet that had a recent experience with a flavoured feed enhanced the preference for that new flavoured feed over the known diet. Previous experiments in rats also reported that social enhancement of feed preferences are higher if the learned feed is unfamiliar than familiar to the observer animals (Galef & Whiskin 2000). In this way, pigs of experiment 3 probably preferred the new diet learned by social interactions over their normal transition diet because adaptive mechanisms to increase variability in the diet.

Our results show, as Galef and Wigmore (1983) previously reported in rats, that pigs were able to learn a flavour preference even without seeing demonstrators consuming the flavoured feed. A post feeding contact was enough establish a learned preference in post-weaning pigs. The direct and brief contact between conspecific animals enabled observers (inexperienced animals) to have contact with flavour cues from a demonstrator (experienced) animal. These cues are presumably released in the snout of the demonstrator as a result of feeding, chewing and of the retro-nasal aroma release that allowed observers to obtain the new feeding behaviour. This way of social influence over pig´s feed behaviour is different to the social impact that Nicol & Pope (1994) and Held et al. (2000) showed before, where inexperienced animals tended to follow to an experienced animal to the new feed location and enhanced their intake. In the same way, Morgan et al. (2001) showed an enhancement of feed intake when animals (naïve and experienced) were housed together. In both situations the interaction between the observer and the demonstrator included the moment of observer consumption or at the moment of demonstrator consumption, reflecting more a learning of the place and the act of feeding than learning associated with the feed previously consumed. Probably the kind of learning observed in our experiments, as well as that Galef demonstrated before, may have evolutionary advantages. Naive (observers) animals in wild or free-range environments can perform other kind of behaviours and activities when demonstrators are searching for feed and learning the consequences of their intake by trial and error. On the other hand, after the social contact, demonstrator can attend to other tasks and do not have to remain during observer consumption. This could improve the effectiveness of the herd increasing the total fitness value. However, a combination of different types of feeding learning strategies may act together, including trial and error and several social tactics to get new intake behaviours.

Classic theories explain that environmental stability has an effect over the way that animals use social contact to learn new behaviours. Social learning is more likely to be adaptive and favoured by selection when environments are relatively stable within and across generations (Boyd & Richerson 1988; Laland et al. 1996) When the rate of environmental change is slow, animals should be more attentive to socially acquired information and social learning strategies become optimal. However, when the environment change rapidly, and dependence on social learning may prevent effective tracking of environmental change, animals need to increase their environment exploration and social learning turns maladaptative because it reflects the past rather than the current conditions. (Laland & Kendal 2003). A mixture of social and individual learning is expected at intermediate rates of environmental change (Laland 1994). Recently, rat’s studies also proved that animals in moderately variable artificial environment increase their social interactions to get new feeding behaviour. Food choices of rats that were moved daily from one cage to another and fed at unpredictable times for unpredictable periods were less affected by demonstrators than were rats maintained in stable environments (Galef & Whiskin 2004).

Pig’s farms are stable environments, but conditions (allocation, mixing groups, or feed composition) may change drastically a number of times in pig’s life. It could be speculated that animals could be more susceptible to learn in a social way what to prefer during the stable environment periods (lactation around 28d, transition around 35d, and growing-fattening around 4-5 months). To obtain good results, social learning of feeding behaviour must be promoted after productive changes are established and familiarity nexus become strong. However establishing feed preferences by social interactions is hard to promote in traditional productive systems were all the animals have the same age and a similar experience with the feed. A planned animal management and handling may be a way to tackle this equal experience problem. Strategies such as weaning by groups or sections of animals would increase littermate interactions between experienced and inexperienced piglets (of the same or different ages). Alternatively, early interactions with a high number of conspecifics could be promoted by mixing litters during early lactation. This could also be a useful strategy to improve social learning of feeding behaviour

Conclusion

Interactions with a familiar conspecific that had a recent experience with a flavoured feed enhanced pigs' preference for that feed. Nevertheless, because of the intensive pig production system, where growing animals are not allowed to interact with younger conspecifics, feeding traditions (learning behaviours that pass between generations) probably disappear and management becomes a fundamental tool to create the conditions to allow this kind of learning to persist.

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Chapter 5

Protein ingredients acceptance conditioned by maternal transference and dietary flavours continuity in nursery pigs.

Abstract

Piglets tend to present neophobia to unfamiliar ingredients at weaning. Acceptance for a novel feed or flavor may be acquired as a result of the associative learning between the volatile compounds of maternal diet that arrive to the womb and mammary glands and the positive consequences of amniotic fluid and milk but also by the familiarity or repetitive exposure of those flavor cues. The aim of the present study was to study the importance of milky flavor continuity in the post-weaning period (Exp 1) and to explore the influence of pre and postnatal exposure to porcine digestive peptides (PDP; Palbio RD62®, Bioibérica S.A., Palafolls, Spain) via maternal diet on the productive performance of post-weaned piglets fed a diet containing the same PDP (Exp 2). A total of 240 male/female post-weaned pigs were used in each experiment. In the first experiment, animals were separated after weaning in 2 groups depending of the presence of lactose inside their diets. Lactose group ate a pre-starter (0-14d) and starter (15-33d) specification with $142g\cdot kg^{-1}$ and $49g\cdot kg^{-1}$ sweet milk whey respectively; control group ate an iso-caloric and iso-proteic diet with $20g\cdot kg^{-1}$ of PDP replacing dairy products. No differences were found during the transition period between the productive parameters of both groups. In experiment 2, half of animals came from mothers that during late gestation (14d) and lactation (28d) ate $20g\cdot kg^{-1}$ PDP in their diets. The other 120 piglets came from mothers fed an iso-caloric and iso-proteic diet without PDP inclusion in these periods. All animals received a common weanling diet containing $20g\cdot kg^{-1}$ of PDP in the pre-starter (0-14d) and starter (15-33d) specification during the transition period. Piglets coming from sow´s diets supplemented with PDP tend to showed higher ADFI during the 15-33d period (740 vs. 693g; $P = 0.07$). Similarly results were observed for ADG in piglets coming from PDP-treated sows during the 0-14d period (144 vs. 137g) and during the 15-33d period (502 vs. 471g; $P < 0.06$). These results suggest that dairy products that supposed to create continuity between the lactation and post weaning period, can be replaced by a high palatable and less cost protein without affecting productive performance of animals and that the pre and postnatal exposure to that protein (PDP) via maternal diet may influence piglet performance because of maternal learning in the presence of the same cues.

Introduction

Modern pigs are capable of a rapid growth, except after weaning when piglets have to eat a new solid feed, which is bulky, dry (usually >88%), less digestible than milk, and composed of ingredients and nutrients (starch and non-starch polysaccharides) that piglet has not previously encountered. For this reason most nutritionists formulate starter diets for weaned piglets with highly digestible ingredients, such as dairy and animal protein ingredients. The inclusion of dairy products and lactose as feed ingredients may smooth the sudden change in feed characteristics that at weaning occurs, and lactose addition to the diet has been described to improve growth performance until approximately 21 to 28 days after weaning, or 10 to 13kg (Pluske et al., 2007).

However, piglets as other mammals may show innate feeding preferences, ie. for Sweet and Umami tastes, and also early learned feeding behaviours which may be acquired before the animal is born (Campbell, 1976; Figueroa et al., 2011). At this respect, Solà-Oriol et al. (2011) showed that dairy products may be less preferred ingredients for nursery piglets than other selected protein sources from animal origin. It was remarkable the high preference showed by piglets to porcine digestible peptides (PDP; Palbio 62SP®;Bioibérica SA, Palafolls, Spain; n=13), a by-product of the heparin industry obtained from hydrolyzed porcine intestinal mucosa during the 4 days choice test that he performed.

