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# Productive behavior of piglets in response to diet composition and the addition of enzymes. Consequences of weaning weight



Cristina Rodríguez Rodríguez <sup>a</sup>

María Alejandra Pérez Alvarado b\*

José Antonio Cuarón Ibargüengoytia† a b

<sup>a</sup> Universidad Nacional Autónoma de México. Facultad de Estudios Superiores – Cuautitlán. Programa de Maestría y Doctorado en Ciencias de la Producción y la Salud Animal. Cuautitlán, Estado de México. México.

<sup>b</sup> Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal. Km 1 carretera a Colón, 76280, Ajuchitlán, Colón, Querétaro. México.

\* Corresponding author: perez.maria@inifap.gob.mx; palvaradoa@hotmail.com

#### **Abstract:**

The response to the addition of  $\alpha$ -amylase, serine protease,  $\beta$ -glucanase, pectinase, and hemicellulase (EXE) to the piglet diet and two soybean use schemes (SOY) was evaluated. With 552 pigs, in 92 experimental units (EU, pen with 6 pigs), with an initial weight and age of 6.14  $\pm$  1.358 kg and 20  $\pm$  0.83 d. There were 4 blocks (monthly production groups) in a randomized complete block design, and the treatments were the factorial arrangement of two levels of EXE (yes or no) and two levels of soybean meal (SOY), low or high, per feeding phase: P1, 12 or 24 % (7 d); P2, 16 or 32 % (14 d), and P3, 20 or 32 % (21 d). Feed intake, weight gain, and feed efficiency were estimated weekly. The consistency of the feces was evaluated daily (from 1 to 5: 1= dry to 5= liquid). Piglets with EXE diets gained more weight (P<0.02), were more efficient (P<0.07), and had better fecal consistency (P<0.04) in the first week. At the end of the assessment, no differences were found (P>0.16) in any of the criteria evaluated. Likewise, no response to SOY (P>0.19) or SOY×EXE interactions (P>0.26) was

observed. When exploring whether piglet weight at weaning influenced the response, two categories (CATs) were established for the initial weight of the EUs: Lightweight, (4.44  $\pm$  0.600 kg, 20 EU) and Heavy (6.73  $\pm$  0.738 kg, 72 EU). The lightweight piglets gained more in the first week and were more efficient throughout the experimental period (P<0.03). Regardless of weaning weight, SOY did not affect productivity, and EXE only provided a temporary advantage.

**Keywords:** Weaning, Exogenous enzymes, Piglets.

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## Introduction

Weaning is one of the most critical stress events in the lives of pigs due to the separation of piglets from their mother and the need to supplement their nutrition with means other than mother's milk<sup>(1,2)</sup>. Milk contains about 20 % dry matter, which in turn is made up of 40 % fat (highly digestible) and 25 % lactose and does not contain starch; in contrast, solid feed is a mixture of animal and plant proteins, non-starchy polysaccharides, anti-nutritional compounds, and starch mainly from cereal grains containing approximately 90 % dry matter<sup>(3)</sup>. Switching to a new feed, based on starch (cereal grains), complex proteins of plant origin (soybean meal), non-starchy polysaccharides, and anti-nutritional compounds, such as phytates, results in a low feed intake<sup>(2,4-7)</sup>, a situation that complicates digestive maturation since the presence of feed is a factor that promotes the development of the intestinal mucosa and stimulates the production of digestive enzymes specific to the animal<sup>(5,8,9)</sup>. It has been estimated that only half of pigs consume feed within the first 8 h after weaning, and 10 % do not consume anything until after 48 h<sup>(10,11)</sup>, with small piglets or those with a lower milk intake during lactation being the best prepared at weaning<sup>(11)</sup> due to the need for an additional feed source during lactation.

In weaned piglets, the change of substrates requires an increase in amylase and proteases (other than renin)<sup>(12,13,14)</sup>. One way to complement endogenous insufficiency is with the addition of exogenous enzymatic activities<sup>(15,16)</sup>.  $\alpha$ -amylase makes possible the hydrolysis of starch, which, if not digested, becomes one of the main causes of loss of osmotic stability in the intestine, giving rise to the phenomenon of "mechanical" diarrhea<sup>(17,18)</sup>. Carbohydrases promote the reduction of digesta viscosity in the small intestine due to the hydrolysis of non-

starch polysaccharides<sup>(19,20)</sup>. The addition of proteases can contribute to the reduction of nitrogenous metabolites (ammoniacal nitrogen and biogenic amines) associated with membrane irritation, which depend on the undigested protein in the diet<sup>(20,21)</sup>. The addition of phytase is justified by the presence of phytates in plant-based ingredients, which, due to their chelation capacity, form insoluble complexes with positively charged minerals and proteins, which interferes with their absorption<sup>(22,23)</sup>.

