

## The use of porcine digestible peptides and their continuity effect in nursery pigs<sup>1</sup>

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**ABSTRACT:** A total of 552 entire male and female nursery pigs were selected to be used in 2 different experiments that aimed to study if milk ingredients can be replaced by highly preferred protein sources (Exp. 1) and if pre- and postnatal exposure of those protein ingredients through the maternal diet may increase pig performance (Exp. 2). In Exp. 1, 240 pigs were separated after weaning (28 d) into 2 groups depending on the presence of lactose in their diets. Pigs ( $n = 120$ ) fed diets with the presence of lactose (lactose +) were given prestarter (0–14 d) and starter (15–33 d) diets with 142 and 50 g/kg of sweet milk whey, respectively; the lactose-free group ( $n = 120$ ) was offered an isoenergetic diet with 20 g/kg of porcine-digestible peptides (PDP; Palbio 62SP; Bioibérica S.A., Palafolls, Spain) and wheat replacing sweet milk whey. Choice and 1-feeder tests were performed in another group of animals ( $n = 72$ ) to evaluate the preference and acceptance for both diets. Pigs preferred ( $P = 0.039$ ) the lactose+ over the lactose-free diet after a 30-min choice test and consumed more ( $P = 0.001$ ) lactose+ than lactose-free diet in a 1-feeder test. However, no difference ( $P > 0.467$ ) in performance was observed between groups

for the entire nursery period. In Exp. 2, 120 animals were obtained from sows that, during late gestation (14 d) and lactation (28 d), were fed diets containing 20 g/kg of PDP and another 120 animals were obtained from sows fed an isoenergetic diet without PDP inclusion. Placenta samples were collected at farrowing to assess the volatile compounds present in the placental fluid of sows. After weaning, all pigs received a feed containing 20 g/kg of PDP in the prestarter and starter diets. A principal components analysis of the total volatile compounds showed the exclusive presence of sulfur-containing compounds and a higher presence of terpene compounds in the placental fluid of PDP-supplemented sows. In addition, pigs coming from sows fed diets supplemented with PDP tended to show a higher ADFI ( $P = 0.07$ ) and ADG ( $P = 0.06$ ) than did pigs coming from control sows during the 15 to 33 d after-weaning period. These results suggest that dietary incorporation of sweet milk whey may be replaced by a specific protein source without affecting performance of pigs after weaning. However, more experiments are needed to elucidate the mechanism for the sow's diets' influence over pig's performance.

**Key words:** familiarity, feed preferences, lactose, maternal diet, pigs, porcine-digestible peptides

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### INTRODUCTION

Weaning is a stressful event for pigs, during which they are challenged to eat a new bulky and dry feed, which is composed mainly of ingredients that the pig has not previously encountered. Most nutritionists formulate starter diets with highly digestible ingredients, such as dairy products and lactose, to enhance feed palatability and smooth over the sudden change in feed

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characteristics. Lactose addition has been described to improve growth performance until approximately 21 to 28 d after weaning (Pluske et al., 2007). However, pigs, as other mammals, may also show preferences for sweet and umami compounds and may exhibit early-learned feeding behaviors that could be acquired before birth (Campbell, 1976; Figuroa et al., 2013). For example, Solà-Oriol et al. (2011) showed that, at 50 g/kg of inclusion, dairy products may be less preferred by nursery pigs than are selected animal protein sources such as porcine-digestible peptides (**PDP**; Palbio 62SP, Bioibérica S.A., Palafox, Spain), a byproduct of the heparin industry obtained from hydrolyzed porcine intestinal mucosa.

On the other hand, mammals may associate volatile compounds present in the amniotic fluid or milk with hedonic or postingested consequences, subsequently showing a preference for the same compounds (Mennella and Beauchamp, 1999; Arias and Chotro, 2007). This may become an adaptive learning strategy for pigs to reduce the negative impact of weaning, as the animals may show a learned feeding behavior related to the ingredient composition of the sow diet. In the present study, we have hypothesized that PDP inclusion in a diet free of dairy products and lactose may maintain the performance of weaned pigs, compared with animals fed diets containing significant amounts of whey and lactose (Exp. 1), and that pre- and postnatal exposure to PDP via the maternal diet may improve feed intake and performance of weaned pigs fed a diet containing PDP (Exp. 2).

## MATERIALS AND METHODS

Two experiments were conducted at the animal research facilities of the Universitat Autònoma de Barcelona (**UAB**; Barcelona, Spain). Experimental procedures were approved by the Ethical Committee on Animal Experimentation of the UAB (Comisión Ética en Experimentación Animal y Humana 1406).

### *Animals and Housing*

A total of 552 male and female pigs (Pietrain × [Large White × Landrace]) were used. Pigs were individually identified by using a plastic ear tag just after birth and were offered an unflavored creep-feed diet (containing dairy products) during lactation from d 10 after birth onward using a commercial pan feeder. Animals were weaned at an average of 28 d and housed in a weanling room with 24 pens. The weanling room had automatic, forced ventilation and completely slatted flooring. Each pen (3.2 m<sup>2</sup> in floor area) was equipped with a hopper feeder with 3 feeding

spaces and an independent water supply to ensure ad libitum feeding and fresh water access.