Dietary flavour cues are known to reach the womb of some species where the foetus may associate flavour cues of the amniotic fluid with the hedonic properties of this liquid, showing a future preference for the flavour cue (Arias and Chotro, 2007; Nolte et al., 1992; Schaal et al., 2000). An adaptive transference in mammals may give to the offspring the ability to recognize and prefer cues in the extra-uterine life, which are now related with safe and nutritious components, becoming a learning strategy to reduce neophobic reaction against the new feed (Mennella and Beauchamp, 1999; Schaal et al., 2004; Hepper and Wells, 2006). Other authors have also shown that milk may also contain flavours cues coming from maternal diet (mainly from vegetal origin) (Désage et al., 1996; Hausner et al., 2008; Guiraudie-Capraz et al., 2005).

Thus, it can be hypothesized that innate feeding behaviour in piglets may be related with the ingredients composition in the sow diet, and may become a learning strategy to reduce the impact of an early weaning in piglets. Oostindjer et al. (2009, 2010) recently described that prenatal exposure to flavours like aniseed reduced stress responses after piglets weaning and increased feed intake, but no preferences changes were observed.

However, in contrast to pure flavours components (Campbell, 1976; Langendijk et al., 2007; Oostindjer et al., 2009, 2010), no information is available about the likely transference of main ingredients flavour cues from the sow diet to the amniotic fluid or milk, or their likely ability to modify feed acceptance for weaned piglets as compared to the dairy ingredients.

The aims of the present study were: 1. - to study if the incorporation of PDP, in a free of dairy products and lactose post-weaning diet may keep the productive performance of weaned piglets as compared to animals fed on diets containing significant amounts of whey and lactose; and 2. - to explore if a pre- and postnatal exposure to PDP via the sow diet may improve feed intake and productive performance of weaned piglets fed on a diet containing PDP.

Materials and methods

Two experiments were conducted at the animal research facilities of Universitat Autònoma de Barcelona (UAB) to evaluate the performance of animals fed on a free lactose diet containing PDP as an ingredients (Experiment 1),and to determine if piglets may acquire a protein-conditioned acceptance during the post-weaning period because of prenatal and postnatal exposure to this protein (PDP, Experiment 2). Experimental procedures were approved by Ethical Committee on Animal Experimentation of the UAB (CEAAH 1406).

Animals and procedure

A total of 240 male/females piglets ([Large White x Landrace] x Pietrain) were weaned in each experiment at an average of 28 days (7.9 ± 1.2 kg; Experiment 1 and 8.4 ± 1.10 kg; Experiment 2). Piglets had been individually identified by using a plastic ear tag just after birth and offered an unflavoured creep feed diet during lactation from day 10 after birth onwards in a commercial pan feeder. Animals were weaned and housed in a room with automatic, forced ventilation and a slatted floor. Each pen (3.2 m^2 in floor area) had a feeder with 3 feeding spaces and an independent water supply. Feed and water was administrated on ad-lib situation.

At weaning animals of Experiment 1 were distributed into four body weight blocks by their initial body weight (Block 1: 6.1 ± 0.58 kg; Block 2: 7.6 ± 0.36 kg; Block 3: 8.5 ± 0.24 kg and Block 4: 9.4 ± 0.25 kg). Within each block piglets were distributed in 6 pens of 10 animals for a balanced body weight distribution (3 pens for each experimental diet). Therefore, animals were randomly allocated into 24 pens and pens distributed into 2 groups depending on the experimental diet (with or without dairy products) offered after weaning.

Another 72 animals (3 weeks after weaning) were used to test the preference or the acceptance for both diets in a short term choice feeding tests. Twelve pen-mates pairs were tested to prefer in a 30 minutes choice test the diet containing dairy products (Lactose diet) or the diet containing PDP rather than dairy products (PDP diet). The other animals were also tested in pen-mates pairs (24) but in this case one unique diet was offered to each pair during 30 minutes to measure the acceptance of the feed (12 pairs with Lactose diet and 12 pairs with PDP diet).

In Experiment 2, 26 sows were selected on day 100 of gestation and split up into two experimental groups which did not differed ($P>0.10$) on their body weight, 265 vs 245 kg; back fat thickness measured in the P2 ,15.2 vs 13.6 mm; parity number, 2.9 vs 3.0; and body condition scoring, 2.8 vs 2.8. Sows were fed during late gestation and lactation with a diet with 20 $g\cdot kg^{-1}$ of the high preferred protein source; PDP or an iso-caloric and iso-proteic diet but without the PDP inclusion (n=13). At weaning, 240 weaned piglets were distributed by groups depending if they came from mothers fed with PDP during late gestation and lactation (n=120; PDP group) or with the control diet (n=120; Control group). To select the experimental animals, piglets of each group were weighted and equilibrated by weaning body weight into four blocks (Block 1: 6.9 ± 0.28 kg; Block 2: 7.9 ± 0.17 kg; Block 3: 8.8 ± 0.25 kg and Block 4: 9.9 ± 0.44 kg). All weaned piglets received ad-lib access to the same diet, an unflavoured common weanling diet containing 20 $g{\cdot}kg^{-1}$ of PDP in the pre-starter (0-14d**)** and starter (15-33d) specification during the transition period.

Feed intake and body weight were weekly measured in both experiments (0, 7, 14, 21, 28 and 33 days after weaning) in order to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed: gain ratio (FGR).

Diets and feeding

Experiment 1

Half of piglets used for the productive trial (120; 12 pens) were offered ad-lib access to an unflavoured common weanling diet without any source of dairy product and containing 20 $g{\cdot}kg^{-1}$ of PDP in the pre-starter (0-14d) and starter (15-33d) diets (PDP Group). The rest of the animals (120 piglets; 12 pens) received an isocaloric, isoproteic and iso AA diet with the inclusion of whey powder as a source of lactose (Lactose Group) Table 1. The same diets were used to perform the preference and acceptance tests.

Table 1. Ingredients (g·kg^{-1}) and calculated composition of the experimental pre-starter and starter pig's diet (as-fed basis). (Experiment 1)

Item	Pre-starter, d0-14		Starter, d15-33	
Ingredient, g·kg^{-1}	Lactose (+)	Lactose (-)	Lactose (+)	Lactose (-)
Maize	260.0	260.0	260	260
Wheat	182.1	300.0	280.8	300
Barley	100.0	100.8	100	139.8
Soybean Meal 44%CP	50.0	50.0	47.3	50
Extruded soya	194.1	191.5	190.6	153.2
Soybean oil	3.1	4.9	0	4.8
Sweet Milk Whey	142.2	0.0	49.8	0
Fish meal LT	25.0	25.0	25	25
Porcine plasma, 80%CP	15.0	15.0	15	15
PDP, 62%CP	0.0	20.0	0.0	20.0
L-Lys HCL	3.6	3.5	3.3	3.3
DL-Met	1.8	1.5	1.3	1.2
L-Thr	1.3	1.4	1.2	1.2
L-Trp	0.3	0.4	0.1	0.2
Calcium carbonate	6.7	7.9	9.7	10.5
Dicalcium phosphate	9.0	12.6	9.3	10.4
Vit-Min GPlus[2]	4.0	4.0	4	4
Sodium bicarbonate	1.5	1.5	1.5	1.5
Sodium chloride	---	---	1.1	---
Cost (€/Tm)	**439.06**	**362.82**	**373.74**	**351.57**
Calculated content, g/kg				
NE, MJ/kg of feed	10.34	10.34	10.26	10.26
CP	197.57	203.8	193.2	193.21
Lysine	14	14	13.1	13.1
Methionine	4.93	4.92	4.34	4.44
Methionine +Cystine	8.3	8.3	7.7	7.7
Threonine	9.1	9.1	8.5	8.5
Tryptophan	2.8	2.8	2.5	2.5
Valine	9.35	9.63	9.11	9.15
Ca	7	7	7.5	7.5
P Total	6.49	6.78	6.18	6.22
P.dig	4	4	3.6	3.6
Lactose	100	0	35	0

[1] Porcine Digestible Peptides, PDP; Palbio 62%PB (Bioibérica, Palafolls, Spain).

