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INFLUENCE OF THE NUTRITIONAL STATUS OF PIGLETS ON THEIR ABILITY TO PERFORM APPROPRIATE DIETARY SELECTION PATTERNS AFTER WEANING

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Certifiquen:

Que la memòria titulada “Influence of the nutritional status of piglets on their ability to perform appropriate dietary selection patterns after weaning”, presentada per **Sergio A. Guzmán Pino** amb la finalitat d’optar al grau de Doctor, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació perquè sigui jutjada per la comissió corresponent.

I perquè consti als efectes oportuns, signen la present a Bellaterra, 28 de Juliol de 2014.

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Summary/Resumen

The present PhD Thesis aims to simulate different physiological or nutritional status that post-weaned piglets face in the intensive production system, e.g., variations in the dietary nutritional content, in the availability of nutrients or feeds, or in the animals' homeostasis. It is hypothesized that commercial piglets may have retained the capacity to perform appropriate dietary selection patterns in relation to their needs, even when the common feeding practices in the pig industry do not allow animals to select their own diet composition.

Chapter 1 assesses whether dietary energy density affects the preference of piglets for protein or carbohydrate sources. One experiment was conducted by using two isoproteic pre-starter diets differing in the digestible energy content, a high-energy (HE) and a low-energy (LE) diet. The LE diet promoted a higher performance than did the HE diet after 14 or 21 days of feeding. Preference was not observed for protein or carbohydrate solutions in piglets fed the LE diet. On the other hand, piglets fed the HE diet showed a higher preference for sucrose solution.

Chapter 2 evaluates whether piglets submitted to a protein-deficiency status are able to select and prefer protein sources to overcome the deficiency. Two isoenergetic pre-starter diets differing in the crude protein content were used in two experiments, a high-protein (HP) and a low-protein (LP) diet. In Experiment 1, piglets showed higher intake and preference for sucrose than for a protein solution, independently of the dietary crude protein content. In Experiment 2, piglets were given eight conditioning sessions with two equally preferred flavors mixed into protein (CSp) or carbohydrate (CSc) solutions. Subsequently, piglets fed the LP diet showed a higher intake and preference for CSp than for CSc, differences being higher for medium and low weight piglets.

Chapter 3 studies whether a long-term exposure to carbohydrate and artificial sweetener solutions has an effect on feeding behavior of piglets. Animals were offered in three different experiments a long-term availability to sucrose 160 g/L, maltodextrin 160 g/L, and saccharin 0.08 g/L plus neohesperidin dihydrochalcone (NHDC) 0.02 g/L solutions as supplement to the maintenance diet. In Experiment 1, piglets showed a higher intake of sucrose 160 g/L than of water and a decrease in feed intake and weight gain. A similar situation occurred in the last days of maltodextrin 160 g/L exposure in Experiment 2. In contrast, animals were not influenced by saccharin 0.08 g/L plus NHDC 0.02 g/L in Experiment 3. After solutions exposure, a reduction in sucrose 20 g/L preference and appetence was observed in Experiments 1 and 2, but not Experiment 3.

Chapter 4 estimates whether dietary electrolyte balance (dEB) influences feed preference, appetence and growth performance of piglets. Seven isoproteic and isoenergetic starter diets differing in the dEB were used in three distinct experiments, diets ranging from -16 mEq/kg to 388 mEq/kg. Productive results of Experiments 1 and 2 showed that low rather than high dEB levels optimized growth performance of piglets. In Experiments 2 and 3 piglets had the opportunity to choose between these diets, being unable to select the diet that optimized their performance neither in short- nor long-term preference tests, showing also a higher appetence for high dEB levels.

It is concluded that piglets might be able to perform appropriate dietary selection patterns in relation to different nutritional status, but critically whether a learning process has been carried out. In the absence of learning, such as in the intensive pig industry conditions at weaning, piglets might be unable to overcome a particular situation that departs from an optimal state just relying on their innate dietary preferences and aversions.

La presente Tesis Doctoral tiene como objetivo simular diferentes estados fisiológicos o nutricionales que enfrentan los lechones destetados en el sistema intensivo de producción, por ejemplo, variaciones en el contenido nutricional de la dieta, en la disponibilidad de nutrientes o piensos, o en la homeostasis de los animales. Se plantea la hipótesis de que los lechones comerciales pueden haber conservado la capacidad de realizar patrones de selección dietéticos adecuados en relación a sus necesidades, incluso cuando las prácticas de alimentación comunes en la industria porcina no permiten a los animales seleccionar su propia composición de dieta.

El Capítulo 1 analiza si la densidad energética de la dieta afecta la preferencia de los lechones por fuentes de proteína o carbohidratos. Se llevó a cabo un experimento mediante el uso de dos dietas pre-estárter isoproteicas que defirieron en el contenido de energía digestible, una dieta alta en energía (HE) y una dieta baja en energía (LE). La dieta LE promovió un rendimiento más alto que la dieta HE después de 14 o 21 días de consumo. En los lechones alimentados con la dieta LE, no se observó preferencia por soluciones de proteína o carbohidratos. Por otro lado, los lechones alimentados con la dieta HE mostraron una mayor preferencia por la solución de sacarosa.

El Capítulo 2 evalúa si lechones sometidos a un estado de deficiencia de proteína son capaces de seleccionar y preferir fuentes de proteína para superar la deficiencia. Se utilizaron en dos experimentos dos dietas pre-estárter isoenergéticas que defirieron en el contenido de proteína bruta, una dieta alta en proteína (HP) y una dieta baja en proteína (LP). En el Experimento 1, los lechones mostraron un mayor consumo y preferencia por sacarosa que una solución de proteínas, independientemente del contenido de proteína bruta de la dieta. En el experimento 2, los lechones recibieron ocho sesiones de condicionamiento con dos *flavors* igualmente preferidos mezclados con soluciones de proteína (CSp) o carbohidratos (CSc). Posteriormente, los lechones alimentados con la dieta LP mostraron una mayor ingesta y preferencia por CSp que CSc, siendo las diferencias mayores en los lechones de pesos mediano y bajo.

El Capítulo 3 estudia si una exposición a largo plazo a soluciones de carbohidratos y edulcorantes artificiales tiene un efecto en el comportamiento alimentario de los lechones. En tres experimentos diferentes, se les ofreció a los animales una disponibilidad a largo plazo a soluciones de sacarosa 160 g/L, maltodextrina 160 g/L y sacarina 0,08 g/L más neohesperidina dihidrocalcona (NHDC) 0,02 g/L, como complemento a la dieta de

mantenimiento. En el Experimento 1, los lechones mostraron una mayor ingesta de sacarosa 160 g/L que de agua, y una disminución en el consumo de pienso y ganancia media de peso. Una situación similar ocurrió en los últimos días de la exposición a maltodextrina 160 g/L en el Experimento 2. Por el contrario, los animales no se vieron influenciados por la sacarina 0,08 g/L más NHDC 0,02 g/L en el Experimento 3. Después de la exposición a las soluciones, se observó una reducción en la preferencia y apetencia por sacarosa 20 g/L en los Experimentos 1 y 2, pero no el Experimento 3.

El Capítulo 4 evalúa si el balance electrolítico de la dieta (dEB) influencia la preferencia y apetencia alimentaria, así como el rendimiento productivo de los lechones. En tres experimentos distintos se utilizaron siete dietas estárter isoproteicas e isoenergéticas que defirieron en el dEB, variando desde -16 mEq/kg a 388 mEq/kg. Los resultados productivos de los Experimentos 1 y 2 mostraron que niveles bajos de dEB en lugar de niveles altos optimizaron el rendimiento de los lechones. En los Experimentos 2 y 3 los lechones tuvieron la oportunidad de elegir entre estas dietas, no pudiendo seleccionar la dieta que optimizó su rendimiento en pruebas de preferencia ni de corto ni largo plazo, mostrando también una mayor apetencia por los niveles altos de dEB.

Se concluye que los lechones pueden ser capaces de realizar patrones de selección dietéticos apropiados en relación a diferentes estados nutricionales, siempre y cuando se haya realizado un proceso de aprendizaje. En ausencia de aprendizaje, como en las condiciones de la industria intensiva de cerdos al destete, los lechones pueden ser incapaces de superar una situación particular que se aparte de un estado óptimo basándose sólo en sus preferencias y aversiones alimentarias innatas.

General introduction

In wild conditions, weaning in piglets is a long and progressive process that occurs between the 9th and 22th weeks of age, allowing animals to learn and adapt to the new conditions (Jensen and Recén, 1989). Piglets grasp at food with their mouths and chew vigorously to mix it with saliva before swallowing the bolus, spending a lot of time rooting in the soil with their snouts (Forbes, 2007). Thus, piglets learn about food and water sources without interruption of milk intake. In addition, social interactions with their mother and experienced conspecifics smooth the transition of feeding behavior patterns necessary for weaning adaption (Graves, 1984).

On the other hand, the weaning process in modern pig husbandry creates an artificial scenario where piglets struggle to overcome the post-weaning adaptation syndrome. Piglets have to be separated from their mothers at a very early age of around three or four weeks of life, making this process the most critical period in the life of a commercial pig with significant consequences on its subsequent performance. Weaning is a period of major stress for the young pig, and is frequently associated with a lag in performance that includes decreased rates of weight gain and feed intake, and higher incidence of disease and mortality. Piglets are removed from the dam and allocated in another pen, where they are mixed with animals from different litters. As the access to the highly-digestible milk from the sows is interrupted, piglets are expected to quickly adapt to the new dry diet with a very different composition, taste and texture. However, the sum of stressor factors normally generates that piglets do not start feed consumption until several hours after weaning (Bruininx et al., 2001). For this reason, during the first days post-weaning animals strongly depend on body energy reserves, as the low feed intake does not meet their energy requirements for maintenance (Cera et al., 1988).

This situation of abrupt weaning of the pig industry, limited also because of housing restrictions, may also affect the acquisition of key mechanisms to piglets that could generate productivity losses until a new effective behavior is established. Piglets are born with innate preferences and aversions to particular flavors. However, innate preferences and aversions cannot be relied on for the rest of the animals' life. For example, a food that has been tasted once or twice in the spring and has been found to be bitter might, by the summer, have become sweet; it might have changed from toxic to nutritious or vice versa. Therefore, pigs and other animals ought to be able to benefit from prior experience to be able to best exploit

its environment, but also to be flexible and to be able to relearn the associations when necessary (Forbes, 2007).

Therefore, learning seems to be a fundamental mechanism that a mammal has to acquire in order to perform an effective feeding behavior to find and consume suitable nutrient sources from the environment (Gieling et al., 2011). Under natural conditions, pigs and other mammals undergo several learning processes that allow animals self-nourish as they growth. However, in the intensive pig industry, piglets face the new environment at a very young stage and without time to learn about it. It is possible that piglets are unable to overcome a particular nutritional situation that departs from an optimal state just relying on their innate dietary preferences and aversions without learning about likely alternatives.

Early weaning in the pig industry is still a major challenge largely unsolved. Overcoming the post-weaning check in piglets may require a multidisciplinary approach, in which consideration for special diets and the nutritional requirements of piglets are one of the cornerstones. Formulating highly-digestible and palatable diets often supplemented with innately preferred flavoring compounds has been a general practice. However, an accurate knowledge of the dietary preferences of piglets at weaning, and the likely mechanisms driving their feeding behaviour, would be additional important features for trying to improve the acceptance of diets of young animals at this stage. Some of these are studied over the present PhD Thesis.

1. Literature review

1.1. Introduction: preference, appetence and palatability

Many of the challenges of modern pig production systems are closely related to feed intake. At weaning, for example, the initiation of feeding is probably one of the most critical points in pig production. Bruininx et al. (2001) reported that some piglets do not start feed consumption after more than 15 hours post-weaning. A delayed feeding at this stage may result in digestive disorders and growth check that may influence the pig's whole productive period. Thus, one nutritional strategy to promote an early feed consumption after weaning (reduced latency time) is the use of highly palatable ingredients in the post-weaning diet, such as milk-derived products, or highly-digestible cereal or animal origin protein sources. However, even though it appears apparently simple; palatability is a complex concept not easy to define.

What do we understand for palatable dietary source? And how is this concept linked to dietary preferences or appetence of piglets at this stage? During the present Thesis such kind of terms will be continuously used being not necessarily related one with the other. For this reason some definitions are given below.

Preference (or aversion) for a particular source (feed or solution) is the behavioral expression obtained as a result of the integration of a multiple distinct sensory, metabolic and physiological inputs processed to generate the overall sensation. Integration of this incoming information in the brain ultimately results in flavor (olfaction and taste) preference or aversion with a strong influence on subsequent perception and behavior. Forbes (2009) suggested that if we offer a choice between two situations (feeds, environments, etc.) to a certain animal, and we observe that something is chosen over the other, then we could say that the preferred one provides the individual with greater comfort, and conversely, the other is avoided because experiencing it causes discomfort. Therefore, preference implies choice, and it is measured by simultaneously offering an animal or group of animals a selection between two sources, and subsequently comparing the amounts selected of each one (source A against source B). This is probably the most widely used method for assessing palatability (Forbes, 2010). However, preference is not necessarily correlated with the total feed intake that the animals will show when offered the two sources separately in time, or with their palatability.

Appetence is here defined as the short-term consumption of a single source when it is first offered. The rate at which animals eat a novel food when it is first offered has been also used as a measure of palatability, because it is an indication of the degree of motivation, which is governed by the degree of hunger and by the anticipation of resulting pleasure or comfort of eating that food. In pigs, the rate of eating during large meals following an enforced fast of several hours is initially rapid, but slows down as the meal progresses (Auffray and Marcilloux, 1983). Both the initial rate of eating and the rate of deceleration are influenced by animal factors, such as degree of hunger, and food factors, such as particle size and flavor (Forbes, 2010).

Palatability, on the other hand, is a complex concept not amenable to concise definition because it depends not only on the organoleptic properties of the offered source, but also on the experience and genetic background of the animal in question and its physiological state, as well as environmental conditions and social context. A comprehensive definition is that given in Wikipedia (2014) and cited by Forbes (2010) in his review: 'palatability is the hedonic reward provided by foods or fluids that are agreeable to the palate in regard to the homeostatic satisfaction of nutritional, water, or energy needs. The palatability of a food or fluid, unlike its flavor or taste, varies with the state of an individual being lower after consumption and higher when deprived. Palatability of foods, however, can be learned'. Precisely, this last point states some of the difficulties in the concept of palatability concerning how a source that can be initially unpalatable (e.g., Bitrex, a bitter taste compound), may become later, at least, acceptable after some exposures based on a learning association (Blair and Fitzsimons, 1970).

Palatability, despite being very widely used is a much-misunderstood term that has not been systematically studied in pigs until date. On the contrary, there is a considerable amount of literature that relates to the dietary preferences of pigs, starting approximately in the 1950s. A chronology of the main pig preference/taste-sensing research conducted until date is presented in Table 1.1. In the beginning, studies on pig preference were basically behavioral-type by using adapted choice test procedures derived from Curt Richter's experiments in rats. More recently, and with the availability of new laboratory techniques and the pig genome sequence, molecular studies related to taste perception mechanisms in pigs are getting momentum.

Table 1.1. Chronology of pig preference/taste-sensing research conducted until date (adapted from Roura, personal communication).

Behavioral studies	Physiological/molecular studies
1956. Salmon-Legagneur and Fevrier: *Sugar preferences*	
1965. Kare et al.: *Preferences for sucrose, glucose and lactose*	
1972. Kennedy and Baldwin: *Preferences for sucrose, glucose and saccharin*	
	1993. Chamorro et al.: *Scanning electron microscopy of fungiform papillae*
1997. Nelson and Sanregret: *Response to bitter compounds*	1997. Mack et al.: *Porcine lingual taste buds*
	1999. Danilova et al.: *Taste perception in pigs (electrophysiological)*
2000. Glaser et al./Tinti et al: *Sweet compounds and amino acids preferences*	
2002. Nofre et al: *Sweeteners preferences*	
2004-2006. Ettle and Roth: *Amino acids and organic acids preferences*	
2005-2009. Sola-Oriol et al.: *Feed ingredients preferences*	2005-2009. Tedó (PhD Thesis): *The umami taste in pigs*
	2006. Kiuchi et al.: *T1R3 characterization in the pig*
Pig genome sequencing SusScrofa9	
	2010. Moran et al.: *T1R2/T1R3/Gustducin/SGLT1/GLUT2 in GIT cells*
	2011. Roura et al.: *T1R1/T1R3 in pigs vs. human and rodents*
	2011. Widmayer et al.: *T1R3/Gustducin/ $PLC_{\beta 2}$/TRPM5 in porcine stomach*
	2011. Simons et al.: *CD36 in porcine taste buds*
	2012. Haid et al.: *GPRC6A/CaSR/GPR92 in porcine stomach*

Table 1.1. Continued.

Behavioral studies	Physiological/molecular studies
	Pig genome sequencing SusScrofa10
2012. Figueroa et al.: *Preferences for conditioned flavors*	2012. Groenen et al.: *Annotation of pig genome with highlights on TRs* 2012. Colombo et al.: *Seven T2Rs and 3 FFA/GPR120 in porcine GIT* 2013. Mazzoni et al.: *Transducin in porcine GIT* 2013. de Jager et al.: *Taste receptor repertoire in pigs*

1.2. Regulation of feed intake in pigs

Under natural conditions, pigs are 'general' feeders that select what they eat from a wide array of foods. Initially, they sample most potential foods but soon they become more selective as they learn the nutritive (or toxic) properties of each source (Forbes, 2007). In the intensive pig production system, however, feed intake is largely controlled through management practices that strive to optimize the economic balance between feed intake and overall animal health and productivity (Carroll and Allee, 2009). Pigs are usually fed single, complete diets intended to fully satisfy nutritional requirements for growth. Animals may decide how much of the feed offered they eat, but not choose or prefer a certain feed according to its palatability or post-ingestive consequences. This condition becomes particularly relevant in young animals during critical stages of growth, such as weaning. The following literature describes the main mechanisms involved in the regulation of feed intake in pigs, either in wild or intensive conditions.

1.2.1. Sensorial perception of feed

The evolution of the chemical senses has resulted in a sensory apparatus for high taste and smell acuity in mammals, and particularly in pigs. These mechanisms are driven by chemosensory codes that sense the nutritional value of available food sources, or their likely riskiness, and translate it into a physiological stimulus that will trigger or discourage feed consumption (Roura et al., 2008).

The ingestion of food simultaneously evokes odor, taste and thermo-mechanical sensations that stimulate appetite for appropriate ingredients to ensure self-nourishment (Roura and Tedó, 2009). Before swallowing occurs, food is scrutinized and discriminated according to several chemical and physical parameters into the oronasal cavity of pigs which determine food palatability, prepares the GIT for its reception and stimulates digestive secretions and gut motility, among others (Forbes, 1998; Katschinski, 2000). Although flavor perception is often described as a combination of smell and taste, characteristics like appearance, texture, temperature or mouth feel play also major roles in such perception. The five senses may influence feed intake and dietary preferences in pigs, although not all of them with the same level of importance. Therefore, a brief description of just olfaction, oral somatosensing and taste will be addressed in this section.

1.2.1.1. Olfaction

The sense of smell seems to be the most developed of the senses in non-primate mammals. This physiological system is understood as a chemosensing mechanism used by animals to find food, detect predators and prey and mark territory (Firestein, 2001). For terrestrial animals, such as pigs, one of the main functions of smell is to identify food sources from distance. Thus, a link between odor-stimulating molecules and the nutritional value of the odorant source might be inferred (Roura et al., 2008). The olfactory sensitivity of pigs has been shown to be extremely high as compared to that of other animal species, like humans (Roura and Tedó, 2009). Pigs can easily detect plant volatile organic compounds while they are seeking for feed. However, just only a small subset of these volatiles generates the ‘flavor

fingerprint' that helps animals to recognize appropriate feeds and to avoid poor or dangerous ones (Goff and Klee, 2006).

Olfaction occurs in the main olfactory epithelium located in the upper wall of the nasal cavity of pigs. The MOE consists of turbinates that increase the surface area, a basement cell membrane and a stratified layer of supporting cells that contain the olfactory sensory neurons (OSN), which are bipolar neurons that project some 20 or 30 cilia that lie in the superficial thin layer of mucus. The OSN are characterized by their ability to express olfactory receptors (OR) on the plasma membrane of the cilia. Ligand binding to the OR transduces the chemical stimulus into an electrical stimulus through the olfactory bulb to the CNS (Firestein, 2001). In comparison to humans, pigs have a higher olfactory epithelium surface, a higher number of OSN, and a lower proportion of pseudogenized (inactive) OR (Roura and Tedó, 2009).

1.2.1.2. Somatosensing

The presence of food into the oral cavity evokes other non-odorous/non-taste chemical, thermal or mechanical sensations that are collectively referred to as somatosensing. Mouth feel sensations, or the thermo-mechanical sensations when feed is introduced into the mouth, are aimed at scrutinizing the physical properties of the feed prior to swallowing (Roura et al., 2008). In pigs, mechanical stimuli such as hardness, fragility and chewing effort have been found to be significantly correlated to feed preference values (Solà-Oriol et al., 2007).

The somatic sensing in the oronasal cavity is linked to the cranial nerve V (trigeminal) and covers all the oronasal epithelium (Witt et al., 2003). Sensory neurons of the trigeminal nerve are part of the pain pathway and are involved in the detection of noxious stimuli such as thermal (low or high temperatures) and pungent (e.g., acids, spices). Both noxious high and low temperatures and pungency are perceived through the stimulation of transmembrane ion channel members of the transient receptor potential family (Dhaka et al., 2006), and may lead to mucosal damage in the GIT. The trigeminal stimulation consequently leads to an alarm response characterized by feed avoidance, a strong stimulation of digestive secretions and an increase in the intestinal motility aimed at protecting the digestive epithelium (Platel and Srinivasan, 2004; Roura et al., 2008).

1.2.1.3. Taste

Taste is the sensory modality designed to inform animals about the nutritional qualities of the food they eat (Janssen and Depoortere, 2013). Thus, animals can recognize a diverse repertoire of nutrient or toxic related chemical entities present in food sources. It is suggested that taste perception has been shaped by the nutritional needs linked to the ecological niches which evolutionary ancestors occupied (Breslin, 2013). Early mammals would have used their sense of taste to identify nutritious food items from their environments and to avoid harmful and potentially lethal ingestion of toxins. Therefore, taste perception may play a critical role in the adaptation of animals to ecological niches and species survival (Roura et al., 2013a).

The current consensus is that mammalian taste sensations can be divided into five qualities: sweet, umami, salty, sour and bitter. Sweet taste is mainly triggered by carbohydrates such as sugars. Umami taste is related to dietary protein and senses some L-amino acids (L-AA), such as glutamic acid (or its sodium salt monosodium glutamate; MSG) and peptides. Salty and sour respond to sodium and protons (or acids). Finally, bitter taste identifies anti-nutritional compounds and other potentially toxic molecules present in the diet (Roura et al., 2013a). However, other candidate taste modalities are currently under scrutiny, such as taste senses linked to fat (primarily free fatty acids), water perception, starch-derived glucose polymers, calcium or carbonation, among others (Bachmanov and Beauchamp, 2007; Roura, 2011; Janssen and Depoortere, 2013).

In general, taste perception mechanisms are associated to mouth papillae. Three main types of papillae (fungiform, cirumvallate and foliate) are primarily present on the tongue, epiglottis and soft palate (Mombaerts, 2000). Papillae contain a high number garlic-clove like organelles named taste buds (Chandrashekar et al., 2006). Type and number of papillae and the number of taste buds in each papillae differ among animal species (Table 1.2). Thus, pigs and cows have the highest number. Pigs have taste buds in all the three different types of papillae, as opposed to other mammals such as cats (no foliate papillae) or cattle (no buds in foliate papillae). In addition, pigs have approximately 3 times more taste buds than humans (Roura and Tedó, 2009). Studies in humans show a positive correlation between the number of taste buds and ability to taste; therefore, it is likely that the sense of taste in pigs is superior to that of humans (Danilova et al., 1999). The cranial nerve IX (glossopharyngeal) innervates

mainly circumvallate and foliate papillae; while the cranial nerve VII (chorda tympani) innervates fungiform papillae (Witt et al., 2003).

Table 1.2. Diversity of taste buds in the oral cavity of different animal species (adapted from Roura et al., 2008).

Animal	Number of taste buds	Reference
Cow	21691	Davies et al., 1979
Pig	19904	Chamorro et al., 1993
Rabbit	17000	Teeter and Kare, 1974
Human	7902	Travers and Nicklas, 1990
Cat	2755	Robinson and Winkles, 1990
Marmoset	2704	Yamaguchi et al., 2001
Dog	1706	Leibetseder, 1980
Rat	1438	Travers and Nicklas, 1990
Hamster	723	Miller and Smith, 1984
Mouse	523	Zhang et al., 2008
Chicken	316	Ganchrow and Ganchrow, 1987

Taste buds are the sensory organelle for taste chemosensing. Each taste bud consists of 50 to 120 taste sensory cells that project a number of microvilli that reach the mucus layer of the tongue (Dulac, 2000). Four different types of cell have been characterized to constitute a taste bud; three taste-type cells (I, II and III) and one basal-type cell believed to be a progenitor of the other three (DeFazio et al., 2006). Type I taste cells are sour-sensing, type II are sweet, umami and bitter sensing, and type III cells play an intermediate signalling role between the true taste cells (type I and II) and the sensory neurons (Firestein, 2001; Romanov and Kolesnikov, 2006). Although each taste bud seems to recognize all basic tastes, any single cell expresses only one family of taste receptor (TR). The TRs are transmembrane proteins expressed in the sensory cell microvilli where they recognize specific soluble taste ligands present in the oral cavity (Dulac, 2000; Mombaerts, 2000). In cell type II, the binding of the appropriate ligand to the TR will trigger an intracellular metabolic cascade followed by an intercellular communication between the taste-sensing cell and the type III cells (Firestein, 2001). Sweet, umami and bitter TRs are part of the G protein-coupled receptors (GPCRs) super-family characterized by 7 transmembrane domains. The TRs have been divided into two classes: T1R and T2R. The T1R is a family of three genes that code for two heterodimeric receptors, the umami composed of T1R1 and T1R3, and the sweet composed of

T1R2 and T1R3 (Li et al., 2002). The T2R is a big family related to bitter taste sensing (Adler et al., 2000; Meyerhoff et al., 2010). The size of the T2R family differs among animals ranging from 3 (chickens), 16 (pigs and dogs), 19 (cattle), 25 (humans) and 35 (laboratory rodents) functional genes (Shi and Zhang, 2006; Roura, 2011; de Jager et al., 2013; Roura et al., 2013a). A comparative between the currently known TR repertoire of humans, pigs and chickens, and the biological sources they sense is shown in Table 1.3.

Table 1.3. Comparative taste receptor repertoire and their ligands in humans, pigs and chickens (adapted from Roura et al., 2013a).

Nutrient/ligand	Human genes	Pig genes	Predicted chicken genes
Energy			
Sugars	T1R2/T1R3	T1R2/T1R3	No T1R2 in chicken
Short-chain FA	CD36, GPR41, GPR43	CD36, GPR41, GPR43	Candidate genes
Med. & long-chain FA	CD36, GPR40, GPR120	CD36, GPR40, GPR120	CD36, GPR120
Protein			
L-Glutamic	T1R1, T1R3, mGluRs	T1R1, T1R3, mGluRs	T1R1, T1R3, mGluRs
L-Phe and L-Trp	CaSR	CaSR	Candidate gene
Other L-AA	GPRC6A	GPRC6A	GPRC6A
Peptones	GPR92/93	GPR92/93	GPR92
Toxins/Anti-nutritional	25 T2R genes	16 T2R genes	3 T2R genes
Minerals			
Calcium	CaSR	CaSR	Candidate gene
Sodium	ENaCs	ENaCs	ENaCs
Organic acids			
High [H+]	PKD1L3, PKD2L1, HCNs	PKD1L3, PKD2L1, HCNs	PKD2L1, HCNs

The binding of ligands that stimulate G-protein TRs activates a well known intracellular transduction pathway (Margolskee, 2002). The transmembrane receptor is coupled with the heterotrimeric gustducin (α-gustducin, β-gustducin ($G\beta_3$), and γ-gustducin ($G\gamma_{13}$)) that, in turn, activates phosphodiesterase to decrease cAMP (mediated by α-gustducin) and phospholipase $C_{\beta 2}$ ($PLC_{\beta 2}$) to generate inositol trisphosphate and diacylglycerol (mediated by transducin; $G\beta_3/G\gamma_{13}$). As a result, the extracellular Ca^{2+} influx and the release from internal stores activates the transient receptor potential channel TRPM5 which leads to taste cell depolarization and signalling to afferent nerves (Roura, 2011).

The existence of a repertoire of proteins in addition to the TRs specifically related to taste perception, such as gustducin, transducin, $PLC_{\beta 2}$ or TRPM5, has been a very important tool in last years in the identification of taste-related sensory cells outside the oral cavity (Roura, 2011). Taste sensory cells present in taste buds of the tongue are part of a family of chemosensory cells found also in non-lingual epithelia of endodermal origin (i.e., respiratory and digestive epithelia). That system has been referred to as the diffuse chemosensory system, characterized by a set of signal transduction components typically found in taste cells, but not aggregated in taste buds (Sbarbati and Osculati, 2005). These solitary chemosensory cells are densely distributed in strategic areas of the body suggesting that they are involved in important physiological processes both in respiratory and digestive systems. Within the GIT, they are located predominantly at the interface among different microenvironments and have been related not only to absorptive and secretory processes, but also to the control of the microbial population and the detection of irritants (Dyer et al., 2007; Gulbransen et al., 2008).

Less than 1% of the cells in the GIT are specialized cells with endocrine functions generically grouped under the enteroendocrine system, which represents the largest endocrine organ in mammals (Janssen and Depoortere, 2013). They produce and secrete a variety of hormonal compounds relevant to the short-term control of feed intake including gastrin, ghrelin, cholecystokinin (CCK), serotonin, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptides (GLPs) and peptide YY (PYY). In recent years, the number of taste-related proteins shown to be expressed in enteroendocrine cells has increased and includes the three subunits of gustducin, T1R1, T1R2, T1R3, TRPM5 and $PLC_{\beta 2}$. Thus, meal-related fluctuations in plasma hormone levels (postprandial increase or decrease) are dependent on the caloric value and the macronutrient composition of the meal which in turn is monitored by chemosensory mechanisms (Janssen and Depoortere, 2013). The mode of action is

schematized in Figure 1.1. Nutrients (sweet, bitter, fat, amino acids) are sensed by different GPCRs as well as transporters in several cell types (endocrine cell, brush cell, enterocyte) of the epithelial lining that cross-regulate each other's expression. The GPCRs induce, via distinct G proteins (e.g., gustducin), the release of second messengers that lead to the release of gut peptides which can communicate directly, via the bloodstream, or indirectly, via the vagal nerve, with the hypothalamus to control feed intake (Janssen and Depoortere, 2013).

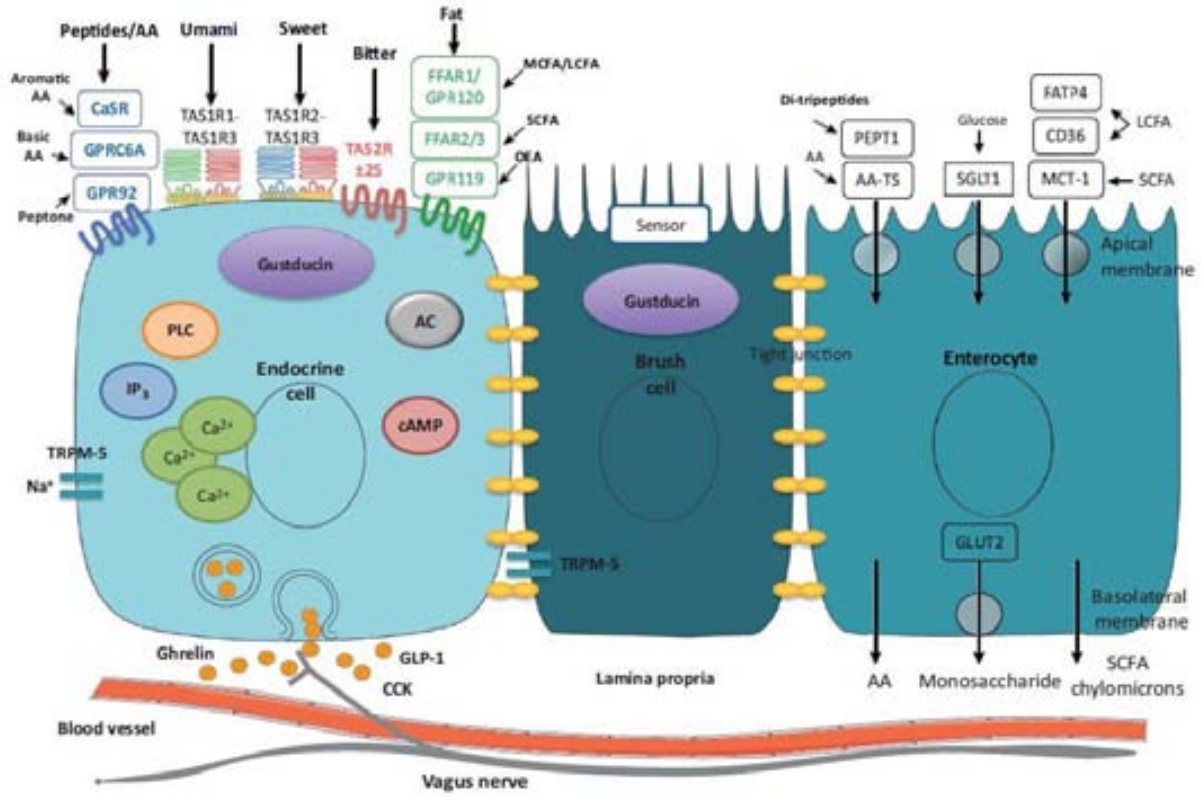

Figure 1.1. Simplified model of the pathways involved in chemosensory signaling in the GIT (from Janssen and Depoortere, 2013).

1.2.2. Post-ingestive effects

The preference of an animal for a certain feed is more than a matter about flavor perception (Myers et al., 2005). Thus, there is an interrelationship between feed's flavor perception and its post-ingestive effects. Post-ingestive and physiological effects leading to meal initiation and termination result as feedback from the cells and organs that modulate the response to a particular flavor, and, as a result, they contribute to determine feed intake and growth of farm animals (Roura et al., 2008). Feedback may be positive (increases palatability) if the feed meets nutritional needs. Feedback may be negative (decreases palatability) if the feed is inadequate or excessive relative to nutritional needs or contains high levels of toxins (Schell et al., 1993).