### *Experiment 1: Weaning without Dairy Ingredients*

Two different cereal-based diets were designed and offered to the pigs after weaning meeting the NRC (2012) standards (Table 1). One unflavored weanling diet without any dairy product source or lactose was incorporated with 20 g/kg of PDP and wheat (300 g/kg) in the prestarter (0–14 d) and starter (15–33 d) periods fed to half of animals (lactose-free group). The other diet was isoenergetic and included sweet milk whey (142 and 50 g/kg) during the prestarter and starter periods, respectively, as a source of lactose (**lactose+**). Porcine-digestible peptides and sweet milk whey compositions are presented in Table 2. Both diets were offered in mash form.

To evaluate pig feeding behavior regarding diets containing lactose or not, 72 pigs (3 wk after weaning) were first used to test their preference (choice test) and acceptance (1-feeder test) for the experimental diets (Figuroa et al., 2012). Twelve pen-mate pairs were used in a 30-min choice test (2 equidistant feeders) to study the preference for these diets. The rest of the pigs were also tested in pen-mate pairs (24), but in this case, 1 unique diet was offered to each pair during 30 min to measure their acceptance for diets (12 pairs with the lactose+ diet and 12 pairs with the lactose-free diet). Feed intake during the preference and acceptance tests was recorded by measuring the initial and final weight of the feeders. Spillage was not significant and was not accounted for when measuring feed consumption. During the preference tests, the positions of the lactose+ and lactose-free diets were counterbalanced across pigs pairs, so each diet appeared equally often on the left and on the right. To perform the test, animals were not feed restricted. However, feed was removed 1 h before the beginning of each test, and it was returned at the end of the choice or acceptance test.

For a performance study with the same diets, a total of 240 pigs were selected at weaning with an average initial BW of 7.9 ± 1.2 kg and were distributed into 4 blocks according their BW (light: 6.1 ± 0.58 kg; middle-light: 7.6 ± 0.36 kg; middle-heavy: 8.5 ± 0.24 kg; and heavy: 9.4 ± 0.25 kg). Within each block, pigs were randomly distributed into 6 pens of 10 animals for a balanced BW. Pens were randomly assigned to 2 experimental groups according to a balanced distribution. Half of the pens (12 pens, 3 pens of each weight block) were ad libitum fed the lactose+ diet, and the rest of the pens ( $n = 12$ ) were fed the lactose-free diet during the whole period (33 d). Feed intake and BW

**Table 1.** Ingredient and nutrient composition of diets used in Exp. 1 by dietary phase (as-fed basis)

Item	Prestarter <sup>1</sup>		Starter <sup>2</sup>	
	Lactose+ <sup>3</sup>	Lactose-free	Lactose+	Lactose-free
Ingredient, g/kg				
Sweet milk whey	142.2	–	49.8	–
PDP <sup>4</sup>	–	20.0	–	20.0
Wheat	182.1	300.0	280.8	300.0
Maize	260.0	260.0	260.0	260.0
Barley	100.0	100.8	100.0	139.8
Soybean meal 44% CP	50.0	50.0	47.3	50.0
Extruded soybean	194.1	191.5	190.6	153.2
Soybean oil	3.1	4.9	–	4.8
Fishmeal	25.0	25.0	25.0	25.0
Animal plasma 80% CP	15.0	15.0	15.0	15.0
L-Lysine HCl	3.6	3.5	3.3	3.3
Dl-Methionine	1.8	1.5	1.3	1.2
L-Threonine	1.3	1.4	1.2	1.2
L-Tryptophan	0.3	0.4	0.1	0.2
Mineral–vitamin mix <sup>5</sup>	4.0	4.0	4.0	4.0
Monocalcium phosphate	9.0	12.6	9.3	10.4
Calcium carbonate	6.7	7.9	9.7	10.5
Sodium bicarbonate	1.5	1.5	1.5	1.5
Sodium chloride	–	–	1.1	–
Calculated nutrient composition, <sup>6</sup> g/kg				
NE, MJ/kg	10.34	10.34	10.26	10.26
CP	197.6	203.8	193.2	193.2
Lysine	14.0	14.0	13.1	13.1
Methionine	4.9	4.9	4.3	4.4
Methionine + cysteine	8.3	8.3	7.7	7.7
Threonine	9.1	9.1	8.5	8.5
Tryptophan	2.8	2.8	2.5	2.5
Valine	9.4	9.6	9.1	9.2
Calcium	7.5	7.5	7.5	7.5
Phosphorus	6.5	6.8	6.2	6.2
Lactose	100.0	–	35.0	–

<sup>1</sup>Prestarter phase diets fed d 0 to 14 after weaning.

<sup>2</sup>Starter phase diets fed d 15 to 33 after weaning.

<sup>3</sup>Lactose+ = diet containing lactose components.

<sup>4</sup>PDP = porcine-digestible peptides (Palbio 62SP; Bioibérica S.A., Palafolls, Spain).