[2] Premix Supplied per kilogram of commercial diet: 7,000 IU of vitamin A (acetate); 500 IU of vitamin D_3 (cholecalciferol); 250 IU of vitamin D (25-hydroxicholecalciferol); 45 mg of vitamin E; 1 mg of vitamin K_3; 1.5 mg of vitamin B_1; 3.5 mg of vitamin B_2; 1.75 mg of vitamin B_6; 0.03 mg of vitamin B_{12}; 8.5 mg of D-pantothenic acid; 22.5 mg of niacin; 0.1 mg of biotin; 0.75 mg of folacin; 20 mg of Fe (chelate of amino acids); 2.5 mg of Cu (sulphate); 7.5 mg of Cu (chelate of glycine); 0.05 mg of Co (sulphate); 40 mg of Zn (oxide); 12.5 mg Zn (chelate of amino acids); 12.5 mg of Mn (oxide); 7.5 of Mn (chelate of glycine); 0.35 mg of I, 0.5 mg of Se (organic); 0.1 mg of Se (sodium).

Experiment 2

Two different cereal based diets were prepared for late gestation and the entire lactation of sows (15 d before farrowing and 28 d after farrowing; Table 2) to contain 9.46 MJ NE·kg^{-1}; 132 g·kg^{-1}of CP and 6 g·kg^{-1} of lysine for late gestation and 10.14 MJ NE·kg^{-1}; 166 g·kg^{-1}of CP and 8 g·kg^{-1} of lysine for the lactation period. Experimental diets contained or not PDP at 20 g·kg^{-1} (n=13); being both diets prepared with the same main ingredients but changing their inclusion in order to keep constant macronutrients and amino acids. Palbio SP62 (PDP) was included as a replacement of soybean meal 44% CP. No external flavours were added into these diets. Sows were fed two equal meals each day (8.00 am and 16.00pm). The total amount of feed offered was individually adjusted according the body condition scoring of each sow during late gestation and during lactation the fed was adjusted to a controlled ad-libitum situation.

After weaning, piglets were *ad libitum* offered an unflavoured pre-starter and a starter cereal based diets (Table 3) to contain 10.55 and 10.26 MJ NE·kg^{-1}; 190 and 185 g·kg^{-1}of CP and 14 and 14.8 g·kg^{-1} of lysine, respectively. Both diets included 20 g·kg^{-1} Palbio SP62 as a protein source**.** The pre-starter diet was offered just after weaning and until 14 d post-weaning. Starter diet was offered from 14 to 33 days post weaning. All of the diets were offered in mash form.

Statistical analysis

Average body weight, ADFI, ADG and FGR were analyzed with ANOVA by using the GLM procedure of the statistical package SAS® taking into account the experimental treatments (group) and body weight block as main effects. The mathematical model used was: $Y_i = \mu + \alpha_i + \beta + \varepsilon_{i,,}$ where Y_i is the body weight, ADFI, ADG or FGR value for the observations; μ is the general mean of all observations; α_i is the effect of the group (piglets fed on PDP or Lactose diets in Experiment 1 and piglets corresponding to the sows fed PDP or Control feed in Experiment 2), β is the effect of the block and ε is the unexplained random error.

Table 2. Ingredients (g·kg^{-1}) and calculated chemical composition of sow's experimental diets (as fed basis). (Experiment 2)

	Late Gestation		Lactation	
Item	Control	PDP	Control	PDP
Ingredient, g/kg of feed				
Maize	250	155.5	250	261.8
Wheat	100	160	200	200
Barley	220	300	170	170
Wheat Bran	180	180	75	75
Gluten feed	80	24.8	50	50
Sugar Beet Pulp	40	40	---	---
Canola Meal	50	50	30	30
Soybean Meal 44%CP	28	3	166	134.7
Soybean hulls	11	23.5	---	---
Palbio 62%PB[1]	---	20	---	20
Animal fat	10	12	26	25.6
L-Lys HCL	0.9	0.5	1.5	1.4
Calcium carbonate	16.3	16.5	13.7	13.7
Dicalcium phosphate	5.2	5.2	7.8	7.8
Vitamins and minerals[2]	4	4	4	4
Sodium chloride	4	4	3	3
Sodium bicarbonate	1	1	3	3
Calculated content, %				
NE, MJ/kg of feed	9.47	9.46	10.14	10.14
CP	132	133	166	165
Lysine	5.97	6.16	8.76	8.98
Methionine	2.24	2.4	2.6	2.79
Methionine +Cystine	4.92	4.98	5.69	5.76
Threonine	4.68	4.8	5.96	6.09
Tryptophan	1.5	1.5	1.95	1.84
Valine	6.23	6.36	7.7	7.76
Ca			7.73	7.65
P Total			5.94	5.96
P.dig			2.71	2.77

[1] Porcine Digestible Peptides, PDP; Palbio 62%PB (Bioibérica, Palafolls, Spain).

[2] Premix Supplied per kilogram of commercial diet: 9,000 IU of vitamin A; 2000 IU of vitamin D_3 (cholecalciferol); 8 mg of vitamin E (alfa-tocoferol); 1.5 mg of vitamin K_3; 4 mg of vitamin B_2; 1.5 mg of vitamin B_6; 0.015 mg of vitamin B_{12}; 10 mg of pantothenic calcium; 22 mg of niacin; 200 mg of Choline chloride; 100 mg of Fe (ferrous carbonate); 10 mg of Cu (sulphate); 0.5 mg of Co (sulphate); 100 mg of Zn (oxide); 80 mg of Mn (oxide); 0.22 mg of Se; 0.5 mg of I, 500 UI of 5-Phytase.

Table 3. Ingredients (g·kg^{-1}) and calculated composition of unique pre-starter and starter pig's diet (d0-d14 Post-weaning). (Experiment 2)

Item	**Pre-starter, d0-14**	**Starter, d15-33**
Ingredient, g/kg of feed		
Maize	189.7	217.7
Wheat	222.7	252.8
Barley	100	100
Wheat bran	---	40
Soybean Meal 44%CP	50	50
Extruded soya	150	114.8
Soybean oil	---	2.9
Sweet Milk Whey	137.1	100
Whet powder	60	40
Fish meal LT	31.4	31.8
PDP, 62%CP	20	20
L-Lys HCL	4.1	6.2
DL-Met	2	1.5
L-Thr	1.7	1.5
L-Trp	0.7	0.5
Calcium carbonate	6.6	10.7
Dicalcium phosphate	17	5.6
Sodium chloride	3	---
Vitamins and minerals[2]	4	4
Calculated content, %		
NE, MJ/kg of feed	10.55	10.26
CP	190.13	185
Lysine	14	14.83
Methionine	5.27	4.73
Methionine +Cystine	8.3	7.7
Threonine	9.1	8.5
Tryptophan	2.8	2.5
Valine	5.31	5.56
Ca	8.3	7.5
P Total	8.5	6.04
P.dig	5.71	3.6

[1] Porcine Digestible Peptides, PDP; Palbio 62%PB (Bioibérica, Palafolls, Spain).

[2] Premix Supplied per kilogram of commercial diet: 7,000 IU of vitamin A (acetate); 500 IU of vitamin D_3 (cholecalciferol); 250 IU of vitamin D (25-hydroxicholecalciferol); 45 mg of vitamin E; 1 mg of vitamin K_3; 1.5 mg of vitamin B_1; 3.5 mg of vitamin B_2; 1.75 mg of vitamin B_6; 0.03 mg of vitamin B_{12}; 8.5 mg of D-pantothenic acid; 22.5 mg of niacin; 0.1 mg of biotin; 0.75 mg of folacin; 20 mg of Fe (chelate of amino acids); 2.5 mg of Cu (sulphate); 7.5 mg of Cu (chelate of glycine); 0.05 mg of Co (sulphate); 40 mg of Zn (oxide); 12.5 mg Zn (chelate of amino acids); 12.5 mg of Mn (oxide); 7.5 of Mn (chelate of glycine); 0.35 mg of I, 0.5 mg of Se (organic); 0.1 mg of Se (sodium).