At the onset of feed ingestion, a number of satiation signals arise from multiples sites in the gut, including the stomach, intestine and pancreas. These signals are conveyed to regions of the brain via stimulation of tension receptors and mechanoreceptors in the gastrointestinal wall, and by the release of peptide hormones by enteroendocrine cells (Cummings and Overduin, 2007). These events ensure the maintenance of the essential interaction between the system that control energy homeostasis (long-term) with those signals governing nutrient intake on a meal-to-meal basis (short-term, Morton et al., 2006). Once food has been swallowed, there are no conscious sensations of its processing through the digestive tract unless it contains toxins or induces excessive distention. Nevertheless, a wealth of information concerning both physical and chemical changes in different parts of the digestive tract is transmitted to the CNS via the automatic nervous system and in the circulating blood as well. Thus, the CNS is made aware of the consequences of eating in terms of physical and chemical effects on the digestive tract, and uses this information in controlling the amounts of food eaten (Forbes, 1998). Forbes (1996) suggested that the information from all these abdominal and metabolic receptors converges, and the CNS is only presented with a general sensation of the degree of "discomfort". This discomfort can then become associated by learning with the sensory properties of the food that was eaten before discomfort was felt.

There is circumstantial evidence of physical factors limiting feed intake in pigs. For example, when food is diluted with indigestible material then daily intake increases in compensation, but there comes a point (sooner in smaller than larger pigs) beyond which the intake of digestible nutrients is no longer sustained (Whittemore et al., 2003). As well as the

capacity of stomach, the bulkiness of the feed plays an important factor in the regulation of intake. Thus, it is reported that one unit increase in the water holding capacity of the feed above 4 g/g is associated with a decrease in feed intake of about 6 g of feed DM per kg of pig live weight (Kyriazakis and Emmans, 1995). In addition, the rate at which the stomach empties is not only relevant to the duration of stomach distension, but also to the fill of duodenum and small intestine that posses stretch receptors. Mechanoreceptors, responsive to distension and muscle contractions, are found in the stomach and intestines and are likely to be responsible for the sensation of fullness that plays a part in limiting feed intake (Forbes, 2009).

Glucose is another major post-ingestive satiating signal for pigs, first in intestine and later in the body proper. Infusion of glucose solution into the duodenum of young pigs markedly reduced feed intake when given just after feed was offered, but not when given 10 minutes before feed was offered, which suggests that glucose may be a very short-lived satiety signal (Gregory, 2002). The total effect of infusing glucose solutions into the duodenum, ileum or jejunum is to depress intake by about the same amount as the energy of the glucose infused, as long as the pigs are allowed free access to drinking water and glucose is infused at physiological rates (Gregory et al., 1987). In the same way, infusion of protein or protein hydrolysates into the stomach, duodenum, jejunum or ileum all decrease intake approximately in proportion to the amount of energy infused, although the extent of the depression varies across different experiments from no effect at all to a two-fold reduction compared to the energy content of the infusion. Moreover, feed intake in pigs is also depressed by infusion of fat emulsions into stomach, duodenum or jejunum and, in the latter site at least, the response is to fatty acids rather than fat (Gregory, 2002). There is some evidence that only monoglycerides have the effect and other that suggests that the response depends on the degree of unsaturation of the fatty acids infused (Forbes, 2009).

There are complex interactions between the stomach and intestine whereby the presence of intake-limiting nutrients in the duodenum causes slowing of gastric emptying with the aim of conserving a constant rate of flow of digesta from stomach to duodenum. Signals from the GIT become integrated with each other, and with signals from other organs, such as the liver, to generate a composite signal that could represent the overall consequences of eating and be used, along with learning, by the hunger/satiety complex of the CNS (Forbes, 2009).

1.2.3. Metabolic and hormonal homeostasis

Despite the fact that there are many peripheral signals that can contribute to feeding behavior and body weight regulation, it is important to recognize that short-term and long-term feed intake and energy balance are regulated through distinct but interacting mechanisms. Some signals, such as nutrients and gastrointestinal hormones, act primarily as determinants of satiety to limit the size of individual meals. These short-term signals have a markedly different function as compared to the long-term regulators of energy homeostasis, which are activated in proportion to body adipose stores and the amount of energy consumed over a more prolonged period of time. These hormones, on the other hand, regulate intake and energy expenditure to ensure that energy homeostasis is maintained and that body weight and adiposity remain relatively constant. Several gastrointestinal hormones have been described for being implicated in the regulation of food/feed intake. Many of these peptides and their receptors are also present in regions of the CNS involved in such regulation, suggesting that their action represents both peripheral and central parallel pathways in modulating feeding behavior (Havel, 2001). A general description of the main hormones involved in the short- and long-term regulation of feed intake in pigs is presented here. More comprehensive reviews can be found in Carroll and Allee (2009) and Black et al. (2009).

One of the hormones first identified in the regulation of energy homeostasis was CCK, which is released from endocrine cells localized in the mucosal layer of the proximal small intestine and also from hypothalamic neurons during feeding. Its release is primarily stimulated by dietary fat, amino acids and small peptides generated during protein digestion. CCK inhibits feed intake by activation of the CCK_1 receptors, reducing meal size. In addition, due to CCK is also a potent inhibitor of gastric emptying, some of its effects to limit food intake may be indirectly mediated by the retention of food in the stomach. Thus, this hormone is mostly involved in the short-term control of feed intake (Havel, 2001; Badman and Flier, 2005).

The preproglucagon gene product yields two important satiety peptides, glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM) (Stanley et al., 2005). They are secreted by the endocrine L cells of the ileum in response to the entry of nutrients into the small intestine. Both peptides inhibit feeding when they are centrally or peripherally administrated, and their repeated administration decrease weight in rodents (Dakin et al., 2004). The actions of GLP-1

and OXM on feeding may be mediated via the GLP-1 receptor, which is expressed in the hypothalamus, brainstem and periphery. However, since GLP-1 is produced in both periphery and hypothalamic neurons, the extent to which GLP-1 from each of these sources participates in the physiological regulation of feeding behavior is unclear (Havel, 2001).

PYY is secreted post-prandially by the L cells of the GIT, especially in the most distal portions of intestine such as the ileum, colon and rectum; correlated with energy intake. Peripheral administration of PYY inhibits feed intake and reduces body weight gain in rodents, primates and humans. PYY crosses the blood barrier and probably exerts its action via the presynaptic Y2 receptor of neuropeptide Y (NPY) neurons in the accurate nucleus of the hypothalamus, inhibiting pro-opiomelacortin neurons and consequently feeding (Batterham et al., 2002).

Gastrin-releasing polypeptide (GRP)/bombesin is a peptide produced by endocrine cells in the gastric mucosa, is the mammalian homologue of a peptide (bombesin) first isolated from glands in the skin of amphibians. GRP not only regulates secretion of gastrin, but also its peripheral administration inhibits food intake in animals, and its intravenous infusion reduces appetite and food intake in humans. It is suggested that GRP-related peptides have a role in the central regulation of food intake (Havel, 2001).

Ghrelin is a twenty eight amino acid acylated hormone mainly synthesized and secreted by the gut in the gastric oxyntic cells at the fundus of the stomach, as well as the duodenum, ileum, caecum, colon and hypothalamus. Ghrelin is derived from a pro-hormone (pro-ghrelin) by post-translational processing, and was first identified based on its stimulation of growth hormone (GH) secretion via a GH secretagogue receptor in animals and humans (Zabielski, 2007). Circulating ghrelin concentration increases during fasting and before meals, it is reduced by the presence of nutrients in the stomach, and it is lower in obese versus lean human subjects. In contrast to the anorexigenic effects of other gastrointestinal hormones, peripheral or central administration of ghrelin increases food intake in rodents. Therefore, ghrelin may have a potential role in the long-term body weight regulation (Havel, 2001; Grove and Cowley, 2005). Like ghrelin, obestatin is a twenty three amino acid product of pro-ghrelin post-translational modification. However, opposite to ghrelin, treatment of rats with obestatin suppressed food intake, inhibited jejunum contraction, and decreased body weight gain. Obestatin does not cross the blood-brain barrier and seems to act solely at the periphery (Pan et al., 2006).

Orexin-A (OXA, hypocretin-1) and orexin-B (OXB, hypocretin-2) are peptides derived from the same amino acid long precursor (prepro-orexin). Orexins and their receptors were first discovered in the rat brain, and soon after in peripheral neural structures and in the enteroendocrine cells, pancreas, stomach and intestinal mucosa. Orexins affect gastrointestinal motility and gastric, intestinal and pancreatic secretions via direct and/or neural mechanisms (Zabielski, 2007). Acute central administration of orexins leads to a robust hyperphagic response in rodents and other vertebrates. Analogous to leptin, orexins also plays a dual role in regulating both homeostatic and hedonic aspects of food intake. Recently, it was shown that appetite, meal frequency and length of a meal were also increased after central administration of orexin-A (Pandit et al., 2011).

Insulin and leptin are the two most important long-term regulators of food/feed intake and energy balance (Havel, 2001). It was first proposed in the early 1970s that insulin is a long-term regulator of food intake, energy balance and body adiposity in humans. Insulin secretion from islet β cells of the endocrine pancreas is stimulated by food ingestion. This is a coordinated effect mediated via activation of the parasympathetic nerves innervating the pancreas, the direct effect of incoming nutrients, specifically glucose and amino acids, and the stimulation by incretin hormones such as GIP and GLP-1, which are released during meal ingestion and absorption. Both fasting plasma insulin levels and insulin responses to meal ingestion are correlated with body adiposity. Insulin can also act indirectly by stimulating leptin production from adipose tissue via increased glucose metabolism. In contrast, dietary fat and fructose do not stimulate insulin secretion and therefore do not increase leptin production. There is also evidence that leptin can inhibit insulin secretion from the pancreas (Havel, 2001).

Leptin is a hormone produced and secreted by adipose tissue, muscles and stomach, and is involved in the regulation of adipose tissue mass, food intake and body weight in neonatal animals. Leptin is also produced in the mammary glands and secreted into the colostrum and milk in humans, mouse, rats and pigs. Active form of leptin receptor is widely distributed in the small intestine mucosa (Zabielski, 2007). Leptin circulating levels reflect both energy stores and acute energy balance and its levels are highly correlated with adipose tissue mass, however, plasma leptin levels decrease independently of modest changes of body fat content during short-term periods of fasting or during restriction in energy intake and they increase after re-feeding or during over-feeding, or by insulin administration (Frederich et al., 1995).

These acute, adiposity-independent decreases of leptin production in response to an energy deficit would be expected to promote increased energy intake and energy conservation before body fat stores become significantly depleted (Havel, 2001).

1.2.4. Central regulation of feed intake

As mentioned before, the brain senses and integrates signals reflecting overall energy stores, recent energy intake, and the presence of specific classes of nutrients (Figure 1.2). Thus, there are connections between the hypothalamus and the hindbrain that form part of the neural circuit that controls feeding.

The hypothalamus, especially the arcuate nucleus, is relatively accessible to circulating factors and inputs from other areas of the brain. Here, signals are received relating to total energy stores in fat and to immediate changes in energy availability, including nutrients within the GIT. These two categories of signals are not exclusive, because signals relating to long-term energy stores, including insulin and leptin, can modulate responses to short-term nutritional inputs. The hypothalamus integrates these peripheral and central signals and exerts homeostatic control over feed intake, levels of physical activity, basal energy expenditure, and endocrine systems, including those that determine reproductive competence (Badman et al., 2005).

Short-term feeding behavior is also controlled by the hindbrain. The nucleus of the tractus solitarius receives input from vagus nerve afferents within the dorsal vagal complex, whereas the area postrema is a target for circulating factors such as amylin and GLP-1 (Badman et al., 2005). Therefore, the caudal brainstem performs the important function of integrating metabolic stimuli and modifying feeding behavior according to nutrient demands. The information of foods is integrated with knowledge gained by the especial senses and committed to memory to serve the animal when it next has to make decisions about how much, or what food, to eat (Forbes, 1998).

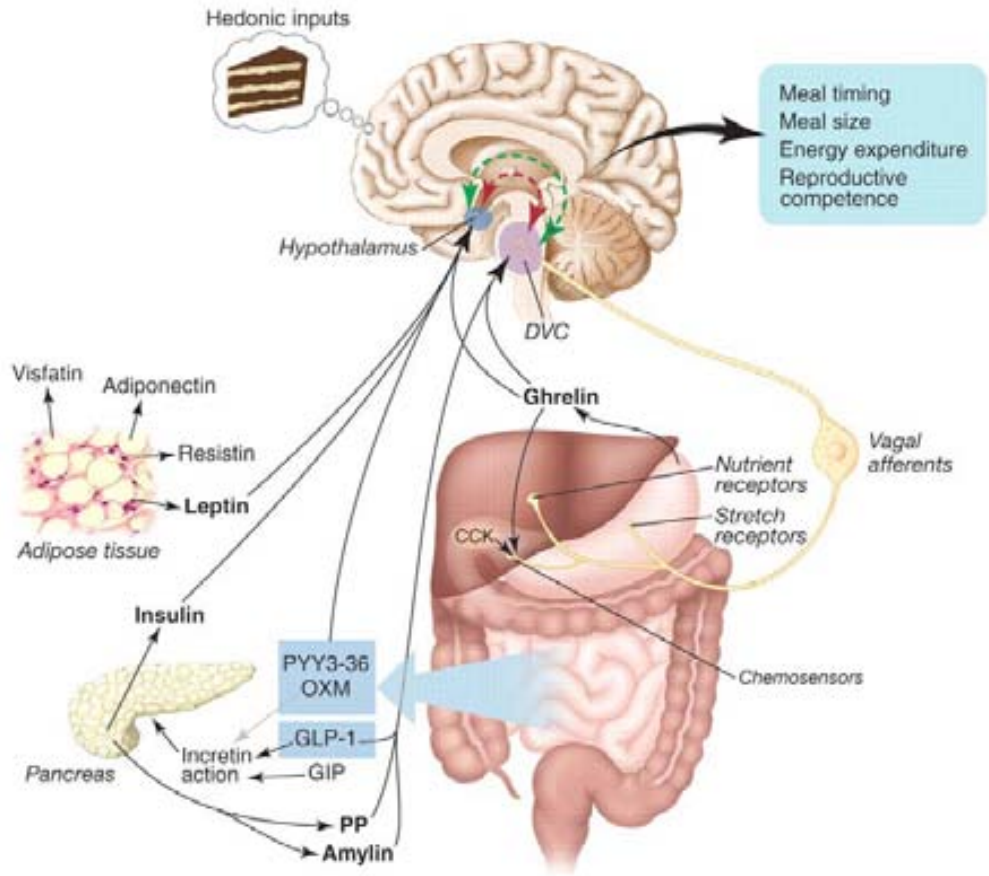

Figure 1.2. Schematic representation of the integration of short- and long-term signals involved in the regulation of feed intake and energy balance (from Badman et al., 2005).

1.2.5. Theories of control of feed intake and diet selection

As reviewed, feed intake is controlled by many different factors, being a challenge to develop methods of integrating these factors into models of the control of intake (Forbes, 2009). The most common assumption has been that whichever factor limits intake the most, then this is the factor in control, other factors being ignored. This principle has been used in young pigs, and apparently validated by Whittemore et al. (2001), related to physical factors that control feed intake (Forbes, 2009).

Forbes (2009) proposed an alternative framework to explain feed intake and diet selection in pigs, known as minimal total discomfort. In that, he proposed that differences in the current rate of supply of a food resource (e.g. energy, protein, bulk) and the optimal supply of that resource generates discomfort, which the animal seeks to minimize. The error (desired supply minus current supply), as proportion of the ideal (proportional shortfall), is squared and the square root of the total for all the resources under consideration is used as a signal of the total discomfort of the animal. Then, total discomfort can be calculated for a range of different feed intake (and choice) levels, and thus the intake (and choice) at which total discomfort is minimum can be determined. Animal and feed parameters can be changed and the new minimal total discomfort is found, with its accompanying intake or choice. Figure 1.3 shows an example of this framework including energy, protein and bulk as the main factors controlling feed intake in the model. The U-shape of the intake/discomfort curve means that there is a range of intakes at which discomfort is close to minimum, and small deviations from optimal values will have relatively small impacts on total discomfort.

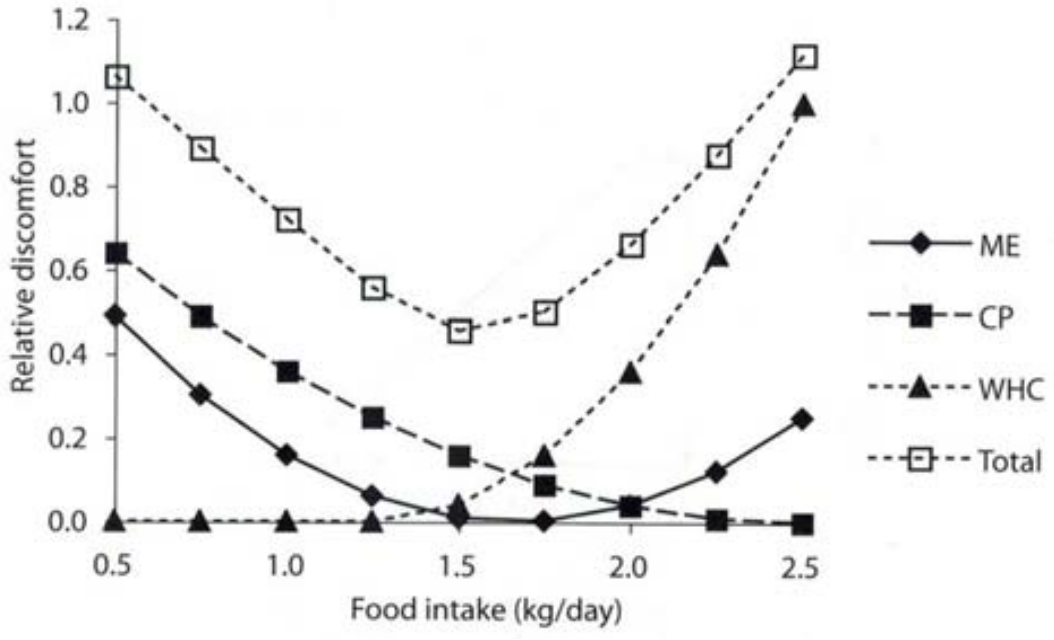

Figure 1.3. Calculations of discomfort due to deviations in the supply of energy (ME), protein (CP) and bulk (WHC) from optimum, and total discomfort (from Forbes, 2009).

1.3. Gustatory preferences and aversions in pigs

1.3.1. Sugars/carbohydrates

As in humans, sweetness is a strong pleasurable taste in pigs (Kennedy and Baldwin, 1972; Glaser et al., 2000; Kittawornrat and Zimmerman, 2010). Sugars, including different types of carbohydrates, polyols and sweeteners, are recognized by the T1R2/T1R3 heterodimeric receptor into the oral cavity and gastrointestinal tract of pigs (Moran et al., 2010; Janssen and Depoortere, 2013). In humans, some D-amino acids such as phenylalanine and tryptophan, but not their L-AA enantiomers, elicit sweet taste (Nelson et al. 2002). Artificial sweeteners also bind to T1R2/T1R3 with high affinity (high-intensity sweeteners); although most of these have been developed according to human sweet perception and they are not equally sensed by pigs (Glaser et al., 2000). Additionally, several proteins have a sweet taste and also bind to T1R2/T1R3 (Bachmanov and Beauchamp, 2007).

The behavioral response of pigs to solutions of sweet taste compounds has been carried out in different classical studies. Kare et al. (1965) examined the taste preferences of young pigs to sucrose, glucose and lactose using concentrations ranging from 5 g/l to 40 g/l and found that pigs preferred all three sugars to water, but sucrose was more attractive than glucose or lactose. Kennedy and Baldwin (1972) showed that ad-libitum fed pigs maintained their preferences for different natural and synthetic sweetener solutions during short- (1 hour) and mid-term (12 hours) choice preference tests. The concentration of each substance tested (sucrose, glucose, saccharin and cyclamate) was progressively increased. Preference for the first three substances increased with concentrations and was similar for both lengths of test. The preference thresholds ranged from 5 mM to 10 mM (1.71 g/l - 3.42 g/l) for sucrose, 10 mM to 30 mM (1.80 g/l - 5.40 g/l) for glucose, and 5 mM to 10 mM (0.92 g/l - 1.83 g/l) for saccharin. Cyclamate did not generate any preference. Glaser et al. (2000) also employed an adapted Richter-type drinking test (Richter, 1936) in growing pigs which were previously accustomed to the test procedure and then offered a large numbers of carbohydrates, polyols and sweeteners. Each choice was performed for 1 minute during which feeding behavior was monitored. All the carbohydrates tested were preferred over water, sucrose being the most strongly preferred. The molar order of effectiveness for the carbohydrates was roughly similar to that for humans: sucrose > D-fructose > maltose = lactose > D-glucose > D-galactose.

Polyols were also attractive for pigs as they are in humans, xylitol being the preferred one, as effective as sucrose. The results for sweeteners were variable, several being less preferred than is the case in humans. Thus, compounds such as aspartame, cyclamate, thaumatin or neohesperidin dihydrochalcone (NHDC) were ineffective at the doses tested, while others such as saccharin, acesulfame-K or sucralose were attractive but with a much weaker efficiency. Similarly, Nofre et al. (2002) investigated the response of pigs to 60 artificial sweetener compounds and found that 35 compounds were attractive to pigs, but less intense than in humans. Lugduname and carrelame, the two most potent sweeteners in humans, were also the most readily accepted by pigs (Kittawornrat and Zimmerman, 2010). However, it is noteworthy that the only sweeteners approved to be included in the diet of piglets according to the European Union legislation (2003) are saccharin and NHDC. All these findings, obtained in preference tests of very short duration (2 minutes - 1 hour) have supported the concept that pigs have an innate preference for sweet taste compounds. Thus, it is suggested that sweetness of a compound might be correlated by the pig with the energy content that this compound possesses.

Cereals account for more than the 60% of porcine diets and play a fundamental role in dietary appetence. Solà-Oriol et al. (2014) recently showed that dietary preferences in pigs were positively correlated with total starch content and in vitro glucose release from the main cereal in the diet (Figure 1.4). Glucose release is higher in small starch granules such as rice starch (Tester et al., 2006), and may result in stimulation of the sweet taste receptor repertoire both in the oral cavity and further down in the GIT (Roura, 2011). The presence of glucose in the GIT stimulates glucose absorption through the T1R2-SGLT1-GLUT2 system and results in activation of the enteroendocrine cells involved in the release of incretins such as GIP and GLP-1, as was previously revised. The sensing of dietary carbohydrates by enteroendocrine cells of the GIT may play an important function in the long-term feed intake of pigs. For example, it is reported that pigs preferred rice instead of corn in a 4-day choice test (Solà-Oriol et al., 2009). Pigs fed rice had higher glycemic index (GI), increased glucose absorption, and a greater and longer serum insulin response than pigs fed corn (Menoyo et al., 2011). Thus, cereals resulting in high GI may increase insulinemia causing a faster clearance of glucose in the blood and a more rapid return to a hunger state, which in turn might result in an increase in feed intake (Menoyo et al., 2011). In contrast to this thought, van Kempen et al. (2007) reported conflicting results showing that low GI diets might be beneficial for weanling

pigs in the long-term, because it results in a 14% feed intake and feed efficiency increase as compared to a diet with fast degrading starch (Roura, 2011).

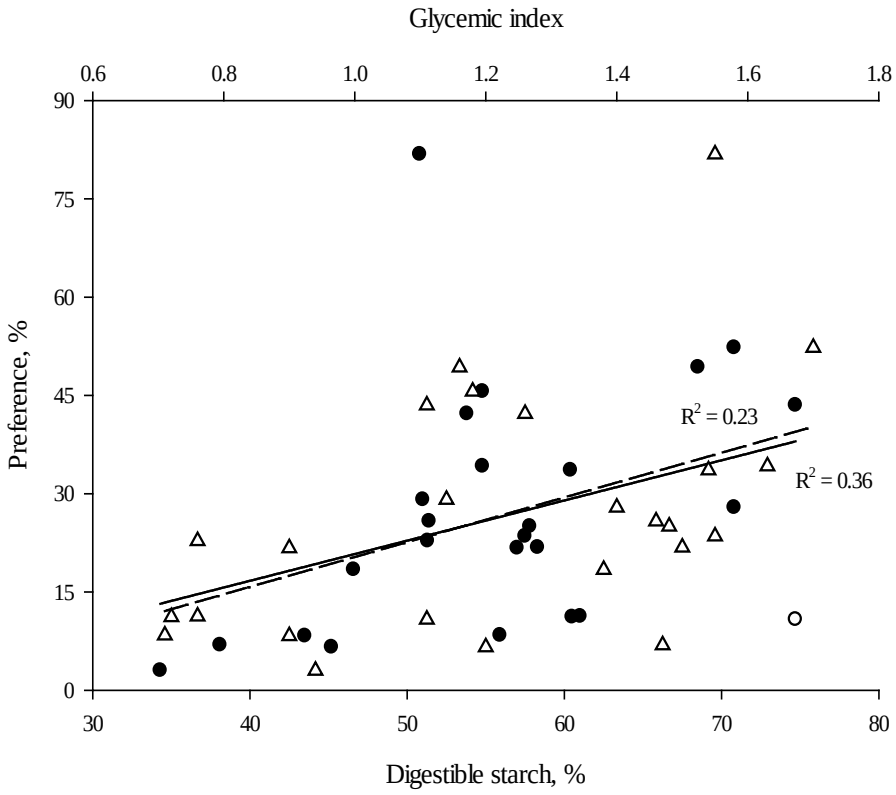

Figure 1.4. Regression lines between contents of digestible starch (circles) and glycemic index (triangles) in cereals, and their previously reported feed preference values (from Solà-Oriol et al., 2014).

1.3.2. Amino acids/proteins

Protein-derived nutrients such as L-glutamic acid (L-Glu), others L-amino acids and peptones trigger umami taste and seem to enhance voluntary feed intake in pigs (Roura and Tedó, 2009). Thus, it is suggested that umami taste, which was first discovered by Ikeda in 1909, is also a hedonic taste in pigs (Tedó, 2009). Most protein-rich dietary ingredients consist of a part of small peptides or unbound amino acids (in mM amounts) that are sufficient to stimulate taste in the tongue. The main substance eliciting umami taste in humans

is L-glutamate, an amino acid widely present in foods. The hedonic potential of L-glutamate in pigs is also enhanced by 5'-ribonucleotide monophosphates such as inosine monophosphate (IMP) and guanosine monophosphate (GMP; Ninomiya, 2002). The primary umami receptor is a heterodimer of T1R1 and T1R3 proteins. In addition to L-Glu, the affinity and array of other L-AA that stimulate T1R1/T1R3 is species dependent. However, additional receptors are also involved in the umami taste sensing, such as metabotropic GPCRs called mGluRs (1 and 4) that recognizes only glutamate and their sensitivity is enhanced by IMP (Chaudhari et al., 2000). In addition, it has recently become apparent that at least three more receptors respond to dietary protein-related nutrients in oral and non-oral tissues: GPRC6A, CaSR and GPR92. These receptors respond to several basic or aliphatic L-AA (GPRC6A), aromatic L-AA such as L-Phenylalanine and divalent cations (CaSR), and peptones (GPR92). The umami/savory receptors described above have been found in stomach, intestines, hypothalamus and heart, among other tissues (Bachmanov and Beauchamp, 2007; de Jager et al., 2013; Foster et al., 2013).

Tinti et al. (2000) employed the same preference test model than did Glaser et al. (2000) to assess gustatory responses of pigs to amino acids and compared them to human responses. Glycine (Gly), L-alanine (L-Ala), L-glutamine (L-Gln), L-hydroxiproline (L-Hyp), L-serine (L-Ser), L-asparragine (L-Asn) and L-threonine (L-Thr) showed the highest preference values in pigs. It is reported that some of these amino acids, such as Gly, L-Ala, L-Ser and L-Thr, are perceived exclusively as sweet and not umami by humans (Tinti et al., 2000). The umami TR repertoire in laboratory rodents is also widely tuned and identifies almost the full repertoire of L-AA (Nelson et al. 2002). A comparative on the L-amino acid sensing between these 3 species is shown in Table 1.4. Based on porcine T1R1/T1R3 nucleotide homologies, pig shares the highest identities with dogs and cats. Humans seem to be half the way between rodents (omnivores) and the dietary highly adapted herbivores (cow) and carnivores (dog and cat). Therefore, based on umami taste, pigs and primates are not a good model the one for the other (Roura and Tedó, 2009).

Table 1.4. Comparative gustatory responsiveness to L-amino acids and the predominant hedonic response in humans, pigs and laboratory rodents (from Roura et al., 2013a).

L-amino acid	Human response	Pig response	Rodents response
Alanine	Sweet	Umami	Umami
Arginine	Bitter	Umami	Umami
Asparagine	Bitter	Umami	Umami
Aspartic acid	Umami, sour	Umami	Umami
Cysteine	Sulphur	NA	Umami
Glutamic acid	Umami, salty	Umami	Umami
Glutamine	Sweet, umami	Umami	Umami
Glycine	Sweet	YES	Umami
Histidine	Bitter	NO	Umami
Hydroxyproline	Sweet	YES	NA
Isoleucine	Bitter	NO	Umami
Leucine	Bitter	NO	Umami
Lysine	Bitter, salty, sweet	YES	Umami
Methionine	Bitter, sulphur, umami	NO	Umami
Phenylalanine	Bitter	NO	Umami
Proline	Sweet, salty	Umami	Umami
Serine	Sweet	YES	Umami
Threonine	Sweet	Umami	Umami
Tryptophan	Bitter	Bitter	NO
Tyrosine	Bitter	NA	NO
Valine	Bitter	NO	NO

NA, not available; YES, means that there is a response but the type of taste has not been identified; NO, means no response.

L-Glu plays a central role in cell metabolism implicated in the transamination and the tricarboxylic acid cycle pathways. In particular, L-Glu is the main energy source in enterocytes. In stomach, L-Glu stimulated gastric secretion and motility in humans. The sensing of aromatic and basic L-AA and peptones results in increased gastrin secretion and increased plasma levels of CCK. In the intestine, L-AA and peptones seem also to be related in the release of incretins such as GIP, GLP-1 and GLP-2. Consequently, L-Glu seems to have the potential to reduce weight gain in humans (Roura, 2011; Janssen and Depoortere, 2013). The porcine stomach seems to be the richest tissue in some TRs expression outside the oral cavity. Overall the knowledge on dietary protein sensing suggests that L-AA and protein hydrolysates are perceived by enteroendocrine cells in the GIT and that they may play an important role in protein-induced satiety (Roura et al., 2013a).

Pigs are able to distinguish and prefer diets better balanced for L-Lys, DL-Met, L-Thr and L-Trp to the same diet deficient in the corresponding amino acid, based on the works of Kirchgessner et al. (1999), Ettle and Roth (2004, 2005), and Roth et al. (2006). Preference for Met was above optimal growth requirements and linked to the Met-source type, where DL-Met was preferred over Methionine Hydroxyl Analog. As suggested by the authors, the driver for amino acid preferences might have been a mechanism of craving for a nutritionally balanced diet, more than a taste perception-related mechanism. Roura (2011), on the other hand, suggested that taste perception seems to be another plausible explanation of those outcomes, based on the results of Tedó et al. (2009) in which 50 mM solutions of L-Lys and L-Met were preferred over plain water, but, on the contrary, L-Trp was significantly rejected. In addition, Suárez et al. (2011) found that the preference of young pigs for DL-Met, and their avoidance for L-Trp, were independent of the nutritional status (deficient, adequate, or in excess). However, the preference for L-Thr developed only after the consumption of the excess treatment. Thus, more than one exclusive mechanism may co-exist related to amino acids/umami compounds preferences in pigs. These mechanisms, and how these are related to different nutritional status of the animals, will be object of study during the present Thesis.

Solà-Oriol et al. (2011) published a systematic study showing that the protein sources from animal origin had the highest preference values. Every 1% change in the inclusion of high-quality proteins between the ranges of 5% to 20% resulted in an average increase of 5.3% over the 50% of preference (neutral value). In contrast, 1% changes in inclusion of highly preferred cereals, fibers or fats sources among the same range resulted in increases of preference of 1.5%, 1.5% and 0.6%, respectively, indicating that protein sources may have a much higher relative impact on feed preference per unit of feed ingredient rather than cereals, fats, or fiber (Solà-Oriol, 2008). In addition, Tokach et al. (2003) reviewed the ingredients that show a direct positive impact on feed intake in piglets, finding dried whey and whey protein concentrate, spray-dried animal plasma and blood meals, dried porcine solubles and high-quality fish meal between them. All these ingredients are void of plant derived anti-nutritional factors and contain a significant amount of sweet and umami active compounds. Overall, pigs seem to have higher taste acuity for amino acids and peptides than for sugars and a potential higher appetite for dietary protein compared to carbohydrates (Roura, 2011).

1.3.3. Lipids/fats

The recognition of fat stimuli was previously believed to rely mostly on textural, olfactory, and post-ingestive cues, but the finding that lipid sensors are present on the tongue suggests that fat can be considered as the 6th taste (Janssen and Depoortere, 2013). Dietary fats are cleaved by lipases to release FFA in the GIT. Short-chain fatty acids (SCFA) are absorbed in the colon by the monocarboxylate transporter isoform 1 (MCT-1), medium-chain fatty acids (MCFA) are absorbed in the intestine by passive diffusion, whereas long-chain fatty acids (LCFA) are absorbed by the fatty acid transporter CD36 and the fatty acid transport protein 4 (FATP4). Activation of a broad range of GPCRs by FFA has recently been discovered. These receptors involved in fatty acid sensing in the GIT include FFAR1 (GPR40), FFAR2 (GPR43), FFAR3 (GPR41) and GPR120 (Wellendorph et al., 2010). They are also expressed in many enteroendocrine cells while being activated by SCFA (FFAR2 and FFAR3), MCFA and LCFA (FFAR1 and GPR120; Janssen and Depoortere, 2013). For example, FFAR1 mediates insulin secretion directly and indirectly via the release of incretins, while GPR120 promotes the secretion of GLP-1, CCK and insulin (Liou et al., 2011). GPR120 has been found to be also expressed in differentiated adipocytes and macrophages, and its dysfunction leads to obesity, glucose intolerance and fatty liver in mouse and humans. Overall, fatty acid sensors seem to have important implications on the physiopathology of metabolic diseases in humans such as diabetes, dyslipidemia or obesity (Blad et al., 2012).

In pigs, there is also evidence of lipid sensing repertoire throughout the GIT (Colombo et al., 2012; de Jager et al., 2013). In addition, the expression of porcine FFAR2 and FFAR3 has been reported in enteroendocrine cells co-expressed with GLP-1, PYY and serotonin (Al-Rammahi et al., 2011). However, it is also suggested that only LCFA and not SCFA may stimulate the release of GLPs from porcine ileum (but not colon) samples (Voortman et al., 2012). Laboratory rodents exhibit a spontaneous attraction for lipid solutions when offered as a choice, suggesting that fat may be considered as an innately preferred taste in these species. Thus, it is likely that the situation is similar in pigs.