<sup>5</sup>Provided per kilogram of diet: 7,000 IU of vitamin A (acetate), 500 IU of vitamin D<sub>3</sub> (cholecalciferol), 250 IU of vitamin D (25-hydroxycholecalciferol), 45 mg of vitamin E, 1 mg of vitamin K<sub>3</sub>, 1.5 mg of vitamin B<sub>1</sub>, 3.5 mg of vitamin B<sub>2</sub>, 1.75 mg of vitamin B<sub>6</sub>, 0.03 mg of vitamin B<sub>12</sub>, 8.5 mg of D-pantothenic acid, 22.5 mg of niacin, 0.1 mg of biotin, 0.75 mg of folacin, 20 mg of Fe (chelate of AA), 2.5 mg of Cu (sulfate), 7.5 mg of Cu (chelate of glycine), 0.05 mg of Co (sulfate), 40 mg of Zn (oxide), 12.5 mg of Zn (chelate of AA), 12.5 mg of Mn (oxide), 7.5 of Mn (chelate of glycine), 0.35 mg of I, 0.5 mg of Se (organic), and 0.1 mg of Se (sodium).

<sup>6</sup>NRC (2012).

were measured on d 0, 7, 14, 21, 28, and 33 after weaning to calculate the ADFI, ADG, and G:F.

**Table 2.** Nutrient composition of porcine-digestible peptides (PDP)<sup>1</sup> and sweet milk whey (as-fed basis)

Calculated nutrient composition, <sup>2</sup> g/kg	PDP	Sweet milk whey <sup>3</sup>
NE, MJ/kg	8.04	9.74
CP	620	125
Lysine	52.2	9.3
Methionine	18	1.9
Methionine + cysteine	21.6	4.3
Threonine	32	7.3
Tryptophan	3	1.8
Isoleucine	27.2	6.6
Valine	33.6	6.3
Arginine	38.4	2.7
Calcium	0.5	8.2
Phosphorus	8.8	6.9

<sup>1</sup>Palbio 62SP (Bioibérica S.A., Palafolls, Spain).

<sup>2</sup>Fundación Española para el Desarrollo de la Nutrición Animal (2010).

<sup>3</sup>Sweet milk whey (Lactalis Ingredients, Bourgbarré, France).

### Statistical Analysis (Experiment 1)

The consumption by pig pairs during the 30-min preference and acceptance tests was analyzed with ANOVA by using the MIXED procedure of the statistical package SAS (9.2; SAS Inst. Inc., Cary, NC), with the diet consumed (lactose+ or lactose-free diet) as the main factor. For the choice test, the experimental unit (pair of pigs) was considered a random effect specifying the covariance structure of the residual matrix as completely general (unstructured). Percent preference for the lactose+ diet was calculated as the lactose+ diet intake divided by total intake × 100. Mean values are presented as least squares means adjusted by Tukey's test and considering a significance level of 5%.

Average pen's BW, ADFI, ADG, and G:F were analyzed with ANOVA by using the GLM procedure of SAS, taking into account the diet consumed (lactose+ or lactose-free diet) and weight block as main effects. The mathematical model used was  $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$ , in which  $Y$  is the BW, ADFI, ADG or G:F value for the observations;  $\mu$  is the general mean of all observations;  $\alpha$  is the effect of the treatment (pigs fed lactose+ or lactose-free diets);  $\beta$  is the effect of the weight block;  $\alpha\beta$  is the interaction between both factors; and  $\epsilon$  is the unexplained random error.

### Experiment 2: Maternal Flavor Transference and Continuity

A total of 26 sows were selected on d 100 of gestation to evaluate the effect of pre- and postnatal maternal transference of protein volatile cues over their litter's parameters after weaning when they were exposed to diets containing the same protein. Sows were

split into 2 experimental groups that did not differ in their BW (265 vs. 245 kg;  $P = 0.11$ ), back fat thickness measured in the P2 (15.2 vs. 13.6 mm;  $P = 0.27$ ), parity (2.9 vs. 3.0;  $P = 0.87$ ), and BCS (2.8 vs. 2.8;  $P = 0.93$ ). Sows were fed during the last 14 d of gestation and the whole lactation period (28 d) with 1 of the 2 experimental diets ( $n = 13$ /treatment). Two different diets were prepared for late gestation (14 d before farrowing) and the entire lactation (28 d) of sows (Table 3). Diets contained 9.46 MJ of NE/kg, 132 g/kg of CP, and 6 g/kg of Lys for late gestation and 10.14 MJ of NE/kg, 166 g/kg of CP, and 8 g/kg of Lys for the lactation period. Experimental diets contained either PDP (20 g/kg) replacing soybean meal or not. Both diets were prepared with the same main ingredients but changing their inclusion level to keep a constant macronutrient and AA composition. No external flavors were added to these diets. Sows were fed 2 equal meals each day at 0800 and 1600 h. The total amount of feed offered was individually adjusted according to the BCS of each sow during late gestation. During lactation, the amount of feed offered was controlled until the fifth day after farrowing, reaching an ad libitum situation from the fifth day until the end of lactation.

Placenta samples from 3 random sows per treatment selected at random were collected at farrowing. Samples were processed to assess the volatile-compounds profile present in the placental fluid of sows fed the control diet or the diet incorporated with 20 g/kg of PDP. Placental fluid was obtained by placenta compression with cheesecloth. This fluid was then centrifuged ( $3,420 \times g$  for 15 min at  $4^\circ\text{C}$ ) and filtered (0.22- $\mu\text{m}$  Millipore filters; Merck KGaA, Darmstadt, Germany). The liquid obtained was stored in 15-mL tubes and kept frozen ( $-20^\circ\text{C}$ ) until analysis.