Consumption of piglets pairs during the 30 minutes choice and acceptance tests were analysed with ANOVA using the MIXED procedure of the statistical package SAS® with the diet consumed (Lactose or PDP diet) as the main factor. For the choice test the experimental unit (pair) was also included as a repeated measure specifying the covariance structure of the residual matrix as completely general (unstructured). The mean values are presented as LSMeans adjusted by Tukey and considering a significance level of 5%.

Results

Experiment 1 (Weaning without dairy ingredients)

The results of the preference and acceptance (one feeder) test are showed in Figure 1. Piglets preferred the lactose (142 g·kg^{-1} of whey) over the PDP (20 g·kg^{-1}) diet after the 30 minutes choice test (211g vs. 77g; P = 0.039; SEM 56g). In the same way, piglets on the one feeder test showed a higher intake when they have the opportunity to eat the lactose than the PDP diet (287g vs. 192g; P = 0.001; SEM 22g).

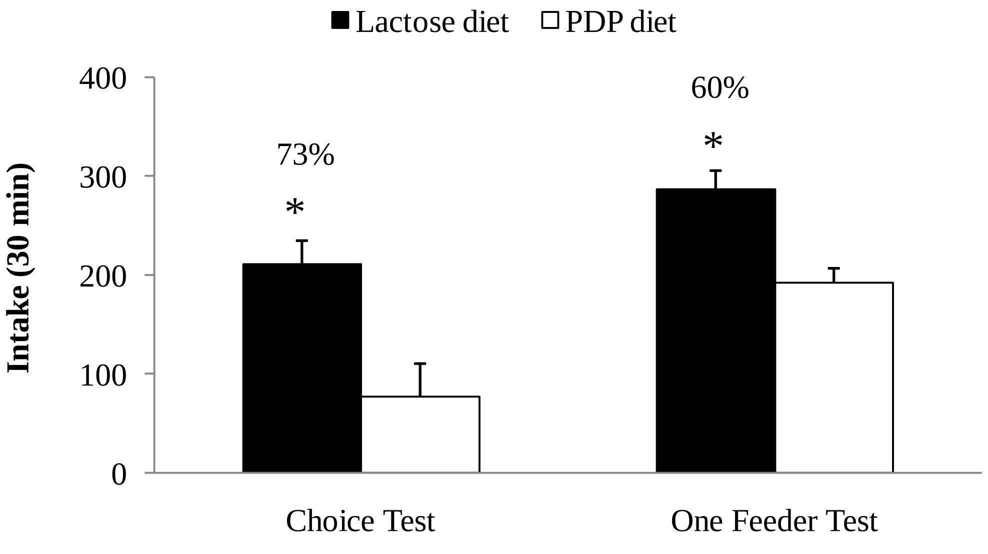

Figure 1: Means (+SEM) feed intakes in a 30 min. choice test or one feeder test of a diet containing lactose (142 g·kg^{-1} of whey) and a diet containing porcine digestive peptides (20 g·kg^{-1} PDP) in post-weaning (49d-old) naive pigs. Numbers in the top of bars indicate the average percent preference for the lactose diet. Asterisks indicate that lactose diet intake is significantly different than PDP diet intake (*P<0.05).

In Table 4 are presented the productive performance of piglets fed on the PDP and lactose diet during the nursery period. No significant differences were observed between experimental treatments. Although it is not shown in the results, during the first week of nursery there were overall differences in the ADG of piglets between block nº 4 or heavier pigs and block nº 1 or small ones (-33g vs. 22g/d; $P < 0.042$; SEM 13.25g) respectively, probably because of the less experience of heaviest piglets with creep feed during lactation. Nevertheless, these differences disappeared during the second week (7-14d PW) when heaviest animals showed a higher feed intake than the smaller pigs (344g vs. 294g/d; $P = 0.05$, SEM 12.39g). There was also a block effect ($F_{3,16} = 5.10$, $P = 0.012$) on AGD and Feed:gain ($F_{3,16} = 3.62$, $P = 0.036$) during the second period when animals changed to a starter diet (14-33d). Animals also presented an effect of the interaction of the blocks x treatments over the ADG ($F_{3,16} = 4.11$, $P = 0.024$) and Feed:gain ($F_{3,16} = 20.26$, $P < 0.001$) during this second period (14-33d) where animals of block 3 (the second in starter weight) presented higher intakes than animals of blocks 2 (1016g vs 866g; $P = 0.032$; SEM 34g) and block 1 (1016g vs 861g; $P = 0.026$; SEM 34g) and tend to present higher feed:gain ratios than animals of block 2 (1.85 vs 1.72; $P = 0.055$; SEM 0.03) and block 1 (1.85 vs 1.73; $P = 0.068$; SEM 0.03).

Experiment 2 (Maternal flavour continuity)

In Table 5 are shown the productive parameters observed during the nursery period. Piglets coming from sow´s supplemented with 20 $g \cdot kg^{-1}$ of PDP in their diets during late gestation and lactation tended to show higher ADFI during the starter phase (15-33d post-weaning) than piglets coming from control sows (740 vs. 693g; $P = 0.07$) when both groups were exposed to 20 $g \cdot kg^{-1}$ of PDP. Similar results were observed for ADG in piglets coming from PDP treated sows during the same period (502 vs. 471g; $P = 0.06$). No differences were observed for FGR along the experimental period. Therefore the final body weight at the end of the starter phase was higher for those animals coming from mothers fed PDP supplemented diets (20.1 vs. 19.3kg; $P = 0.14$). However, the differences were not statistically significant. No interactions were observed between treatments and blocks over in none of the productive parameters.

Table 4. Effect of the lactose content (incorporation of dairy products) on growth performance of piglets from day 0 to day 33 post-weaning.

	Treatment			*P*-Value		
	[1]Lactose (+)	[2]Lactose (-)	SEM	Lactose	Block	Lactose x Block
BW, kg						
d0	7.906	7.910	0.0057	0.637	<.0001	0.648
d14	9.779	9.703	0.1395	0.705	<.0001	0.648
d33	20.919	21.132	0.3033	0.626	<.0001	0.775
ADFI, kg/d						
d0-14	0.214	0.209	0.0070	0.603	0.126	0.418
d14-33	0.618	0.631	0.015.1	0.467	0.012	0.024
ADG kg/d						
d0-14	0.134	0.128	0.0099	0.692	0.595	0.666
d14-33	0.518	0.531	0.0135	0.517	0.165	0.607
Feed:Gain						
d0-14	1.66	1.68	0.08	0.870	0.223	0.796
d14-33	1.77	1.77	0.02	0.937	0.036	<.0001

[‡] From ANOVA analysis including the effects of lactose inclusion (Lactose (+) or (-)), Block (4 Body weight groups/treatment) and the interaction between Lactose inclusion x Block. Treatment n=12. The mean values are presented as LSMeans adjusted by Tukey and considering a significance level of 5%.

[1] Post-weaning unflavoured diet including whey powder as a source of lactose to meet the requirements of lactose recommended by the NRC for weanling piglets (Lactose Group).

[2] Post-weaning isocaloric, isoproteic and iso AA unflavoured diet without any source of dairy product and containing 20 $g \cdot kg^{-1}$ of Porcine Digestive Peptides (PDP) in the pre-starter (0-14d) and starter (15-33d) periods (PDP Group).

Table 5. Effect of the incorporation of Porcine Digestive Peptides (20 g·kg^{-1} PDP) into sow's diet during late gestation and lactation (PDP group) on growth performance of piglets from day 0 to 33 post-weaning.