1.3.4. Minerals and acids

Sodium (salty) and protons (sour) may penetrate cell membranes through ion or ligand gated protein channels or other mechanisms that include potential intracellular targets. Sodium is recognized by the epithelial sodium channel ENaC, a trimeric receptor composed of three subunits (α, β, γ) highly selective towards sodium and present in porcine taste receptor cells (Beauchamp and Stein, 2008). Sour taste is known for acid perception and is sensed by hydrogen gated channels (PKD1L3 and PKD2L1; Ishimaru et al., 2006). However, only a moderate correlation exists between the hydrogen ion concentration of a food and its perceived sourness. Weak organic acids such as acetic, propionic, formic and lactic are able to penetrate cell membranes in an undissociated form and then dissociate inside the taste cell. Acidifying the neuronal cytosol of the trigeminal nerve in the oral cavity causes the stimulation of nociceptors leading to pungency and pain. However, strong acids such as citric and tartaric are inert at a trigeminal level resulting in no or insignificant pungency responses (Roura, 2011). In electrophysiological studies in pig taste nerves performed by Danilova et al. (1999), citric acid elicited the largest response among a wide group of taste agonists.

It has been described that in the case of deficiency of some minerals, such as sodium, calcium, or phosphorus, animals such as rodents, poultry or cattle are able to almost instantaneously select a food supplemented with the nutrient without previous experience with that food in order to reestablish homeostasis (Denton, 1982; Blair-West et al., 1992; Leshem, 1999). There is strong evidence of inherent mechanisms that act to motivate animals deficient in sodium or water, for example, to ingest avidly the substance they need once it is encountered (Galef, 1999). In a series of classic studies carried out during the 1930s and 1940s, Richter demonstrated that, when challenged either by artificially induced nutrient deficiencies or by homeostatic perturbations resulting from spontaneous changes in physiological state, rats would alter their patterns of food selection so as to redress any disturbance to internal homeostasis that they experienced (Galef, 1999). He found that adrenalectomized rats that die from sodium loss in a few days if fed only water and a standard rodent diet, they survive indefinitely if are also given access to concentrated sodium solutions that normal rats find so unpalatable that refuse to ingest (Richter, 1936). Thus, Richter suggested that ingestion by adrenalectomized rats of concentrated sodium solutions was the result of innate systems detecting sodium deficiency, identifying sources of sodium in the

external world, and motivating ingestion of sodium (Galef, 1999). Many other species such as the pig appear to similarly inherently prefer the taste of salt, and therefore it seems likely that animals use also such systems of sodium detection to maintain a constant internal milieu.

1.3.5. Anti-nutritional factors, drugs and toxins

The bitter taste system is regarded as a basic mechanism of defense against anti-nutritional, drugs or potentially toxic compounds present in the diet. The immediate result of bitter sensing is a decrease in food ingested. However, many of the undesired substances make it through the oral cavity and into the GIT where chemosensors will trigger gastrointestinal defense mechanisms such as increased neutralizing secretions (e.g. saliva), gut motility and regulation of blood flow. In addition, vomiting and food aversive behaviors might be also developed as protective responses to highly deleterious compounds (Roura, 2011). Similar to other TRs families, T2Rs have also been found in non-taste tissues throughout the GIT. Thus, bitter compounds in the stomach decreased gastric emptying and resulted in an increase of CCK release (Rozengurt, 2006). In contrast, in the large intestine T2Rs mediate a rapid passage of bitter compounds by evoking anion secretion (Cl and HCO_3) following bitter agonist stimulation (Kaji et al., 2009). Both mechanisms seem to be complementary as part of a host defense mechanisms. In addition, recent findings have disclosed the potential involvement of T2Rs against bacterial infections in the respiratory tract (Tizzano et al., 2010).

Pigs are innately averse to bitter compounds and substances. It has been reported that pigs elicit avoidance responses to antibiotics and quinine HCl, denatonium benzoate (Bitrex) and caffeine among other compounds (Blair and Fitzsimons, 1970; Nelson and Sanregret, 1997; Danilova et al., 1999). The bitter compound phenylthiocarbamide (PTC) also significantly decreased gastric emptying and increased the nutrient transport in the small intestine when added to the diet (Mani et al., 2012). Overall, the involvement of bitterness in gut motility, CCK secretion and satiety underpins a high potential for bitter compounds to manipulate feed intake in mammals (Roura et al., 2013a).

1.4. Learning and diet selection in pigs

Learning refers to the process by which experiences change the nervous system of animals and hence their behavior. Normally, we refer to these changes as 'memories'. From a physiological point of view, learning changes the way animals perceive, act, think and feel by producing changes in the nervous system in the circuits responsible for perception, in those responsible for the control of movement, and in connections between the two (Carlson, 2007).

Animals learn to associate the sensory properties of a food with the metabolic and/or toxic consequences of eating that food and to use such associations to guide subsequent feeding behavior, both in terms of the amount eaten and the choice between foods (Forbes, 1998). Animals are born with innate preferences and aversions to particular flavors (as it has been previously reviewed; Forbes, 2007). These preferences may be developed in the womb and can be manifest even before birth. For example, Hepper (2005) informed that around 15 to 16 weeks after conception, human fetuses show their sugar appreciation by swallowing more amniotic fluid when it is sweet, and less when bitter. However, innate preferences and aversions cannot be relied on for the rest of the animals' life. Thus, a food that has been tasted once or twice in the spring and has been found to be bitter might, by the summer, have become sweet; it might have changed from toxic to nutritious or vice versa. Therefore, an animal ought to be able to benefit from prior experience to be able to best exploit its environment, but also to be flexible and to be able to relearn the associations when necessary (Forbes, 2007).

In a series of studies conducted in the 1990s, Kyriazakis et al. (1990, 1991a,b) showed that growing pigs were able to control their protein intake when given a cafeteria between a high- and a low-protein diet. Thus, pigs appeared to be able to select a balanced diet that met their protein requirements avoiding excesses of protein intake. The authors observed that pigs may have changed their choice as they grew, reflecting their changing requirements (Kyriazakis et al., 1990); females selected a diet of a lower protein content than males did (Kyriazakis and Emmans, 1991), and animals were also able to correct previous underfeeding with protein by the composition of the diet that they selected (Kyriazakis et al., 1991b). In all these studies, pigs were previously given the opportunity to experience the feeds given as a choice, showing considerable variation in the selection when the previous experience was not offered (Kyriazakis et al., 1991a). This training period, with alternate exposure to the offered

feeds, may have required over six days associating the feeds with the nutritional consequences, but it may have been reduced to only around three days by using a trained individual in the group (Morgan et al., 2003).

Later, Kyriazakis et al. (1999) proposed an integrative framework of feeding behavior and diet selection for farm animals. This framework considers feeding behavior as part of a continuous close-looped system (Figure 1.5). Feeding behaviour, both in terms of food intake and diet selection, influences, and is in turn influenced by, an animal's internal state and knowledge of its feeding environment. The internal state of an animal is not a static but instead a dynamic process, being the outcome of physiological changes such as those accompanying growth and pregnancy, and the direct effects of past or current feeding. The framework suggests that feeding behaviour of animals will depend largely on learning, since learning would make the animal more effective in adapting to the temporal and spatial changes in its feeding environment. The rate at which animals learn about foods and the period during which this association is retained depends largely on the extent to which a previous disturbance has affected the animal's internal state, and on the extent of the post-ingestive consequences induced by the food. Thus, the greater the departure from an appropriate internal state the greater will be the reinforcing properties of the food and the faster the learning.

Figure 1.5. A framework for considering the way in which learning and the animal's internal state affect feeding behavior (adapted from Kyriazakis et al., 1999).

Learning can take at least four basic forms: perceptual learning, motor learning, relational learning and stimulus-response learning. Perceptual learning consists primarily of changes in perceptual systems that make possible the recognition of stimuli so that animals can respond to them appropriately. Motor learning, although it may primarily involve changes within neural circuits that control movement, is guided by sensory stimuli being a form of stimulus-

response learning. Relational learning, which is the most complex form of learning, includes the ability to recognize objects through more than one sensory modality, to recognize the relative location of objects in the environment, and to remember the sequence in which events occurred during particular episodes. Finally, stimulus-response learning consists in the ability of animals to learn to perform a particular behavior when a particular stimulus is present. Thus, it involves the establishment of connections between circuits involved in perception and those involved in movement. Stimulus-response learning includes two major categories that have been extensively studied: classical and instrumental (operant) conditioning. For the purpose of this Thesis, just stimulus-response learning will be revised now. For a complete and detailed description of the other types of learning, see the chapter of Carlson (2007).

Instrumental conditioning, also called operant conditioning, is a learning procedure whereby the effects of a particular behavior in a particular situation increase (reinforce) or decrease (punish) the probability of the behavior. That is, when a behavior is followed by favorable consequences, the behavior tends to occur more frequently; when it is followed by unfavorable consequences, it tends to occur less frequently. Instrumental conditioning involves an association between a response and a stimulus. Classical conditioning, on the other hand, is a form of learning in which an unimportant stimulus acquires the properties of an important one. It involves an association between two stimuli. A stimulus that previously had little effect on behavior becomes able to evoke a reflexive, species-typical behavior (Carlson, 2007). Classical conditioning is usually viewed as a form of Pavlovian conditioning, in which a particular flavor (the conditioned stimulus, CS+) is associated with the oral and/or post-oral properties of nutrients (the unconditioned stimulus, US; Sclafani and Ackroff, 2012). Flavor-taste (or flavor-flavor) learning refers to the process by which a preference or aversion develops for a neutral target flavor (e.g., almond flavor) that is mixed with an already preferred or avoided taste (e.g., sweet or bitter taste). Flavor-post-oral (or flavor-consequence) learning refers to the process by which a preference or aversion develops for a flavor that is associated with positive (e.g., nutrient feedback from sucrose) or negative (e.g., visceral discomfort from a food toxin) post-oral consequence. It is adaptive in allowing humans and other animals to select nutrient-rich foods and avoid potentially dangerous ones. Normally, both forms of conditioning may operate during an eating experience (Sclafani and Ackroff, 2012).

Flavor-avoidance learning has been extensively studied and revised (Sclafani and Ackroff, 2012). For example, feeding or injecting lithium chloride causes nausea in a wide range of animals and a novel food offered for a short time after such treatment causes aversion to that food after a few sessions. Similarly, aversion to some toxic substances commonly found in the diet can be conditioned by association with novel flavours; oxalic acid is present in the leaves of many root crops and conditioned taste aversions to it can persist for at least 60 days in sheep. In chickens, injection with CCK followed by eating a colored food causes conditioned aversion to that color (Forbes, 2010). On the contrary, flavors that have been associated with positive oral or post-oral effects tend to be preferred in future exposures. Thus, sweet and fatty taste compounds can normally reinforce preferences for arbitrary flavor cues when they are mixed together during conditioning sessions (Elizalde and Sclafani, 1990). However, conditioned flavor preferences can even convert a normally avoided flavor, such as bitter, into a preferred one following intra-gastric infusions of nutrients (Drucker et al., 1994). The effectiveness of intra-gastric infusions of nutrients to condition flavor preferences has been demonstrated in numerous experiments carried out in laboratory rodents in which the consumption of a novel flavor (CS+) is paired with a nutritive infusion (e.g., MSG), while another flavor (CS-) is paired with water or saline infusions as control. Then, in a subsequent choice test between CS+ and CS-, animals typically display a strong and persistent preference for CS+ over CS- (Myers et al., 2005; Ackroff and Sclafani, 2011). In pigs, it has been recently demonstrated how they can be conditioned to acquire and show preferences for new flavor cues associated with the post-ingestive effects of different protein sources, such as soybean protein concentrate or porcine digestible peptides (Figueroa et al., 2012a,b).

1.5. Summary and implications

The initiation of feeding at weaning is probably one of the most critical points in pig production systems. However, feed intake and diet selection in pigs is a complex issue involving several factors in its control and regulation. Firstly, it involves those mechanisms that allow animals to regulate meal initiation, mainly dependent in sensorial perception mechanisms that act before ingestion starts. Thus, pigs have been described to possess a high olfactory sensitivity to detect food sources from the environment. Mechanical stimuli of feeds, such as hardness, fragility and chewing effort, also influence dietary preferences. In

addition, taste sensitivity of pigs has been found to be greatly higher as compared to other animal species, like humans. Then, feed intake is regulated by post-ingestive factors that give information about nutrients and the physiological consequences after the ingestion of feed. Between them, physical factors and the contents of glucose, protein or fat released after a meal affect the amount ingested. Finally, the control of ingestion in pigs is also regulated by short- and long-term signals that act as determinants of satiety limiting the size of individual meals and maintaining energy homeostasis, such as those provided by the hormones CCK, GLP-1, insulin, leptin, among others. The CNS ultimately integrates all this different information to determine subsequent feeding behavior of animals.

Sweet taste compounds are strong pleasurable for pigs. It has been demonstrated in previous studies that pigs show a short-term preference for a large list of carbohydrates, polyols and sweeteners when tested against water, sucrose being the most strongly preferred compound in an extent similar to humans. It is suggested that pigs may have an innate preference for sweetness, but the degree of such preference in the long-term is not known. Similarly, umami taste compounds, mainly triggered by protein-derived nutrients such as L-glutamic acid and others L-amino acids are reported to be highly hedonic for pigs. Thus, amino acids such as Gly, L-Ala, L-Ser and L-Thr are also preferred by pigs when tested against water. However, the ability of pigs to select and discriminate between these pleasant sweet or umami compounds if they are both offered as a choice depending on their nutritional status has not been previously studied. Pigs appear to be able to control their protein intake by selecting a balanced diet that meets their protein requirements, avoiding a deficiency or an excess of protein intake. Nonetheless, it has not been assessed before whether pigs may perform an appropriate dietary choice to overcome a particular situation such as a protein deficiency once the status has been established. In addition, the association between the sensory properties and the post-ingestive consequences generated by the consumption of the offered sources is described to be fundamental in the development of an effective feeding behavior. Nevertheless, current conditions of the pig industry may not allow pigs to acquire the necessary learning and this fact is important to be elucidated. These and other questions will be addressed throughout this PhD Thesis. Based on the reviewed literature, the hypothesis and objectives proposed for this work are given in the next section.

2. Hypothesis and objectives

The general hypothesis tested in the present PhD Thesis was that post-weaned piglets are able to perform appropriate dietary selection patterns in relation to different physiological or nutritional status, e.g., variations in the dietary nutritional content, in the availability of nutrients or feeds, or in the animals' homeostasis.

The main objectives proposed for this Thesis were:

1. To assess whether dietary energy density affects the preference of piglets for protein or carbohydrate sources.

2. To evaluate whether post-weaned piglets submitted to a protein-deficiency status are able to select and prefer protein sources to overcome protein deficiency.

3. To study whether a long-term exposure to carbohydrate and artificial sweetener solutions has an effect on feeding behavior of post-weaned piglets.

4. To estimate whether dietary electrolyte balance influences feed preference, appetence and growth performance of post-weaned piglets.

Nine different experiments were designed to achieve these objectives, and the results are included in the following sections Chapters 1 to 4.

In Chapter 1, one experiment was conducted by using 2 isoproteic pre-starter diets differing in the digestible energy content, a high-energy (3.90 Mcal DE/kg) and a low-energy (3.35 Mcal DE/kg) diet. The objective was to assess its effect on the short-term preference of piglets for protein (porcine digestible peptides 20 g/L) or carbohydrate (sucrose 20 g/L) solutions after 14 or 21 days of feeding.

In Chapter 2, two isoenergetic pre-starter diets differing in the crude protein content were used in two experiments, a high-protein (204 g CP/kg) and a low-protein (142 g CP/kg) diet. Experiment 1 studied the ability of piglets to innately prefer a protein (porcine digestible peptides 40 g/L) instead of a carbohydrate (sucrose 40 g/L) solution when animals were fed for 8 days the low-protein diet. Experiment 2, on the other hand, assessed the role of associative learning in the appropriate selection of piglets depending on their nutritional status, by evaluating the preference of the animals for conditioned stimuli previously associated to protein or carbohydrate consequences during 18 days of protein restriction.

In Chapter 3, piglets were offered in three different experiments a long-term availability to sucrose 160 g/L, maltodextrin 160 g/L, and saccharin 0.08 g/L plus neohesperidin dihydrochalcone 0.02 g/L solutions as supplement to the standard maintenance diet. The aim was to study whether a long-term exposure for 12 days might modify the feed intake and growth performance of piglets, as well as their preference and appetence for a sweet (sucrose 20 g/L) over a protein (animal plasma 20 g/L) solution, which was assessed before and after the experience with the carbohydrate and artificial sweetener solutions.

Finally, in Chapter 4, seven isoproteic and isoenergetic starter diets differing in the dietary electrolyte balance were used in three distinct experiments. The dietary levels offered ranged from -16 mEq/kg to 388 mEq/kg. Experiment 1 was designed to evaluate changes in the acid-base status, nutrient metabolism and growth performance of piglets associated with variations on the electrolyte levels. Experiment 2 aimed to further explore the effect of diets differing in electrolyte balance on growth performance and the short-term preference of piglets. Experiment 3, in contrast, studied the long-term preference and appetence of piglets for different electrolyte balance diets

3. Chapter 1

Dietary energy density affects the preference for protein or carbohydrate solutions and piglet performance after weaning

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3.1. Abstract

Physiological state or dietary nutrient content can be determinants of the sensory perception with consequences for feed preferences. The aim of the present study was to assess whether the preference for protein or carbohydrate of piglets is affected by dietary energy density. In total, 240 weanling piglets (28 d-old, initial BW 7.2 kg ± 1.1 kg) were allocated to 24 pens (10 pigs/pen) according to BW. Piglets were split up into 2 groups and had ad libitum access to a high energy (HE, 3.90 Mcal DE/kg, crude fat 129 g/kg) or a low energy (LE, 3.35 Mcal DE/kg, crude fat 60 g/kg) diet with similar CP content (190 g/kg). Piglet performance and preference for protein [porcine digestible peptides (PDP, Palbio 62SP, Bioibérica, Palafolls, Spain) 20 g/L] or carbohydrate (sucrose 20 g/L) solutions were measured on d 14 and 21 after weaning using a double-choice test (DCHT). The LE diet promoted a higher (P < 0.05) ADFI and ADG than HE diet. Final BW on d 21 was higher (P < 0.001) for piglets fed the LE diet than piglets fed the HE diet (12.8 kg vs. 11.5 kg). Preference (P > 0.05) was not observed for protein or carbohydrate solutions on d 14 or 21 in piglets fed the LE diet. On the other hand, piglets fed the HE diet had higher (75% on d 14 and 65% on d 21, P < 0.01) preference for the sucrose solution. Dietary energy level and consequent nutrient imbalances, such as dietary protein-to-energy ratio, may affect feed preference for protein or carbohydrate solutions in piglets.

3.2. Introduction

Pigs have a complex and sophisticated biological system that allow them to regulate their feed selection, intake and self-nourishment in accordance to different nutritional or physiological states. This system is as an interconnected network that involves different organs and tissues of the body integrated by the central nervous system (Forbes, 2007; Black et al., 2009), and result in different actions of pigs towards feeds, ultimately expressed as preference or aversion. Thus, when a particular feed is consumed their post-ingestive feedback may triggers metabolic signals to alter feeding behavior.

The ability of pigs to accurately select diets to satisfy their nutritional requirements has been documented by using diets differing in CP content (Kyriazakis et al., 1991) or dietary essential AA (Kirchgessner et al., 1999; Roth et al., 2006). In these situations pigs were able to perform sensible choices to avoid the nutrient deficiency. However, evidence does not exist that pigs are able to adapt these choices depending on current nutritional status, for example through unbalances in the dietary protein-to-energy ratio. The aim of the present study was to assess whether preference for protein or carbohydrate is affected by dietary energy density in piglets.

3.3. Materials and methods

All procedures described in this study were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (UAB) and were approved by Ethical Committee on Animal Experimentation of the UAB (CEAAH 1406).

3.3.1. Diets and feeding

Two pre-starter diets differing in DE content, a high energy (HE, 3.90 Mcal DE/kg, crude fat 129 g/kg) and a low energy diet (LE, 3.35 Mcal DE/kg, crude fat 60 g/kg) with similar CP content (190 g/kg) were offered ad libitum to the piglets in mash form starting at weaning and

for 21 d. The HE diet was formulated to exceed the DE requirements of pigs by adding soybean (*Glycine max*) oil (60 g/kg) to a diet containing maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and extruded soybean (312 g/kg, 100 g/kg, 100 g/kg, and 131 g/kg, respectively). The LE diet was formulated to contain a sub-optimal DE content by adding sepiolite (29.3 g/kg; Myta, Zaragoza, Spain) to a diet containing maize, barley, wheat, and extruded soybean (105 g/kg, 350 g/kg, 120 g/kg, and 90 g/kg, respectively). Sweet milk whey (150 g/kg), soybean meal (44% CP; 50 g/kg), synthetic AA, and a vitamin and mineral premix had the same inclusion in both diets. Diets resulted in different protein-to-energy ratios, 48.7 g CP/Mcal DE and 56.7 g CP/Mcal DE for HE and LE diet, respectively. Total lysine:energy ratio, 4.1 g Lys/Mcal DE, was maintained in both diets; and Met, Met + Cys, Thr, and Trp were balanced to Lys. The AA to DE ratio was lower in the HE diet than in the LE diet for Ile (1.9 g vs. 2.2 g Ile/Mcal DE, respectively) and Val (2.3 g vs. 2.9 g Val/Mcal DE, respectively), and lower than requirements (2.1 g Ile and 2.7 g Val/Mcal DE) according to NRC (1998).

3.3.2. Animals, facilities and experimental design

In total, 240 piglets [Pietrain × (Landrace × Large White)] were weaned at 28 d of age with an average initial BW of 7.2 kg ± 1.1 kg (mixed sexes). Piglets were distributed just after weaning according to their BW into 4 blocks and allocated in a weanling room with 24 pens (10 piglets/pen). The weaning room had forced ventilation and completely slatted flooring. Each pen was equipped with one feeder and a commercial drinker. Pens were randomly assigned to the experimental treatments: either the HE or LE diet.

During the first 2 weeks after weaning, piglets were trained to the presence of 2 pans containing 800 mL of tap water for 30 min. On days 14 and 21 after weaning, the preference of 4 piglets per pen was assessed by using a 3 min double-choice test (DCHT) protocol (Solà-Oriol et al., 2009) in which protein and carbohydrate water-based solutions were tested [20 g/L porcine digestible peptides (PDP; Palbio 62SP, Bioibérica, Palafolls, Spain) vs. 20 g/L sucrose]. Solution position was rotated within pens. Feed disappearance and BW gain was monitored days 0 to 14 and days 15 to 21.

3.3.3. Calculations and statistical analysis

Preference for protein or carbohydrate solution was measured as the percentage of each solution of the total fluid intake and was compared to the neutral value of 50% by using a Student's *t*-test (SAS Inst. Inc., Cary, NC). Solution intake and the productive performance results were analyzed taking into account the dietary DE content, BW block, and their interaction with ANOVA by using the GLM procedure of SAS. Average values were compared by least square means with Tukey adjustment for multiple comparisons with an α-level of 0.05.

3.4. Results

Pigs fed the LE diet had a higher ($P < 0.05$; Table 3.1) ADFI, ADG, and BW than pigs fed the HE diet during days 0 to 14 and days 14 to 21. Differences in energy intake and G:F were not observed between groups. Final BW on d 21 was 1.31 kg higher ($P < 0.001$) for pigs fed the LE diet than the HE diet. Piglets fed the LE diet did not prefer ($P > 0.05$) PDP or sucrose (Figure 3.1). Piglets fed the HE diet preferred ($P < 0.01$) sucrose to PDP solution (75% on d 14 and 65% on d 21).

Table 3.1. Growth performance of post-weaned piglets fed the experimental diets[1]

Item	HE[2] diet	LE[3] diet	SEM	*P*-value
Days 0 to 14				
Initial BW, kg	7.17	7.18	0.005	0.42
ADG, g	155[a]	198[b]	9	< 0.01
ADFI, g	230[a]	282[b]	11	< 0.01
EI[4], Mcal DE/d	0.90	0.94	0.04	0.43
G:F	0.67	0.70	0.01	0.11
Final BW, kg	9.50[a]	10.14[b]	0.127	< 0.01
Days 15 to 21				
ADG, g	335[a]	446[b]	13	< 0.01
ADFI, g	536[a]	636[b]	28	0.02
EI, Mcal DE/d	2.09	2.13	0.10	0.79
G:F	0.64	0.71	0.03	0.13
Final BW, kg	11.51[a]	12.82[b]	0.185	< 0.001

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[1]Least square means n=12 (10 pigs per replicate).
[2]HE = high energy.
[3]LE = low energy.
[4]EI = energy intake.

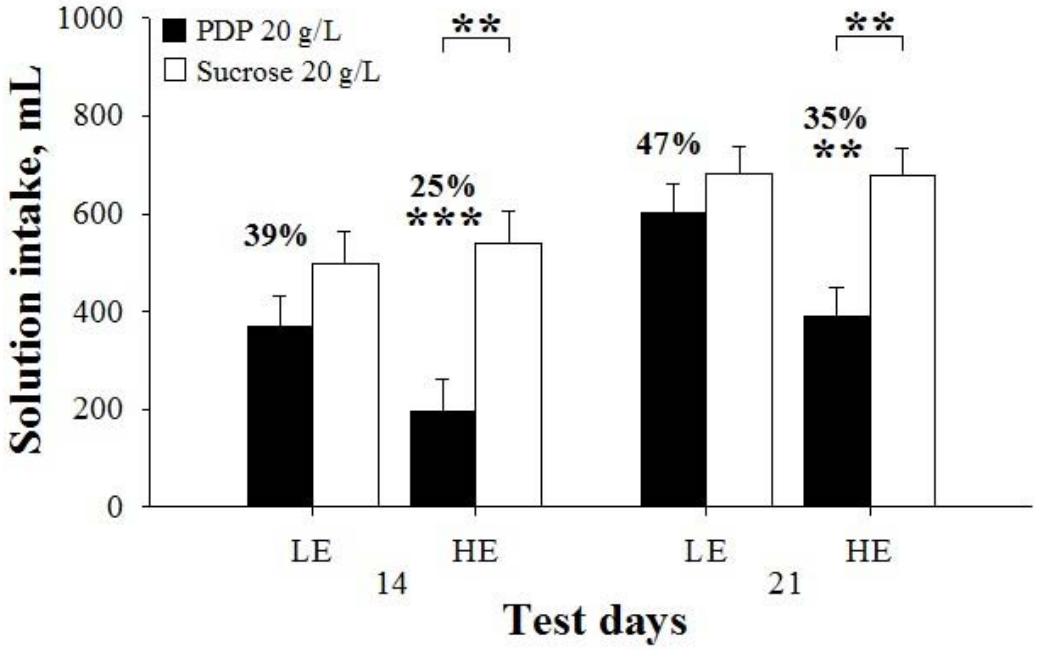

Figure 3.1. Effect of feeding a high energy (HE) or a low energy (LE) diet on intake and preference of piglets for porcine digestible peptides (PDP; Palbio 62SP, Bioibérica, Palafolls, Spain) or sucrose solutions at days 14 and 21 post-weaning. Clasps (]) indicates that the intakes of those solutions differed ($P < 0.01$). Numbers atop bars represent preference for PDP, lower (** = $P < 0.01$; *** = $P < 0.001$) than 50% in piglets fed the HE diet.

3.5. Discussion

We designed the experimental diets to generate a clear difference in the dietary energy:protein balance between 2 groups of weanling piglets. Growth performance confirmed that a HE diet (low protein-to-energy ratio) decreased the growth rate of the animals as compared to those fed an AA-balanced but LE diet. The similar energy intake between diets confirmed that the animals regulated feed intake to obtain similar daily energy intake, with consequences, such as a daily protein restriction in the HE diet. Piglets fed LE diets may have the capacity to increase feed intake, and their performance is less likely to be impaired (Beaulieu et al., 2009).

The unexpected sucrose preference by piglets fed on the HE diet indicates that piglets were unable to express an innate preference for protein in the case of the protein deficient status. On the other hand, sucrose was preferred by pigs fed the HE (high fat) diet, likely reflecting changes in the perception of sweet taste by these pigs. Moreover, the absence of a learning process in this time framework (Kyriazakis et al., 1991), where an association could be made by pigs between the sensory properties of tested feeds and the physiological consequences of eating them, might have reduced the ability of the pigs to make sensible choices. This should be considered for future research on this topic.

4. Chapter 2

Influence of the protein status of piglets on their ability to select and prefer protein sources

Physiology & Behavior 2014;129:43–9

4.1. Abstract

Pigs may have retained the capacity to choose feeds based on their nutritional requirements, even after decades in which they are not allowed to select their diet composition due to the common feeding systems of the intensive pig industry. We used 480 early-weaned piglets in two experiments to assess their ability to select and prefer protein-related sources, depending on their protein status. Piglets were fed after weaning with two isoenergetic diets formulated to contain an optimal or sub-optimal crude-protein (CP) content, a high-protein (HP, 204 g CP/kg as-fed) or a low-protein diet (LP, 142 g CP/kg), respectively. In Experiment 1, the preference of piglets was assessed by using a choice test between protein (porcine digestible peptides [PDP] 40 g/L) and carbohydrate (sucrose 40 g/L) water-based solutions for a period of three minutes. Piglets showed higher intake and preference for the sucrose 40 g/L than for the PDP 40 g/L solution, independently of the dietary CP content (9.8 mL/kg body weight [BW] vs. 3.7 mL/kg BW and 10.4 mL/kg BW vs. 4.3 mL/kg BW in HP and LP pigs, respectively). In Experiment 2, piglets were given eight training sessions in which two equally preferred flavors were mixed with protein (porcine animal plasma 60 g/L, CSp) or carbohydrate (maltodextrin 60 g/L, CSc) solutions. In the subsequent choice test, piglets fed the HP diet showed a tendency to a higher intake of CSc than of CSp (6.5 mL/kg BW vs. 5.4 mL/kg BW). On the other hand, piglets fed the LP diet showed a higher intake and preference for CSp than for CSc (15.5 mL/kg BW vs. 10.2 mL/kg BW), differences being higher for medium and low BW piglets than for heavy ones. The results show that piglets are unable to express a specific appetite for protein to correct previous underfeeding with it; however, they may show an appropriate dietary selection pattern in order to overcome protein deficiency through associative learning.

4.2. Introduction

Pigs in the intensive industry are usually fed single, complete diets intended to fully satisfy nutritional requirements for growth. Animals may decide how much of the feed offered they eat, but not choose or prefer a certain feed according to its palatability or post-ingestive consequences. Nonetheless, some seminal references on this topic report that pigs have retained the capacity to choose feeds based on their nutritional requirements. When giving pigs a long-term choice between a pair of feeds, a combination of which is not limiting, pigs appear to select a balanced diet that meets their protein requirements and avoids an excess of protein intake (Kyriazakis et al., 1990, 1991a). Pigs may change their choice as they grow, to reflect their changing requirements (Kyriazakis et al., 1990); females select a diet of lower protein content than males do (Kyriazakis and Emmans, 1991), and animals are also able to correct previous underfeeding with protein by the composition of the diet that they select (Kyriazakis et al., 1991b). In the same way, pigs have shown specific selection for diets differing in the levels of lysine (Kirchgessner et al., 1999), methionine (Roth et al., 2006), threonine (Ettle and Roth, 2005) or tryptophan (Ettle and Roth, 2004).

The wide range of scenarios in which pigs make appropriate choices, concerning different diets and rapid compensatory growth rates after abrupt diet changes, suggests that the rate of metabolism of the young pig rapidly responds to dietary changes in the protein content or in its quality (Kyriazakis and Emmans, 1990). It is remarkable that pigs showed appropriate choices when two diets were previously tested or with familiar feedback (Kyriazakis et al., 1990, 1991a,b; Kyriazakis and Emmans, 1991), and they showed considerable variation when the previous experience was not offered (Kyriazakis et al., 1991a). The closer in nutritional composition the two feeds were, the less able were the animals to discriminate between them (Staddon, 1983; Solà-Oriol et al., 2009). It seems that pigs associate the properties of the feeds, such as their odor, taste or texture, with the nutritional feedback signals during the previous single-diet experience period, as we have also observed when a new flavor was associated with the consumption of different protein sources (Figueroa et al., 2012a,b).

However, some results have also shown that pigs may show innate large differences in the choice between pairs of feeds when the animals do not have previous separate contact with the diets (Solà-Oriol et al., 2009, 2011). The high preference also displayed for sweet solutions in short-term tests suggests that pigs may innately detect this hedonic flavor in the

environment by different mechanisms that probably evolved through years to favor the intake of highly caloric foods (Dulac, 2000), sucrose being the most strongly preferred carbohydrate for pigs (Glaser et al., 2000). Similarly, the umami taste mainly elicited by the amino acid L-glutamate evokes hedonic responses in pigs and may drive animals for the detection of protein sources from the environment (Beauchamp, 2009).

There is no certainty whether pigs may immediately change their hedonic reactions and feeding preferences when they experience a non-optimal internal state. However, alliesthesia may explain that specific compounds could generate more pleasure when the internal status of the animal needs that element (Cabanac, 1971). On the other hand, pigs may require a learning period with the feedback signals from the gastrointestinal tract and metabolism to increase the acceptance or preferences for the restricted nutrient. In the present study, we propose the hypothesis that growing pigs will shift in preference to protein intake in order to correct a previous protein underfeeding, and this will be performed by exclusively using the intrinsic flavors of a highly palatable protein source (Experiment 1). In the scenario that they were not able to show this rapid response, we aim to test the hypothesis that piglets with previous underfeeding with protein will acquire a preference for new flavor cues through associative learning with the post-ingestive consequences of a protein source (Experiment 2).

4.3. Material and methods

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

4.3.1. Animals and housing

In total, 480 male and female piglets (Pietrain × [Landrace × Large White]) were selected to be used in two experiments. Piglets were weaned at 28 days of age, with an average initial body weight (BW) of 7.2 kg ± 1.10 kg (mean ± S.D.) in Experiment 1, and 7.2 kg ± 1.08 kg in

Experiment 2. In each experiment, 240 piglets were distributed into four blocks of weight of 60 animals each (Light: 5.7 kg ± 0.06 kg, Middle-light: 6.8 kg ± 0.01 kg, Middle-heavy: 7.6 kg ± 0.02 kg, and Heavy: 8.7 kg ± 0.01 kg). These were further distributed into six pens of 10 piglets in a weanling room with 24 pens. Within each weight class, three pens were randomly assigned to a high-protein diet (HP) and three to a low-protein diet (LP). The division into blocks of weight reduced the experimental variability and allowed for studying the effect of the interaction between the BW category at weaning and the experimental treatments. The weaning room had automatic, forced ventilation and completely slatted flooring. Each pen (3.2 m^2 in floor area) was equipped with a feeder with three feeding spaces and an independent water supply to ensure *ad libitum* feeding and freshwater access.

4.3.2. Experimental diets and feeding

During lactation, piglets were supplemented with a creep-feed diet from 10 days of age until weaning. The term “creep-feed” refers to the milk-replacer feed offered to the piglets (litters) during the suckling period in order to familiarize the animals with solid feed as early as possible. Creep-feed was formulated without the addition of supplemental flavors.

