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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.

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^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.

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
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, 14,000 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.

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.

2.3. Experimental design

2.3.1. 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 min. This procedure intended to stimulate the approach of the animals during testing, as was reported in a previous study conducted in our group [12]. Then, the choice test was performed for the 10 piglets of each pen with two pans placed for 3 min 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 [14]. 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 [16,20,21].

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 1 h 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 s 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 [13], 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.

2.3.2. 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. [22] 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 [23]. 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 3 min. 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.

2.4. 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.

3. Results

3.1. 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 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).

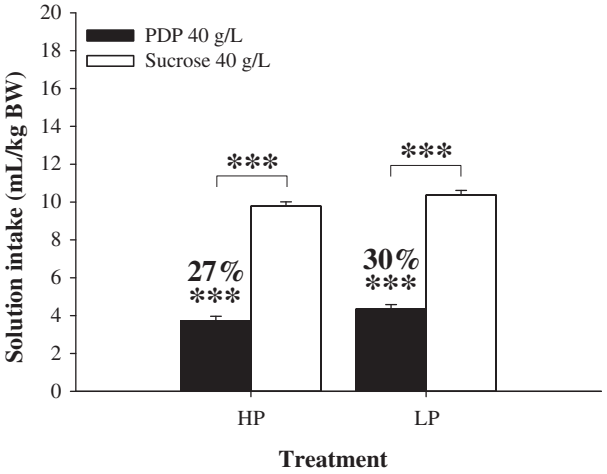

Fig. 1. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on intake and preference of piglets for porcine digestible peptides (PDP) 40 g/L or sucrose 40 g/L solutions during the choice test conducted in Experiment 1. Clasps indicate different intakes between both solutions (***$P < 0.001$).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

3.2. Experiment 1

3.2.1. 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; Fig. 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 [$t = -0.50$, $df = 19$, $P = 0.62$].

Table 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
Day 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.

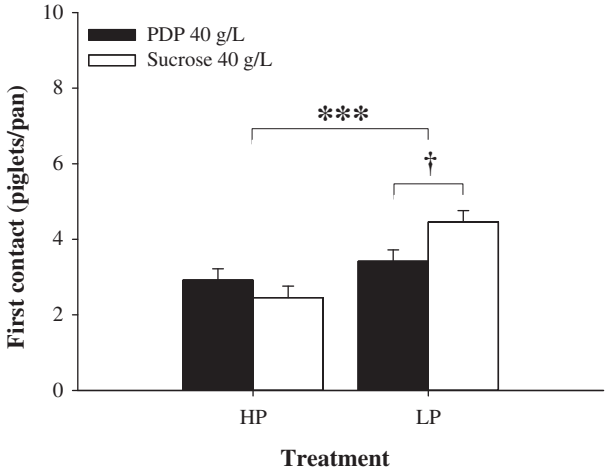

Fig. 2. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on the first contact of piglets with porcine digestible peptides (PDP) 40 g/L or sucrose 40 g/L solutions during the choice test conducted in Experiment 1. Clasps indicate different first contact score between either solutions or experimental treatments (†$P < 0.1$, ***$P < 0.001$).

3.2.2. 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 [$F(1,19) = 3.89$, $P = 0.06$; Fig. 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, $F(1,43) = 15.26$, $P < 0.001$].

3.3. Experiment 2

3.3.1. Choice test

The choice performed by the piglets fed the HP and LP diets for CSp or CSc after training sessions is shown in Fig. 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 [$F(3,5) = 9.73$, $P < 0.05$; Fig. 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].

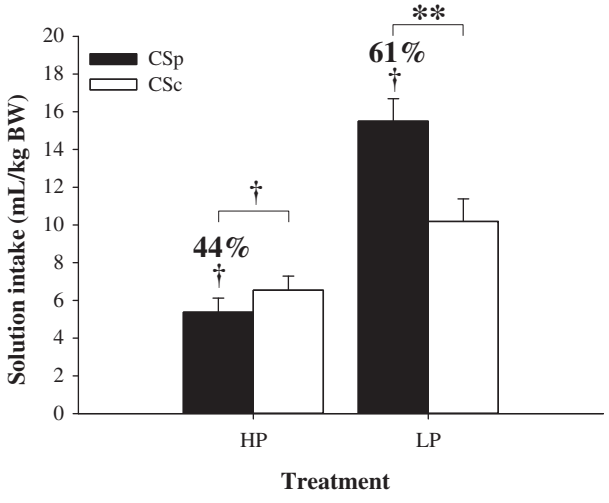

Fig. 3. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on intake and preference of piglets for conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the choice test conducted in Experiment 2. Clasps indicate different intakes between both solutions (†$P < 0.1$, **$P < 0.01$). Numbers on top of the bars represent percent intake of CSp and its difference from the neutral value of 50% (†$P < 0.1$).