	Treatment			*P*-Value		
	[1]Control	[2]PDP	SEM	Treatment	Block	Treatment x Block
BW, kg						
d0	8.442	8.437	0.0057	0.53	<.0001	0.701
d14	10.395	10.516	0.1645	0.61	<.0001	0.361
d33	19.347	20.063	0.3260	0.14	<.0001	0.166
ADFI, kg/d						
d0-14	0.229	0.235	0.0123	0.73	0.984	0.856
d14-33	0.693	0.740	0.0175	0.07	0.131	0.147
ADG kg/d						
d0-14	0.137	0.144	0.117	0.67	0.895	0.312
d14-33	0.471	0.502	0.110	0.06	0.213	0.139
Feed:Gain						
d0-14	1.62	1.65	0.57	0.73	0.629	0.254
d14-33	1.47	1.47	0.014	0.96	0.224	0.105

‡From ANOVA analysis including the effects of Maternal gestational diet (Control or PDP) Block (4 Body weight groups/treatment) and the interaction between Maternal gestational diet x Block. Treatment n=12. The mean values are presented as LSMeans adjusted by Tukey and considering a significance level of 5%.

[1] Piglets coming from sows that eats during the late gestation an unflavoured control diet (Control Group).

[2] Piglets coming from sows that eats during the late gestation an unflavoured diet with 20 g·kg^{-1} Palbio SP62 (PDP) included as a replacement of soybean meal 440 g·kg^{-1} CP (PDP Group).

*Piglets of both treatments were offered *ad libitum* access to an unflavoured pre-starter and a starter cereal based diets. Both diets included 20 g·kg^{-1} of Palbio SP62 (PDP) as a protein source**.**

Discussion

We confirmed in Experiment 1 that the inclusion of a high hedonic animal protein source (PDP) promoted a similar productive performance of weaned piglets as compared to those animals fed on diets containing significant amounts of whey and lactose in the pre-starter and starter diet. It is generally accepted that including dried whey or dairy ingredients in a starter diet improves performance of pigs weaned at 3 to 4 wk of age (Graham et al., 1981). However, there is still controversy about which is the reason(s) why early weaned pigs respond to the inclusion of dairy ingredients in the diet. Some authors found that a diet containing dried whey or skimmed milk may be higher in DM and energy digestibility than a cereal-vegetable protein diet (Tokach et al., 1989). Giesting et al. (1985) or Tokach et al. (1989) indicated that protein (lactoalbumin and casein) fractions of dried whey and skim milk are important, but its carbohydrate fraction (lactose) is responsible for most of the response in growth rate and feed intake (Mahan, 1992). More recently O´Doherty et al. (2005) have described that high lactose inclusion (300 g/kg) in starter diets increased daily gain, improved feed efficiency and eliminated the necessity for in-feed antibiotics.

Lactose is among the principal sugars that escape digestion in the small intestine and a large proportion may be fermented to lactic acid (Pierce et al., 2006). Different authors have recently indicated that increasing concentration of lactose or dietary vegetable fiber (Hermes et al., 2009) in the weanling diets may increase the piglet performance in high protein diets, but not in low protein diets, associated to increases in the lactobacilli and Bidifidobacterium concentration in feces, and significant decreases in enterobacteria counts in feces. Thus, recent evidence suggest that much of the nutritional value of whey resides in its carbohydrate fraction (Mahan, 1992), suggesting that if another protein source along with a highly digestible carbohydrate is provided, the replacement of milk products may be possible. In our study, the sweet milk whey (142 $g \cdot kg^{-1}$) was replaced by wheat (118 $g \cdot kg^{-1}$) and PDP (20 $g \cdot kg^{-1}$) and no lactose was contained in this diet. Our results could reflect that non-starch polysaccharides contained in the wheat and the rest of vegetable ingredients could have promoted similar responses to those suggested for lactose.

Mahan (1993) also suggested that the pig growth responses to dried whey in comparison to a corn-soybean meal diet without lactose depend on the quality of the dried whey. When, edible-grade dried whey was fed to weaned pigs, improved growth rate were achieved, whereas a feed-grade or low quality source of dried whey did not improved pig gains.

Most nutritionists include also dairy products and lactose in the weanling piglet's diets to smooth the sudden change in feed characteristics that at weaning occurs, letting in some way a progressive change from sow´s milk to a dry diet. Also milky flavours have been used several times to increase the acceptance of new diets because of the previous association of the milk smell with the positive post-ingestive and hedonic characteristics of milk ingestion during the suckling period. However, other authors have suggested that palatability, rather than novelty, may be responsible for greater consumption of pre-starter and starter diets (Ermer et al., 1994). Nevertheless, this is not totally true because even high palatable diets are not preferred at first time because of neophobia (Figueroa et al., 2010) and linked adaptive cues are needed in each transition feed stage.

At this respect, we observed that, naive animals showed a preference for the diet including a 142 $g \cdot kg^{-1}$ of dry whey to the PDP diet (20 $g \cdot kg^{-1}$ PDP) in short term choice feeding studies (30 min), in a similar way than previous studies where new components as PDP tended to present neophobia to pigs (Figueroa et al., 2010, 2012). When we gave animals the option to consume only one of these diets during 30 minutes (one feeder test), pigs showed also a higher acceptance for the feed that included whey. Both components (2% PDP plus wheat diet vs 14% whey diet) may differ in their familiarity in post-weaning piglets. Even if it is derived from cow milk, whey may create a generalization of the milky flavour creating a link between the pre and post-weaning periods.

However, these results are in contrast to those described previously by Solà-Oriol et al. 2011 when fifteen protein sources were evaluated at three different levels (50, 100 and 200 g/kg) in comparison to a common reference diet containing soybean meal protein concentrate (56% CP) in 4 days choice feeding test. Feeds with fish meal and dried porcine hydrolyzed protein at 50 $g \cdot kg^{-1}$ were the most preferred (72 and 76 % of total feed intake, $P < 0.05$) compared to the reference feed. These results were relevant if we compare them to dairy products that did not show differences or even reduced preference when included at 100 or 200 $g·kg^{-1}$. It is remarkable to explain that those trials performed choice tests of 4 days as compared to our 30 min test, this cause than other factors rather than a possible palatability or innate preferences are affecting the final results. In that experiment also PDP was included at higher levels (50-200 g/kg) than in our choice test where a practical comparison was performed between diet containing 20 $g·kg^{-1}$ of PDP vs 142 $g·kg^{-1}$ of whey.

However, clear differences on feed preference were not enough to promote differences between PDP and lactose groups in productive parameters when only one diet was provided during the pre-starter and starter period. These results may indicate that other factors rather than an early acceptance or preference differences between both ingredients affects the productive performance during the nursery period. It has been described that a PDP flavoured solutions at 20 $g·kg^{-1}$ is equally consumed than flavoured water when it is offered to piglets at a first time (Figueroa et al., 2012). However, when animals got experience with the PDP they shows higher acceptance for the protein solution over water. The reduction of neophobia and also a possible positive post-ingestive effect that could increase progressively the acceptance of a new ingredient if it promotes a valuable postingestive effect (Sclafani, 2004). It is describe also than the positive effects of a previous association, in this case the postingestive value of PDP, could increase the future palatability of the compound (Dwyer et al., 2008, 2009).

In Experiment 2 we evaluated if a pre- and postnatal exposure to PDP via the sow diet may improve feed intake and productive performance of weaned piglets fed on a diet containing PDP. Piglets may present difficulties to initiate dry feed intake if they are abrupt weaned and learning processes of the new environmental cues are not allowed (Bruininx et al., 2001). Our results (Trial 2) showed that piglets tended to increase consumption of feed containing PDP when this ingredient was included previously in the sow diet. Curiously, the response was observed from day 14 to 33 and not the first two weeks after weaning, which could indicate that stressful factors as new pen-mates, transportation, or new environment may have decreased feed intake of both groups of animals, independently of prenatal exposure.