Two isoenergetic pre-starter diets differing in crude-protein (CP) content, a HP and a LP diet were formulated and offered to the animals from weaning to 18 days post-weaning (Table 4.1). The HP diet was formulated to satisfy the CP requirements of pigs, whereas the LP diet was formulated to contain a sub-optimal CP content to support potential growth of piglets and thus to promote a severe deficiency for some essential amino acids. A total lysine/digestible energy ratio of 4.1 g Lys/Mcal DE was maintained in both diets; and the content of methionine, methionine + cysteine, threonine, and tryptophan was balanced to lysine according to ideal ratios for protein accretion (NRC, 2012). However, the content of isoleucine, valine and other essential dietary amino acids were not balanced to lysine, and their contributions in the LP diet were lower (1.6 g Ile and 1.4 g Val/Mcal DE) than were the requirements for weanling pigs (2.2 g Ile and 2.8 g Val/Mcal DE; NRC, 2012). This strategy in the design of the LP diet was performed attempting to simulate what occurs when low-protein diets are designed with the supplementation of available synthetic amino acids, but

that may become deficient in other essential amino acids such as isoleucine, valine or arginine. Both diets were offered *ad libitum* in mash form.

Table 4.1. Composition, chemical analysis and estimated nutrient content of the pre-starter diets used in the experiments.

	High-protein diet	Low-protein diet
Ingredients (g/kg DM)		
Maize	105.3	450.0
Barley	122.5	117.2
Wheat	300.0	107.0
Soybean oil	2.1	5.8
Extruded soybean	150.0	100.0
Soybean meal 44% CP	50.0	-
Fishmeal LT	25.0	15.0
Animal plasma 80% CP	50.0	15.3
Sweet milk whey	174.0	146.0
Calcium carbonate	7.9	6.5
Monocalcium phosphate	4.9	12.4
L-Lysine-HCl	2.5	9.8
DL-Methionine	1.3	3.8
L-Threonine	0.5	4.1
L-Tryptophan	0.1	1.3
Mineral-vitamin mix[a]	4.0	4.0
Salt	-	1.8
Chemical analysis (g/kg DM)		
Dry Matter	906.1	897.4
Crude protein	204.1	141.9
Neutral detergent fiber	8.2	7.6
Fat	60.1	65.3
Ash	57.8	47.5
Estimated nutrient content (g/kg DM)		
Digestible energy (Mcal/kg)	3.60	3.60
Lysine	14.8	14.8
Methionine	4.5	6.0
Methionine + cysteine	8.7	8.7
Threonine	9.6	9.6
Tryptophan	2.9	2.9
Isoleucine	8.8	5.6
Valine	6.8	4.9

[a] Supplied per kg of feed: 3 mg of ethoxiquin, 14000 UI of vitamin A, vitamin D 1000 UI as vitamin D_3 and 500 UI as 25-hydroxycholecalciferol, vitamin E 50 mg as alpha-tocopherol acetate and 40 mg of RRR-alpha-tocopherol, 2 mg of vitamin K_3, 3 mg of vitamin B_1, 7 mg of vitamin B_2, 3.5 mg of vitamin B_6, 0.06 mg of vitamin B_{12}, 45 mg of nicotinic acid, 17 mg of pantothenic acid, 0.2 mg of biotin, 1.5 mg of folic acid, 40 mg of Fe, Cu 5 mg as cupric sulfate pentahydrate and 15 mg as cupric chelate of glycine, Zn 80 mg as zinc oxide and 25 mg as zinc chelate of glycine, Mn 25 mg as manganese oxide and 15 mg as manganese chelate of glycine, 0.7 mg of I, Se 0.1 mg as organic selenium and 0.2 mg of sodium selenite, 0.1 mg of Co.

4.3.3. Experimental designs

4.3.3.1. Preference of piglets for protein sources in a protein-deficiency status (Experiment 1)

The experimental design included a pre-training of piglets to the presence of two pans in each pen during the first week after weaning, a choice test and first-contact (FC) measure between protein and carbohydrate solutions on Days 8 and 9, and the assessment of pig performance from weaning to Day 18.

Piglets fed the HP and LP diets were familiarized to the weanling room and pre-trained with two pans containing 800 mL of tap-water in each pen for 30 minutes. This procedure intended to stimulate the approach of the animals during testing, as was reported in a previous study conducted in our group (Figueroa et al., 2012a). Then, the choice test was performed for the 10 piglets of each pen with two pans placed for three minutes in the front of the pens containing 800 mL of either 40 g/L of porcine digestible peptides (PDP; Palbio 62SP, Bioibérica; Palafolls, Spain) as protein solution (0.0248 g of CP, 0.13 kcal DE/mL) or 40 g/L of commercial sucrose as carbohydrate solution (0.16 kcal DE/mL). The rationale for the use of PDP 40 g/L as protein solution was because it is a high digestible protein source (620 g of CP/kg), whose amino acid composition contains a great amount of glutamic acid (14%), which is the main substance eliciting umami taste. In addition, it has been reported in previous studies that the addition of PDP promotes strong preferences, as compared to soybean meal, when added to the weanling diets of pigs (Solà-Oriol et al., 2011). In turn, the use of sucrose 40 g/L as carbohydrate solution was decided due to the fact it promotes high hedonism and preference responses in short-term tests against water (Kennedy and Baldwin, 1972; Baldwin, 1996; Glaser et al., 2000).

The testing situation was conducted on the pen group (12 pens per treatment) rather than on an individual animal in order to avoid changes in the location of the piglets during the test and the consequent fearful behavior of piglets in isolated conditions. To control for side preference, solution position inside the pen was changed within pens and between days of the test, i.e., the protein solution was offered on the left side of the pen and the carbohydrate solution on the right side for half the pens of each diet group on Day 8 after weaning. On Day 9 after weaning, the left-right solution position was rotated in relation to the position of the

previous day. Piglets were not water-restricted during the tests. However, the feeders were removed from the pens approximately one hour before the test and were re-offered just after finishing it. This action ensured the attention of the animals by the time the choice test was performed. The number of piglets at a pan during the first 15 seconds after offering the solutions (first contact, FC) was recorded as a measure of the palatability of each solution. This is an observational measure also reported previously (Figueroa et al., 2012a), in which one observer (the same for both test's days) stayed at the front of each pen and registered the number of animals that effectively ingested the protein or carbohydrate solutions.

Feed disappearance and BW of piglets was monitored from weaning to Day 8 and from Days 8 to 18 post-weaning in order to calculate the average daily feed intake (ADFI), average daily gain (ADG) and feed:gain ratio (FGR) during the experimental period.

4.3.3.2. The value of associative learning on diet selection of piglets in a protein-deficiency status (Experiment 2)

Piglets fed with the HP and LP diets were given eight alternate training sessions from Days 10 to 17 after weaning in this experiment. Two different flavors (conditioned stimulus, CS) were mixed with protein or carbohydrate water-based solutions (unconditioned stimulus, US) and were offered to the animals in an extra container with a total amount of 5000 mL. Porcine animal plasma (60 g/L; AP820, APC; Ankeny, USA) was used as protein solution (0.042 g of CP, 0.2325 kcal DE/mL), while spray-dried maltodextrin (60 g/L; dextrose equivalent 12 to 16, C*Dry MD 01910, Cargill Inc.; Minneapolis, USA) was used as carbohydrate solution (0.24 kcal DE/mL). Porcine animal plasma represents, as well as does PDP, an animal protein ingredient commonly used in swine diets (700 g of CP/kg). The amino acid composition of porcine animal plasma, in general, does not differ much from that of PDP, with an approximately 1% increase in the contents of lysine, threonine and tryptophan, and an approximately 2% increase in the contents of cysteine and glutamic acid (as-fed basis). In relation with the carbohydrate solution, maltodextrin with a low dextrose equivalent value was used, rather than the high-hedonic sucrose, in order to focus on the association with the post-ingestive consequences of the carbohydrate. A recent study of Roura et al. (2013) shows that piglets did not show a significantly higher preference for a

maltodextrin solution below the concentration of 30 g/L, but they showed a preference for a 60 g/L concentration, compared against water, in a 2-minute choice test. In addition, another study of flavor conditioning in pigs by using a maltodextrin solution reported no conditioned preference for flavors paired with 22.5 g/L of maltodextrin (Clouard et al., 2012). This information was taken into account when determining the dose of maltodextrin during the training period, in order to avoid a failure in the perception of maltodextrin by piglets.

Two water-soluble flavors (strawberry and creamy-cheese, 0.4 g/L; Lucta SA; Montornès del Vallès, Spain) were used as CS and counterbalanced across the replicates of each treatment (n=12 per treatment) to act as CS related to protein (CSp) or carbohydrate (CSc) consequences. Flavors used were previously tested to be equally preferred by pigs (data not shown). The stimuli were counterbalanced across diets and pens. For half the pens of each diet group, strawberry was the CS for the maltodextrin solution and creamy-cheese was the CS added to the animal plasma; for the other pens, the flavor-solution pairs were strawberry-protein and creamy-cheese maltodextrin. For half the pens of each diet group, the maltodextrin solution was presented on Days 1, 3, 6 and 8 of training, and the protein on Days 2, 4, 5 and 7; the other pens received protein on Days 1, 3, 6 and 8, and carbohydrate on Days 2, 4, 5 and 7. Training sessions lasted until the containers were empty, without an accurate estimation of the individual solution intake of each piglet.

After the training period, from Days 18 to 21 after weaning, a preference test between CSp and CSc, and an appetence test by using a one-pan test were performed. These tests were non-reinforced, i.e., just flavored water was offered to the animals. Four piglets of each pen were randomly selected to be tested as a group over two days, either in the choice test or in the one-pan test (the same four animals for each test). The reason behind testing a group of four instead of 10 animals, as in Experiment 1, was due to the fact that for this experiment the piglets were older and with a higher ingestive capacity, in comparison with the animals of the previous one. Therefore, by using this design, a likely lack in the total fluid offered during tests was avoided. The choice test was conducted by offering the animals two different pans containing 800 mL of CSp and CSc for three minutes. As in Experiment 1, to control for side preference, solution position inside the pen was changed within pens and between days of the test. To perform the one-pan test, a single pan containing 800 mL of one of the conditioned flavors (CSp or CSc) was offered to the piglets on alternate days. The order of testing first CSp or CSc on the days was changed within pens, as well as the order of testing first the

choice or one-pan test that was randomized within replicates of each treatment. Feed disappearance and BW of piglets in this experiment was also monitored from weaning to Day 8 and from Days 8 to 18 post-weaning.

4.3.4. Calculations and statistical analysis

Solutions intakes measured for each pen during the 2-day choice test were averaged and the mean value was considered for the analysis. Then, these values, as well as the one-pan test's registers, were averaged for the number of piglets that performed each test (10 piglets in the choice test of Experiment 1, and four piglets in the choice and one-pan tests of Experiment 2), and were standardized to the different weights of the animals in each treatment and experiment by dividing by the registered BW on the test days. The standardization aimed to make the solution intake registered for animals with different BW comparable; therefore, it diminishes differences in consumption due to different ingestive capacities of the animals.

Choice-test data were analyzed with ANOVA by using the MIXED procedure of SAS (version 9.2, SAS Institute; Cary, USA), taking into account the dietary CP content (HP or LP diet), block of weight (light, middle-light, middle-heavy or heavy), and their interaction as main factors. When the interaction between diet and block did not reach significance in a first analysis, it was removed from the final model. The pen of 10 and the group of four piglets were considered the experimental unit and entered into the model as a repeated measure, specifying the covariance matrix structure as compound symmetry (which yielded the lowest Bayesian information criteria). In addition to the intake registers, the preference values for the protein solution in Experiment 1 or CSp in Experiment 2 were measured as the percentage of each solution of the total fluid intake and were compared to the neutral value of 50% of preference and between each treatment by using a Student's *t*-test.

Data from first contact with the pans in Experiment 1, one-pan test in Experiment 2, and feed intake and growth performance (BW, ADG and FGR) in both experiments were analyzed with a statistical model considering the same main factors previously described with ANOVA by using the GLM procedure of SAS. For all of the analysis, average values were compared by least-squares means with the Tukey adjustment for multiple comparisons. The alpha level

used for the determination of significance was 0.05, and tendencies for $0.05<P<0.1$ are also presented.

4.4. Results

4.4.1. Feed intake and growth performance of piglets

The effect of the dietary CP content on feed intake and growth performance of piglets in both experiments is shown in Table 4.2. In Experiment 1, piglets fed the LP diet had a lower feed intake [$F(1,18)=8.15$, $P=0.01$] and BW [$F(1,18)=21.31$, $P<0.001$] than did piglets fed the HP diet on Day 8 post-weaning. Accordingly, lower ADG [$F(1,18)=24.19$, $P<0.001$] and worse FGR [$F(1,18)=14.67$, $P=0.01$] were achieved for the piglets fed the unbalanced diet during the experimental period. A similar situation was observed in Experiment 2, with lower feed intake [$F(1,19)=21.09$, $P<0.001$], BW [$F(1,19)=75.85$, $P<0.001$], ADG [$F(1,19)=73.16$, $P<0.001$] and higher FGR [$F(1,19)=76.59$, $P<0.001$] for the piglets fed the LP diet than for piglets fed the HP diet during training sessions (Days 10 to 17 post-weaning) as well as tests days (from Day 18 after weaning).

Table 4.2. Feed intake and growth performance of piglets fed with the high-protein (HP) and low-protein (LP) diets in Experiments 1 and 2 during the experimental periods.

	Experiment 1				Experiment 2			
	HP	LP	SEM	*P*-value	HP	LP	SEM	*P*-value
Weaning to Day 8								
Initial BW, kg	7.17	7.19	0.014	0.30	7.18	7.18	0.004	0.61
ADFI, g/d	181.6	146.8	8.8	< 0.05	199.9	149.8	7.7	< 0.001
ADG, g/d	140.9	75.5	9.6	< 0.001	160.0	63.9	7.9	< 0.001
FGR	1.36	2.02	0.126	< 0.01	1.26	2.45	0.096	< 0.001
Final BW, kg	8.30	7.80	0.078	< 0.001	8.46	7.69	0.062	< 0.001
Days 8 to 18								
ADFI, g/d	455.5	268.6	8.0	< 0.001	418.4	272.8	14.6	< 0.001
ADG, g/d	304.9	136.7	11.4	< 0.001	364.0	164.4	7.2	< 0.001
FGR	1.51	1.99	0.084	< 0.001	1.15	1.65	0.036	< 0.001
Final BW, kg	11.42	9.16	0.098	< 0.001	12.46	9.50	0.119	< 0.001
Weaning to Day 18								
ADFI, g/d	334.9	214.4	6.2	< 0.001	326.4	221.0	9.8	< 0.001
ADG, g/d	235.7	109.5	5.5	< 0.001	278.1	122.1	6.3	< 0.001
FGR	1.44	1.97	0.047	< 0.001	1.18	1.81	0.028	< 0.001

BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; FGR, feed:gain ratio.

4.4.2. Experiment 1

4.4.2.1. Choice test

When piglets fed with the HP or LP diet were given the opportunity to choose between PDP 40 g/L and sucrose 40 g/L, they showed a higher intake of sucrose 40 g/L than of the PDP 40 g/L solution, independently of the dietary CP content [9.8 mL/kg BW vs. 3.7 mL/kg BW, $F(1,8)=555.99$, $P<0.001$ in HP pigs, and 10.4 mL/kg BW vs. 4.3 mL/kg BW, $F(1,5)=268.46$, $P<0.001$ in LP pigs; Figure 4.1]. The preference observed for the protein solution, 27% in piglets fed the HP diet and 30% in piglets fed the LP diet, was significantly lower than the neutral value of 50% in both groups of animals [$t=-8.74$, $df=11$, $P<0.001$ in HP pigs, and $t=-5.98$, $df=8$, $P<0.001$ in LP pigs] and was not significantly different between them [$t=-0.50$, $df=19$, $P=0.62$].

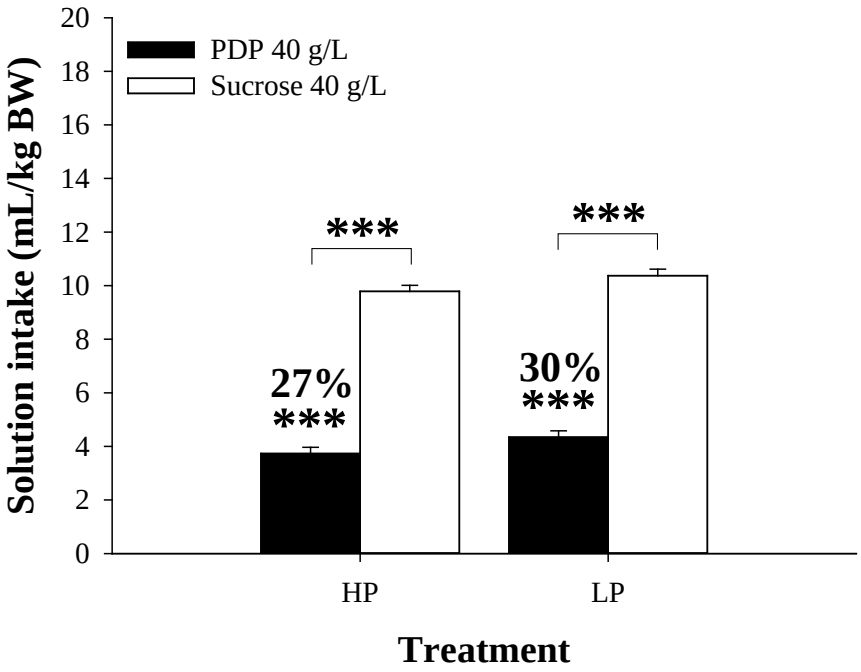

Figure 4.1. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on intake and preference of piglets for porcine digestible peptides (PDP) 40 g/L or sucrose 40 g/L solutions during the choice test conducted in Experiment 1. Clasps indicate different intakes between both solutions (***=P<0.001). Numbers on top of the bars represent percent intake of PDP and its difference from the neutral value of 50% (***=P<0.001).

4.4.2.2. First contact of piglets

Piglets fed the LP diet showed a statistical tendency for more FC with the sucrose 40 g/L than with the PDP 40 g/L solution [F(1,19)=3.89, P=0.06; Figure 4.2]. No differences were observed in the FC of piglets fed the HP diet with the protein or carbohydrate solution [F(1,19)=1.91, P=0.18]. Overall, the FC score of piglets fed the unbalanced diet was higher than was that of piglets fed the balanced one [3.9 piglets/pan vs. 2.7 piglets/pan, F(1,43)=15.26, P<0.001].

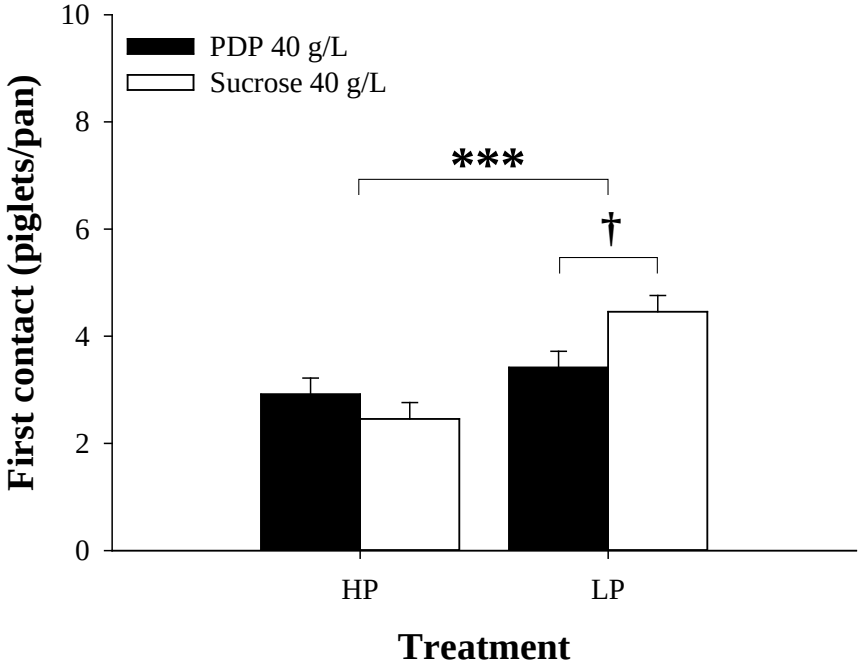

Figure 4.2. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on the first contact of piglets with porcine digestible peptides (PDP) 40 g/L or sucrose 40 g/L solutions during the choice test conducted in Experiment 1. Clasps indicate different first contact score between either solutions or experimental treatments (†=P<0.1, ***=P<0.001).

4.4.3. Experiment 2

4.4.3.1. Choice test

The choice performed by the piglets fed the HP and LP diets for CSp or CSc after training sessions is shown in Figure 4.3. Piglets fed the HP diet showed a tendency for a higher intake of CSc than of CSp [6.5 mL/kg BW vs. 5.4 mL/kg BW, F(1,7)=3.57, P=0.1]. The preference observed for CSp also showed a tendency to be lower than the neutral value [44%, t=-2.12, df=10, P=0.06]. On the other hand, piglets fed the LP diet showed a higher intake of CSp than of CSc [15.5 mL/kg BW vs. 10.2 mL/kg BW, F(1,5)=20.67, P<0.01]. The preference for CSp tended to be higher than the neutral value in this case [61%, t=2.06, df=8, P=0.07], and was significantly higher than was the preference in piglets fed the HP diet [t=-2.96, df=18,

P<0.01]. No interaction was observed concerning the values of flavor choice for the different blocks of BW in the HP group [*F*(3,7)=0.63, *P*=0.62], however, piglets fed the LP diet showed dissimilar intakes of CSp and CSc, depending on their BW [*F*(3,5)=9.73, *P*<0.05; Figure 4.4]. Thus, no different intakes of CSp or CSc were observed in heavy and middle-heavy piglets [*F*(3,5)=3.28, *P*=0.12, and *F*(3,5)=3.95, *P*=0.09, in heavy and middle-heavy piglets, respectively]. Nevertheless, middle-light and light piglets fed the unbalanced diet showed the higher intakes of CSp in comparison with those of CSc [*F*(3,5)=6.41, *P*=0.04, and *F*(3,5)=19.12, *P*<0.01, in middle-light and light piglets, respectively].

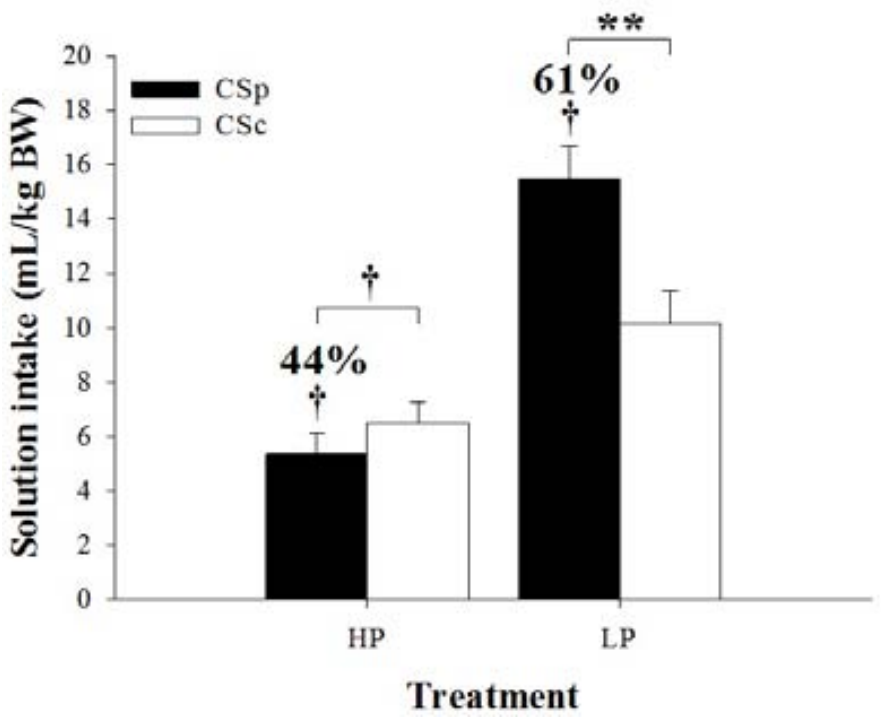

Figure 4.3. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on intake and preference of piglets for conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the choice test conducted in Experiment 2. Clasps indicate different intakes between both solutions (†=*P*<0.1, **=*P*<0.01). Numbers on top of the bars represent percent intake of CSp and its difference from the neutral value of 50% (†=*P*<0.1).

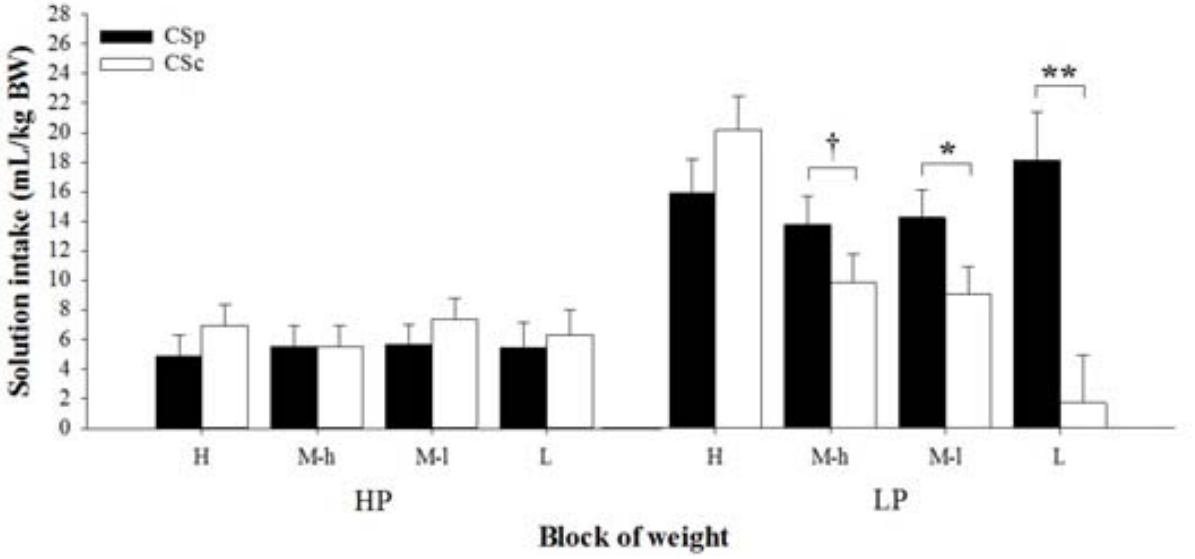

Figure 4.4. Effect of the block of weight (light [L], middle-light [M-l], middle-heavy [M-h] and heavy [H]) of piglets fed with a high-protein (HP) or a low-protein diet (LP) on their intake of conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the choice test conducted in Experiment 2. Clasps indicate different intakes between both solutions (†=P<0.1, *=P<0.05, **=P<0.01).

4.4.3.2. One-pan test

No different intakes of CSp or CSc were observed in piglets fed the HP and LP diets during the one-pan access [F(1,18)=0.70, P=0.41 in HP pigs, and F(1,18)=0.23, P=0.64 in LP pigs; Figure 4.5]. Overall, the intake of CSp and CSc in piglets fed the LP diet was higher than was the intake of flavors in piglets fed the HP diet [21.2 mL/kg BW vs. 10.4 mL/kg BW, F(1,41)=26.00, P<0.001].

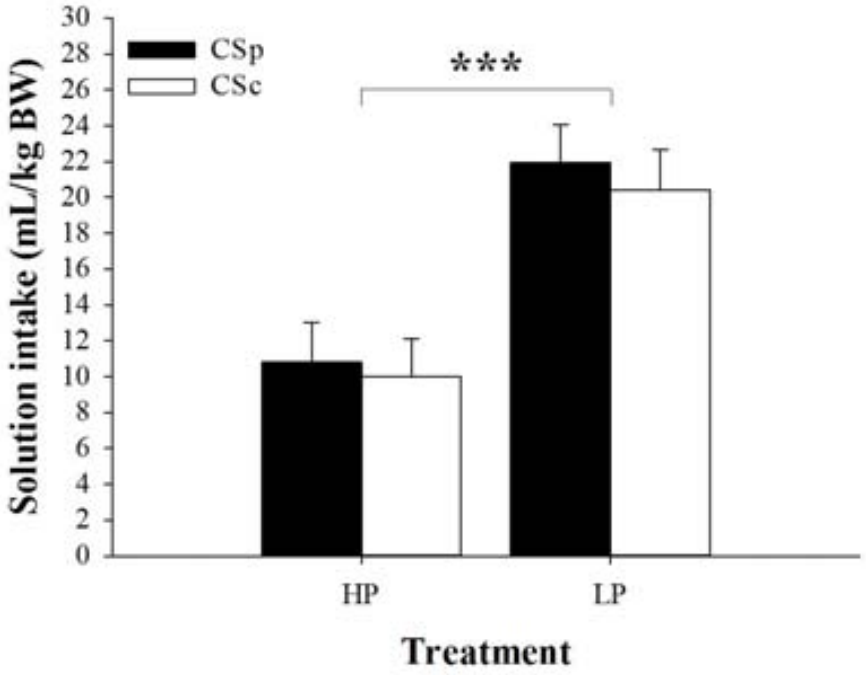

Figure 4.5. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on the intake of piglets of conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the one-pan test conducted in Experiment 2. Clasp indicates different intakes between experimental treatments (***=P<0.001).

4.5. Discussion

The present work gives support to the concept that pigs are able to detect metabolic changes caused by underfeeding the amount or quality of protein, and they modify their evaluation of flavors through associative learning (Experiment 2). Pigs do not appear to be able to select and prefer almost instantaneously a protein source after a period of underfeeding with protein (when tested against sucrose, Experiment 1).

Differences in the physiological or nutritional status of pigs may frequently occur in the intensive pig industry, especially at weaning or along the nursery period, with significant impact on later growth performance of the animals. In the present study, the LP diet (39.4 g CP/Mcal DE) decreased the growth rate of the animals, as compared to those fed the HP diet (56.6 g CP/Mcal DE), even when diets contained similar amounts of lysine (14.8 g/kg),

methionine + cysteine (8.7 g/kg), threonine (9.6 g/kg) and tryptophan (2.9 g/kg). The protein-to-energy ratio of the LP diet was lower than the range of 53 g CP/Mcal DE to 71 g CP/Mcal DE described by NRC to prevent an influence on the performance of starter pigs (NRC, 2012). Experimental evidence shows that specific protein selection by pigs may be evoked by diets varying in their overall protein content (Kyriazakis et al., 1990, 1991a), and also related to the quality of the protein source as reflected by the appetite for some specific amino acids (Kirchgessner et al., 1999; Ettle and Roth, 2004, 2005; Roth et al., 2006).

However, our results show a higher intake and preference for sucrose 40 g/L than for the PDP 40 g/L solution during the choice test conducted in Experiment 1, suggesting that piglets were unable to express a rapid or specific appetite for protein to correct previous underfeeding with it by exclusively using the intrinsic flavors of the offered sources. These results are in close agreement with a previous study conducted in our group, in which piglets fed a high-caloric-content diet (high fat, low protein-to-energy ratio) were not able to express an innate change in the preference for protein (PDP solution), as compared to sucrose, in a short-term choice study (Guzmán-Pino et al., 2012). It has been described that in the case of deficiency of some nutrients such as sodium, calcium or phosphorus, animals are able to select almost instantaneously a food supplemented with the nutrient without previous experience with that food in order to reestablish homeostasis (Denton, 1982; Blair-West et al., 1992; Leshem, 1999). The suggestion involves the idea that animals may use “specific appetites” to select appropriate diets. In contrast, our results in a 3-minute choice test and those in the literature are contradictory. Early studies proposed that protein-deprived rats have an unlearned preference for the odor of some dietary proteins (Deutsch et al., 1989; Heinrichs et al., 1990). However, an increase in monosodium glutamate preference in protein-deficient rats has not been demonstrated (Torii et al., 1986), and therefore there is no compelling evidence of an innate-specific appetite for protein (Galef, 1999).

On the other hand, there is a large number of reports that suggest that pigs are able to change their feeding behavior in response to previous protein underfeeding. The dietary selection was appropriate when pigs were first given the opportunity to experience the feeds given as a choice (Kyriazakis et al., 1990, 1991a,b; Kyriazakis and Emmans, 1991), or after long-term choices studies (Kirchgessner et al., 1999; Ettle and Roth, 2004, 2005; Roth et al., 2006). It is suggested that animals learn what and how much to eat by forming associations. The training process (alternate exposure to the feeds) may require over six days in naive pigs

to associate the feeds and their intrinsic flavors with their nutritional consequences (Kyriazakis et al., 1990), but it may be reduced to only around three days by using a trained individual in the group (Morgan et al., 2003). The period may be longer when two feeds are given as a choice and the animals find it difficult to untie, or associate, each feed with their nutritional consequences (Kyriazakis et al., 1991a).

The first contact of piglets with the pans as a measure of palatability was performed to observe the capacity of attraction during the first seconds of consumption in piglets under commercial productive conditions. However, palatability, despite being widely used, is a much-misunderstood term (Forbes, 2010) that has not been systematically studied in pigs. There is general agreement, particularly in rodents, that taste reactivity and lick microstructure analysis can be indicators of an animal's hedonic reaction to palatable substances (Dwyer, 2012). The rate at which animals eat a novel source when first offered has also been used as a measure of its palatability. However, the simultaneous offer of the two solutions prevents judging the palatability of each source independently and, rather than the pleasure during the consumption, this measure gives us an idea of the animal´s motivation to select one or the other solution during the beginning of the intake. There were no differences in the first contact in piglets fed the LP diet, but a higher number of piglets was observed at both pans in piglets under the protein-deficiency status. These results could indicate the degree of motivation of these animals, driven by their degree of hunger and by the anticipation of resulting pleasure or comfort of eating these sources (Forbes, 2010).

In our second experiment, a protocol was designed to evaluate how piglets can acquire preferences for new flavor cues by their association with the nutritional consequences of high-protein or high-caloric ingredients. In order to avoid the hedonic influence of sucrose on flavor association, we decided to use maltodextrin instead of sucrose solution in the design of training sessions. The mechanisms behind the sensory perception of maltodextrin by pigs are not totally understood yet. However, the lower preference threshold reported for sucrose (5 g/L - 10 g/L; Kennedy and Baldwin, 1972; Glaser et al., 2000) than that for maltodextrin (30 g/L; Roura et al., 2013b) might be an indicator of the higher hedonic value of the former in piglets. Inclusion levels of 60 g/L - 70 g/L of maltodextrin such as that used for this experiment generated significant preferences in piglets (64%) in comparison with water (Roura et al., 2013b). Nevertheless, it is not clear if this preference is due to a specific taste sensation or other characteristics of the maltodextrin solution, such as its viscosity. The

training protocol included two flavors equally preferred by piglets mixed with a protein (CSp) or maltodextrin (CSc) solution for eight alternate days. The results obtained in the subsequent choice test for CSp vs. CSc indicate that piglets submitted to a protein-deficiency status were able to express a higher preference for CSp, suggesting that they may use and reinforce flavor preference to show an appropriate diet-selection pattern to overcome the deficiency through associative learning. Our results are in close agreement with previous studies of protein-based flavor preferences in protein-restricted environments conducted in hamsters (DiBattista and Mercier, 1999) and humans (Gibson et al., 1995). To our knowledge, this work is the first report in pigs of a new flavor conditioning under protein-deficiency status.