3.3.2. 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; Fig. 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, $F(1,41) = 26.00$, $P < 0.001$].

4. 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 [19]. Experimental evidence shows that specific protein selection by pigs may be evoked by diets varying in their overall protein content [1,2], and also related to the quality of the protein source as reflected by the appetite for some specific amino acids [5–8].

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 [24]. 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 [25–27]. 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 [28,29]. However, an increase in monosodium glutamate preference in protein-deficient rats has not been demonstrated

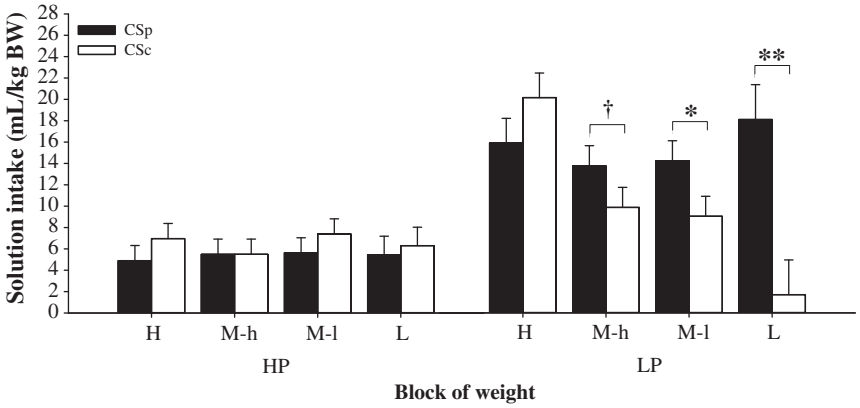

Fig. 4. Effect of the block of weight (light [L], middle-light [M-l], middle-heavy [M-h] and heavy [H]) of piglets fed with a high-protein (HP) or a low-protein diet (LP) on their intake of conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the choice test conducted in Experiment 2. Clasps indicate different intakes between both solutions (†$P < 0.1$, *$P < 0.05$, **$P < 0.01$).

[30], and therefore there is no compelling evidence of an innate-specific appetite for protein [31].

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 [1–4], or after long-term choices studies [5–8]. 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 [1], but it may be reduced to only around three days by using a trained individual in the group [32]. 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 [2].

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 [33] 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 [34]. 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 were 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 [33].

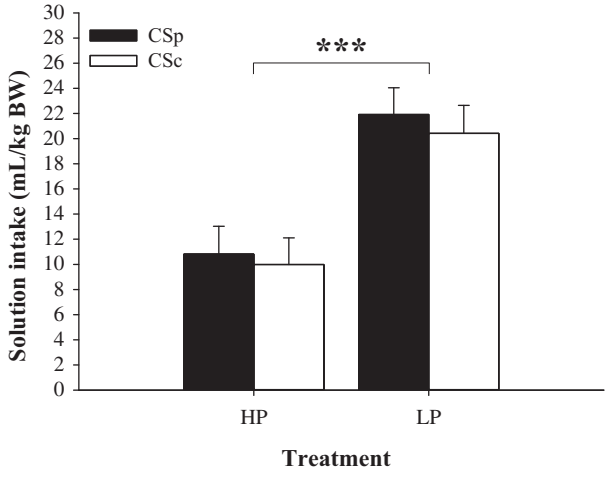

Fig. 5. Effect of feeding a high-protein (HP) or a low-protein diet (LP) on the intake of piglets of conditioned stimulus related to protein (CSp) or carbohydrate (CSc) solutions during the one-pan test conducted in Experiment 2. Clasp indicates different intakes between experimental treatments (***$P < 0.001$).

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) [16,20] than that for maltodextrin (30 g/L) [22] 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 [22]. 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 [35] and humans [36]. 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. [37]. 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 [38]. 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 [37]. 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.

5. 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.

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