A total of 240 pigs ( $8.4 \pm 1.10$  kg) were selected and distributed by groups depending on whether they came from mothers fed with PDP during late gestation and lactation ( $n = 120$ ; PDP group) or from mothers fed the control diet ( $n = 120$ ; Control group). Pigs of each group were weighed and equalized by BW into 4 blocks (light:  $6.9 \pm 0.28$  kg; middle-light:  $7.9 \pm 0.17$  kg; middle-heavy:  $8.8 \pm 0.25$  kg; and heavy:  $9.9 \pm 0.44$  kg) and were allocated into 24 pens (10 pigs/pen). All selected animals ad libitum received a prestarter (0–14 d) and starter (15–33 d) diet in mash form, containing 20 g/kg of PDP as a protein source (Table 4). The nutrient composition of the prestarter and started diets was 10.55 and 10.26 MJ of NE/kg, 190 and 185 g/kg of CP, and 14.0 and 13.8 g/kg of Lys, respectively. As in Exp. 1, feed intake and BW were measured weekly (d 0, 7, 14, 21, 28, and 33 after weaning) to calculate the ADFI, ADG, and G:F. The statistical analysis of

**Table 3.** Ingredient and nutrient composition of diets of sows used in Exp. 2 by dietary phase

Item	Late gestation <sup>1</sup>		Lactation <sup>2</sup>	
	Control	PDP <sup>3</sup>	Control	PDP
Ingredient, g/kg				
PDP	–	20.0	–	20.0
Maize	250.0	155.5	250.0	261.8
Wheat	100.0	160.0	200.0	200.0
Barley	220.0	300.0	170.0	170.0
Wheat bran	180.0	180.0	75.0	75.0
Gluten feed	80.0	24.8	50.0	50.0
Sugar beet pulp	40.0	40.0	–	–
Canola meal	50.0	50.0	30.0	30.0
Soybean meal 44% CP	28.0	3.0	166.0	134.7
Soybean hulls	11.0	23.5	–	–
Lard	10.0	12.0	26.0	25.6
L-Lysine HCl	0.9	0.5	1.5	1.4
Mineral–vitamin mix <sup>4</sup>	4.0	4.0	4.0	4.0
Monocalcium phosphate	5.2	5.2	7.8	7.8
Calcium carbonate	16.3	16.5	13.7	13.7
Sodium bicarbonate	1.0	1.0	3.0	3.0
Sodium chloride	4.0	4.0	3.0	3.0
Calculated nutrient composition, <sup>5</sup> g/kg				
NE, MJ/kg	9.47	9.46	10.14	10.14
CP	132.0	133.0	166.0	165.0
Lysine	6.0	6.2	8.8	9.0
Methionine	2.2	2.4	2.6	2.8
Methionine + cysteine	4.9	5.0	5.7	5.8
Threonine	4.7	4.8	6.0	6.1
Tryptophan	1.5	1.5	2.0	1.8
Valine	6.2	6.4	7.7	7.8
Calcium	8.6	8.7	7.7	7.7
Phosphorus	5.9	5.7	5.9	6.0

<sup>1</sup>Late-gestation phase diets fed d 100 to 114 of gestation.

<sup>2</sup>Lactation phase diets fed from farrowing to d 28 after farrowing.

<sup>3</sup>PDP = porcine-digestible peptides (Palbio 62SP; Bioibérica S.A., Palafolls, Spain).

<sup>4</sup>Provided per kilogram of diet: 9,000 IU of vitamin A, 2,000 IU of vitamin D<sub>3</sub> (cholecalciferol), 8 mg of vitamin E (alpha-tocopherol), 1.5 mg of vitamin K<sub>3</sub>, 4 mg of vitamin B<sub>2</sub>, 1.5 mg of vitamin B<sub>6</sub>, 0.015 mg of vitamin B<sub>12</sub>, 10 mg of pantothenic calcium, 22 mg of niacin, 200 mg of choline chloride, 100 mg of Fe (ferrous carbonate), 10 mg of Cu (sulfate), 0.5 mg of Co (sulfate), 100 mg of Zn (oxide), 80 mg of Mn (oxide), 0.22 mg of Se, 0.5 mg of I, and 500 IU of 5-phytase.

<sup>5</sup>NRC (2012).

these data was similar to that previously described for pig's parameters in Exp. 1.

### **Volatile Compounds Analysis (Experiment 2)**

Headspace sampling of volatile compounds was performed with a CombiPal auto-sampler (CTC Analytics AG, Zwingen, Switzerland) equipped with a 50/30- $\mu\text{m}$  divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). The fiber was preconditioned in the heated and purged Fiber

**Table 4.** Ingredient and nutrient composition of diets of pigs used in Exp. 2 by dietary phase (as-fed basis)

Item	Prestarter <sup>1</sup>	Starter <sup>2</sup>
Ingredient, g/kg		
Maize	189.7	217.7
Wheat	222.7	252.8
Barley	100.0	100.0
Wheat bran	–	40.0
Soybean meal 44% CP	50.0	50.0
Extruded soybean	150.0	114.8
Soybean oil	–	2.9
Sweet milk whey	197.1	140.0
Fishmeal	31.4	31.8
PDP <sup>3</sup>	20.0	20.0
L-Lysine HCl	4.1	6.2
DL-Methionine	2.0	1.5
L-Threonine	1.7	1.5
L-Tryptophan	0.7	0.5
Mineral–vitamin mix <sup>4</sup>	4.0	4.0
Monocalcium phosphate	17.0	5.6
Calcium carbonate	6.6	10.7
Sodium chloride	3.0	–
Calculated nutrient composition, <sup>5</sup> g/kg		
NE, MJ/kg	10.55	10.26
CP	190.1	185.0
Lysine	14.0	13.8
Methionine	5.2	4.7
Methionine + cysteine	8.3	7.7
Threonine	9.1	8.5
Tryptophan	2.8	2.5
Valine	5.3	5.6
Calcium	8.3	7.5
Phosphorus	8.5	6.0

<sup>1</sup>Prestarter phase diet fed d 0 to 14 after weaning.