The higher intake and preference for CSp in piglets fed the LP diet is in accordance with the framework of diet selection proposed by Kyriazakis et al. (1999). In that theory, the authors suggest that the feeding behavior of animals will largely depend on learning, since learning would make the animal more effective in adapting to the temporal and spatial changes in its feeding environment. In this context, when growing pigs are offered a choice of two feeds with different protein contents, they could choose proportions of these feeds that provide the optimum dietary protein-to-energy ratio based on learned preferences (Forbes, 2009). We observed in the protein-deficient group that in comparison with heavier piglets, middle-light and particularly light piglets showed the greatest differences in the intake between CSp and CSc. These results suggest that the reinforcement properties of protein conditioning may vary among pigs, having a greater impact in piglets which have been deprived of nutrients and protein more (low rather than high BW at the same age). This suggestion is also based on the diet-selection framework, which indicates that the rate at which animals learn about foods depends on the extent of the animal's deficiency and on the extent of the post-ingestive consequences induced (Kyriazakis et al., 1999). In the same way, it is also worth stating that piglets fed the LP diet showed a pronounced increase in the appetence or motivated consumption of CSp and CSc when they were offered the separate solutions during the one-pan test, as compared to those animals fed the HP diet.

4.6. Conclusion

It is concluded that piglets may be able to select and prefer flavors conditioned by the post-ingestive consequences of a protein source and show an appropriate selection pattern to overcome a protein-deficiency status based on associative learning. On the other hand, they appear to be unable to express a specific appetite for protein to correct a previous underfeeding with it by using exclusively the intrinsic flavors of the protein source against the high hedonic values of sucrose.

5. Chapter 3

Effect of a long-term exposure to carbohydrate and artificial sweetener solutions on the preference, appetence, feed intake and growth performance of post-weaned piglets

5.1. Abstract

Commercial pigs display an innate attraction for sweet taste compounds. However, the impact of long-term availability to complementary carbohydrate or artificial sweetener solutions on their general feeding behaviour has not been examined. Here we assess the effect of 12-days exposure to sucrose 160 g/L, maltodextrin 160 g/L, or saccharin 0.08 g/L plus neohesperidin dihydrochalcone (NHDC) 0.02 g/L solutions on the preference (choice test) and appetence (one-pan test) of piglets for protein (animal plasma 20 g/L) or sweet (sucrose 20 g/L) solutions. Piglets showed higher intake and preference for sucrose 20 g/L than for animal plasma 20 g/L solution in an initial choice test. In Experiment 1, piglets were then free-offered sucrose 160 g/L as a complement to their diet, showing a higher intake of it than water and a decrease in feed intake and weight gain. A similar situation occurred in the last days of maltodextrin 160 g/L exposure in Experiment 2. In contrast, animals were not influenced by the availability of saccharin 0.08 g/L plus NHDC 0.02 g/L in Experiment 3. After solution exposure, a reduction in the sucrose 20 g/L preference and appetence was observed in Experiments 1 and 2, but not Experiment 3. It is concluded that long-term exposure to sucrose 160 g/L or maltodextrin 160 g/L, but not saccharin 0.08 g/L plus NHDC 0.02 g/L, reduces feed intake and growth performance of piglets and also reverses their innate preference and appetence for sweet over protein solutions.

5.2. Introduction

Laboratory rodents have been traditionally used as models for humans in order to study and better understand eating-related disorders such as human obesity. The behavioural and physiological factors that regulate sugar appetite in rats and mice have been extensively studied, as they may represent good models for motivational systems based on orosensorial stimulation (Baldwin, 1996). Thus, when offered a highly palatable sucrose 320 g/L solution as a complement to their nutritionally complete diet, adult rats overeat and gain excessive weight in a phenomenon described as obesity by choice (Sclafani and Springer, 1976; Ackroff and Sclafani, 1988; Ackroff et al., 2007). However, larger mammals such as the pig deserve further consideration as a suitable nutritional model for humans, because their anatomical and functional features are more similar to those of human beings than are those of rats (Danilova et al., 1999; Roura et al., 2011).

The omnivorous diet of the pig in wild conditions shares significant similarities with human dietary habits not seen in other omnivorous species, such as the rat or the mouse (Roura et al., 2011). Dietary preferences are intimately linked to taste perception mechanisms, which are also shared and similar between pigs and humans (Roura et al., 2008). Among the basic currently accepted tastes, sweet and umami compounds are strongly pleasurable for pigs. Sugars, including different types of carbohydrates, polyols and sweeteners, are recognized by the T1R2/T1R3 heterodimeric receptor into the oral cavity and gastrointestinal tract of pigs (Moran et al., 2010; Janssen and Depoortere, 2013). Pigs show an innate attraction and preference for solutions of sucrose, glucose, lactose and sodium saccharin when compared in short-term preference tests against water (Baldwin, 1976, 1996). The attraction is similar to that showed by humans, reflecting a trait that has probably evolved through years to signal highly caloric carbohydrate-rich nutrients (Dulac, 2000). From Glaser et al. (2000), it is known that sucrose and fructose response intensities are identical in both species, sucrose being the most strongly preferred carbohydrate for pigs (Glaser et al., 2000). These compounds added in-feed at levels of around 50 g/kg also increased feed intake and weight gain of weanling animals (Lewis et al., 1955). However, there is no conclusive literature concerning how and in which intensity pigs sense other oligosaccharides or more complex carbohydrates, such as maltodextrin, which is important as, unlike rats, humans perceive maltodextrin as an almost tasteless compound (Clouard et al., 2012). It is certainly possible

that the hedonic intensity of maltodextrin solutions in pigs is lower than that reported for sucrose, because they showed a higher preference threshold for maltodextrin (30 g/L) than sucrose (5 g/L - 10 g/L) when tested against plain water in a recent study (Roura et al., 2013b). In contrast, artificial sweeteners defined as high-intensity by humans trigger less intensive sweet taste responses in the pig tongue. Thus, pigs respond to sodium saccharin solutions but with an approximately 65 times lower efficiency than humans do (Glaser et al., 2000). In the same way, pigs may not respond to neohesperidin dihydrochalcone (NHDC) solutions below the concentration of 0.6 g/L, a semi-natural sweetener often combined with saccharin (Moran et al., 2010). Saccharin and NHDC are the only sweeteners approved to be included in the diet of piglets in the European Union (2003). Nevertheless, their inclusion at 150 mg/kg is not reported to produce a significant performance increase (Sterk et al., 2008).

Kennedy and Baldwin (1972) observed in a 12-hour choice test against water that young pigs showed increases in sucrose solution intake of concentrations of approximately 3 g/L to 77 g/L with concomitant decreases in water intake – but there was no assessment of sucrose availability on feed intake (Kennedy and Baldwin, 1972). Since that study, no other report has evaluated the possible effects of a long-term availability to a highly hedonic and more concentrated complementary carbohydrate or artificial sweetener solution on the feeding behaviour of pigs. In humans, there is a general concern about the detrimental impact on public health of a long-term consumption of caloric drinks. Indeed, the increased prevalence of obesity in children has coincided with an increase in the consumption of sugar-sweetened beverages (Hill et al., 2003; Cota et al., 2006; de Ruyter et al., 2012). In the present work, we examined post-weaned piglets as another feasible and practical animal model for the understanding of human dietary ingestive patterns such as those observed in obesity. Previously, we observed that piglets fed ad libitum a high-fat-content diet regulated their feed intake and daily energy intake but preferred a sweet (sucrose 20 g/L solution) instead of a protein source in a short-term choice test conducted after the feed exposure, which indicates that these animals were not able to correct their nutritional imbalance through the dietary selection (Guzmán-Pino et al., 2012). Here, in order to further explore the hedonic motivation of piglets we used a concentrated sucrose solution (160 g/L, Experiment 1) to expose the animals with a highly hedonic sweet compound which also has considerable caloric post-ingestive effects. The aim was to assess whether a long-term exposure (12 days) might modify the feed intake and growth performance of piglets, as well as their preference and appetence for a sweet (sucrose 20 g/L) over a protein (animal plasma 20 g/L) solution. In

order to discriminate between the influence of sweetness and the contribution of the caloric load, we used a low dextrose equivalent maltodextrin solution (160 g/L, Experiment 2). Finally, in order to study the influence of the sweet sensation without the post-ingestive consequence on the response, a combination of a calorie-free saccharin (0.08 g/L) plus NHDC (0.02 g/L) solution was used (Experiment 3). We hypothesized that, similar to rodents, pigs may show a high-affinity pattern towards a palatable solution if it is freely offered as a complement to the diet, based on their innate attraction with sweet taste compounds. In addition, the long-term exposure to solutions that are hedonically preferred to the maintenance feed may have a negative effect on the feed intake of the animals and may also reduce their preference for less hedonically valuable low-concentration sweet solutions.

5.3. Material and methods

All procedures described in this study were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (UAB). Experimental procedures were approved by the Ethical Committee on Animal Experimentation of the UAB (CEAAH 1406). The treatment, management, housing and husbandry conditions conformed to European Union Guidelines (1986).

5.3.1. Animals, diets and housing

In total, 162 male and female piglets (Pietrain × [Landrace × Large White]) from 14 to 35 days post-weaning were selected to be used in four experiments. Experiments 1, 2 and 3 were performed with a total of 108 piglets, with 36 piglets in each. Experiment 4 was conducted by using a total of 54 piglets. During lactation, piglets were supplemented with a creep-feed diet from 10 days of age until weaning. Piglets were weaned at 28 days of age. In Experiments 1, 2 and 3, at the beginning of the starter period on Day 14 after weaning piglets were distributed according to their body weight (BW) and were further allocated into 12 pens of three piglets per pen. In Experiment 4, on Day 35 after weaning piglets were allocated into 18 pens of three piglets per pen. In all experiments, piglets were fed a single, commercial starter diet

(Table 5.1) formulated to provide a complete and equilibrated nutrient content in order to maximize growth potential of animals, according to NRC (2012). This diet was offered ad libitum in mash form. The weaning room had automatic, forced ventilation and completely slatted flooring. Each pen (3.2 m^2 in floor area) was equipped with a feeder with three feeding spaces and an independent water supply to ensure ad libitum feeding and freshwater access.

Table 5.1. Composition and estimated nutrient content of the starter diet used in the experiments.

	g/kg DM
Ingredients	
Maize	350.0
Barley	187.1
Wheat	180.0
Extruded soybean	109.0
Soybean meal 44% CP	58.9
Fishmeal LT	50.0
Whey powder 50% fat	25.0
Commercial nucleus[a]	10.0
Monocalcium phosphate	8.8
Calcium carbonate	7.0
L-Lysine-HCl	5.2
L-Threonine	2.2
DL-Methionine	1.8
L-Tryptophan	0.5
Salt	4.5
Estimated nutrient content	
Dry matter	890.6
Net energy (MJ/kg)	10.4
Crude protein	179.8
Crude Fibre	31.5
Fat	59.3

[a] Supplied per kg of feed: 3060 μg of retinol, 52.5 μg of cholecalciferol, 39.9 mg of α-tocopherol, 3 mg of menadione, 2 mg of thiamin, 3 mg of riboflavin, 3 mg of pyridoxine, 0.025 mg of cyanocobalamin, 20 mg of calcium pantothenate, 60 mg of nicotinic acid, 0.1 mg of biotin, 0.5 mg of folic acid, 150 mg of Fe, 156 mg of Cu, 0.5 mg of Co, 120 mg of Zn, 49.8 mg of Mn, 2 mg of I, 0.3 mg of Se.

5.3.2. Experimental designs

5.3.2.1. Experiments 1, 2 and 3: Long-term solution exposure in piglets

These experiments were designed to evaluate the effect of a long-term free availability of an extra carbohydrate or artificial sweetener solution on the innate preference and higher appetence of piglets for sweet solutions, and also on their feed intake and growth performance. The experimental design included an initial choice test on Day 14 after weaning, an ad libitum solution exposure period from Days 14 to 26 during which feed intake and growth performance were recorded, and a final choice test on Day 26 and one-pan test on Days 27 and 28 after weaning.

5.3.2.1.1. Initial and final choice test

During the first two weeks after weaning, piglets were familiarized to the weanling room and pre-trained with two pans containing 800 mL of tap-water in each pen for 30 minutes. The innate preference of piglets for sweet solutions was assessed at the beginning of the experimental period (Day 14 after weaning) by using a single choice test between protein and carbohydrate water-based solutions for 3 minutes. This choice between protein and carbohydrate was also assessed at the end of the experimental period (Day 26 after weaning). The test was performed for the 3 piglets of each pen, with 2 pans placed in the front of the pens containing 800 mL of either 20 g/L of porcine animal plasma (AP820, APC; Ankeny, USA) as protein solution (0.014 g of CP, 0.324 kJ DE/mL) or 20 g/L of commercial sucrose as carbohydrate solution (0.335 kJ DE/mL). To control for side preference, solution position inside the pen was counterbalanced between pens, i.e., the protein solution was offered on the left side of the pen and the carbohydrate solution on the right side for half the pens and vice versa.

5.3.2.1.2. Ad libitum solution exposure

Pens were randomly assigned to a control or experimental group after the initial choice test, and each one was provided with an extra container with a total capacity of 5 L placed on the middle of the pen as a complement to the diet and normal water supply. Thus, the control group (six pens) was provided with an extra supply of tap-water, while the experimental group (six pens) was provided with one of the carbohydrate or artificial sweetener solutions used for 12 consecutive days. During this period, containers were regularly checked and refilled at least daily in order to provide an ad libitum exposure to the solutions.

In Experiment 1, 160 g/L of commercial sucrose was offered to the piglets in order to expose them to a highly hedonic sweet solution which also provides considerable caloric post-ingestive effects (2.678 kJ DE/mL). The same concentration, 160 g/L, of spray-dried maltodextrin (C*Dry MD 01910, Cargill Inc.; Minneapolis, USA) was supplied to the animals in experimental group in Experiment 2. The maltodextrin product used had a low dextrose equivalent value (12 to 16), providing similar caloric effects than those of the sucrose 160 g/L solution (2.678 kJ DE/mL) without the same hedonic effects of the sweet taste of a similarly concentrated sucrose solution. Therefore, maltodextrin solution focuses on the post-ingestive effects of that solution. The solution used in Experiment 3 was a combination of 0.08 g/L of sodium saccharin plus 0.02 g/L of NHDC (references 07106 and 06838, respectively, Lucta SA; Montornès del Vallès, Spain). These doses were determined by reference to the maximum incorporation levels for saccharin and NHDC allowed by the European Union to the diet of piglets, 150 mg/kg (2003). A saccharin:NHDC relation of 4:1 was used in this experiment. Even though it is possible that pigs may not sense NHDC solutions lower than 0.6 g/L alone (Glaser et al., 2000), they are routinely combined with saccharin because NHDC may block some of the bitterness of saccharin when they are mixed (Moran et al., 2010). Animals were individually weighed in each experiment on Days 14, 21 and 26 after weaning, and the depletion from the feeders was also monitored on the same days in order to calculate the average daily feed intake (ADFI), average daily gain (ADG), feed:gain ratio (FGR) and energy:gain ratio (EGR) of piglets during these experimental periods.

5.3.2.1.3. One-pan test

The higher appetence of piglets for a sweet than for a protein solution was assessed after the ad libitum period, and the final preference test, in the control and experimental group of each experiment by using a one-pan test, over two consecutive days. A single pan containing 800 mL of the animal plasma 20 g/L or the sucrose 20 g/L solutions was offered to the piglets for 3 minutes each day. The order of testing first the protein or carbohydrate solutions on Days 27 or 28 after weaning was counterbalanced across pens of each group.

5.3.2.2. Experiment 4: Piglets preference for carbohydrate or artificial sweeteners solutions

Experiment 4 was conducted in order to better understand the innate preference values of piglets for the solutions used in Experiments 1-3 (sucrose 160 g/L, maltodextrin 160 g/L, and saccharin 0.08 g/L plus NHDC 0.02 g/L) when tested against sucrose 20 g/L solution as reference.

Naive piglets were fed the same commercial starter diet than in prior experiments and had no previous contact with any additional solution or related flavour all across the nursery period in this experiment. On Day 35 after weaning, the three piglets of each pen were offered two pans placed in the front of the pens containing 800 mL of the solutions tested for three minutes, in a single choice test procedure as described for the previous experiments. Three comparisons were conducted, with six randomly assigned pens for each: (i) sucrose 160 g/L vs. sucrose 20 g/L, (ii) maltodextrin 160 g/L vs. sucrose 20 g/L, and (iii) saccharin 0.08 g/L plus NHDC 0.02 g/L vs. sucrose 20 g/L. Piglets were individually weighed after finishing the choice test.

5.3.3. Calculations and statistical analysis

Solution intakes measured for each pen during the choice and one-pan test were averaged for the number of piglets that performed each test (3 piglets), and were standardized to the different weights of the animals in each group and experiment by dividing by the registered BW on the test days. The standardization aimed to make the solution intake registered for animals with different BW comparable; therefore, it diminishes differences in consumption due to different ingestive capacities of the animals.

Choice-test data were analyzed for the initial and final tests separately with a two-way ANOVA by using the GLM procedure of SAS (version 9.2, SAS Institute; Cary, USA), taking into account a within-subject factor of solution (animal plasma 20 g/L vs. sucrose 20 g/L), a between-subject manipulation of solution exposure (control, water vs. experimental, sucrose 160 g/L/maltodextrin 160 g/L/saccharin 0.08 g/L plus NHDC 0.02 g/L), and their interaction as main factors (only included when significant). The pen of three piglets was considered the experimental unit. The same statistical model was used for the analysis of one-pan test data. Preference values for the protein solution in the initial and final choice test of Experiments 1-3; and for sucrose 160 g/L, maltodextrin 160 g/L, and saccharin 0.08 g/L plus NHDC 0.02 g/L solutions in Experiment 4 were measured as the percentage that each target solution comprised of the total fluid intake and were compared between each treatment and test (Experiments 1-3) and to the neutral value of 50% of preference (Experiment 4) by using a Student's *t*-test.

Solution intakes during the 12-day ad libitum period were monitored daily in order to establish a net balance of energy intake per kg of BW. Intake values were averaged for the number of piglets that consumed them, and their contribution on the daily energy intake of piglets was considered. These data, as well as feed intake and growth performance data (BW, ADG and FGR) were analyzed with a one-way ANOVA considering the exposure to water or the experimental solutions as the main factor, by using the GLM procedure of SAS. For all of the analysis, average values were compared by least-squares means with the Tukey adjustment for multiple comparisons. The alpha level used for the determination of significance was 0.05, and tendencies for $0.05<P<0.1$ are also presented.

5.4. Results

5.4.1. Experiments 1, 2 and 3

5.4.1.1. Ad libitum solution exposure

The effect of a 12-day free availability of an extra sucrose 160 g/L (Experiment 1), maltodextrin 160 g/L (Experiment 2), and saccharin 0.08 g/L plus NHDC 0.02 g/L (Experiment 3) solution on the solution intake, feed intake and growth performance of piglets in periods Days 1 to 7 and Days 7 to 12 is shown in Tables 5.2, 5.3 and 5.4, respectively. Piglets with free access to the sucrose 160 g/L solution showed a higher intake of it in comparison with water intake of piglets in the control group during the period Days 1 to 7 [$F(1,10)=7.74$, $P=0.019$]. A lower ADFI [$F(1,10)=15.06$, $P=0.003$] and BW [$F(1,34)=8.03$, $P=0.008$] was registered in piglets with access to the sucrose 160 g/L solution during all the experimental periods. Accordingly, a lower ADG was observed in this group of animals during the period Days 1 to 7 [$F(1,34)=19.79$, $P<0.001$]. The FGR of piglets exposed to the carbohydrate solution tended to be higher than that of control animals during the period Days 1 to 7 [$F(1,9)=4.09$, $P=0.074$], but it was not significantly different during the subsequent period Days 7 to 12 [$F(1,10)=3.05$, $P=0.111$]. Nonetheless, when considering the total of energy ingested by both feed and solution, piglets complemented with the carbohydrate solution showed a less efficient conversion of energy into body weight as observed by higher EGR in both periods as compared than those of control pigs [$F(1,10)=4.87$, $P<0.052$].

Table 5.2. Solution intake, feed intake and growth performance of piglets with access to an extra supply of water (control) or sucrose 160 g/L solution for 12 consecutive days (Experiment 1).

	Control	Sucrose 160 g/L	SEM	*P*-value
Days 1 to 7				
Initial BW, kg	10.33	10.32	0.169	0.993
ADSI, mL/d	655.8[a]	1274.9[b]	157.3	0.019
ADFI, g/d	448.0[a]	255.7[b]	31.2	0.001
ADEI, MJ/d				
Sucrose	-	3.35 (SEM 0.38)	-	-
Feed	6.53[a]	3.72[b]	0.46	0.002
ADG, g/d	254.2[a]	111.6[b]	22.7	<0.001
FGR	1.79	2.11	0.116	0.074
EGR, kJ/g	26.04[a]	55.37[b]	3.860	<0.001
Final BW, kg	12.11[a]	11.11[b]	0.247	0.007
Days 7 to 12				
ADSI, mL/d	889.6	1312.9	183.7	0.134
ADFI, g/d	570.7[a]	367.2[b]	37.1	0.003
ADEI, MJ/d				
Sucrose	-	3.43 (SEM 0.42)	-	-
Feed	8.28[a]	5.36[b]	0.54	0.003
ADG, g/d	424.5	327.6	37.9	0.080
FGR	1.44	1.12	0.128	0.111
EGR, kJ/g	20.86[a]	27.06[b]	1.990	0.052
Final BW, kg	14.23[a]	12.74[b]	0.370	0.008

BW, body weight; ADSI, average daily solution intake; ADFI, average daily feed intake; ADEI, average daily energy intake; ADG, average daily gain; FGR, feed:gain ratio; EGR, energy:gain ratio.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Piglets with free access to the maltodextrin 160 g/L solution showed no significantly higher intake in comparison with water intake of piglets provided with the extra supply, during the different experimental periods [$F(1,10)=1.11$, $P>0.317$]. Nevertheless, a numerical increase of 25% in maltodextrin 160 g/L solution consumption was observed during the period Days 7 to 12. A lower ADFI [$F(1,10)=10.65$, $P=0.009$] and average daily energy intake (ADEI) due to feed consumption [$F(1,10)=10.65$, $P=0.009$] was registered in those animals supplemented with the carbohydrate solution during the period Days 7 to 12, without significant differences in the BW between both groups of piglets after the solution exposure, all over the experiment [$F(1,34)=0.85$, $P>0.364$]. Nonetheless, the ADG of maltodextrin piglets was lower than that of control piglets during the period Days 7 to 12 [$F(1,34)=7.23$,

P=0.011], affecting the way that animals convert energy into weight gain as observed by a higher EGR in this period [*F*(1,10)=11.36, *P*=0.007].

Table 5.3. Solution intake, feed intake and growth performance of piglets with access to an extra supply of water (control) or maltodextrin 160 g/L solution for 12 consecutive days (Experiment 2).

	Control	Maltodextrin 160 g/L	SEM	*P*-value
Days 1 to 7				
Initial BW, kg	10.41	10.43	0.219	0.945
ADSI, mL/d	594.2	520.8	102.5	0.624
ADFI, g/d	493.5	455.2	16.7	0.135
ADEI, MJ/d				
Maltodextrin	-	1.38 (SEM 0.21)	-	-
Feed	7.15	6.61	0.25	0.135
ADG, g/d	343.7	335.5	24.8	0.817
FGR	1.47	1.38	0.094	0.489
EGR, kJ/g	21.38	24.12	1.460	0.214
Final BW, kg	12.82	12.78	0.321	0.937
Days 7 to 12				
ADSI, mL/d	759.0	947.0	126.1	0.317
ADFI, g/d	617.9[a]	514.0[b]	22.5	0.009
ADEI, MJ/d				
Maltodextrin	-	2.47 (SEM 0.29)	-	-
Feed	9.00[a]	7.49[b]	0.33	0.009
ADG, g/d	483.6[a]	395.0[b]	23.3	0.011
FGR	1.30	1.31	0.064	0.901
EGR, kJ/g	18.84[a]	25.41[b]	1.378	0.007
Final BW, kg	15.23	14.75	0.368	0.364

BW, body weight; ADSI, average daily solution intake; ADFI, average daily feed intake; ADEI, average daily energy intake; ADG, average daily gain; FGR, feed:gain ratio; EGR, energy:gain ratio.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Piglets with free access to the saccharin 0.08 g/L plus NHDC 0.02 g/L solution showed no significantly different intake as compared to water intake during any experimental period [*F*(1,10)=0.15, *P*>0.706]. In the same way, no significantly different results were observed between piglets offered with either the artificial sweetener or water solutions regarding the ADFI, BW, ADG or FGR in this experiment [*F*(1,10)=0.22, *P*>0.05].

Table 5.4. Solution intake, feed intake and growth performance of piglets with access to an extra supply of water (control) or saccharin 0.08 g/L plus NHDC 0.02 g/L solution for 12 consecutive days (Experiment 3).

	Control	Saccharin 0.08 g/L plus NHDC 0.02 g/L	SEM	*P*-value
Days 1 to 7				
Initial BW, kg	10.70	10.71	0.278	0.987
ADSI, mL/d	586.7	579.1	63.6	0.935
ADFI, g/d	416.5	392.7	24.7	0.513
ADG, g/d	273.6	248.1	23.9	0.448
FGR	1.53	1.64	0.085	0.404
Final BW, kg	12.62	12.40	0.350	0.648
Days 7 to 12				
ADSI, mL/d	1070.2	998.2	131.3	0.706
ADFI, g/d	604.9	572.7	48.3	0.647
ADG, g/d	443.6	445.2	35.8	0.974
FGR	1.38	1.29	0.056	0.274
Final BW, kg	14.84	14.62	0.419	0.713

BW, body weight; ADSI, average daily solution intake; ADFI, average daily feed intake; ADG, average daily gain; FGR, feed:gain ratio.

5.4.1.2. Initial and final choice test

Figure 5.1 shows a summary of consumption in the preference tests before and after the free access to the additional solutions in Experiments 1-3. In these, a higher intake and preference for the sucrose 20 g/L solution in comparison with the animal plasma 20 g/L solution was observed in the initial choice test conducted at the beginning of the experimental period [$F(2,21)=5.05$, $P=0.005$ in Experiment 1; $F(2,15)=7.05$, $P=0.016$ in Experiment 2; and $F(2,17)=2.69$, $P=0.039$ in Experiment 3). Subsequently, after receiving an extra supply of water for 12 days, piglets in control groups, in general, maintained their solution selection pattern despite the fact that no significantly different intakes were observed in the final choice test at the end of the experimental period in these animals (a tendency to a higher intake of sucrose 20 g/L than of animal plasma 20 g/L solution was observed in Experiment 3, $F(1,10)=3.04$, $P=0.112$). Importantly, the preference values observed for the animal plasma 20 g/L solution were not significantly different with those observed at the onset of the experiments in the initial choice test, i.e., 37% vs. 27% in Experiment 1 [$t=1.07$, $df=16$, $P=0.299$], 37% vs. 29% in Experiment 2 [$t=0.72$, $df=13$, $P=0.483$], and 35% vs. 36% in Experiment 3 [$t=-0.06$, $df=14$, $P=0.955$].

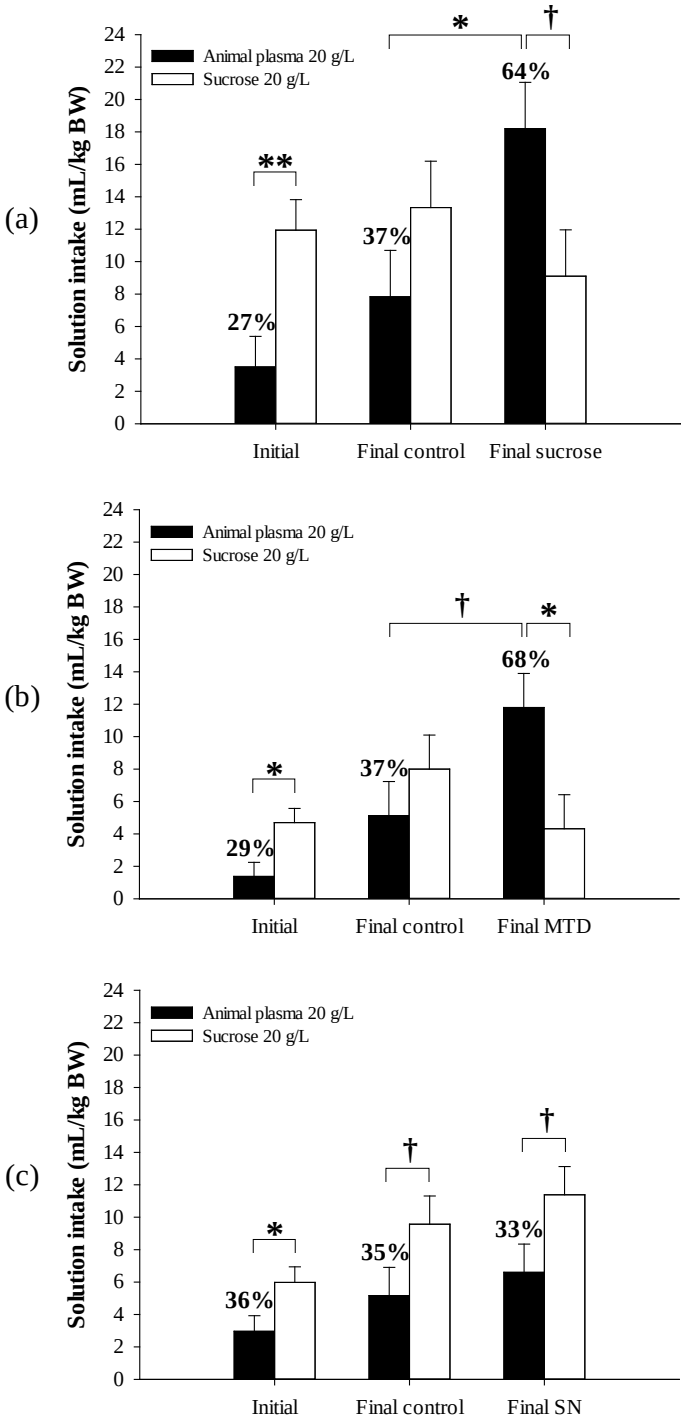

Figure 5.1. Intake and preference of piglets for animal plasma 20 g/L or sucrose 20 g/L solutions during the initial or final choice tests, conducted 12 days after the exposure to an extra supply of water (final control), or sucrose 160 g/L (final sucrose, (a)), maltodextrin 160 g/L (final MTD, (b)) or saccharin 0.08 g/L plus neohesperidin dihydrochalcone 0.02 g/L (final SN, (c)) solutions. Error bars represent the SEM. Clasps indicate different intakes between both solutions ($^{\dagger}P<0.1$, $*P<0.05$, $**P<0.01$). Numbers on top of the bars represent percent intake of animal plasma 20 g/L.

In Experiment 1 (Figure 5.1(a)), a significant interaction among the within-subject factor of test solution type and the between-subject factor of solution exposure was observed [$F(3,20)=2.69$, $P=0.019$]. Piglets offered the sucrose 160 g/L solution for 12 consecutive days showed a significant higher intake of animal plasma 20 g/L solution in comparison with animals in control group previously exposed to water [$F(1,10)=5.22$, $P=0.046$]. The intake of the protein solution also tended to be higher than the intake of sucrose 20 g/L solution in the final choice test of piglets pre-offered the highly concentrated carbohydrate solution [$F(1,10)=3.60$, $P=0.087$]. In addition, the animal plasma 20 g/L preference of 64% was significantly different from the 37% of protein preference showed by the animals in the control group [$t=-2.27$, $df=10$, $P=0.047$] and the 27% of preference displayed in the initial choice test [$t=3.47$, $df=16$, $P=0.003$].

In Experiment 2 (Figure 5.1(b)), a similar interaction than that in Experiment 1 between test solution type and solution exposure was observed [$F(3,18)=2.23$, $P=0.030$]. A tendency towards a higher intake of animal plasma 20 g/L solution was observed in piglets which had previously been offered free access to the maltodextrin 160 g/L solution, in comparison with piglets in control group [$F(1,9)=3.34$, $P=0.101$]. Animal plasma 20 g/L consumption in the final choice test of maltodextrin piglets was also significantly higher than that of sucrose 20 g/L solution [$F(1,8)=5.85$, $P=0.042$]. The preference for the protein solution was 68% in this case and was significantly different from the 37% of protein preference showed by piglets in the control group [$t=-2.27$, $df=9$, $P=0.050$] and the 29% of preference in the initial choice test [$t=3.43$, $df=12$, $P=0.005$].

A statistical tendency to a higher intake of sucrose 20 g/L solution as compared to animal plasma 20 g/L solution was observed in the final choice test of those animals supplemented with saccharin 0.08 g/L plus NHDC 0.02 g/L solution in Experiment 3 [$F(1,10)=3.98$, $P=0.074$; Figure 5.1(c)]. No interaction between test solution and the experimental manipulation was observed in this case [$F(3,20)=2.61$, $P=0.913$]. Piglets with long-term access to the artificial sweetener solution showed a subsequently 33% of preference for animal plasma 20 g/L when offered as a choice, which was not different from the 35% of preference showed for the same solution by control piglets [$t=0.24$, $df=10$, $P=0.819$] and the 36% of protein preference at the beginning of the experimental period [$t=-0.44$, $df=14$, $P=0.668$].

5.4.1.3. One-pan test

The appetence of piglets for animal plasma 20 g/L and sucrose 20 g/L solutions in the control and experimental groups in the different experiments is shown in Figure 5.2. After receiving only the extra supply of water, piglets in the control groups in the three experiments exhibited a higher appetence for the sucrose 20 g/L than for the animal plasma 20 g/L solution, as measured by the one-pan access during two alternate days [$F(1,34)=6.52$, $P=0.015$]. In contrast, no significant differences in appetence for the protein or carbohydrate sources were observed in the experimental groups after the 12-day exposure to their respective experimental solutions [$F(1,10)=2.90$, $P>0.120$]. However, it is important to note that while a significant interaction [$F(3,20)=1.85$, $P=0.033$] and a tendency to the same interaction [$F(3,20)=0.99$, $P=0.107$] between test solution type and solution exposure were observed in Experiments 1 and 2, respectively, no interaction was observed in Experiment 3 [$F(3,20)=1.37$, $P=0.431$]. Thus, while the direction of the consumption in the saccharin 0.08 g/L plus NHDC 0.02 g/L exposed group was maintained, piglets long-term offered sucrose 160 g/L and maltodextrin 160 g/L solutions numerically reversed this pattern. In fact, a higher appetence for animal plasma 20 g/L solution was observed after the exposure to sucrose 160 g/L when compared with the protein appetence of piglets in control groups [$F(7,64)=2.40$, $P=0.051$].