Flavour continuity is described in several species (Nolte et al., 1992; Mennella et al., 1995; Schaal et al., 2000; Nicolaïdis, 2008) and may reduce neophobia when animals find solid feed with the same o similar cues that they experience before. This neophobia inhibition, through maternal diet, may due to a familiar learning effect, in which animals could face a certain stimulus with repeated prenatal (amniotic fluid) or postnatal (milk) experience, but also could be due to an associative learning effect during pregnancy or lactation, in which the amniotic fluid and comfort of the womb as well as the hedonic and post-ingestive characteristics of milk could be associated with one specific sensory stimulus creating preferences or even acceptance for the associated cue (Myers and Sclafani, 2006).

During gestation and lactation sows as other mammal’s mothers teach in a passive way to their offspring the future volatile cues that they are going to find in their environment. However, when no-natural conditions are placed to increase animal’s performance in intensive systems, this flavour continuity not always occurs. Diets components of gestational and lactation sows feeds may present different ingredients as compared to the first piglets solid diets. Differences diets design pursues to meet animal requirements and productive performance in each stage. However, this strategy may break flavour continuity and increase an already problematic weaning period.

Conclusion

The results of the present study suggest that pre and postnatal exposure to feed ingredients via maternal diet may influence piglet performance in the presence of the same cues probably due to the positive association of the uterine or milk experience with these feed components. Our results and previous studies, suggest that a planned learning strategy may be used to reinforce feed intake of weanling piglets by the reduction of stress (Oostindjer et al., 2009) or by the change in animals acceptance (trial 2). The use of dairy products appears to be not necessary if a high palatable protein source is offered during nursery

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General Discussion

Mammals in their natural environment or even in intensive production systems have to be able to satisfy their nutritive requirements through food intake. For this objective, most of species innately recognize and prefer foods with a high amount of energy (Sweet taste; Pérez et al. 1995), protein (umami taste; Rolls 2009) or even electrolytes (salty taste; Wald & Leshem 2003). Other tastes or flavours that an animal discover inside the environment are identified as a challenge and uncertainty about its possible consequences and intake tend to be reduced or stopped. In chapter 1, for example, it was described that a simple creep feed diet (without added flavours compounds) was preferred (63%) over a commercial creep feed flavoured diet (synthetic flavours). Also the results observed during the training periods of conditioning trials of chapter 2 were similar; Protein flavoured solutions of porcine digestible peptides (PDP) and soybean meal concentrate (SPC) were consumed in similar amounts than a flavoured solution (experiment 1) or tap water (experiment 2) during the first sessions despite their hedonic and post-ingestive characteristics. However, once the animals learn the positive (hedonic or post-ingestive) or negative (unpalatable or toxic) consequences of the intake of unknown feeds, their nervous system will create an associative learning between the cue and the consequence of the intake. This situation will facilitate the future searching and recognition of beneficial feeds, making it more efficient and adaptive.

Therefore, learning is a fundamental mechanism that a mammal has to acquire effective feeding behaviours to find and consume necessary nutrients from the environment. It has been demonstrated that pigs have several learning abilities while flavours cues seems to be the more effective to create associative learning (Gieling et al. 2011). In this way, pigs as other species, may learn what kind of feed to eat through the maternal learning of feed cues before or after birth (Mennella et al. 2001; Chapter 1 and 5), through their personal intake experience or trial and error learning (Dwyer et al. 2009; Myers & Sclafani 2006; Chapter 2 and 3) and finally because of the social learning of the feeding behaviour that they can acquire with the direct or indirect contact with conspecifics (Galef & Whiskin 2004; Chapter 4).

Feeding behaviour learning becomes more necessary around the weaning period where animals have to feed by themselves. Without human intervention, piglets become independent animals in terms of feed intake during a progressive and long weaning (10-17 weeks). During this lactation time and with their mothers, littermates and conspecifics company, piglets learn new feeding behaviours. However, piglets in the intensive pig industry are restricted to express their learning abilities because of the conditions imposed. Piglets are weaned at an early age (21-28 days) decreasing the opportunities to learn patterns of feeding behaviour (Oostindjer et al. 2011). One possible solution to this short lactation period is the incorporation of solid feed during the suckling days (creep feed), but piglets tend to show an irregular intake (Bruininx et al. 2002). As a consequence, piglets shows neophobic reactions to the first pre-starter diets after the weaning despite of the industry efforts to increase feed palatability and nutritive value by the incorporation of highly digestible and palatable ingredients like dairy products. This neophobia joins other weaning stressors as environment changes, transportation, and mixing with unknown animals. The situation generates a decrease in the feed intake and anorexia that create health and productive difficulties (Pluske et al. 2007). In the present study, we hypothesised that neophobic reactions could be reduced if piglets could have the opportunity to develop their cognitive abilities during an early stage by linking the new flavours or ingredient cues with positive consequences (Ackroff & Sclafani 2011; Hepper & Wells 2006; Mennella & Beauchamp 2009; Oostindjer et al. 2009).

We evaluated in our experiments different learning strategies of the feeding behaviour in pigs that could change or modify flavours preferences or acceptance. Associative learning of one neutral flavour with the positive consequences of amniotic fluid (Chapter 1), milk (Chapter 5), proteins (Chapters 2 and 3), sucrose (Chapter 3) and even conspecific contact (Chapter 4), created in most cases long-lasting preferences for a that flavour cue. This could be explained because of the adaptive value of all this interactions.

The adaptive value of the maternal learning of feeding behaviour is that mothers may transfer to their foetuses valuable information of the environmental cues. This knowledge will allow newborn to recognize and prefer nutritive feed stuffs during their first solid meals that without this learning could create neophobia (Miller & Holzman 1981). In the same way trial and error learning shows an adaptive value because stronger associative learning is produced with the highest nutritive and hedonic feeds. Finally the social learning of the feeding behaviour may be especially valuable where the environment remains stable, enabling animals to learn adaptive feeding behaviours while spending less energy than acquiring those behaviours by trial and error learning.

Maternal learning of feeding behaviour

In the first chapter we observed that piglets learned to prefer flavour cues because of a prenatal experience with those cues. It has been shown that prenatal learning may be established by a prenatal familiarity with the new flavour or an associative learning of the flavour with the positive value given by the opioid system, which it is activated by the amniotic fluid especially during the last period of gestation (Arias & Chotro 2007). This learning may help newborn animals to know what kind of safety and nutritive feed are available in the external environment and probably explain the long-lasting preferences observed even after weaning in our experiment. There is also some evidence to indicate that the amniotic fluid itself induces unconditioned responses of appetence and orientation (Marlier et al. 1998; Schaal et al. 1995, 1998; Varendi et al. 1996). Some flavours from the mother's diet are described to reach the amniotic fluid, being swallowed by the foetus, and have in contact with the olfactogustatory apparatus that is functional in foetuses (Nicolaïdis 2008). It seems to be also a sensitive period (during late gestation) in which the exposure to certain flavours produce enduring preferences that ensure the acquisition of important information about which foods are safe, appropriate, and nutritious (Mennella & Beauchamp 1998). In experiment 2 of chapter 1 we used aniseed and vanilla flavours as the specific cues during the late gestation of sows (2 weeks before farrowing) to study prenatal learning in pigs. These animals were able to prefer those flavours during lactation and after weaning (2d). However, when it was studied if prenatal contact with flavours enhanced the feed intake of flavoured diets (experiment 3 of chapter 1) animals did not show the same behaviour. Feeds, rather than volatile cues impregnated in strips (experiment 2 chapter 1), are a complex matrix of different ingredients with their own characteristics and flavours. Main ingredients in the simple diet (corn, barley, wheat or soybean protein concentrate) were all contained in the sows diet and probably piglets recognized those cues in the same way that the recognized the other artificial flavours, affecting in this way their further preferences.