<sup>2</sup>Starter phase diet fed d 15 to 33 after weaning.

<sup>3</sup>PDP = porcine-digestible peptides (Palbio 62SP; Bioibérica S.A., Palafolls, Spain).

<sup>4</sup>Provided per kilogram of diet: 7,000 IU of vitamin A (acetate), 500 IU of vitamin D<sub>3</sub> (cholecalciferol), 250 IU of vitamin D (25-hydroxycholecalciferol), 45 mg of vitamin E, 1 mg of vitamin K<sub>3</sub>, 1.5 mg of vitamin B<sub>1</sub>, 3.5 mg of vitamin B<sub>2</sub>, 1.75 mg of vitamin B<sub>6</sub>, 0.03 mg of vitamin B<sub>12</sub>, 8.5 mg of D-pantothenic acid, 22.5 mg of niacin, 0.1 mg of biotin, 0.75 mg of folacin, 20 mg of Fe (chelate of AA), 2.5 mg of Cu (sulfate), 7.5 mg of Cu (chelate of glycine), 0.05 mg of Co (sulfate), 40 mg of Zn (oxide), 12.5 mg of Zn (chelate of AA), 12.5 mg of Mn (oxide), 7.5 of Mn (chelate of glycine), 0.35 mg of I, 0.5 mg of Se (organic), and 0.1 mg of Se (sodium).

<sup>5</sup>NRC (2012).

Conditioning Station (CTC Analytics AG, Zwingen, Switzerland) at 270°C for 1 h. Samples (0.5 g of feed or 1 mL of placental fluid) were placed in 10-mL amber-glass vials for headspace sampling. Samples were incubated at 60°C for 5 min and then extracted for 1 h at the same temperature. The extraction was performed with agitation at 250 rpm in cycles of 5 s on and 2 s off. Desorption took place in the injection port at 250°C for 1 min in splitless mode.

The gas chromatography–mass spectrometry analyses were performed using an Agilent 6890 N gas chromatograph fitted with a mass spectrometer Agilent Technologies 5973 system (both from Agilent Technologies, Inc., Palo Alto, CA). The column was a TR-WAX from Teknokroma (Sant Cugat del Vallès, Spain) 60 m by 0.25 mm i.d. with a 1- $\mu$ m film thickness. The temperature program was as follows: 35°C for 8 min, then raised by 4°C/min up to 150°C, and then raised by 20°C/min up to 260°C and then maintained for 15 min. The carrier gas was He at a constant flow of 1.3 mL/min. The mass detector was operated at 150°C in electron impact mode at 70 eV, and the ion source temperature was 230°C. The chromatograms were recorded by monitoring the total ion currents in a mass range of 33 to 350  $m/z$  without solvent delay. Peak identification was based on the comparison of their mass spectra with those supplied by the National Institute of Standards and Technology (NIST08; 5973 MSD Agilent Technologies) and Wiley library (Wiley 7N edition, Wiley and Sons Ltd.; West Sussex, England) databases.

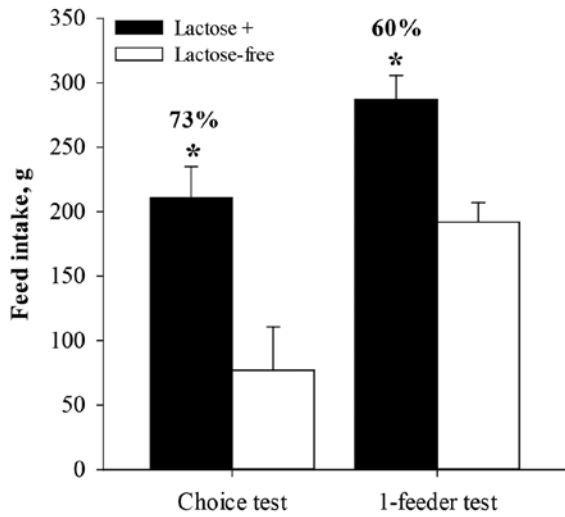
As the different volatile compounds present in the volatile profile studied were not independent, the relationships among volatile compounds in feed and in placental fluid were studied with principal components (PC) analysis, by using the PRINCOMP procedure of SAS.

## RESULTS

### Experiment 1: Weaning without Dairy Ingredients

The results of the preference (choice test) and acceptance (1-feeder test) tests are shown in Fig. 1. Pigs preferred ( $P = 0.039$ ) the lactose+ (142 g/kgA of sweet milk whey) over the lactose-free (20 g/kg of PDP) diet after the 30-min choice test (211 g vs. 77 g [SEM 56]). In the same way, pigs on the 1-feeder test showed a higher ( $P = 0.001$ ) intake when they had the opportunity to eat the lactose+ diet than the lactose-free diet (287 g vs. 192 g [SEM 22]).