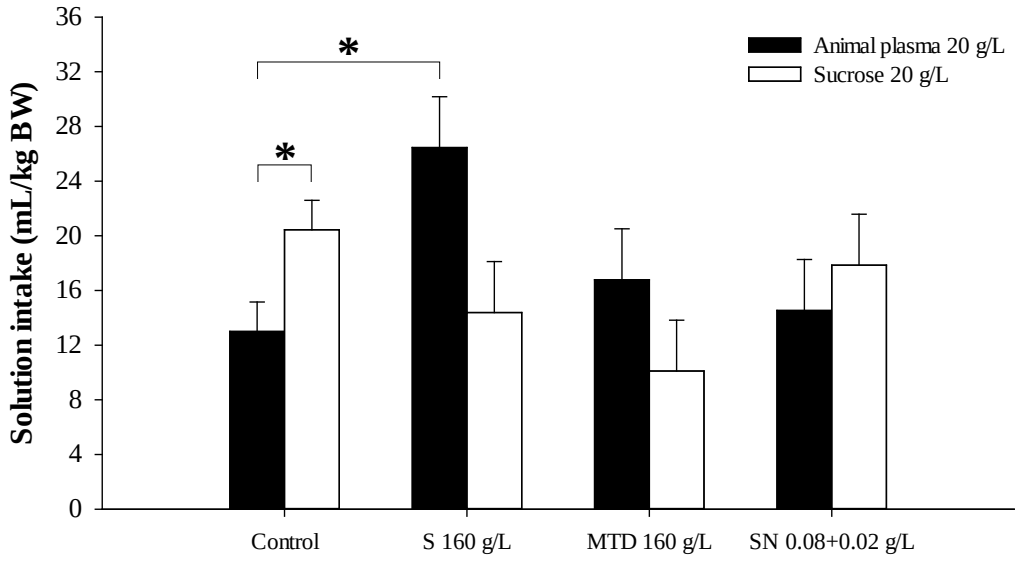

Figure 5.2. Intake of piglets of animal plasma 20 g/L and sucrose 20 g/L solutions during the one-pan test conducted 12 days after the exposure to an extra supply of water (control), sucrose 160 g/L (S 160 g/L), maltodextrin 160 g/L (MTD 160 g/L) or saccharin 0.08 g/L plus neohesperidin dihydrochalcone 0.02 g/L (SN 0.08+0.02 g/L) solutions. Error bars represent the SEM. Clasps indicate different intakes between both solutions (*P<0.05).

5.4.2. Experiment 4

Figure 5.3 shows the results of the three comparisons conducted in this experiment. In the first, naive piglets showed a higher intake of sucrose 160 g/L than of sucrose 20 g/L solution [F(1,8)=8.06, P=0.022; Figure 5.3(a)]. Indeed, the 66% preference observed for sucrose 160 g/L solution was significantly higher than the neutral value of 50% [t=3.79, df=4, P=0.019]. In the second comparison, a statistical tendency towards higher intake of sucrose 20 g/L was observed when it was tested against maltodextrin 160 g/L solution [F(1,10)=4.07, P=0.071; Figure 5.3(b)]. The 27% preference registered for maltodextrin 160 g/L solution was significantly lower than the neutral value in this case [t=-2.52, df=5, P=0.054]. Finally, a tendency towards higher intake of sucrose 20 g/L over saccharin 0.08 g/L plus NHDC 0.02 g/L solution was observed in the third choice test [F(1,8)=3.81, P=0.087; Figure 5.3(c)]. The

35% preference for the artificial sweetener solution observed here was statistically lower than 50% [t=-2.95, df=4, P=0.042].

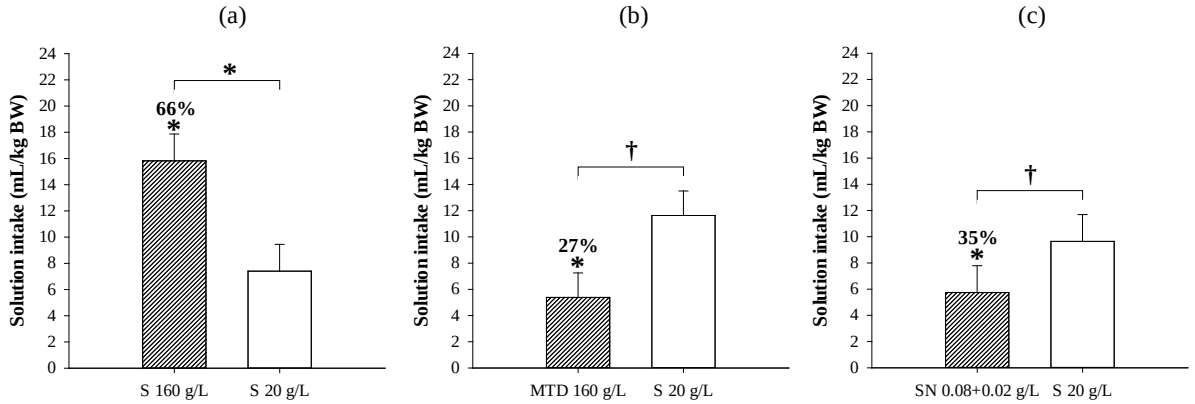

Figure 5.3. Intake and preference of piglets for sucrose 160 g/L (S 160 g/L, (a)), maltodextrin 160 g/L (MTD 160 g/L, (b)) and saccharin 0.08 g/L plus neohesperidin dihydrochalcone 0.02 g/L (SN 0.08+0.02 g/L, (c)) or sucrose 20 g/L (S 20 g/L) solution. Error bars represent the SEM. Clasps indicate different intakes between both solutions ($^{\dagger}P$<0.1, *P<0.05). Numbers on top of the bars represent percent intake of the corresponding solution and its difference from the neutral value of 50% (*P<0.05).

5.5. Discussion

In humans, the widespread availability of tasty, inexpensive, energy-dense foods, typically rich in fat and sugar, is thought to contribute to the increasing prevalence of obesity (Hill et al., 2003). The present work illustrates for the first time the feeding behaviour of post-weaned piglets when they offered long-term access to highly hedonic and/or caloric compounds in their diet. Similar to the response observed in rats (Sclafani and Springer, 1976; Ackroff and Sclafani, 1988; Ackroff et al., 2007), weanling piglets exhibited a high-affinity pattern towards a concentrated sweet and caloric sucrose 160 g/L solution when it was freely offered as a complement to the nutritionally complete diet (Experiment 1). Piglets did not initially show the same ingestive behaviour when offered an almost tasteless (to humans) but densely caloric maltodextrin 160 g/L solution, although an increase in maltodextrin solution

consumption was observed during the later exposure days (Experiment 2). Animals appeared not to be influenced by the availability of an extra non-caloric saccharin 0.08 g/L plus NHDC 0.02 g/L solution for 12 consecutive days (Experiment 3).

Previous studies conducted by Kennedy and Baldwin (1972) and Glaser et al. (2000) in naive pigs have reported preferences for sweet solutions when they are tested against water in short- (2 minutes - 1 hour) or mid-term (12 hours) preference tests. These findings, together with those obtained by Kare et al. (1965) and McLaughlin et al. (1983), have supported the concept that pigs have an innate preference for sweet taste compounds. Here, we tested a sweet solution (sucrose 20 g/L) against a protein solution (animal plasma 20 g/L) in the initial choice tests for Experiments 1-3. We observed, in all of them, a higher intake and preference for the sweet solution when animals had no previous contact with the solutions. These results are in line with our previous observations that growing pigs preferred sucrose solutions over protein sources even under conditions of protein-deficiency (Guzmán-Pino et al., 2012, 2014). The innate sweet preference of piglets observed in the 3-minute choice test set the starting point to investigate the effect of the long-term exposure to carbohydrate and artificial sweetener solutions.

In Experiment 1, giving piglets ad libitum access to the additional sucrose 160 g/L solution reduced feed intake and weight gain of the animals at Days 7 and 12 of exposure, in comparison with piglets supplied only with additional water. The effects on growth performance were severe, with an 11% of BW reduction in the animals supplemented with carbohydrate. In contrast to rats, which become obese when offered free access to additional sucrose, piglets did not increase their total energy intake but consumed, on average, 44% of their calories from the additional solution. The absence of additional calorie consumption suggests that they regulated their feed consumption in response to the calories ingested from the solution in order to avoid excessive energy intake. Although the situation is a complex one, these results are consistent with the theory of energy control of feed intake described in previous studies in pigs (Beaulieu et al., 2009; Black et al., 2009).

In Experiment 2, we observed a 25% of increase in maltodextrin 160 g/L solution consumption during Days 7 to 12 of the exposure period. The mechanisms underlying maltodextrin perception in pigs are not yet known: In rats, maltodextrin is perceived as a palatable taste, while for humans it is an almost tasteless and is primarily detectable in solution due to differences in viscosity compared to water (Sclafani, 2004; Dwyer, 2008).

Pigs do prefer maltodextrin solutions above the concentration of 60 g/L - 70 g/L when tested against water (Roura et al., 2013b), but it is not clear if the preference is due to a specific taste sensation or the physicochemical properties of the solution – although it is noteworthy that the preference thresholds for sweet sucrose solutions are far lower (Kennedy and Baldwin, 1972; Glaser et al., 2000). In the current Experiment 4 a concentrated maltodextrin 160 g/L solution was innately less preferred than a much less concentrated sucrose 20 g/L solution. In Experiment 2, an increment observed in maltodextrin consumption was observed later in the exposure phase which generated a reduction on the feed intake of the animals, and thus a reduction on their BW gain, presumably due to the caloric load provided by the solution. Based on this consumption pattern, it could be suggested that the low dextrose equivalent maltodextrin solution was not initially hedonically positive to the piglets but that the animals increased the intake once they have learned about the positive post-ingestive consequences of the consumption (caloric intake).

In Experiment 3, the feeding behaviour of piglets was not affected by the availability of the saccharin 0.08 g/L plus NHDC 0.02 g/L solution. The response intensity to artificial sweeteners in pigs is weaker than that in humans, which is attributed to the absence of one (or more) steric interaction (s) or steric fit (s) in the porcine receptor (Glaser et al., 2000). As noted in the materials section, this dose was chosen to fit within the maximum level allowed by European Union regulation concerning the incorporation of these sweeteners into the piglets' diets (2003). The most likely reason why saccharin plus NHDC was not considered as an attractive sweet solution that could promote ingestion was because of its lack in taste stimulation, i.e., doses used appear to be too weak to be detected by piglets. There are some differences in the previously reported preference thresholds for saccharin by piglets, with indifference to saccharin concentrations below 0.4 g/L (Glaser et al., 2000), or a range of detection thresholds between 0.92 g/L and 1.83 g/L (Kennedy and Baldwin, 1972); regardless, all such concentrations are higher than the one used here. The same applies for NHDC, with evidence that it can be detected by pigs at above 0.6 g/L (Glaser et al., 2000). The threshold of saccharin seems to be lower in other species such as rats or mice, starting from 0.02 g/L (Clouard et al., 2012). It is important to note at this point that we have conducted two other experiments concerning the effects of saccharin plus NHDC solutions which increased the dose of the sweeteners used (data not shown). The concentrations used were saccharin 3.2 g/L plus NHDC 0.8 g/L in one experiment (total dose 4 g/L, a solution 40 times more concentrated than in Experiment 3), and saccharin 12.8 g/L plus NHDC 3.2 g/L in the other

(total dose 16 g/L, 160 times more concentrated than Experiment 3). The solutions were freely offered to the piglets for 12 consecutive days, following the same experimental procedure as reported here. In both these additional experiments we observed a lower saccharin plus NHDC solution intake in comparison with water intake of control piglets, as well as a significant reduction in the feed intake and/or weight gain of the animals during the total or partial experimental periods [$P<0.05$]. These results appear somewhat inconsistent with those reported by Kennedy and Baldwin (1972), who observed strong preferences for saccharin solutions among concentrations of 1.83 g/L and 18.31 g/L in a 12-hour choice test (Kennedy and Baldwin, 1972). They inferred that the rejection threshold for saccharin was above the concentration of 18.31 g/L, which is approximately 5.7 times higher than the level that was rejected by pigs in our long-term exposure experiments.

Despite that fact that saccharin 0.08 g/L plus NHDC 0.02 g/L did not promote a significant increase in the solution consumption; some effects on the performance of animals could have been expected in the light of recent studies. Moran et al. (2010) showed that the expression of the Na^+/glucose co-transporter (SGLT1), a receptor involved in intestinal glucose absorption, was up-regulated in piglets supplemented for 3 days with saccharin 0.05 g/L plus NHDC 0.01 g/L solution, and suggested that this enhanced intestinal capacity of piglets to absorb glucose may promote growth (Moran et al., 2010). However, the results of offering a more concentrated saccharin plus NHDC solution for a longer period in the current Experiment 3 do not support the suggestion by Moran et al. (2010). The effect of a long-term exposure to artificial sweeteners on weight gain of animals has also been studied in rats: Davidson et al. (2011) reported increased feed intake and body weight gain in rats after exposure to a saccharin 3 g/L solution for 14 days. Because these effects were only observed when rats had access to a sweet supplementary or maintenance diet, they suggest that the effects of saccharin exposure were due to the experience of a sweet taste without calories weakening the predictive relationship between sweet taste and the caloric consequences of eating (Davidson et al., 2011). Taken together, these results suggest that a long-term exposure to an artificial sweetener solution could increase weight gain of animals only if it does not reduce feed intake directly and if the base diet is sweet already.

Piglets provided with the extra supply of water or the saccharin 0.08 g/L plus NHDC 0.02 g/L solution maintained their innate sweet preference for sucrose 20 g/L over animal plasma 20 g/L in the final choice test at the end of the experiments. In contrast, long-term exposure to

sucrose 160 g/L or maltodextrin 160 g/L solutions reversed this initial preference. One possible explanation of this change could be by an enhancing of the value of the protein solution. As discussed, sucrose 160 g/L and maltodextrin 160 g/L intakes generated a reduction in the feed intake of the animals. While piglets reached and covered their energy needs with the caloric load provided by the solution consumption, the intake of other nutrients, such as amino acids, were not fully covered meaning that the animals self-generated a protein-deficiency status. We have previously investigated this topic by submitting piglets to a protein-deficiency condition through varying diet composition, either by lowering the total crude protein content or increasing the digestible energy content of the diet (by manipulating the fat content). Perhaps surprisingly, we observed that piglets are unable to select and prefer a protein source based exclusively on its intrinsic flavour, and that in order to perform an appropriate selection pattern a learning process in which the sensory properties of the source solution is associated with the post-ingestive consequences of its consumption is needed (Guzmán-Pino et al., 2012, 2014). In the current experiments, the simultaneous short-term offer of sucrose 20 g/L and animal plasma 20 g/L solutions during the initial choice test did probably not generate this learning memory in the piglets. Therefore, although sucrose 160 g/L and maltodextrin 160 g/L exposure probably did produce a protein deficiency, the rejection of sucrose 20 g/L in the choice tests is unlikely to be exclusively due to an increase in the value of the alternative protein plasma solution.

Given that the choice behaviour of pigs exposed to sucrose or maltodextrin was not only due to an increase in the value of the protein solution, it must instead be also due to a decline in the value of the sucrose 20 g/L solution after the long-term sucrose 160 g/L or maltodextrin 160 g/L solution exposure. Critically, the response to a particular stimulus is not a fixed function of that stimulus, but instead is partially governed by previous and current exposure to other similar stimuli (Flaherty, 1996). In this way, the reduction in the sucrose 20 g/L preference in the final choice test might be due to a successive negative contrast effect in which sucrose 20 g/L seemed less valuable to the piglets than sucrose 160 g/L after the 12 days exposure, and as a result the consumption of sucrose 20 g/L was reduced. This hypothesis is supported by the results of Experiment 4, where, as expected, a higher intake and preference for sucrose 160 g/L than for sucrose 20 g/L solution was observed. The importance of taste similarity is consistent with previous results where, despite a protein deficiency generated by the incorporation of soybean oil in the diet (60 g/kg), piglets preferred sucrose 20 g/L solution over a protein solution in a 3-minute choice test (Guzmán-

Pino et al., 2012). In this case, the nutritional imbalance was not produced by a compound with the same basic taste as that tested (soybean oil vs. sucrose, i.e., fatty vs. sweet), and so the value of sucrose 20 g/L was not reduced in the subsequent choice test. Moreover, simultaneous negative contrast could have contributed to the reduction in feed consumption observed when piglets had concurrent access to a more palatable sucrose solution. Maltodextrin 160 g/L was also less preferred than sucrose 20 g/L in Experiment 4, supporting the idea that naive piglets do not have an innate preference for concentrated maltodextrin, especially if it is tested against an innately preferred solution such as sucrose. However, the hedonic value of maltodextrin 160 g/L might have been enhanced once the animals become familiar with the solution, and its post-ingestive consequences. Once this higher hedonic value for maltodextrin is established by experience it could then have reduced the attractiveness for sucrose 20 g/L due to a contrast effect after the long-term exposure.

Results obtained in the appetence tests were, in general, in line with those from the preference tests. That is, we observed significantly higher appetence for sucrose 20 g/L than for animal plasma 20 g/L solution in control piglets, a difference which was not present, and partially reversed, in animals with access to the sucrose 160 g/L and maltodextrin 160 g/L solutions, but not in piglets pre-exposed to saccharin 0.08 g/L plus NHDC 0.02 g/L. In fact, even though the low number of replicates in experimental groups (n=6), long-term access to sucrose 160 g/L solution produced a significantly higher appetence for the protein source when compared with the appetence for protein in animals in control groups.

More generally, the results reported in this work represent a potentially rewarding new approach for the study of human nutritional disorders such as obesity, especially in children. The increasing prevalence of human obesity has coincided with an environmental change in the availability of highly hedonic and/or caloric nutrients, e.g., sugar-sweetened beverages (Hill et al., 2003; Cota et al., 2006; de Ruyter et al., 2012). While the classic animal model of adult rats corresponds to a biological system that is inaccurate in the control of energy intake and weight gain (as it also typically seen in adult humans), the growing pig represents a behavioural model that finely regulates their energy homeostasis, even in the presence of a food-rich environment. Commercial pigs are mammals that have been genetically selected over the last decades for a high and efficient growth rate and lean mass deposit, which may explain the why they respond differently to access to high-calorie diet supplements. Given that children are also in stages of growth the weaned piglet may comprise a more appropriate

model than adult rats. Moreover, understanding the better caloric compensation observed in pigs than in rats or humans might provide a valuable insight into how exposure to highly sweet and caloric solutions contributes to the development of obesity and how this may be avoided.

5.6. Conclusion

The feeding behaviour of post-weaned piglets is affected by long-term exposure to complementary carbohydrate solutions, either sucrose 160 g/L or maltodextrin 160 g/L, but not by the free-availability of an extra saccharin 0.08 g/L plus NHDC 0.02 g/L solution. The effects include reductions in feed intake, growth performance, and the innate preference and appetence of the animals for sweet over protein. These data speak against the practicality of carbohydrate solution supplementation in pig diets, and suggest that piglets may represent a feasible and practical alternative animal model for the study of human eating disorders, such as obesity.

6. Chapter 4

Influence of dietary electrolyte balance on feed preference and appetence, and growth performance of post-weaned piglets

6.1. Abstract

A total of 672 male and female piglets (21 d post-weaning; approximately 13 kg BW) were selected to be used in three different experiments to assess the influence of dietary electrolyte balance (dEB; Na + K – Cl, in mEq/kg of diet) on feed preference, appetence, and growth performance. In Exp. 1, piglets were fed four isoenergetic and isoproteic starter diets differing in dEB level: two calcium-chloride diets (calcium chloride 10 g/kg; calcium carbonate 3.6 g/kg) ranging from 16 mEq/kg to 133 mEq/kg, based on the different incorporation of sodium bicarbonate (without or with 10 g/kg, respectively), and two calcium-carbonate diets (10.6 g/kg) ranging from 152 mEq/kg to 269 mEq/kg, based on the similar sodium-bicarbonate addition. Piglets fed the 16 mEq/kg and 133 mEq/kg diets achieved a higher ADG ($P < 0.04$), BW ($P < 0.04$), and apparent total-tract digestibility of CP and Zn ($P < 0.05$) than did piglets fed the 269 mEq/kg diet. The 16 mEq/kg level also reduced blood TCO_2 ($P < 0.01$), bicarbonate ($P < 0.01$), and base-excess ($P < 0.02$) concentrations, as compared with the rest of the dietary treatments. Three diets differing in dEB were designed for Exp. 2 and 3: -16 mEq/kg (calcium chloride 12.2 g/kg), 151 mEq/kg (basal), and 388 mEq/kg (calcium carbonate 8.6 g/kg; sodium bicarbonate 20 g/kg) diets. In Exp. 2, higher ADFI ($P = 0.03$), BW ($P = 0.02$), ADG ($P < 0.001$), and G:F ($P < 0.01$) were observed for piglets fed the -16 mEq/kg than those of the 388 mEq/kg diet. Subsequently, their short-term preference for these diets was assessed by using a 2-d choice-test protocol (30 min). Piglets showed a higher ($P < 0.001$) intake and preference for the 388 mEq/kg than they did for the -16 mEq/kg diet, independently of the dietary treatment they received before. Pigs also showed an innate rejection ($P < 0.001$) for the -16 mEq/kg when compared to the 151 mEq/kg diet. Exp. 3 assessed the long-term preference and appetence for the -16 mEq/kg and 388 mEq/kg diets. Similarly to Exp. 2, animals showed a higher ($P < 0.001$) intake of the 388 mEq/kg than they did of the -16 mEq/kg diet during both the preference (14 d) and appetence (2h) evaluation conducted in this experiment. Results show that low (-16 mEq/kg–133 mEq/kg) rather than high (269 mEq/kg–388 mEq/kg) dEB levels optimize growth performance of piglets. When they have the opportunity to choose, they are unable to select the diet that optimizes their performance, neither in short- nor long-term preference tests, showing also a higher appetence for high instead of low dEB levels.

6.2. Introduction

Feeds in the animal industry are usually fortified with minerals, such as calcium (limestone), phosphorus (calcium phosphate), and sodium and chloride (salt, sodium bicarbonate). Through fortification, feed provides the necessary mineral requirements, but it may also modify the dietary electrolyte balance (dEB), which is the net balance between fixed cations and anions (Na + K – Cl, in mEq/kg of diet). It represents, in part, the net acid or alkaline load contributed by the diet (Patience and Chaplin, 1997), and may significantly alter the acid-base status and, consequently, the performance of pigs (Patience et al., 1987; Dersjant-Li et al., 2002). It is generally accepted that a negative dEB, usually provided by an excess of chloride ions, results in reduced feed intake, while dEB in a positive range, i.e., supplementing feeds with sodium bicarbonate, optimizes pig growth (Patience et al., 1987; Patience and Wolynetz, 1990; Dersjant-Li et al., 2001). However, to our knowledge, the ability of pigs to select and prefer diets with high, instead of low, dEB values has not been previously tested.

On the other hand, minerals such as calcium carbonate or sodium bicarbonate show a high acid-binding capacity in the digestive tract which may increase gastric pH of weanling piglets when added to the post-weaning diets (Lawlor et al., 2005). Therefore, feed mineral fortification may also affect the digestive process and general status of the animals, with effects that may be divergent in relation to the animal's requirements. The general hypothesis tested in this study is that post-weaned piglets are able to choose diets differing in dEB and mineral ingredients, preferring those levels that optimize their growth performance. The objectives are to define feed preference and appetence, and changes in nutrient metabolism attributed to dEB, as distinct from those due to specific mineral sources, either calcium chloride, calcium carbonate, or sodium bicarbonate.

6.3. Materials and methods

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

6.3.1. Animals and housing

In total, 672 male and female piglets [Pietrain × (Landrace × Large White)] were selected to be used in three different experiments. Exp. 1 was performed with a total of 240 piglets, Exp. 2 with a total of 336 animals, with Exp. 3 using 96 piglets. Piglets were weaned at 28 d of age, and then, on d 21 after weaning, were distributed with an average initial BW of 13.4 kg ± 1.17 kg (mean ± S.D.) in Exp. 1, 11.3 kg ± 1.31 kg in Exp. 2, and 13.3 kg ± 0.90 kg in Exp. 3. In Exp. 1 and 2 piglets were distributed according to their BW into three blocks of weight of 80 animals each (Light: 12.1 kg ± 0.64 kg, Middle: 13.4 kg ± 0.45 kg, and Heavy: 14.7 kg ± 0.41 kg in Exp. 1, and Light: 9.9 kg ± 0.53 kg, Middle: 11.3 kg ± 0.44 kg, and Heavy: 12.8 kg ± 0.74 kg in Exp. 2). The animals in each block were further distributed into eight pens of 10 piglets in a weanling room with 24 pens. Within each weight class, two pens were randomly assigned to receive one of the four experimental diets designed for Exp. 1, while four pens were randomly assigned to one of two diets formulated in Exp. 2. In Exp. 3, animals were randomly distributed into 24 pens of four piglets each. The weaning room had automatic, forced ventilation and completely slatted flooring. Each pen (3.2 m^2 in floor area) was equipped with a hopper feeder with three feeding spaces and an independent water supply to ensure ad libitum feeding and freshwater access.

6.3.2. Experimental diets and feeding

In Exp. 1, four isoenergetic and isoproteic starter-diets differing in dEB level were formulated and offered to the animals from 21 to 37 d post-weaning (Table 6.1). A basal diet mainly containing maize (350 g/kg), barley (200 g/kg), wheat (111 g/kg), and extruded soybean (166.9 g/kg) was mixed with different concentrations of calcium chloride, calcium carbonate, and sodium bicarbonate in order to generate a range of dEB levels. Thus, two calcium-chloride diets (calcium chloride 10 g/kg; calcium carbonate 3.6 g/kg) ranged from 16 mEq/kg to 133 mEq/kg, based on the different incorporation of sodium bicarbonate (without or with 10 g/kg, respectively), and two calcium-carbonate diets (10.6 g/kg) ranged from 152 mEq/kg to 269 mEq/kg, based on the similar sodium-bicarbonate addition. Titanium dioxide (3 g/kg) was also used as the indigestible marker in all diets.

Table 6.1. Composition, chemical analysis and estimated nutrient content of the starter diets used in Exp. 1 (as-fed basis).

	Dietary electrolyte balance, mEq/kg			
Item	16	133	152	269
Ingredients, g/kg				
Maize	350.0	350.0	350.0	350.0
Barley	200.0	200.0	200.0	200.0
Wheat	111.0	111.0	111.0	111.0
Extruded soybean	166.9	166.9	166.9	166.9
Soybean meal 44% CP	50.0	50.0	50.0	50.0
Fishmeal LT	50.0	50.0	50.0	50.0
Sweet milk whey	25.0	25.0	25.0	25.0
L-Lysine-HCl	3.5	3.5	3.5	3.5
DL-Methionine	1.5	1.5	1.5	1.5
L-Threonine	2.7	2.7	2.7	2.7
L-Tryptophan	0.4	0.4	0.4	0.4
Monocalcium phosphate	5.6	5.6	5.5	5.5
Mineral-vitamin mix[1]	4.0	4.0	4.0	4.0
Salt	2.7	2.7	2.7	2.7
Calcium chloride	10.0	10.0	–	–
Calcium carbonate	3.6	3.6	10.6	10.6
Sodium bicarbonate	–	10.0	–	10.0
Diatomite	10.0	–	13.0	3.0
Phytase	0.2	0.2	0.2	0.2
Titanium dioxide	3.0	3.0	3.0	3.0
Chemical analysis, g/kg				
Dry matter	890.4	890.5	897.1	893.6
Crude protein	185.7	186.4	185.1	188.0
Neutral detergent fiber	9.9	9.5	10.5	10.2
Fat	52.6	53.3	52.5	55.0
Ash	67.9	59.4	79.5	65.1
pH	6.0	6.5	6.1	6.8
Acid-binding capacity, mEq/kg	340.0	373.3	380.0	426.7
Buffering capacity, mEq/kg	113.3	107.3	120.9	112.3
Estimated nutrient content, g/kg				
Digestible energy, Mcal/kg	3.41	3.41	3.41	3.41
Sodium	1.8	4.5	1.8	4.5
Potassium	7.2	7.2	7.2	7.2
Chloride	8.8	8.8	4.0	4.0
Calcium	7.5	7.5	7.5	7.5
Zinc, mg/kg	138.7	138.7	139.4	139.4

[1]Supplied per kg of feed: 3 mg of ethoxiquin, 14000 UI of vitamin A, vitamin D 1000 UI as vitamin D_3 and 500 UI as 25-hydroxycholecalciferol, vitamin E 50 mg as alpha-tocopherol acetate and 40 mg of RRR-alpha-tocopherol, 2 mg of vitamin K_3, 3 mg of vitamin B_1, 7 mg of vitamin B_2, 3.5 mg of vitamin B_6, 0.06 mg of vitamin B_{12}, 45 mg of nicotinic acid, 17 mg of pantothenic acid, 0.2 mg of biotin, 1.5 mg of folic acid, 40 mg of Fe, Cu 5 mg as cupric sulphate pentahydrate and 15 mg as cupric chelate of glycine, Zn 80 mg as zinc oxide and 25 mg as zinc chelate of glycine, Mn 25 mg as manganese oxide and 15 mg as manganese chelate of glycine, 0.7 mg of I, Se 0.1 mg as organic selenium and 0.2 mg of sodium selenite, 0.1 mg of Co.

Table 6.2. Composition, chemical analysis and estimated nutrient content of the starter diets used in Exp. 2 and 3 (as-fed basis).

	Dietary electrolyte balance, mEq/kg		
Item	-16	151	388
Ingredients, g/kg			
Maize	350.0	350.0	350.0
Barley	200.0	200.0	200.0
Wheat	125.0	100.7	100.7
Extruded soybean	162.3	170.9	170.9
Soybean meal 44% CP	50.0	50.0	50.0
Fishmeal LT	50.0	50.0	50.0
Sweet milk whey	25.0	25.0	25.0
L-Lysine-HCl	3.5	3.4	3.4
DL-Methionine	1.4	1.5	1.5
L-Threonine	2.7	2.7	2.7
L-Tryptophan	0.9	0.4	0.4
Monocalcium phosphate	9.9	9.9	9.9
Mineral-vitamin mix[1]	4.0	4.0	4.0
Salt	3.0	3.0	3.0
Calcium chloride	12.2	–	–
Calcium carbonate	–	–	8.6
Sodium bicarbonate	–	–	20.0
Diatomite	–	28.6	–
Chemical analysis, g/kg			
Dry matter	893.3	894.7	897.0
Crude protein	194.1	193.4	193.3
Neutral detergent fiber	9.6	9.6	9.4
Fat	48.3	52.0	52.3
Ash	54.5	56.2	64.6
pH	5.8	5.9	6.8
Acid-binding capacity, mEq/kg	186.7	237.8	426.7
Buffering capacity, mEq/kg	65.8	81.3	112.2
Estimated nutrient content, g/kg			
Digestible energy, Mcal/kg	3.44	3.39	3.39
Sodium	1.9	1.9	7.3
Potassium	7.2	7.2	7.2
Chloride	10.1	4.2	4.2
Calcium	7.5	7.5	7.5
Zinc, mg/kg	139.3	138.4	138.4

[1]Supplied per kg of feed: 3 mg of ethoxiquin, 14000 UI of vitamin A, vitamin D 1000 UI as vitamin D_3 and 500 UI as 25-hydroxycholecalciferol, vitamin E 50 mg as alpha-tocopherol acetate and 40 mg of RRR-alpha-tocopherol, 2 mg of vitamin K_3, 3 mg of vitamin B_1, 7 mg of vitamin B_2, 3.5 mg of vitamin B_6, 0.06 mg of vitamin B_{12}, 45 mg of nicotinic acid, 17 mg of pantothenic acid, 0.2 mg of biotin, 1.5 mg of folic acid, 40 mg of Fe, Cu 5 mg as cupric sulphate pentahydrate and 15 mg as cupric chelate of glycine, Zn 80 mg as zinc oxide and 25 mg as zinc chelate of glycine, Mn 25 mg as manganese oxide and 15 mg as manganese chelate of glycine, 0.7 mg of I, Se 0.1 mg as organic selenium and 0.2 mg of sodium selenite, 0.1 mg of Co.

The same diet composition was used for Exp. 2 and 3 (Table 6.2). Three isoenergetic and isoproteic starter-diets differing in dEB level were offered to the animals starting at 21 d post-weaning. The dEB levels offered were -16 mEq/kg, 151 mEq/kg, and 388 mEq/kg, with the -16 mEq/kg diet containing calcium chloride (12.2 g/kg), and the 388 mEq/kg diet containing calcium carbonate (8.6 g/kg) and sodium bicarbonate (20 g/kg). The basal diet, 151 mEq/kg, mainly contained maize (350 g/kg), barley (200 g/kg), wheat (100.7 g/kg), and extruded soybean (170.9 g/kg), and was incorporated with diatomite (28.6 g/kg) instead of calcium chloride, calcium carbonate, or sodium bicarbonate. All diets were offered ad libitum in mash form.

6.3.3. Experimental designs

Exp. 1 was designed to evaluate changes in the acid-base status, nutrient metabolism and growth performance of piglets associated with variations on dEB levels. Piglets were fed the 16 mEq/kg, 133 mEq/kg, 152 mEq/kg, and 269 mEq/kg dietary treatments for a period of 16 d, at which time feed disappearance and BW gain was monitored. There were six pens of 10 piglets per treatment; the pen was considered the experimental unit. After 7 d of feeding, one representative fecal sample per pen was collected in order to measure apparent total-tract crude protein and zinc digestibility. The samples consisted of a homogeneous mixture from more than 50% of the piglets of each pen, which were then stored at -20ºC prior to analysis. Later, on d 12 of feeding, the acid-base status of animals was assessed. Five venous blood samples per treatment from piglets of different pens were collected via jugular venipuncture into 3-mL lithium heparinized vacuum tubes, and then analyzed for pH, partial pressure of CO_2 (PCO_2), total carbon dioxide (TCO_2), bicarbonate (HCO_3), base excess and sodium, potassium and chloride by using an i-STAT Portable Clinical Analyzer with EC8+ cartridges (i-STAT Corp.; Princeton, NJ).

The objective of Exp. 2 was to further explore the effect of diets differing in dEB levels on growth performance of piglets and also on their subsequent short-term preference for these diets. Two extreme dietary treatments, -16 mEq/kg and 388 mEq/kg diets, were designed to be offered to the animals for 12 d, at which time productive performance was evaluated. Twelve pens (experimental unit) were assigned for each experimental diet. After the single-

diet feeding period, the short-term preference of piglets for the -16 mEq/kg or the 388 mEq/kg diets was assessed by using a 2-d choice-test protocol for 30 min (Guzmán-Pino et al., 2012, 2014). Four piglets of each pen were randomly selected to be tested as a group over 2 d, with two feeders placed in the front of the pen. Each feeder contained 1.4 kg ± 0.19 kg of each diet, on average. To control for side preference, diet position inside the pen was changed within pens and between days of the test, i.e., the -16 mEq/kg diet was offered on the left side of the pen and the 388 mEq/kg diet on the right side for half the pens of each diet group the first day of testing, and the second day the left-right diet position was rotated in relation to the position of the previous day. At the same time, the short-term preference of naive piglets for the -16 mEq/kg and the 388 mEq/kg diets against the 151 mEq/kg level as reference diet was also assessed in this experiment by following the same procedure previously described.