With the same pre-natal strategy and also with a post-natal learning through the milk, piglets were able to recognize and increase their acceptance for porcine digestive peptides (PDP) diet during the nursing period (chapter 5). Animals that came from sows fed PDP during gestation and lactation tended to show higher ADFI and ADG during the nursery period (15-33d) than piglets that came from sows fed the control diet (without PDP).

Like amniotic fluid, milk has positive consequences (hedonic and post-ingestive) to act as Unconditioned stimuli (US) during associative learning with feed cues. This could reinforce the preference of milky flavours. In fact, feed flavours that arrive to amniotic fluid from maternal diet during gestation are similar to flavours that may arrive to milk during lactation and this could help flavour continuity. Maternal transferences of flavour cues can generate subsequent preferences for these stimuli in several mammals' species (Hepper & Waldman 1992; Mennella et al. 2001; Nicolaïdis 2008; Simitzis et al. 2008; Schaal et al. 2000). In humans for example, neonates born from anise-consuming mothers displayed a persistent preference for anise odor, whereas those born from non–anise-consuming mothers showed aversion or neutral responses in front of anise (Schaal et al. 1995). In pigs, Oostindjer et al. (2009) described effects of prenatal flavour exposure over stress and intake after weaning, but only a few pigs studies have been proved that preferences can be modified by a prenatal experience (Campbell 1976). The adaptive value of this kind of learning could facilitate weaning process in pigs if continuity between maternal diet and the first solid diets of piglets is established. However, this condition is usually not taken into account by the swine industry.

Volatile compounds of nutritive (Chapter 5) and no nutritive (Chapter 1) ingredients may be transferred from maternal diets to amniotic fluid and milk but there is no evidence that maternal behaviour do not change during the gestation to increase diet variability allowing to their offspring to get experience with a higher number of cues. In this way, flavour learning of the feeding behaviours before birth could not be a passive way to get experience with external flavour cues but a way of maternal teaching where mothers may change their behaviour to increase the benefit of their offspring. However, new research is needed to prove this hypothesis.

By taking advantage of their mother's experience, offspring gain a larger spectrum of food and gives them a better chance of survival in nature. Maternal gestational and lactation diets may affect the hedonic choices of their offspring and may help to explain the variety of feed preferences among animals and the transmission of taste preferences from one generation to the next, which provides the basis for cultural differences on feed preferences (Nicolaïdis 2008).

Trial and error learning of feeding behaviour

Pigs have cognitive abilities to learn by trial and error the consequences of a new food (Gieling et al. 2011). However, during the last decades, pigs have been used only in a few classical conditioning trials to study their feeding behaviour (Clouard et al. 2012). No references were neither available about the use of protein sources as reinforces for flavour preferences for pigs. In other species like rats innately preferred nutrients as sucrose and proteins may facilitate the intake of neutral flavours by an associative learning with their hedonic or post-ingestive effects (Ackroff & Sclafani 2011). Pairing also novel flavours with recovery from illness (Green & Garcia 1971), fats (Lucas & Sclafani 1999), social interactions (Galef & Whiskin 2001), maternal fluids (Arias & Chotro 2007) or palatable tastes as saccharin (Holman 1975) may increase the preference or acceptance of new flavours in rats.

In the second and third chapters it was observed that piglets can be conditioned to prefer a neutral flavour (CS+) because of the previous association of this cue with the positive consequences (US) of proteins (chapter 2 and 3) and carbohydrates (chapter 3). The hedonic value of these nutrients and their post-ingestive effects may change preferences during the nursery period. The acquisition of feeding behaviours through trial and error learning is essential to establish intake patterns. As it was observed in our experiments, conditioned preferences can be remarkably persistent even when the positive consequences on which the preference was originally based are no longer present (Capaldi et al. 1983; Harris et al. 2004; Myers & Sclafani 2006) which suggests that other processes rather than classic conditioning might be involved (Dwyer 2005). By trial and error learning, pigs were also able to add the preferences of a CS+ with the innate preferences of a protein (US). In this way CS+/US solutions were preferred over

US solutions during the nursery period (Experiment 2 of Chapter 2). As previously reported with sweet USs, flavours paired with protein solutions may acquire a “protein” taste quality (Sclafani 2002; Stevenson et al. 2000; Warwick & Weingarten 1996). In this way, flavour of the CS+ may have enhanced the protein taste quality of the PDP (US).

Changes on the palatability of the flavours conditioned by the positive effects of nutrients are also described in literature (Dwyer et al. 2009; Myers & Sclafani 2001b). Harris et al. (2004) suggested that consuming a CS with sucrose served to pair the CS with the hedonic reaction elicited by the sucrose, which, in turn, resulted in the CS’s having the same hedonic properties than sucrose. In experiment 2 of chapter 2, we attempted to measure these palatability changes by measuring the first contacts with solutions (pigs/pen/15 sec.). The first contact observed in the conditioned group shows results in the same direction than other palatability measures like taste reactivity test or licks clusters size studies where animals change their hedonic perception especially during the first tests, having a short extinction time. In this way, palatability changes do not explain the long lasting preferences of flavour conditioned trials (Dwyer et al. 2009).

The reward quality of food and nutrients is determined by its orosensory properties and its post-oral nutritive consequences (Sclafani 1997). Both kinds of rewards normally act together during the intake (Warwick & Weingarten 1994; Zellner et al. 1983). Post-ingestive and hedonic effects of food over flavour conditioned preferences play different roles and can be dissociated by different techniques (Warwick & Weingarten 1994). By matching the hedonic value of two protein or carbohydrate solutions that differed in their post-ingestive effects (Chapter 3) we observed that the post-ingestive effects of PDP (4%) and sucrose (4%) were strong enough by themselves to create flavour conditioned preferences in pigs.

Mammals can perceive food as pleasant or unpleasant stimuli according to their current physiological state (Cabanac 1971). High growing potential animals like pigs may detect feeds ingredients like proteins or carbohydrates as powerful stimulus to create flavors preferences. Several brain structures are implicated in post-ingestive carbohydrate conditioning but the specific brain mechanisms that mediate protein conditioning have yet to be investigated. The ability of flavour-nutrient learning to create persistent preferences could decrease post-weaning neophobia. However, due to the abrupt changes imposed by early weaning, it seems more useful if the conditioned preferences are established during the pre-weaning period

Social learning of feeding behaviour

Social learning in pigs and other species may influence the future feeding behaviour of a naive animal (Galef 1995; Gieling et al. 2011; Held et al. 2000; Hopper et al. 2011; Laland 2004). Observer animals may copy a new feeding pattern that may or may not be adaptive by the observation of the feeding place of conspecifics (Held et al. 2000; Nicol & Pope 1994), when an inexperienced piglet is housed with an experienced conspecific (Morgan et al. 2001) or even after brief interactions where olfactory cues are transmitted (Galef & Whiskin 1997, 2003). It was observed in chapter 4 that the direct contact (30 min) with familiar conspecifics that previously consumed a flavoured feed (demonstrators) modified naive observers piglet´s preferences. Piglets exposed to social interactions preferred the flavoured feed that the conspecific ate before.

As it was described previously in rats, social learning may act primarily to introduce a new feeding behaviour into an individual’s repertoire; while the experience of their consequences determine the persistence of that behaviour (Galef & Whiskin 2001). In this way, piglets that socially learn new feeding behaviours, may present a social facilitation to the flavour exposed, which it is not as resistant as associative learning with nutrients. However, they need also to learn by trial and error that the flavour is safe and for this reason it is not as resistant as associative learning with nutrients because the experience could easily change the direction of the preferences. Opportunities to learn a-socially to eat a food other than that preferred reduced the stability of a food preference especially if the previous learned preference is not reinforced anymore or is less rewarded (maladaptive preference) than the other foods (Galef & Whiskin 2004). Nevertheless, social interactions in pigs could help non-experienced animals to learn a new feeding behaviour without spending time and energy exploring the environment by trial and error learning inside productive facilities.