Table 5 presents the performance of pigs fed the lactose+ or the lactose-free diets. No differences ( $P > 0.467$ ) were observed in feed intake or growth performance among animals fed the experimental diets. There was an interaction between diet consumed and weight block in ADFI ( $P = 0.024$ ) and G:F ( $P < 0.001$ ) during the d-15-through-33 period, when animals changed from the prestarter to starter diet. Middle-heavy pigs tended ( $P = 0.066$ ) to show a higher intake of lactose-free than of lactose+ diet when offered as a single option, whereas no differences ( $P > 0.893$ ) in consumption were observed in light, middle-light, and



**Figure 1.** Mean (and SEM) feed intakes in a 30-min choice or 1-feeder test of a diet containing lactose (lactose+, 142 g/kg of sweet milk whey) or a diet without dairy products (lactose-free, 20 g/kg of porcine-digestible peptides [Palbio 62SP; Bioibérica S.A., Palafolls, Spain]) in nursery pigs. Numbers at the top of bars indicate the average percent preference for the lactose+ diet. Asterisks (\*) indicate that the lactose+ diet intake is different ( $P < 0.05$ ) from that of the lactose-free diet.

heavy animals during this period. However, a lower ( $P = 0.001$ ) G:F was also registered in middle-heavy pigs when they were fed the lactose-free diet, in comparison with the animals of the same weight block but fed the lactose+ diet.

**Experiment 2: Maternal Flavor Transference and Continuity**

The parameters of pigs are shown in Table 6. Pigs coming from sows supplemented with 20 g/kg of PDP in their diets during late gestation and lactation tended ( $P = 0.077$ ) toward a higher ADFI during the starter phase (15–33 d after weaning) than did pigs coming from control sows when both groups were exposed to 20 g/kg of PDP after weaning. Similar results ( $P = 0.062$ ) were observed for ADG in pigs coming from PDP-supplemented sows during the same period. No differences ( $P > 0.363$ ) were observed in G:F, and no interactions ( $P > 0.115$ ) were observed between groups and blocks along the experimental period.

The PC analysis of the total volatile compounds in the placental fluid showed negative correlations between sows fed the control diet and sows fed the diet supplemented with 20 g/kg of PDP during late gestation and a strong covariation within the PDP-supplemented sows. Nevertheless, higher dispersion was observed for the animals fed the control diet, indicating greater individual variability or lower dietary effect on the volatile compounds present in the placental fluid (Fig. 2). This figure also confirms the observa-

**Table 5.** Effect of lactose content on feed intake and growth performance of pigs in Exp. 1<sup>1</sup>

Item	Diet <sup>2</sup>			P-value		
	Lactose+	Lactose-free	SEM	Diet	Block	Diet × block
<b>BW, kg</b>						
d 0	7.90	7.91	0.006	0.637	0.0001	0.648
d 14	9.78	9.70	0.140	0.705	0.0001	0.648
d 33	20.92	21.13	0.303	0.626	0.0001	0.775
<b>ADFI, kg/d</b>						
d 0–14	0.21	0.21	0.007	0.603	0.126	0.418
d 15–33	0.62	0.63	0.015	0.467	0.012	0.024
<b>ADG, kg/d</b>						
d 0–14	0.13	0.13	0.009	0.692	0.595	0.666
d 15–33	0.52	0.53	0.014	0.517	0.165	0.607
<b>G:F</b>						
d 0–14	0.62	0.61	0.028	0.838	0.375	0.738
d 15–33	0.57	0.57	0.007	0.873	0.073	<0.0001

<sup>1</sup>From ANOVA analysis, including the factors of lactose inclusion (lactose+ or lactose-free diets), weight block (4 BW groups/treatment), and the interaction. Treatment  $n = 12$ . Mean values are least squares means with a significance level of  $P < 0.05$ .

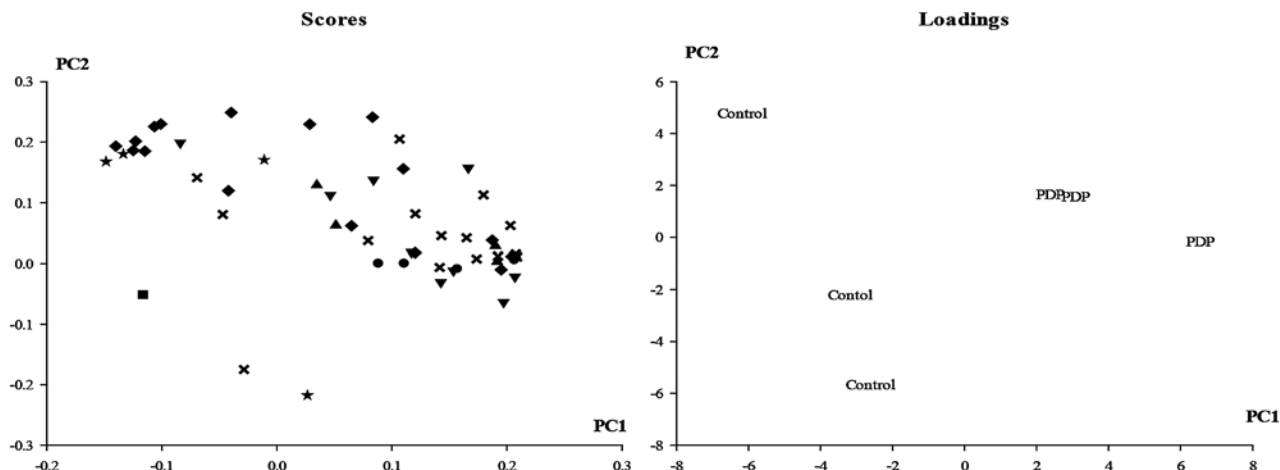
<sup>2</sup>Lactose+ = diet containing lactose components, including sweet milk whey (142 and 50 g/kg during the prestarter and starter periods, respectively) as source of lactose to meet the requirements recommended by the NRC (2012) for weaning pigs; Lactose-free = isoenergetic diet without any source of dairy products and containing 20 g/kg of porcine digestive peptides (Palbio 62SP; Bioibérica S.A., Palafolls, Spain) in the prestarter and starter periods.