Exp. 3 aimed to evaluate the long-term preference and appetence of piglets for two different electrolyte-balanced diets. In this study, a total of 24 pens of four piglets each were used. Firstly, 12 pens were offered the -16 mEq/kg and 388 mEq/kg diets in two feeders placed in the front of the pens for a total period of 14 d, starting at d 21 post-weaning. During this period, feeders were regularly checked and refilled at least daily in order to provide an ad libitum exposure to the experimental diets. As in Exp. 2, in order to avoid an undesirable side preference, the left-right diet position inside the pen was counterbalanced between pens. Subsequently, the appetence of naive piglets for the -16 mEq/kg and the 388 mEq/kg diets was assessed with the remaining 12 pens by using a one-feeder test protocol over two alternate days (Guzmán-Pino et al., 2014), starting at d 35 post-weaning. A single feeder containing 1.0 kg of each diet was offered to the piglets for 2h each day. The order of first testing the different dEB diets on the two days of testing was counterbalanced across pens.

6.3.4. Calculations and statistical analysis

Feed intake measured for each pen during the 2-d (30 min/d) choice-tests of Exp. 2, it was averaged, and the mean value was considered for the analysis. Feed-intake values measured for each pen during the 14-d choice-test of Exp. 3 were averaged by week and the mean value was considered. Then, these values, as well as the one-feeder-test's results of Exp. 3, were averaged for the number of piglets that performed each test (four piglets) and were

standardized to the different weights of the animals in each treatment and experiment by dividing by the registered BW on the test days. The standardization aimed to make the feed intake registered for animals with different BW comparable; therefore, it diminishes differences in consumption due to different ingestive capacities of the animals.

Choice-test data were analyzed with ANOVA by using the MIXED procedure of SAS (9.2; SAS Inst. Inc.; Cary, NC), taking into account the dEB level (-16 mEq/kg, 151 mEq/kg, or 388 mEq/kg diets), block of weight (only in Exp. 2; light, middle, or heavy), and their interaction as main factors. When the interaction between dEB and block did not reach significance in a first analysis, it was removed from the final model. The group of four piglets was considered the experimental unit and was entered into the model as a repeated measure, specifying the covariance matrix structure as compound symmetry (which yielded the lowest Bayesian information criteria). In addition to the intake registers, the preference values for the 151 mEq/kg or the 388 mEq/kg diets were measured as the percentage that this feed comprised of the total feed intake and were compared to the neutral value of 50% of preference by using a Student's *t*-test.

Apparent total-tract digestibility values of Exp. 1 were calculated based on the nutrient and titanium-dioxide contents of feces relative to dietary contents using the index method (Adeola, 2001). These data, data from the acid-base assessment in Exp. 1, and feed-intake and growth-performance data in Exp. 1 and 2 were analyzed with a statistical model considering the same main factors previously described with ANOVA by using the GLM procedure of SAS. For all of the analysis, average values were compared by least-squares means with the Tukey adjustment for multiple comparisons. The α level used for the determination of significance was 0.05, and tendencies for $0.05 < P < 0.1$ are also presented.

6.4. Results

In Exp. 1, piglets fed the 16 mEq/kg diet tended ($P = 0.06$) to show higher ADFI than did piglets fed the 269 mEq/kg diet (Table 6.3). A higher ADG ($P < 0.04$) and BW ($P < 0.04$) at the end of the exposure were also achieved for the animals fed the calcium-chloride diets, both the 16 mEq/kg and 133 mEq/kg diets, than for those fed the 269 mEq/kg diet. Piglets fed the 16 mEq/kg diet had lower blood TCO_2 ($P < 0.01$), bicarbonate ($P < 0.01$), and base excess

($P < 0.02$) levels than did piglets fed the 133 mEq/kg, 152 mEq/kg, or 269 mEq/kg diets (Table 6.4). No differences ($P > 0.15$) were observed in pH, PCO_2, sodium, or potassium concentrations among dietary treatments. However, blood chloride concentration was higher ($P = 0.02$) in piglets fed the 16 mEq/kg than those fed the 152 mEq/kg diet. The apparent digestibility of crude protein and zinc was lower ($P < 0.05$) in piglets fed the 269 mEq/kg diet when compared to that of animals fed the 16 mEq/kg or 133 mEq/kg diets (Table 6.5).

Table 6.3. Feed intake and growth performance of piglets fed the experimental diets in Exp.1.

	Dietary electrolyte balance, mEq/kg					*P*-value	
Item	16	133	152	269	SEM	dEB	Block
Initial BW, kg	13.22	13.49	13.66	13.16	0.204	0.34	< 0.001
ADFI, g/d	835.2	776.9	770.1	749.8	22.3	0.06	< 0.01
ADG, g/d	361.9^{a}	373.3^{a}	318.2^{ab}	243.4^{b}	28.1	< 0.04	0.76
G:F	0.44^{ab}	0.48^{a}	0.41^{ab}	0.33^{b}	0.033	0.02	0.41
Final BW, kg	19.01^{a}	19.46^{a}	18.75^{ab}	17.05^{b}	0.463	< 0.04	< 0.02

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

Table 6.4. Acid-base profile of piglets fed the experimental diets in Exp. 1.

	Dietary electrolyte balance, mEq/kg					*P*-value	
Parameter	16	133	152	269	SEM	dEB	Block
pH	7.30	7.36	7.35	7.36	0.02	0.18	0.79
PCO_2, mmHg	61.5	57.7	59.8	58.7	2.71	0.72	0.30
TCO_2, mmol/L	32.0^{a}	35.2^{b}	35.8^{b}	35.0^{b}	0.53	< 0.01	0.06
Bicarbonate, mmol/L	30.0^{a}	33.3^{b}	33.8^{b}	33.3^{b}	0.60	< 0.01	0.10
Base excess, mmol/L	3.0^{a}	7.7^{b}	7.8^{b}	7.3^{b}	0.88	< 0.02	0.77
Sodium, mmol/L	140.2	142.0	140.7	141.0	0.87	0.42	0.34
Potassium, mmol/L	6.5	6.0	6.5	7.3	0.40	0.15	0.84
Chloride, mmol/L	103.9^{a}	102.9^{ab}	100.7^{b}	102.7^{ab}	0.71	0.02	0.73

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

Table 6.5. Apparent total-tract crude protein and zinc digestibility for the experimental diets used in Exp. 1.

	Dietary electrolyte balance, mEq/kg					*P*-value	
Apparent digestibility, %	16	133	152	269	SEM	dEB	Block
Crude protein	73.3^{ab}	74.6^{a}	69.6^{bc}	68.2^{c}	1.2	< 0.04	0.70
Zinc	9.1^{a}	13.0^{a}	10.6^{a}	1.1^{b}	2.1	< 0.05	0.26

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

In Exp. 2, a higher ADFI (P = 0.03), BW (P = 0.02), ADG (P < 0.001), and G:F (P < 0.01) was observed for piglets fed the -16 mEq/kg diet than for piglets fed the 388 mEq/kg diet (Table 6.6). When animals were given the opportunity to choose between the two diets during the subsequent short-term preference test, they showed a higher (P < 0.001) intake of the 388 mEq/kg than of the -16 mEq/kg diet, independently of the dietary treatment they were fed before (Figure 6.1). Thus, the preferences values observed for the high dEB level, 68% and 70%, were higher (P < 0.001) than was the neutral value of 50% in both groups of animals. When these diets were tested against the 151 mEq/kg diet as reference, no significantly different (P = 0.24) intake was observed between the 388 mEq/kg and the 151 mEq/kg diets (Figure 6.2). On the other hand, a higher (P < 0.001) intake of the 151 mEq/kg diet was observed when it was tested against the -16 mEq/kg diet. The preference for the basal diet of 82% was significantly higher (P < 0.001) than was the neutral value of 50%, in this case.

Table 6.6. Feed intake and growth performance of piglets fed the experimental diets in Exp.2.

	Dietary electrolyte balance, mEq/kg			*P*-value	
Item	-16	388	SEM	dEB	Block
Initial BW, kg	11.27	11.34	0.172	0.78	< 0.001
ADFI, g/d	904.8[a]	859.0[b]	14.0	0.03	< 0.01
ADG, g/d	503.7[a]	438.5[b]	11.4	< 0.001	0.04
G:F	0.56[a]	0.51[b]	0.009	< 0.01	0.81
Final BW, kg	17.32[a]	16.60[b]	0.199	0.02	< 0.001

[a,b]Within a row, means without a common superscript differ (P < 0.05).

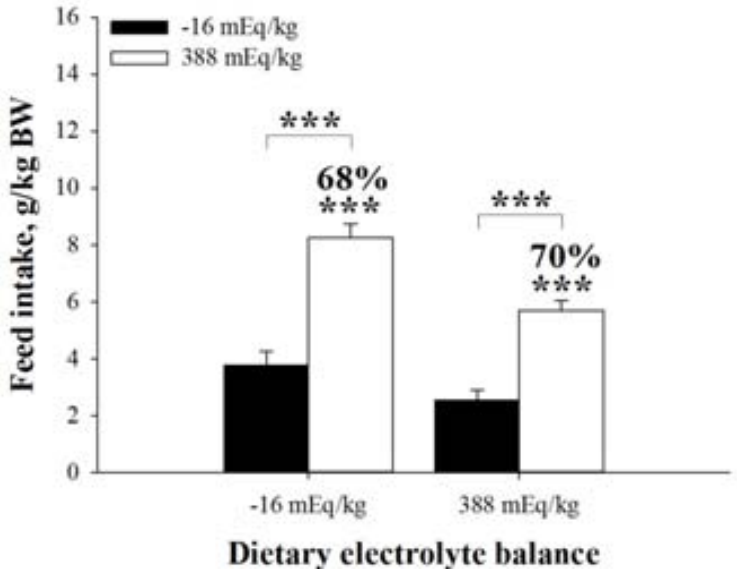

Figure 6.1. Effect of feeding diets differing in their electrolyte balance (-16 mEq/kg and 388 mEq/kg diets) on intake and preference of piglets for the same diets during the short-term choice test conducted in Exp. 2. Clasps indicate different ($P < 0.001$) intakes between both diets. Numbers on top of the bars represent percent intake of this diet and its difference ($P < 0.001$) from the neutral value of 50%.

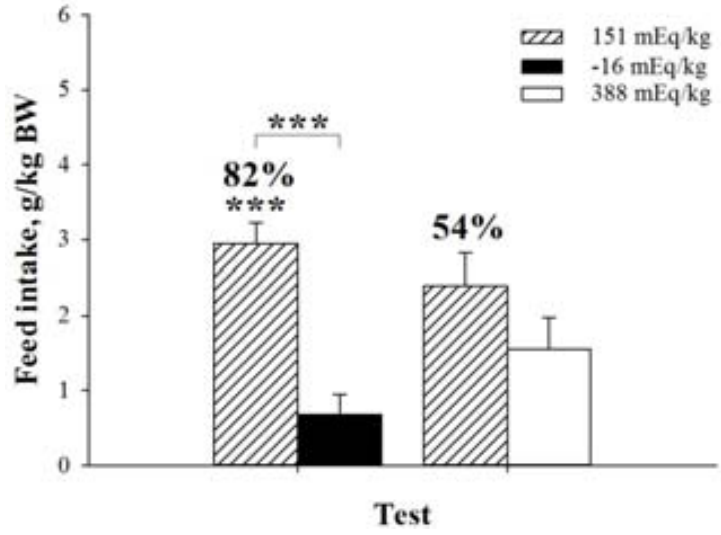

Figure 6.2. Intake and preference of piglets for diets differing in their electrolyte balance (-16 mEq/kg, 151 mEq/kg, and 388 mEq/kg diets) during the short-term choice test conducted in Exp. 2. Clasp indicates different ($P < 0.001$) intakes between both diets. Numbers on top of the bars represent percent intake of this diet and its difference ($P < 0.001$) from the neutral value of 50%.

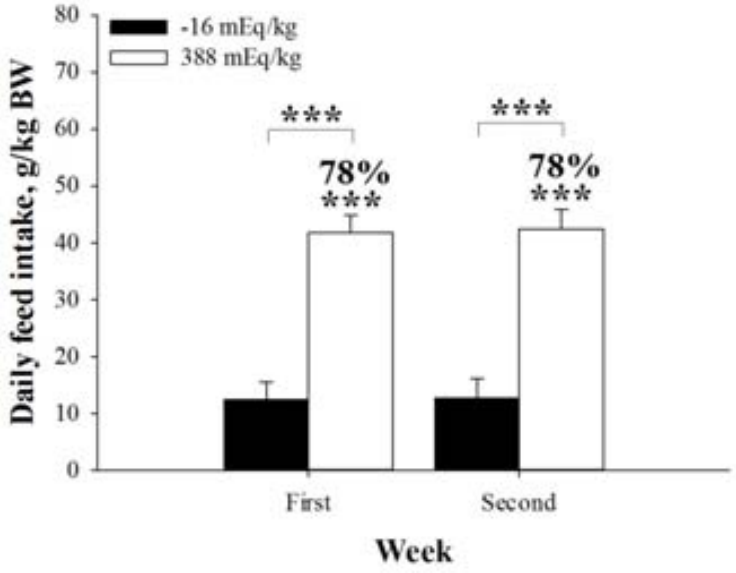

Figure 6.3. Intake and preference of piglets for diets differing in their electrolyte balance (-16 mEq/kg and 388 mEq/kg diets) during two weeks in the long-term choice test conducted in Exp. 3. Clasps indicate different ($P < 0.001$) intakes between both diets. Numbers on top of the bars represent percent intake of this diet and its difference ($P < 0.001$) from the neutral value of 50%.

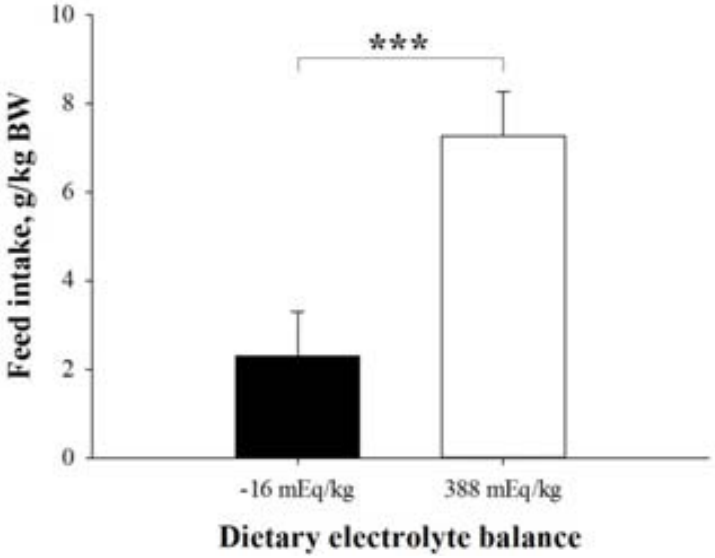

Figure 6.4. Intake of piglets of diets differing in their electrolyte balance (-16 mEq/kg and 388 mEq/kg diets) during the one-feeder test conducted in Exp. 3. Clasp indicates different ($P < 0.001$) intake between both diets.

In Exp. 3, a higher ($P < 0.001$) intake of the 388 mEq/kg diet, in comparison with the intake of the -16 mEq/kg diet, was observed during the long-term (14 d) preference assessment (Figure 6.3). Thus, the high dEB level achieved a preference value of 78%, which was higher ($P < 0.001$) than the 50% of preference throughout the experiment. Piglets also showed a higher ($P < 0.001$) intake of the 388 mEq/kg diet than of the -16 mEq/kg diet during the one-feeder access in this experiment (Figure 6.4).

6.5. Discussion

The experimental diets were designed to generate a set of different dEB values; from 16 mEq/kg to 269 mEq/kg (Exp. 1), and from -16 mEq/kg to 388 mEq/kg (Exp. 2 and 3). Different diets were obtained by varying the sources of calcium and/or sodium supplemented into the basal diet of each experiment. Thus, in order to reduce the dEB value, calcium carbonate (supplied as limestone) was replaced by calcium chloride, maintaining an optimal and constant calcium concentration in the diets (7.5 g/kg) to meet requirements of growing pigs between 11 kg and 25 kg (7.0 g/kg; NRC, 2012), but increasing chloride concentration. In contrast, sodium bicarbonate was supplied to increase the dietary sodium concentration and dEB, as previously referred to by other authors (Patience et al., 1987; Patience and Wolynetz, 1990; Patience and Chaplin, 1997; Dersjant-Li et al., 2001). Chloride and/or sodium concentrations were not presented in toxic quantities in the diets (NRC, 2012).

In Exp. 1, it was observed that piglets fed the 16 mEq/kg diet for 12 d had lower blood bicarbonate, base excess, and TCO_2 concentrations, in comparison with piglets fed the rest of the dietary treatments. These results reflect the acidogenic nature of the 16 mEq/kg diet and the influence of dEB on the acid-base profile of pigs, which has been previously documented. For example, Patience et al. (1987) showed a decrease in bicarbonate and base excess levels by feeding pigs diets with dEB values lower than 175 mEq/kg. Similarly, Patience and Chaplin (1997) and Dersjant-Li et al. (2002) reported that pigs fed a -20 mEq/kg and a -100 mEq/kg diet reduced blood pH, bicarbonate, and base excess, when compared with a 163 mEq/kg and a 200 mEq/kg diet, respectively.

As reported by NRC (2012), the optimal electrolyte balance in the diet for pigs is about 250 mEq/kg. However, growth-performance results in the literature are contradictory. Austic

et al. (1983) suggested that dEB values ranging from 100 mEq/kg to 300 mEq/kg allowed for optimal performance of pigs and, similarly, Patience et al. (1987) reported that optimal growths were obtained with diets of around 175 mEq/kg. Dersjant-Li et al. (2001) reported the best growth rates when pigs were fed diets with 200 mEq/kg and 500 mEq/kg, as compared to pigs fed a -100 mEq/kg diet. In the same way, Haydon et al. (1990) showed a linear increase in daily feed intake with increasing the dEB value from 25 mEq/kg to 400 mEq/kg. Thus, it has been generally accepted that acidogenic diets with a high concentration of chloride ions and low dEB values reduce the performance of young pigs. Nonetheless, Patience and Chaplin (1997) reported a tendency to a faster growth and an improved gain:feed ratio when piglets were fed a -20 mEq/kg diet instead of a 104 mEq/kg or a 163 mEq/kg diet. Here, it was observed in both Exp. 1 and 2 that the best performances were obtained by feeding animals diets with low, instead of high, dEB values. Piglets fed the 16 mEq/kg and 133 mEq/kg diets in Exp. 1, or the -16 mEq/kg diet in Exp. 2, showed a higher feed intake, weight gain, gain:feed ratio, and BW at the end of the exposure, in comparison with piglets fed the 269 mEq/kg or 388 mEq/kg diets, respectively. This suggests that post-weaned piglets can develop optimal growth rates even with diets with low dEB values, lower than the optimal range suggested by NRC (2012).

The poor performance observed in piglets fed high dEB levels in these experiments may be explained by the digestibility results of Exp. 1. The highest electrolyte-balanced diet (269 mEq/kg) reduced the apparent digestibility of crude protein and zinc, as compared to diets with dEB levels lower than 133 mEq/kg. Both, amino acids and zinc, are considered key nutrients for the growth of the animal (Harper et al., 1970; Hahn and Baker, 1993; D'Mello, 2003). It was observed that piglets at weaning have a low acid secretion in the stomach that, in addition to other factors such as low concentration of lactic acid and/or irregular consumption of large meals, may result in an elevated gastric pH, even higher than 5.0 (Kidder and Manners, 1978; Lawlor et al., 2005). In this respect, dietary incorporation of sources such as calcium carbonate or sodium bicarbonate, which show a high acid-binding capacity value, may raise the stomach pH of weanling pigs (Bolduan et al., 1988; Lawlor et al., 2005). The effect of a high gastric pH after weaning is detrimental for protein digestion, as pepsinogen could barely be converted into pepsin at a pH greater than 5.0 in the stomach (Kidder and Manners, 1978; Yen, 2001). In addition, the increased gastric pH may have also affected the solubility of other minerals, such as calcium, phosphorus, and zinc, by promoting the generation of Zn-Ca-phytate precipitates (Simpson and Wise, 1990).

The hypothesis tested in the short-term preference test of Exp. 2 was that piglets previously fed the -16 mEq/kg and 388 mEq/kg diets for 12 d would select and prefer the diet that best fits to balance their internal milieu, and this would be performed by exclusively using the intrinsic properties of the offered sources. It is well-known that animals have a constant tendency to maintain their acid-base homeostasis, and this may be reflected through the dietary selection pattern they perform (Forbes, 1998). In addition, it has been described that in the case of deficiency of some nutrients, such as sodium, calcium, or phosphorus, animals are able to almost instantaneously select a food supplemented with the nutrient without previous experience with that food in order to reestablish homeostasis (Denton, 1982; Blair-West et al., 1992; Leshem, 1999). This makes special sense in the case of piglets fed the low dEB level, whose dietary sodium concentration (1.9 g/kg) was lower than the sodium requirements (2.8 g/kg) according to NRC (2012). The suggestion, which has been studied mainly in laboratory rodents, but extrapolated to all mammals, involves the idea that animals may use unlearned "specific appetites" to select appropriate diets, and particularly when they are sodium-deficient (Schulkin, 1982). However, contrary to what was expected, we observed that when offered as a choice, both groups of animals showed a higher intake and preference for the 388 mEq/kg than for the -16 mEq/kg diet independently of the dEB they had previously received. It could be suggested that in these tests the distinct taste of the 388 mEq/kg diet, i.e., its high sodium concentration, was naturally preferred over that of the -16 mEq/kg diet, which was high in chloride. Animals recognize sodium by a highly specific sodium channel in taste-receptor cells that elicit salty taste (Beauchamp and Stein, 2008). In fact, many species appear to like the taste of salt, and for this reason salt has normally been used as food flavor-enhancer (Desmond, 2006). Nonetheless, the most likely rationalization for this result may rely on an innate rejection mechanism for chloride, rather than an inherent preference for sodium. Taste properties of divalent salts such as calcium chloride are complex, but they are characterized primarily by bitter taste, with additional sensations being described as metallic, astringent, and sour (Lawless et al., 2003, 2004). Bitter taste compounds are innately rejected by animals, as they may signal the presence in the diet of anti-nutritional factors, drugs or potentially toxic compounds (Blair and Fitzsimons, 1970; Janssen and Depoortere, 2013). Thus, in this experiment it was also observed that naive piglets showed a rejection for the -16 mEq/kg diet when it was tested against the basal 151 mEq/kg diet, which supports the idea of the innate aversion for the calcium-chloride diet.

The ability to appropriately choose feeds or nutrient sources based on any particular nutritional status has been studied in pigs in previous studies conducted by our group, especially when related to nutritional imbalances in the dietary protein-to-energy ratio (Guzmán-Pino et al., 2012, 2014). Thus, we have observed that pigs are able to perform appropriate dietary-selection patterns to correct previous underfeeding or a particular deficiency status in order to re-establish homeostasis when a learning process occurred associated with positive stimuli. Learning lets the animals associate the sensory properties of the offered sources with the metabolic post-ingestive consequences induced by consumption (Forbes, 1998). The short-term exposure to the experimental diets during preference tests of Exp. 2 probably did not allow piglets to generate a learning memory between the organoleptic and metabolic properties of the feeds. However, long-term exposure during the preference test of Exp. 3 probably did allow it. Therefore, it could be speculated that the proportions of the -16 mEq/kg and 388 mEq/kg diets selected during this test reflected the proportion that provided the animals with the optimum acid-base balance, based on a learned association.

The higher appetence observed for the 388 mEq/kg rather than for the -16 mEq/kg diet in the one-feeder test of Exp. 3 confirms the dissimilar (higher) initial orosensorial motivation of piglets for the consumption of diets incorporated with sodium bicarbonate than for those with calcium chloride that may be innately aversive when they are given as a choice. The appetence reflected the initial consumption over 2h, and was not related with the total feed consumption when diets were offered as single diets over 12 d or 16 d. However, studies in pigs show that a bitter compound that is initially aversive and rejected once detected may be normally consumed after some exposures as long as it does not generate discomfort or toxic consequences to the animals after the ingestion (Blair and Fitzsimons, 1970; Forbes, 2010), which might be the case in this study.

In conclusion, dEB significantly influences the productive performance and acid-base status of post-weaned piglets, however, differing from what has been traditionally accepted, we observed that low (from -16 mEq/kg to 133 mEq/kg) rather than high (from 269 mEq/kg to 388 mEq/kg) dEB levels optimized growth performance. The effects may be attributed to a lower apparent digestibility of crude protein and zinc when piglets are fed single diets with high dEB levels, due to the predominant supplementation with minerals such as calcium carbonate and sodium bicarbonate, which have a great acid-binding capacity in the stomach. When piglets had the chance to select diets with low or high dEB values, they preferred those

with a high dEB either in short- or long-term preference tests. Similarly, piglets showed a higher appetence for the high dEB level, probably due to inherent mechanisms that favor and motivate animals for the detection and ingestion of sodium instead of chloride.

7. General discussion

As it is generally accepted, weaning is the most critical period for the pig life under the current intensive production conditions. The situation in the intensive system (early weaning at 3-4 weeks of life) has a wide contrast with how weaning take place in the wild. From 9th to 22th weeks of age, piglets get used to the new condition as they learn about food and water sources without interruption of milk intake (Jensen and Recén, 1989). In addition, social interactions with their mother and experienced conspecifics smooth the transition of feeding behavior patterns necessary for weaning adaption (Graves, 1984). On the contrary, early weaning generates a set of stressor factors to the piglets that may negatively influence feed intake and growth performance for the rest of their life.

The present PhD Thesis aimed to simulate different physiological or nutritional status that post-weaned piglets face in the intensive production system. We hypothesized that commercial piglets may have retained the capacity to perform appropriate dietary selection patterns in relation to their needs, even when the common feeding practices in the pig industry do not allow animals to select their own diet composition. The hypothesis says that when given the opportunity to choose among sources of different nature (e.g., carbohydrate or protein sources), piglets will be able to select and prefer those sources that best fit to their nutritional requirements. It was thought that an accurate knowledge of the dietary preferences of piglets at weaning, and the likely mechanisms driving their feeding behaviour, would be key components to try to improve the acceptance of young animals' diets at this stage.

First of all, we examined the effects of the dietary macronutrient composition on the ability of piglets to choose appropriate sources. This was performed by manipulating the dietary contents of digestible energy (Chapter 1) and crude protein (Chapter 2) during the pre-starter phase. Then, we studied more in depth the innate attraction of piglets for sweet taste compounds by assessing the effect of a long-term access to an extra carbohydrate or artificial sweetener solution during the starter phase (Chapter 3). Finally, the effects of the mineral composition, resulting in different dietary electrolyte balance offered to the animals, were evaluated during the same stage with the aim of the appropriate selection (Chapter 4).

7.1. The response of the animals to a protein-deficiency condition

The strategy of designing diets with an optimal and a sub-optimal dietary digestible energy and crude protein contents was studied in Chapters 1 and 2, respectively. Protein deficiency was, as a result, artificially generated by feeding animals in two ways: (1) by offering a diet with high energy density (Chapter 1), and (2) by offering a diet with low protein content (Chapter 2). This context set the ideal scenario to study whether piglets in a protein-deficiency condition may have the ability to overcome the deficiency through dietary selection by using their highly-developed sensorial perception mechanisms.

One diet (high-energy [HE] diet in Chapter 1, and high-protein [HP] diet in Chapter 2) was formulated to exceed the nutritional requirements of piglets on energy and protein during the pre-starter phase, respectively; while the other diet (low-energy [LE] diet in Chapter 1, and low-protein [LP] diet in Chapter 2) was designed to have a lower energy and protein content, according to NRC (2012). Thus, diets formulated for the experiments of these chapters provided different dietary protein-to-energy ratios to the animals. As shown in Table 7.1, HE and LP diets provided a protein-to-energy ratio below requirements (Cuaron et al., 1981).

Table 7.1. Dietary protein and energy contents, recommended contents and protein-to-energy ratio offered in the diets of Chapters 1 and 2.

Diet	CP content[1]	Recommended[1,2]	DE content[3]	Recommended[2,3]	Protein-to-energy ratio[4]
HE	190.0	175.0	3.90	3.54	48.7
LE	190.0	175.0	3.35	3.54	56.7
HP	204.1	175.0	3.60	3.54	56.6
LP	141.9	175.0	3.60	3.54	39.4

[1]g/kg
[2]NRC (2012)
[3]Mcal/kg
[4]g CP/Mcal DE

Experimental diets were designed to provide the same lysine-to-energy ratio of 4.1 g Lys/Mcal DE. According to NRC (2012), the optimal lysine-to-energy ratio for growing pigs between 7 kg and 11 kg is around 3.8 g Lys/Mcal DE. The contents of methionine, methionine plus cysteine, threonine and tryptophan were then balanced to lysine according to ideal ratios for protein accretion (NRC, 1998). Therefore, in our experiments, sub-optimal diets were designed to promote a deficiency in the content of essential amino acids such as isoleucine, valine or arginine, but not in the content of the first four limiting amino acids for pigs. The present approach may provide a conceptually more global protein-deficiency condition rather than a specific dietary deficiency for main limiting amino acids in pig diets, as it was evaluated in previous studies for lysine (Kirchgessner et al., 1999), methionine (Roth et al., 2006), threonine (Ettle and Roth, 2005) and tryptophan (Ettle and Roth, 2004).

The productive results confirmed that performance was in accordance to the protein-to-energy ratio offered to the piglets (Figure 7.1). For example, when considering a period of 18 days of feeding, the highest ADFI and ADG were promoted by the LE diet, which offered the highest protein-to-energy ratio. Piglets fed this diet and those of the HP group (the second highest protein-to-energy ratio) showed higher BW than did piglets fed the HE and LP diet (the worst ratios). As suggested by NRC (1998), there is an optimal protein-to-energy ratio for young pigs, and, consequently, nutrient requirements often are expressed as the amount of nutrients per Mcal of DE. This ratio might not be fixed during the animal's life; instead it could change as the relative maintenance and gain requirements vary (NRC, 1998). Nevertheless, it is not fully clarified which this ratio is, or if, on the contrary, there is an optimal range supporting growth. Our observations are in line with those of Cuaron et al. (1981), which reported that dietary protein-to-energy ratios within the range of 53 g CP/Mcal DE to 71 g CP/Mcal DE did not influence the performance of starter pigs. It could be suggested that independently of the nature of the nutrient promoting imbalance, dietary protein-to-energy ratio is a major factor determining post-weaning piglet performance.

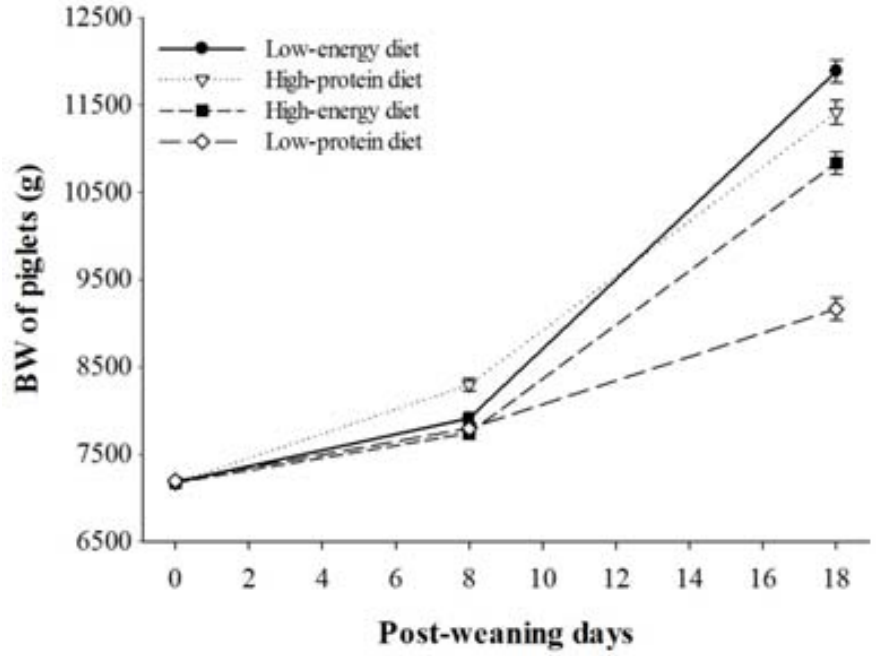

Figure 7.1. Body weight of piglets fed the diets offered in Chapters 1 and 2.

In Chapter 1, it was observed that piglets fed the HE (high fat) diet were unable to express an innate preference for the protein source (porcine digestible peptides [PDP] 20 g/L solution) after a period of protein restriction for 14 days or 21 days. On the other hand, results showed that when piglets were fed the unbalanced HE diet, they significantly increased the preference for sucrose 20 g/L solution in comparison with piglets fed a LE diet but that possessed an appropriate protein-to-energy ratio (Cuaron et al., 1981). It is intriguing why piglets that suffer a protein-to-energy restriction show a higher preference for the sucrose solution. In another study conducted in our group we also observed a similar increase in the preference for a sweet and caloric sucrose solution in piglets after a period of 4h fasting, in contrast to piglets with normal access to the feed (Annex 1). It seems that in both situations of imbalance or necessity, piglets are not able to naturally choose an option that helps to equilibrate their milieu, and, on the contrary, they choose the hedonically more preferred source. This is in agreement with studies in humans, which show that after periods of food restriction the appetence for high-calorie foods is increased (Goldstone et al., 2009).

7.2. Protein deficiency by a free-availability of highly-hedonic solutions

Results obtained in Chapters 1 and 2 in which a sucrose solution was inherently preferred over a protein solution even in a protein-deficiency condition promoted the study of the hedonic attraction of piglets for sweet taste compounds. Previous studies conducted in laboratory rodents reported that when adult rats were exposed to a long-term availability of concentrated sucrose solutions, they were unable to control their energy intake becoming obese due to the additional normal consumption of feed (Sclafani and Springer, 1976; Ackroff and Sclafani, 1988; Ackroff et al., 2007). Previous studies in pigs reported a similar attraction than that of rats for sweet compounds during short- or mid-term (from 2 minutes up to 12 hours) preference tests against water (Kennedy and Baldwin, 1972; Glaser et al., 2000). Therefore, in Chapter 3 we decided to adapt the experimental conditions of the rat model into the commercial pig system to assess the extent of the sweet attraction in piglets after weaning. However, it is clearly established that dietary nutrient requirements strongly differ between adult rats and growing pigs, as nutrient requirements are directly influenced by the animal's physiological state, performance potential and environmental conditions (NRC, 2012). The rate of protein accretion is higher in commercial piglets during their growing period than is for adult rats, whose dietary nutrients are mainly used for maintenance and fat deposition purposes.

