



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



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## HIGHLIGHTS

- Post-weaned piglets were submitted to a protein-deficiency status through the diet.
- In a naive 3-minute choice test they preferred carbohydrate despite protein status.
- Piglets were trained with flavors mixed into protein or carbohydrate solutions.
- After training, they showed a higher intake of the protein-conditioned flavors.
- Piglets may show an appropriate selection pattern through associative learning.

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## 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 3 min. 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.

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## 1. 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 [1,2]. Pigs may change their choice as they grow, to reflect their changing requirements [1]; females select a diet of lower protein content than males do [3], and animals are also able to correct previous underfeeding with protein by the composition of the diet that they select [4]. In the same way, pigs have shown specific selection for diets differing in the levels of lysine [5], methionine [6], threonine [7] or tryptophan [8].

The wide range of scenarios in which pigs make appropriate choices, concerning different diets and rapid compensatory growth rates after

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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 [9]. It is remarkable that pigs showed appropriate choices when two diets were previously tested or with familiar feedback [1–4], and they showed considerable variation when the previous experience was not offered [2]. The closer in nutritional composition the two feeds were, the less able were the animals to discriminate between them [10,11]. 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 [12,13].

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 [11,14]. 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 [15], sucrose being the most strongly preferred carbohydrate for pigs [16]. 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 [17].

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 [18]. 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).

## 2. 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).

### 2.1. 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<sup>2</sup> 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.

### 2.2. 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 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 [19]. 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) [19]. This strategy in the design of the LP diet was performed attempting to simulate what occurs when low-protein diets are designed with the

**Table 1**

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

	High-protein diet	Low-protein diet
<i>Ingredients (g/kg DM)</i>		
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 <sup>a</sup>	4.0	4.0
Salt	–	1.8
<i>Chemical analysis (g/kg DM)</i>		
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
<i>Estimated nutrient content (g/kg DM)</i>		
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

<sup>a</sup> Supplied per kg of feed: 3 mg of ethoxyquin, 14,000 IU of vitamin A, vitamin D 1000 IU as vitamin D<sub>3</sub> and 500 IU as 25-hydroxycholecalciferol, vitamin E 50 mg as alpha-tocopherol acetate and 40 mg of RRR-alpha-tocopherol, 2 mg of vitamin K<sub>3</sub>, 3 mg of vitamin B<sub>1</sub>, 7 mg of vitamin B<sub>2</sub>, 3.5 mg of vitamin B<sub>6</sub>, 0.06 mg of vitamin B<sub>12</sub>, 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.

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