The maintenance and propagation of “traditions” of food preferences depend on several factors besides the conspecific contact taking place within complex environmental and social situations. Factors like feeding time, population replacement rate, individual learning and more rewarded alternative feeds may affect directly the success of a new feeding behaviour learned socially. The familiarity of the model animal or demonstrator conspecific could be another important factor that could affect the transmission of a new feeding behaviour. We observed in experiment 1 of chapter 4 that pigs learned the flavour from familiar conspecifics demonstrators (same transition pen) but not from unfamiliar demonstrators. Interaction with familiar but not with unfamiliar (next-door pen) pig demonstrators was sufficient to induce a preference for the feed that demonstrators had previously eaten. Piglets also recognized and learned from lactation litter-mates after 3 weeks of the weaning and then of physical separation (experiment 2 of chapter 4).

Familiar subjects are more likely to be genetically related and also to share a common environment, because of that, also feeding behaviours are common and adaptive to observer’s fitness (Laland 2004). In contrast to pigs, in other species like rats, familiarity with the demonstrator is not important to show a flavour preference after the social interaction (Galef & Whiskin 2008; Saggerson 2006) probably because rats extend more oro-nasal recognition contact with an unfamiliar animal and “attentional modulation” that made observer put less attention to a familiar conspecific. Nevertheless, the effect of the demonstrator familiarity over feeding behaviour in pigs may be explained also by the negative interactions between unfamiliar conspecifics, and selective exploration that animals perform to the unknown cues when they interact with a conspecific.

Social interactions could also override neophobic reactions, unlearned aversions or change previous preferences for a known or highly preferred feed (Galef 1986; Nicol 2004). In experiment 3 of chapter 4 it was observed that social interactions in pigs enhanced the preference for demonstrators consumed feed over their known diet. The flavoured feed, which in most cases causes neophobic reactions during the first meal, was preferred over an unflavoured and also known diet. In this way, pigs of experiment 3 probably preferred the new diet learned by social interactions over their normal transition diet due to the adaptive mechanisms to increase variability in the diet (Galef

& Whiskin 2000). Social interactions could help pigs to override neophobia towards a particular ingredient or flavour after interacting with a conspecific that has previously eaten these components.

A new feeding behaviour transmitted by social interactions may persist in animal's populations as a feeding "tradition". With optimal conditions, it has been observed feed preference tradition in laboratory colonies of Norway rats can survive for several generations (Galef & Allen 1995). However, "traditions" studies in laboratory animals have been successfully replicated only few times under natural conditions (Galef 2010; Galef &Allen 1995; Müller & Cant 2010). Pig's productive environments where there is not contact with older or more experienced conspecifics, create difficult conditions for the transmission of feeding behaviours through generations. Even inside the same productive period. Nevertheless, social learning may be a valuable strategy to reduce neophobia to new diets if some management is included to improve the cultural transmission and introduce new behaviours into an individual's repertoire. Strategies such as weaning by groups of animals or to increase early interactions with conspecifics would increase interactions between experienced and unexperienced piglets.

For omnivores, such as pigs, it is important that there not be too many genetically determined restrictions on what constitutes an acceptable food. The three ways of feeding behaviour learning observed in this thesis demonstrate that pigs have several cognition abilities to acquire new patterns of alimentary behaviours. Flavour preferences learned through maternal transference, associative learning with nutrients and social contact could be maintained during a long time inside pig's population, increasing their options to fit against new environment feed cues. These three ways of learning could act also together increasing the adaptive value of the learned feed. However, housing, feeding and even management conditions create restrictions to acquire, transmit and preserve feeding behaviours. In this way feeding behaviours could not stay within the group of animals as a cultural pattern. These poor and constant conditions, causes that each generation of pigs have to look again the few tools available in the environment to learn the most effective eating behaviours. Management appears to be a fundamental key for piglets to develop their innate potential cognitive abilities without altering the normal production conditions and facilitating the transmission ways of feeding behaviour.

Conclusions

1. Piglets are able to discriminate between their mother’s amniotic fluid and amniotic fluid smell from other sows during their first weeks of life. Moreover, adding a new flavour into the sow’s diet during late gestation transfers flavour cues to the unborn piglets, which allow them to change their flavour preferences.

2. Post-weanling piglets can acquire a significant preference for a flavour paired with a protein source (CS+), which likely evokes the positive hedonic or post-ingestive memories of the unconditioned stimulus (US). The protein-paired CS+ flavour can also increase the preference and consumption of the protein US when the CS+ is combined with the US.

3. Piglets can acquire flavour preferences through an associative learning with the post-ingestive effects of sucrose and porcine digestive peptides (PDP).

4. A brief social interaction with a familiar conspecific pig that has recently consumed a flavoured feed (demonstrator) enhances naive pigs' (observers) preference for that feed over a novel or even already known diet. Familiarity of the demonstrator (penmates or littermates) is important to establish feed preferences by social interactions in pigs.

5. Pre and postnatal exposure to feed ingredients (PDP) through the sow diet may influence piglet performance when they are offered the same cues in the diet after weaning, probably due to the positive association with these feed components during the uterine or milk experience.

6. Inclusion of a high hedonic animal protein source (2% PDP) resulted in a productive performance of weaned piglets similar to that of piglets fed on diets containing significant amounts of whey (14%) and lactose in pre-starter and starter diets.

7. A planned learning strategy may be used in the pig industry to develop flavour preferences in weanling piglets by the associative learning of one neutral flavour with the positive effects of maternal fluids and nutrients, or by promoting brief social interactions with conspecifics.

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Annex

Author Education and Training

List of Publications

Author Education and Training

Jaime Figueroa Hamed was born in 1981, in Santiago de Chile. He completed his degree at the School of Veterinary Medicine of the Universidad de Chile in 2006. Thereafter, he continued his studies in Barcelona, Spain and he obtained his Master degree in Animal Science at the School of Veterinary Medicine of Universitat Autònoma de Barcelona in 2009. He is currently a PhD student in Animal Production at Universitat Autònoma de Barcelona with emphasis on Pigs Nutrition. He has been developing a research line about different aspects of feeding behaviour of commercial pigs, specially learning strategies to understand and modified animals' preferences and acceptances for different feed cues. Part of his work was developed at the Department of Psychology at the Brooklyn College of the City University of New York, USA and at the Psychology School in Cardiff, Wales. This work was the basis of the current thesis submitted to obtain a PhD degree in Animal Production (Nutrition) at the Faculty of Veterinary Medicine of Universitat Autònoma de Barcelona in 2012.

List of Publications

Scientific Papers

Figueroa, J., D. Solà-Oriol, E. Borda, A. Sclafani, J. F. Pérez. 2012. Flavour preferences conditioned by protein solutions in post-weaning pigs. Physiology & Behavior, 107: 309-316.

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Figueroa, J., D. Solà-Oriol, X. Manteca, J. F. Pérez. 2012. Social learning of feeding behaviour in weaned pigs: effects of neophobia and familiarity with the demonstrator conspecific. Animal Behaviour. Under revision.

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Figueroa, J., D. Solà-Oriol, S. L. Vinokurovas, X. Manteca, J. F. Pérez. 2012. Flavour preferences in piglets conditioned by prenatal flavour exposure through the maternal gestation diet. Under submission

Guzmán-Pino, S., D. Solà-Oriol, **J. Figueroa**, J. F. Pérez. 2012. The energy source and their availability affect the preferences for protein or carbohydrate solutions and piglet performance after weaning. Journal of animal science. Accepted.

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S. Guzmán-Pino, D. Solà-Oriol, **J. Figueroa**, J. F. Pérez. 2012. The energy source and their availability affect the preferences for protein or carbohydrate solutions and piglet performance after weaning. 12th International Symposium of Digestive Physiology of Pigs. Keystone, Colorado, USA.

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