**Table 6.** Effect of the incorporation of porcine-digestible peptides (PDP) into the sow’s diet on feed intake and growth performance of pigs in Exp. 2<sup>1</sup>

Item	Group <sup>2</sup>			P value		
	Control	Pre-exposed	SEM	Group	Block	Group × block
<b>BW, kg</b>						
d 0	8.44	8.44	0.006	0.537	0.0001	0.701
d 14	10.40	10.52	0.165	0.612	<0.0001	0.361
d 33	19.35	20.06	0.326	0.141	<0.0001	0.166
<b>ADFI, kg/d</b>						
d 0–14	0.23	0.24	0.012	0.731	0.984	0.856
d 15–33	0.69	0.74	0.018	0.077	0.131	0.147
<b>ADG, kg/d</b>						
d 0–14	0.14	0.14	0.117	0.672	0.895	0.312
d 15–33	0.47	0.50	0.110	0.062	0.213	0.139
<b>G:F</b>						
d 0–14	0.62	0.61	0.023	0.787	0.643	0.274
d 15–33	0.68	0.68	0.007	1.000	0.363	0.115

<sup>1</sup>From ANOVA analysis, including the factors of maternal diet (Control or PDP [Palbio 62SP; Bioibérica S.A., Palafolls, Spain] diets), weight block (4 BW groups/treatment), and the interaction. Treatment  $n = 12$ . Mean values are least squares means with a significance level of  $P < 0.05$ .

<sup>2</sup>Control = pigs coming from sows fed a control diet and offered post-weaning diets including 20 g/kg of PDP as a protein source; Pre-exposed = pigs coming from sows fed a diet incorporated with 20 g/kg of PDP and offered postweaning diets including the same amount of the protein source.



**Figure 2.** Principal component (PC) analysis of the volatile compound in the placental fluid of sows fed a control diet (Control) or the control diet supplemented with 20 g/kg of porcine-digestible peptides (PDP; Palbio 62SP; Bioibérica S.A., Palafolls, Spain) of the 7 volatile-compound families: acids (■), alcohols (▼), carbonyl ketones (★), esters (▲), phenyl compounds (◆), sulfur-containing compounds (●), and terpenes (×). Principal component 1 explained 40.4% of the total variation, and PC2 explained 23.2% of the variation. Scores: orientation of the volatile-compound families relative to the PC. Loadings: orientation of sow placental fluid relative to PC.

tions for the volatile compounds analyses showing the presence of sulfur-containing compounds only in the placental fluid of sows fed the PDP-supplemented diet and a higher number of terpene compounds in sows fed the PDP diet compared with those fed the control diet. The first 4 PC had eigenvalues above 1 (22.6, 12.9, 10.2, and 7.04), and these explained 40.3, 23.2, 18.3, and 7.04% of total variability, respectively.

## DISCUSSION

### *Effect of Dairy Ingredients in the Nursery Period on Pig Performance*

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

the necessity for in-feed antibiotics. Lactose is among the principal sugars that escape digestion in the small intestine, and a large proportion may be fermented to lactic acid (Pierce et al., 2006). Different authors have recently indicated that lactose or dietary vegetable fiber may increase pig performance in weanling diets using high- but not low-protein diets (Hermes et al., 2009), associated with increases in *Lactobacillus* and *Bifidobacterium* concentrations and significant decreases in Enterobacteriaceae counts in feces. Nevertheless, Mahan (1992) has suggested that if another protein source with a highly digestible carbohydrate is provided, the replacement of milk products may be possible. In our study, sweet milk whey (142 g/kg) was totally replaced by PDP (20 g/kg) and wheat (118 g/kg), where nonstarch polysaccharides contained in wheat and the rest of vegetable ingredients could have promoted responses similar to those suggested for lactose. Mahan (1993) also suggested that the pig-growth responses to dried whey in comparison with a corn-soybean meal diet without lactose depend on the quality of the dried whey. Therefore, when edible-grade dried whey was fed to weaned pigs, improved growth rates were achieved, whereas a feed-grade or low-quality source of dried whey did not improve pig gains.