It was observed that the exposure for 12 days to sucrose 160 g/L and maltodextrin 160 g/L solutions (0.64 kcal DE/ mL) also produced a protein deficiency in those animals. In this case, and in contrast to Chapters 1 and 2, the protein-deficiency status was auto-generated by the high avidness of piglets for the consumption of a caloric solution (mainly sucrose) that reduced the amount of feed (and amino acids) ingested during the experimental period. It is reported that glucose is one of the major post-ingestive satiating signals for pigs, as different authors have reported that duodenal infusions after feed offering markedly reduced feed intake (Gregory, 2002). The total effect of infusing glucose solutions may allow decreasing intake by about the same amount of energy of the infused glucose (Gregory et al., 1987). As it was also observed in Chapter 1 with the HE diet, piglets did not increase their total energy intake and a 44% of their calculated caloric intake was loaded from the additional sucrose 160 g/L solution. The absence of additional calorie consumption, in contrast to the phenomenon observed in adult rats in maintenance, suggests that piglets may regulate feed consumption to

avoid an excessive energy intake. From the Literature Review section of this Thesis it has been evident that feed intake in pigs is a complex issue controlled by several counterbalanced factors. However, maintaining other factors as a constant, the results observed in these experiments are consistent with the theory of energy control of feed intake (Black et al., 2009). In the case of maltodextrin 160 g/L solution, it was observed a lower initial preference by pigs as that promoted by the sucrose 160 g/L solution. Nonetheless, maltodextrin became hedonically preferred as pigs learned the positive consequences of their consumption.

Literature reports that some artificial sweeteners such as saccharin are also preferred by pigs in short-term test as compared against water (Kennedy and Baldwin, 1972; Glaser et al., 2000). However, the response intensity in pigs is weaker than that in humans, mainly attributed to the absence of one (or more) steric interaction (s) or steric fit (s) in the porcine receptor (Glaser et al., 2000). Saccharin and NHDC are the only sweeteners approved to be included in the diet of piglets in the European Union, with a maximum incorporation level of 150 mg/kg. Nevertheless, when this dose was extrapolated to an additional drinking solution, the final concentration (saccharin 0.08 g/L plus NHDC 0.02 g/L) was below the detection thresholds previously reported for these compounds (from 0.4 g/L to 1.83 g/L for saccharin, and 0.6 g/L for NHDC) according to the observations of Kennedy and Baldwin (1972) and Glaser et al. (2000). Anyway, we also evaluated higher concentrations of saccharin and NHDC within values reported to promote a high preference in pigs during 12-hour preference test (from 1.83 g/L to 18.31 g/L; Kennedy and Baldwin, 1972) and they were ineffective and even counterproductive. Saccharin 3.2 g/L plus NHDC 0.8 g/L and saccharin 12.8 g/L plus NHDC 3.2 g/L reduced solution consumption and feed intake and/or body weight gain when they were offered for a period of 12 days, suggesting that the rejection threshold for saccharin may be lower than the previously reported data.

Based on the results obtained in Chapter 3, it was proposed that the growing pig may represent an alternative animal model for the study of human feeding behaviour, especially for the infant population. The weaned piglet may comprise a more appropriate model than adult rats, given the fact that piglets and children share significant similarities in dietary habits not seen in rats (Roura et al., 2011), and also both are in stages of growth with a similar utilization of dietary nutrients.

7.3. An integration of the protein deficiency and the dietary selection based on the framework of minimal total discomfort

Due to the fact that throughout this Thesis piglets experienced a protein-deficiency condition due to different causes, a theoretical model that integrates all the information covered in these experiments was developed. This model was done based on a adaptation of the framework of minimal total discomfort proposed by Forbes (2009) to explain feed intake and diet selection in pigs (page 21 in the Literature Review section), and allows to estimate the degree of protein restriction between the different experiments as well as how the restriction is released when animals choose the proper diet.

For the purpose of this analysis, the protein intake (g of CP/day) during the different experiments was used as the limiting resource included into the model. First of all, it has to be taken into account the following assumptions:

- The optimal rate of protein intake for a growing pig is close to 75.0 g of CP/day for piglets between 7 kg and 11 kg, and 131.3 g of CP/day for piglets between 11 kg and 25 kg, as reported by NRC (2012).

- Based on the registered BW when piglets were protein-deficient and offered the choice of protein, the piglets in our trials were classified in the 7-11 kg category for Chapters 1 and 2; and in the 11-25 kg category for Chapter 3.

- It was also assumed that the protein solution offered during choice tests or conditioning procedures was totally and equally consumed by individual piglets in each group. Thus, when a group of 4 piglets consumed 800 mL of protein solution, we assumed that each animal ingested 200 mL (Chapters 1 and 3); when the pen of 10 piglets consumed 5000 mL of protein solution, each animal ingested 500 mL (Chapter 2).

Piglets were determined to have a daily protein intake of 43.7 g of CP/day when fed the HE diet (Chapter 1), 38.7 g of CP/day when fed the LP diet (Chapter 2, Experiment 2), 66.0 g of CP/day when they had access to the additional sucrose 160 g/L solution (Chapter 3, Experiment 1), and 94.4 g of CP/day when they were offered the maltodextrin 160 g/L solution (Chapter 3, Experiment 2), based on the registered feed intake and the corresponding dietary CP content of each feed.

This allowed calculating the difference between the observed protein intake and the optimal CP intake of reference, according to the BW of the piglets in each experiment, e.g. piglets fed the LP diet had a difference of 75.0 – 38.7 = 36.3 g of CP/day of protein deficit. This difference, as proportion of the optimal intake, is understood as the proportional shortfall. Thus, in the example, piglets fed the LP diet had a proportional shortfall of 36.3 / 75.0 = 0.48. Then, according to the model of Forbes (2009), by squaring and summing the proportional shortfalls of the different factors included in the model, the relative discomfort is obtained by subsequently applying the square root to the total sum. However, as in this exercise we are just considering protein as limiting factor, the relative discomfort and the proportional shortfall are the same, 0.48. The consumption of the protein solution (animal plasma 60 g/L) during training sessions of piglets fed the LP diet provided the animals an additional protein intake of 21 g of CP/day. Therefore, the intake of additional protein reduced the difference from the optimal CP intake to 75.0 – 59.7 = 15.3 g of CP/day, which in turn decreased the proportional shortfall to 15.3 / 75.0 = 0.20 and thus the relative discomfort. The same calculation was performed for each protein-deficiency condition promoted by each experiment and is shown in Figure 7.2.

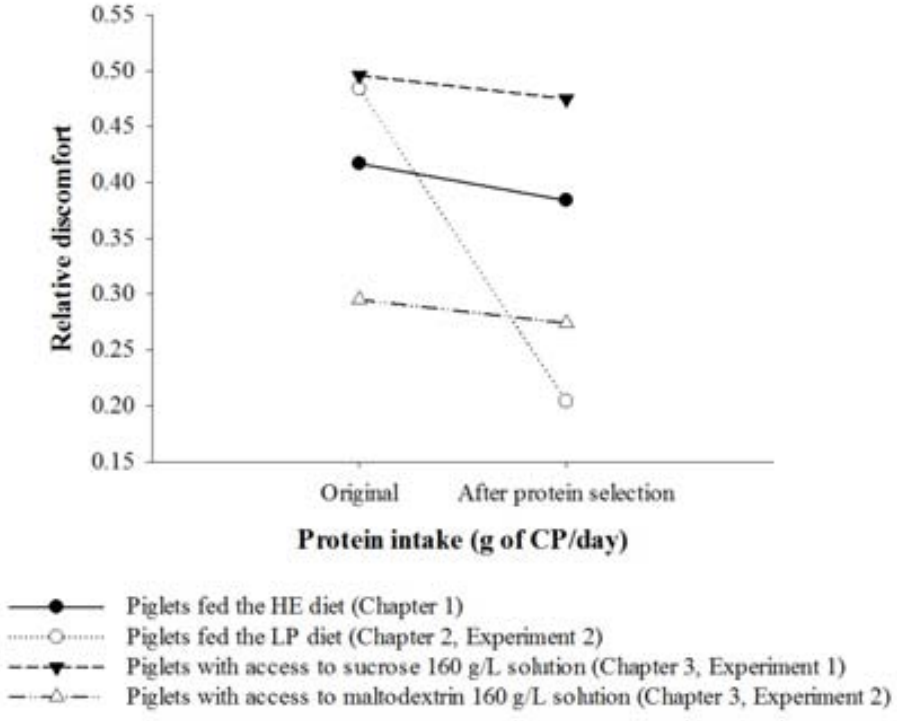

Figure 7.2. Theoretical change in the relative discomfort after the selection of the protein source by piglets in Chapters 1, 2 and 3.

The calculation of protein discomfort suggests that deficiency states were not equal among the different experiments. Thus, it is remarkable that piglets that had access to the additional sucrose 160 g/L solution experienced a level of protein discomfort similar to that of piglets that were fed the LP diet. This is important, as indicates that the attraction for hedonic and concentrated sweet compounds in piglets is of an extent that may generate a protein deficiency to the animals similar to that observed by a 19% of reduction of the optimal dietary CP content (141.9 g of CP/kg in the LP diet vs. 175.0 g of CP/kg as optimal; NRC, 2012). This information adds a potential new concept to the previous literature regarding the sweet taste attraction in pigs. Sucrose solutions are not only preferred from 3 g/L to 77 g/L during 1 hour or 12 hours choice tests against water (Kennedy and Baldwin, 1972), or of 5 g/L during 1 minute (Glaser et al., 2000), they are also preferred in the long-term when 160 g/L solutions are offered over 12 days of exposure, generating a substantial protein deficiency status to the animals.

It has been suggested that humans show feeding behaviour patterns that instead of being wise according to the nutritional conditions, are more likely to be compulsive towards highly palatable substances, such as sweet taste or high-fat sources (Cota et al., 2006). Here, it was observed that pigs may also follow a compulsive pattern towards concentrated sucrose solutions when offered ad libitum. Reward and motivation have been extensively studied in the context of drug addiction, and recent evidence has suggested that addiction to food and to drugs may be based on overlapping neuronal pathways (Volkow and Wise, 2005). Recently, it has been suggested that the endogenous cannabinoid and opioid systems play a key role both in feeding and in reward, and might functionally interact with each other (Cota et al., 2003, 2006). These systems appear not to be related with the general control of energy homeostasis; instead they process appetitive and hedonic aspects of food intake, and are capable of overriding homeostatic control acting as stimulus even in the absence of energy deficit (Harrold et al., 2012). In animals, the inhibitory effects of opioid and endocannabinoid antagonists on food intake have been well documented in rodents (Kirkham and Williams, 2001; Harrold et al., 2012) and also in calves (Montoro et al., 2012). The basic effect is a selective inhibition in the consumption of palatable foods and drinks such as sucrose solutions. However, there is no available evidence yet of endogenous cannabinoid or opioid systems controlling the sensory pleasure derived from feed consumption in pigs. This set a promising opportunity for the conduction of future studies in the field of CNS regulation of appetite in pigs.

The second important aspect to highlight from this calculation relates to the strength of the protein solution to minimize discomfort. We realized that selection of the protein source by piglets in Chapters 1 and 3 would have just slightly reduced the protein discomfort in these animals, while piglets in Chapter 2 may have undergone an almost total relief of the discomfort after the consumption of the offered protein. The main factors that influenced this difference were: (1) the amount of solution offered, and (2) the protein concentration. Forbes (2009) suggested that in order to minimize discomfort, an animal is able to move from its current (sub-optimal) to its desired (optimal) status experimenting with the different rates of intake and gaining knowledge as it goes. The knowledge gained strongly depends on the characteristics of the experimented sources. Small deviations in the relative discomfort, such as those generated by the protein selection in piglets in Chapters 1 and 3 appear to have relatively small impacts on the total perception of the animals (Forbes, 2009).

In addition, it is suggested that the speed at which animals gain knowledge about food sources, and the period during which this association is retained, depends largely on the extent to which a previous disturbance has affected the animal's internal state, and, importantly, on the extent of the post-ingestive consequences induced by the source (Kyriazakis et al., 1999). Therefore, although theoretical and lacking in real confrontation, this model shows that the protein-deficiency states observed across the present PhD Thesis were not equally overcome by the selection of the protein source offered as a choice. This fact may have had a significant effect on the outcomes here discussed, and proposes a possible improvement to the methodology employed in the experiments. In a later section, we will explore how the piglets were able to learn and to modify their feeding behavior depending on the nutritional status and training periods.

7.4. An imbalance in the acid-base homeostasis

The effect of the dietary macromineral composition, and the consequent imbalances in the acid-base status of piglets, were examined in Chapter 4. Similarly to Chapters 1 and 2, in which diets were designed to contain an optimal or sub-optimal energy or protein composition, the sources of calcium and/or sodium supplemented to the diet were manipulated in Chapter 4. Thus, dietary concentrations of the chloride anion and the sodium cation changed across different treatments to modify the dEB offered to the animals.

It was observed that piglets fed for 12 days a low dEB diet (16 mEq/kg) had lower blood bicarbonate, base excess, and TCO2 concentrations in comparison with piglets fed higher dEB levels (from 133 mEq/kg to 269 mEq/kg). This phenomenon was described in previous studies in pigs that have already documented the influence of dEB on their acid-base status (Patience et al., 1987; Patience and Chaplin, 1997; Dersjant-Li et al., 2002). It was also observed that altering the dEB affected the productive performance. Thus, piglets fed diets from -16 mEq/kg to 133 mEq/kg showed higher feed intake, weight gain, gain:feed ratio and BW at the end of the experimental periods, in comparison with piglets fed diets from 269 mEq/kg to 388 mEq/kg. Unexpectedly if we consider previous references in the literature (Austic et al., 1983; Patience et al., 1987; Haydon et al., 1990; Dersjant-Li et al., 2001), diets with a high dEB value (high sodium content) decreased performance of post-weaned piglets.

In Chapter 4, the sources used to vary the dEB (calcium chloride, calcium carbonate and sodium bicarbonate) also influenced the digestive process of piglets, as it was observed a reduction in the apparent digestibility of crude protein and zinc when piglets were fed a high electrolyte-balanced diet (269 mEq/kg). This diet was supplemented with calcium carbonate and sodium bicarbonate, which are compounds that show a high acid-binding capacity in the stomach (Lawlor et al., 2005). It is suggested that the use of such compounds in the post-weaning diets is detrimental for protein digestion, as they may contribute to raise the stomach pH of weanling pigs to values greater than 5.0 at which pepsinogen could barely be converted into pepsin (Kidder and Manners, 1978; Yen, 2001).

It was observed in these experiments that the sources used to vary the dEB were sensory perceived in a different way by the animals. Piglets always preferred the high-sodium diets, despite the fact that when given as a single diet they promoted a lower growth. The preference values observed for the high dEB level during short- or long-term choice test were highly significant of approximately 70% or more. Then, the hypothesis that piglets are able to choose diets differing on dEB, preferring those levels that optimize their growth performance was rejected.

In Chapters 1, 2 and 3 of this Thesis the solutions offered as a choice during preference tests were of protein (umami) or carbohydrate (sweet) nature, which both are known as highly-pleasant compounds for piglets. Nonetheless, in Chapter 4, feed was supplemented with a mineral such as calcium chloride that is likely to be perceived as bitter by piglets as it is by humans. Indeed, taste properties of divalent salts such as calcium chloride are complex, but they are characterized primarily by bitter taste, with additional sensations described as metallic, astringent, and sour (Lawless et al., 2003, 2004). The bitter taste system is regarded as a basic mechanism of defense against anti-nutritional factors, drugs or potentially toxic compounds present in the diet. The immediate result of bitter sensing is a decrease in food ingested, however, others defensive mechanisms such as increased saliva secretion, a lag in gastric emptying, an increase in CCK release, a regulation of blood flow and/or vomiting has been previously registered in humans and rats (Rozengurt, 2006; Kaji et al., 2009; Janssen and Depoortere, 2013). Pigs have been reported to elicit avoidance responses to antibiotics and quinine HCl, denatonium benzoate (Bitrex) and caffeine among other compounds (Blair and Fitzsimons, 1970; Nelson and Sanregret, 1997; Danilova et al., 1999). In consequence, feed supplemented with calcium chloride during short- and long-term preference tests was

innately rejected. The ability of piglets to select and prefer the diet that best fits to equilibrate their internal milieu through their feed selection may have been overridden by the taste properties of the offered sources in these experiments.

7.5. The importance of learning in the ability of piglets to perform an appropriate dietary selection pattern

Learning is a fundamental mechanism that a mammal has to acquire in order to perform an effective feeding behavior to find and consume suitable nutrient sources from the environment (Gieling et al., 2011). Under natural conditions, pigs and other mammals undergo several learning processes that allow animals self-nourish as they growth. However, in the intensive pig industry, piglets face the new environment at a very young stage and without time to learn about it. It is possible that piglets are unable to overcome a particular nutritional situation that departs from an optimal state just relying on their innate dietary preferences and aversions without learning about likely alternatives.

Animals are born with innate preferences and aversions to particular flavors (Forbes, 2007). Some of these preferences may be developed and established in the womb and can be manifest even before birth. For example, human fetuses show their sugar appreciation around 15 to 16 weeks after conception by swallowing more amniotic fluid when it is sweet and less when bitter (Hepper, 2005). However, innate preferences and aversions cannot be relied on for the rest of the animals' life (Forbes, 2007). Thus, it has been previously demonstrated that pigs and other species may learn feeding behavior by vertical transmission from the mother before or after birth (Mennella et al., 2001; Oostindjer et al., 2009; Figueroa et al., 2013a), by their own experiences with foods once ingestion starts (Myers et al., 2005; Ackroff and Sclafani, 2011; Figueroa et al., 2012a,b), and by horizontal transmission from social experiences with conspecifics (Galef, 1986; Galef and Whiskin, 2001; Figueroa et al., 2013b). Once learning has been established, animals can associate the sensory properties of a certain food with the metabolic and/or toxic consequences of eating that food, and they can use such associations to guide subsequent feeding behavior, both in terms of the amount eaten and the choice between foods (Forbes, 1998).

In Chapter 1 and Experiment 1 of Chapter 2 of this Thesis we observed that piglets were unable to show an innate preference for the protein source offered in the protein-deficiency condition. It is suggested that the absence of an effective learning process with the protein source did not allow the animals to express an appropriate selection pattern to overcome the dietary deficiency. The suggestion is in close agreement with previous observations in pigs of Kyriazakis et al. (1990, 1991a,b), who showed in a series of studies that growing pigs were able to control their protein intake when given a long-term choice between a high- and a low-protein diet. Critically, in all these studies pigs were previously given the opportunity to experience the feeds given as a choice for at least 6 times, and considerable variation was observed in the selection when the previous experience was not offered.

However, previous literature in this respect had already questioned the fact whether animals may have a specific appetite for protein to reestablish homeostasis; as opposed to what is reported, and apparently evident, for other compounds such as salt or calcium that have been described to be innately preferred (Denton, 1982; Blair-West et al., 1992; Leshem, 1999; Galef, 1999). In addition, in our studies the sources offered as options during choice tests were not only of protein nature or with different CP concentrations, but also they were of sweet nature (sucrose 20-40 g/L solutions). This made that piglets had to choose the protein source over the highly-hedonic carbohydrate compound (Kare et al., 1965; Kennedy and Baldwin, 1972; Glaser et al., 2000). As indicated previously, feeding behavior in humans and animals is not governed solely by homeostatic processes. Instead, pleasure and reward mechanisms play a central role in the control of food/feed intake (Kringelbach et al., 2012).

On the other hand, in Experiment 2 of Chapter 2 it was observed that piglets submitted to a protein-deficiency condition were able to express a higher preference for a new flavor previously conditioned to protein post-ingestive consequences. Thus, piglets fed the LP diet showed a higher intake of CSp than of CSc, in contrast to piglets fed the HP diet that tended to a higher intake of CSc than of CSp. This suggests that piglets may use and reinforce flavor preference to show an appropriate diet-selection pattern to overcome the deficiency through stimulus-response learning, which is in line with the basis of flavor-consequence learning that is adaptive in allowing animals to select nutrient-rich foods and avoid potentially dangerous ones (Sclafani and Ackroff, 2012). Kyriazakis et al. (1990, 1991a,b) observed that pigs were able to select a balanced diet that met their protein requirements avoiding deficiencies or excesses of protein intake. However, the ability of piglets to perform an appropriate dietary

choice to overcome a particular situation such as a protein deficiency once this status is established had not been assessed yet in pigs. Our results are in close agreement with previous studies of protein-based flavor preferences in protein-restricted environments conducted in other species, such as hamsters (DiBattista and Mercier, 1999) and humans (Gibson et al., 1995). In addition, in this experiment it was also observed that piglets fed the LP diet showed dissimilar intakes of CSp and CSc depending on their BW. In comparison with heavier piglets, middle-light and particularly light piglets in the protein-deficient group showed the greatest differences in the intake between CSp and CSc. It is suggested that the reinforcement properties of protein conditioning may vary among pigs, having a greater impact in piglets which have been largely deprived of nutrients and protein (low rather than high BW at the same age). This is in accordance with the diet-selection framework proposed by Kyriazakis et al. (1999) and the framework of minimal total discomfort proposed by Forbes (2009) as it was previously commented, highlighting the importance of the extent of a deficiency and the extent of the post-ingestive consequences induced on the effectiveness of an offered source in promoting learning.

In Chapter 3, long-term exposure to sucrose 160 g/L or maltodextrin 160 g/L solutions reversed the initial preference of piglets for sucrose 20 g/L over animal plasma 20 g/L solutions. Piglets offered the sucrose 160 g/L solution ad libitum for 12 consecutive days tended to a higher intake of animal plasma 20 g/L solution in the final choice test, and similarly, protein consumption in the final choice test of maltodextrin piglets was significantly higher than that of sucrose 20 g/L solution. Nevertheless, it is unlikely that in these experiments the enhancing in the value of the protein solution that promoted a higher intake may be attributed to learned associations with the post-ingestive consequences of that protein stock. Sucrose 20 g/L and animal plasma 20 g/L solutions were simultaneously offered one time during the initial choice test for a very short period of time (3 minutes), which was with high probability ineffective in promoting an association. Choice tests allow animals to have experience with the two options at the same time (actually rats can mix fluids in their mouth). In addition, previous studies in pigs required a minimal number of six (Clouard et al., 2012; Figueroa et al., 2012a,b) or three (with a trained conspecific; Morgan et al., 2003) independent training sessions to generate learning memory. Therefore, it is suggested that in this case other mechanisms different from learning drove the animals for the protein selection. It could be suggested that a decline in the value of the sucrose 20 g/L solution due to a successive negative contrast effect could have affected the sucrose

perception (Flaherty, 1996; Dwyer, 2012). In this sense, sucrose 20 g/L solution seemed less valuable to the piglets than sucrose 160 g/L or maltodextrin 160 g/L after the 12 days exposure, and as a result the consumption of sucrose 20 g/L was reduced.

A similar situation was observed in Chapter 4. The short-term exposure to the experimental diets during preference tests of Experiment 2 probably did not allow piglets to associate the sensory and metabolic properties of the feeds. However, long-term exposure for 14 days during the preference test of Experiment 3 probably did allow it. Then, it could be speculated that the proportions of the -16 mEq/kg and 388 mEq/kg diets selected during this test may have reflected the proportion that provided the animals with the optimum acid-base balance. In addition, in these experiments piglets may have learned about the absence of additional discomfort or new toxic consequences by the consumption of a diet with an innately aversive bitter taste, such as that supplemented with calcium chloride (-16 mEq/kg diet). This feature has been pointed out in previous studies in pigs such as that of Blair and Fitzsimons (1970) and the much-quoted example of Bitrex, and emphasizes some of the difficulties in the concept of palatability of feeds concerning how a source, that was initially strongly unpalatable and rejected, may become later normally accepted and even consumed after some exposures based on a learning association.

Finally, it is important to remark this difference between preference and long-term feed consumption. As indicated, the high-chloride diet was rejected against the high-sodium diet during the preference tests conducted in Chapter 4. However, when offered as unique option, piglets showed higher intake of the high-chloride than of the high-sodium diet. Another clear example of this was obtained in previous studies of our group (Davin et al., 2011). When offered as a choice, piglets rejected diets supplemented with 3000 ppm of zinc oxide against a control, unsupplemented diet. Nonetheless, despite of its low preference, dietary supplementation with zinc has been described to increase feed intake and performance when a single diet is provided. This fact illustrates that it may not be necessarily accurate to extrapolate the outcomes of preference tests into the standard conditions of the intensive pig industry.

8. Conclusions

The following conclusions are obtained from the present PhD Thesis:

1. Feeding piglets diets with a low dietary protein-to-energy ratio (either by increasing the energy and fat content or by decreasing the protein content), or offering the animals a long-term availability to a concentrated sucrose solution, decreases the growth of piglets in a scenario that was called of protein deficiency.

2. The supplementation with minerals such as calcium carbonate and sodium bicarbonate in diets with high dEB levels may decrease the growth performance of piglets, which is associated to the lower apparent digestibility of some nutrients such as crude protein and zinc.

3. Piglets show higher preference and appetence for sucrose solutions than for protein solutions, as well as higher preference and appetence for high dEB levels than for low dEB levels containing calcium chloride, even when they may impair growth performance.

4. When offered a choice between two opposite options, piglets are unable to select the option that optimizes their performance neither in short- nor long-term preference tests, showing also higher appetence for sucrose instead of protein solutions and for high instead of low dEB levels.

5. Post-weaned piglets might be able to perform appropriate dietary selection patterns in relation to different nutritional status, but critically whether a learning process has been carried out. In the absence of learning, such as in the intensive pig industry conditions at weaning, piglets might be unable to overcome a particular situation that departs from an optimal state just relying on their innate dietary preferences and aversions. This should be considered when designing programs aimed to improve the acceptance of diets of young animals at this stage.

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10. Annexes

10.1. Annex 1

The preference for carbohydrate or protein is affected by the feeding status in post-weaned piglets

Abstract presented at The Nutrition Society Winter Meeting 2011

Proceedings of the Nutrition Society 2011;70:E368

Pigs, like other mammals, have a complex biological system that allows them to control their voluntary feed intake and self-nourishment in accordance to different physiological states [(1)]. The integration by the central nervous system of multiple signals arising from organs and tissues depending on feeding status may result in different response of pigs towards different nutrients, basically as a preference or an aversion[(2)]. The aim of the present study was to assess whether the preference for a carbohydrate or protein source is affected by the feeding status (fasted or satiated) in post-weaned piglets.

A total 120 post-weaned piglets (56 days old) were distributed according to body weight into 12 pens (10 piglets/pen) and fed with a commercial weanling diet. On days 28, 29, 35 and 36 after weaning those animals were submitted to an alternated sequence of *ad libitum* feeding or 4 h fasting. At the same days, the preferences for a carbohydrate (sucrose 0.0292 mol/l) or protein (porcine digestible peptides (PDP, Palbio 62SP®) 20 g/l) solutions were evaluated by using a double-choice test (DCHT) protocol. Two different bottles (5 litres) were simultaneously offered for a period of 5 min. Solution intakes and the corresponding preference values (measured as the percentage contribution of this solution to the total volume intake) were analyzed with ANOVA. Preference values were also compared to the neutral value of 50 % by using a Student's *t*-test.

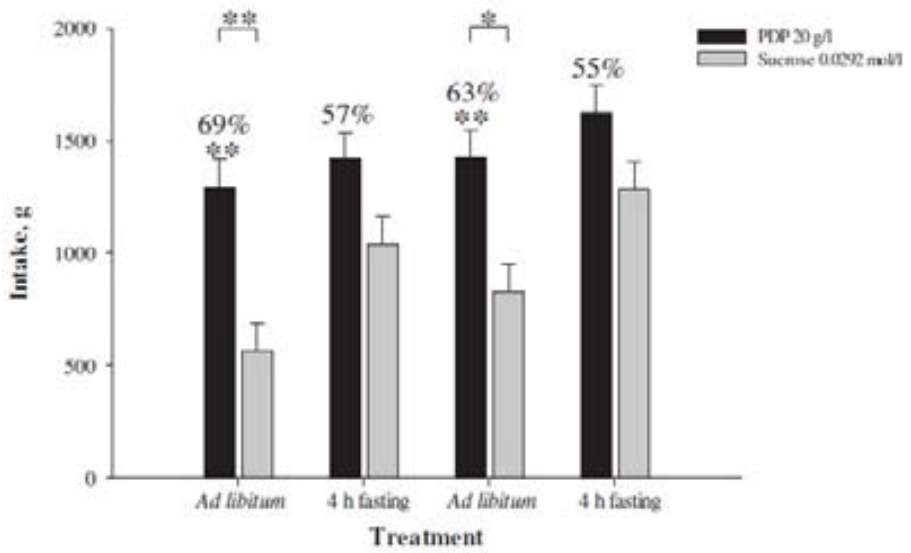

Figure 10.1. Effect of ad libitum feeding or 4 h fasting on intake and preference of piglets for porcine digestible peptides (PDP) 20 g/l or sucrose 0.0292 mol/l solutions.

When piglets had free access to feed they showed a higher volume intake (clasps, **P*<0.05, ***P*<0.01) and preference (numbers on top of the bars, ***P*<0.01) for the PDP solution than for sucrose solution, while no significant preference (*P*>0.05) was observed for the tested solutions when piglets were fasted for a period of 4 h. These results show that weanling piglets may change their preferences for a carbohydrate or a protein source depending on the feeding status.

1. Forbes JM (1998) *Appl Anim Behav Sci* **57**, 287-297.

2. Myers KP (2007) *Appetite* **48**, 123-127.

10.2. Author education and training

Sergio A. Guzmán Pino was born in 1985, in Santiago de Chile. After graduating from Instituto Nacional in 2002, he completed his Degree of Veterinary Medicine at Universidad de Chile in 2008. Thereafter, he was granted with a “Becas Chile” research fellowship from the Chilean Government to pursue doctoral studies at Universitat Autònoma de Barcelona, Spain. He started his Master degree studies at Universitat Autònoma de Barcelona in 2010, obtaining his Master of Science in 2011. Currently, he is a PhD student in Animal Production at the Animal and Food Science Department of Universitat Autònoma de Barcelona, with emphasis on Animal Nutrition. During his PhD, he has been developing a research line focused in evaluating the capacity and the mechanisms involved in the dietary choices of piglets, and its relation with particular physiological or nutritional status of the animals. Part of his training was conducted at the Centre for Nutrition and Food Sciences of The University of Queensland, Australia. This work is the basis of the current PhD Thesis submitted to obtain a PhD degree in Animal Production (Nutrition) at Universitat Autònoma de Barcelona in 2014.

10.3. List of publications

Scientific Papers

S. A. Guzmán-Pino, D. Solà-Oriol, J. Figueroa, and J. F. Pérez. 2014. Influence of the protein status of piglets on their ability to select and prefer protein sources. **Physiology & Behavior**, Vol. 129, pp. 43-49. doi: http://dx.doi.org/10.1016/j.physbeh.2014.02.029. Impact factor (JCR, 2013): 3.160. Area: Behavioral Science (Q1).

S. A. Guzmán-Pino, D. Solà-Oriol, J. Figueroa, D. Dwyer, and J. F. Pérez. 2014. Effect of a long-term exposure to carbohydrate and artificial sweetener solutions on the preference, appetence, feed intake and growth performance of post-weaned piglets. **Physiology & Behavior** (under review).

S. A. Guzmán-Pino, D. Solà-Oriol, R. Davin, E. G. Manzanilla, and J. F. Pérez. 2014. Influence of dietary electrolyte balance on feed preference and appetence, and growth performance of post-weaned piglets. **Journal of Animal Science** (under review).

S. A. Guzmán-Pino, D. Solà-Oriol, J. Figueroa, E. Borda, and J. F. Pérez. 2012. Dietary energy density affects the preference for protein or carbohydrate solutions and piglet performance after weaning. **Journal of Animal Science**, Vol. 90, pp. 71-73. doi: 10.2527/jas.49994. Impact factor (JCR, 2011): 2.096. Area: Agriculture, Dairy & Animal Science (Q1).

J. Figueroa, D. Solà-Oriol, **S. A. Guzmán-Pino**, E. Borda, and J. F. Pérez. 2012. Flavor preferences conditioned by post-ingestive effect of sucrose and porcine digestive peptides in postweaning pigs. **Journal of Animal Science**, Vol. 90, pp. 381-383. doi: 10.2527/jas.51308. Impact factor (JCR, 2011): 2.096. Area: Agriculture, Dairy & Animal Science (Q1).

Conference Proceedings

S. A. Guzman-Pino, M. Fu, and E. Roura. 2014. Dietary bitterness reduces feed intake in piglets with or without gastrointestinal discomfort conditioning. XXIVth International Conference of European Chemoreception Research Organization. September 10-13, 2014. Dijon, France. Type of presentation: Poster.

S. A. Guzmán-Pino, D. Solà-Oriol, R. Davin, E. G. Manzanilla, C. Torrente, and J. F. Pérez. 2014. A high dietary electrolyte balance reduces growth performance and CP and Zn total tract apparent digestibility in weanling piglets. ASAS Annual Meeting. July 20-24, 2014. Kansas City, MO, USA. Type of presentation: Poster.

R. Davin, **S. A. Guzmán-Pino**, D. Solà-Oriol, E. G. Manzanilla, and J. F. Pérez. 2014. Calcium level and dEB affect the protein and mineral digestibility of lactating sows. ASAS Annual Meeting. July 20-24, 2014. Kansas City, MO, USA. Type of presentation: Poster.

J. Figueroa, D. Solà-Oriol, R. Davin, E. Borda, **S. A. Guzmán-Pino**, and J. F. Pérez. 2014. Effect of porcine digestive peptides as sweet milk whey replacer for piglets diets: preferences, acceptance and performance during the nursery period. ASAS Annual Meeting. July 20-24, 2014. Kansas City, MO, USA. Type of presentation: Poster.

S. A. Guzmán-Pino, D. Solà-Oriol, and J. F. Pérez. 2013. Influence of dietary electrolyte balance on feed preference, appetence and performance in post-weaned pigs. XIV Biennial Conference of the Australasian Pig Science Association (APSA). November 24-27, 2013. Melbourne, VIC, Australia. Type of presentation: Oral presentation. **Manipulating Pig Production XIV. Proceedings of the Fourteenth Biennial Conference of the Australasian Pig Science Association (APSA)**, pp. 41.

S. A. Guzmán-Pino, D. Solà-Oriol, J. Figueroa, and J. F. Pérez. 2013. Pig preference for flavours conditioned by post-ingestive consequences under protein deficiency after weaning. XIV Biennial Conference of the Australasian Pig Science Association (APSA). November 24-27, 2013. Melbourne, VIC, Australia. Type of presentation: Oral presentation. **Manipulating Pig Production XIV. Proceedings of the Fourteenth Biennial Conference of the Australasian Pig Science Association (APSA)**, pp. 40.

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