Most nutritionists also use dairy products and lactose to smooth over the sudden change in feed characteristics that takes place at weaning, allowing, in some way, a progressive change from sow's milk to a dry diet. Milky flavors have been used several times to increase the acceptance of new diets, due to their palatability or to the animal's association of the flavor with the positive oral or postingested character-

istics of milk ingestion during the suckling period. In this respect, we observed that naïve animals showed a preference (choice test) and appetite (1-feeder test) for the diet including sweet milk whey over the PDP diet during the short-term feeding tests (30 min). Both ingredients may also differ in their familiarity to postweaning pigs, with whey containing milk-derived volatile compounds that may create a generalization of the maternal milky flavors, creating a link between the pre- and postweaning periods. Nonetheless, contrary to our observations, Solà-Oriol et al. (2011) previously described a higher preference for PDP than for dairy products commonly used in pig diets. It is worth mentioning that in these tests, PDP and dairy products were evaluated at the same inclusion level of 50 g/kg and no preference was observed when these ingredients were assessed at 100 or 200 g/kg. In addition, the tests were performed over 4 d compared with our 30-min tests, which may have caused repetitive exposure or familiarity, rather than short term preference or palatability, to likely affect the adaptation of pigs to the new diet.

Despite the clear differences observed on feed preference and acceptance between diets with or without lactose in short-term tests, the parameters registered were similar when only 1 of these diets was provided throughout the whole nursery period. As in a 4-d choice test, a longer exposure with the protein source may enable pigs to learn the positive consequences of its ingestion, making initial intake differences disappear. Repetitive exposures to the protein source and a likely positive associative learning between the hedonic and postingestive effects and the volatile compounds (Figuroa et al., 2012) may progressively increase the preference for and acceptance and palatability of new ingredients (Sclafani, 2004; Dwyer et al., 2009).

#### ***Pre- and Postnatal Exposure of Protein Ingredients (Porcine-Digestible Peptides) through Maternal Diet***

Pigs may have difficulty initiating feed intake if they are abruptly weaned and a process of learning new environmental cues is not allowed (Bruininx et al., 2001). In Exp. 2, pig's performance was studied when PDP was previously included in the sow diet. During the second half of the experimental period (d 15 to 33) but not during the first 2 wk after weaning, animals tend ( $P < 0.1$ ) to present a higher feed intake and BW gain. It could be suggested that the different stressful factors affecting pigs at weaning may have decreased feed intake of both groups of animals, independently of their maternal diets.

Flavor continuity has been described in several mammalian species such as ewes (Nolte et al., 1992;

Désage et al., 1996), rabbits (Coureaud et al., 2002), rats (Arias and Chotro, 2007), humans (Mennella et al., 1995; Hausner et al., 2008; Nicolaïdis, 2008), and pigs (Oostindjer et al., 2009; Figuroa et al., 2013). It may reduce neophobia when animals find solid feed with cues similar to those their mothers have experienced before. This may be attributable to a familiar learning effect, in which animals could face a certain stimulus with repeated prenatal (amniotic fluid) or postnatal (milk) experience. It could be also due to an associative learning effect during pregnancy or lactation, in which the hedonic effect of amniotic fluid, as well as the hedonic and postingested characteristics of milk, could act as unconditioned positive stimuli that, associated with one specific sensory-conditioned stimulus (such as taste or odor), could generate preferences or even increase animal acceptance for an associated sensory cue (Myers and Sclafani, 2006; Arias and Chotro, 2007).

There is a biochemical and chemical support for flavor continuity through maternal fluids in pigs (Guiraudie-Capraz et al., 2005). Recently, Figuroa et al. (2013) showed that pigs born from sows offered flavored diets (anise or milky-cheese flavors) during late gestation (100–114 d of gestation) preferred those flavors during lactation and the first days after weaning. Oostindjer et al. (2009, 2010, 2011) did not find any marked preference for flavors previously offered in sows' diet over naïve flavors. However, the authors described that preexposure to flavors increased the total intake and weight of animals, latency time to start a fight, and decreased diarrhea of preexposed pigs compared with unexposed animals during the reexposure to the flavor after weaning. Moreover, the volatile-compounds analysis of our study showed that sows fed the PDP-supplemented diet during late gestation differentially transferred sulfur-containing compounds to their amniotic fluid compared with sows fed the control diet without PDP. Porcine digestible peptides are a protein hydrolysate byproduct of the heparin industry that contain a high content of inorganic and organic sulfates derived from glycosaminoglycans, such as chondroitin sulfate, heparan sulfate, and dermatan sulfate. The presence in the diet of these compounds inhibits methanogenesis and promotes the growth of sulfate-reducing bacteria in the colon of mammals (Gibson et al., 1988; Christl et al., 1992). The action of sulfate-reducing bacteria on organic sulfates generates sulfur-containing compounds and sulfoxides, among others (Gibson et al., 1993). On the other hand, the reduction of methanogenesis promotes the absorption of these sulfur compounds into the colonic mucosa (Christl et al., 1992), which may explain their increase in the placental fluid of PDP-supplemented sows. Previous reports suggest that maternal transference may provide



a useful strategy to increase the feed intake of pigs after weaning. However, the present data do not support that conclusion, although interesting trends were observed in animals' feed intake and BW gain.

Dairy products such as sweet milk whey may help to increase feed intake and mask some ingredients that could create neophobic reactions during the first days after weaning. However, their use appears to not be necessary and might be skipped when a highly valuable animal protein source such as PDP is offered in the diet. Several studies suggest an effect of pre- and postnatal contact with maternal feed cues over newborn animals' feeding behavior. Nevertheless, more experiments are needed to elucidate a possible learning of new protein cues coming from sow diets over pig's performance in front of the same protein cues.

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