This work was conducted under the hypothesis that adding an  $\alpha$ -amylase, a serine protease, and a multienzyme complex with activity of  $\beta$ -glucanase, hemicellulase, and pectinase could mitigate the impact of two strategies of inclusion of soybean meal (low and high) in the diet of newly weaned piglets in addition to minimizing the potential challenges in the transition period represented by weaning. Besides the evaluation, the consequences of weaning weight on the productive response to treatments were explored.

### Material and methods

The handling of the animals and the experimental procedures were carried out in compliance with the guidelines of NOM-062-ZOO-1999, technical specifications for the production, care, and use of laboratory animals (DOF, 2001)<sup>(24)</sup>.

The work was conducted at the Swine Livestock Unit of the National Center for Disciplinary Research in Animal Physiology and Improvement, INIFAP. It is located in the locality of Ajuchitlán, municipality of Colón, Querétaro, Mexico, at 20° 41' 42" N, 100° 00' 54" W, at an elevation of 1,969 m asl. The average temperature during the experiment was 19.2 °C with a recorded minimum and maximum of 4.5 and 31 °C, and 22.8 % average relative humidity.

A total of 552 piglets were used, which were from four blocks (consecutive groups of monthly production) split into 92 experimental units (EUs, the experimental unit was a pen made up of 6 piglets with a balanced mix of females and castrated males per treatment; 3 males and 3 females per EU); there were 20 EU for the first block and 24 EU for the 3 subsequent blocks. In all blocks, piglets were identified at birth by permanent ear markings (notches) and they did not have access to preweaning feed. Piglets were weighed the day before weaning as a randomization procedure to treatments based on litter of origin, sex, and body weight. The average age and weight of the pigs at the beginning of the experiment (weaning) was  $6.14 \pm 1.358$  kg in weight and  $20 \pm 0.83$  d of age.

The experiment was conducted for 42 d divided into two stages of 21 d each in order to create two stressful situations, one at the time of weaning and the other due to the change of housing,

environmental management, and feeding. During the first 21 d, the piglets were housed in a weaning room, an enclosed building with an environment controlled by a gas heater and natural ventilation. The building has 24 raised cages of plastic slatted floor with an effective surface area of 1.36 m². Each cage is provided with a nipple drinker and a 7-slot trough. At weaning, piglets were received with the thermostat set at a minimum of 30 °C and it was gradually reduced by 4 °C per week. From the fourth to the sixth week, the piglets were housed in an open-front type building with no environmental control other than the use of curtains. The building has 24 pens with solid concrete floors, with an effective area of 5.40 m². Each pen has a wet hopper feeder that includes a nipple drinker on the feeding pan and an additional nipple drinker on the opposite side of the feeder. The identity of the EUs was maintained throughout the evaluation.

The feeding program consisted of three phases (Table 1): Phase 1, lasting 7 d from weaning; Phase 2, for 14 d, and Phase 3, for the last 21 d of the assessment. The experiment was conducted according to a randomized complete block design, in which the treatments consisted of a factorial arrangement (2×2), resulting from 2 inclusion strategies (low or high) of soybean meal (SOY) in the diet: Phase 1, 12 % (low SOY) or 24 % (high SOY); Phase 2, 16 % (low SOY) or 32 % (high SOY); and Phase 3, 20 % (low SOY) or 32 % (high SOY); and the addition or not of exogenous enzymes (EXE), resulting in a total of four treatments.

**Table 1:** Experimental diets and their nutritional composition\*

Ingredients, kg/t	Pha	Phase 1		Phase 2		3
Level of soybean meal,	12.00	24.00	16.00	31.70	20.00	32.00
Sorghum, grain (8.5 %)	26.90	23.48	30.43	21.25	43.33	37.00
Corn, yellow (8 %)	28.00	24.00	28.00	28.00	20.00	20.00
Soybean, meal (47 %)	12.00	24.00	16.00	31.70	20.00	32.00
Milk, whey	16.20	16.20	8.00	8.00		
Fish, meal	7.00		7.00		7.00	
Rapeseed, meal (36 %)	3.00	3.00	4.00	4.00	5.00	5.00
Rapeseed, oil	3.50	5.10	3.50	3.50		
Suet	-		-		2.20	2.60
Phosphate, mono and dicalcium	0.55	1.24	0.57	1.23	0.24	0.91
L-Lysine·HCl	0.69	0.70	0.55	0.45	0.43	0.44

Calcium, carbonate	0.53	0.75	0.49	0.72	0.54	0.78	
Salt	0.40	0.40	0.40	0.40	0.36	0.36	
L-Threonine	0.40	0.40	0.18	0.40	0.14	0.13	
DL-Methionine	0.20	0.23	0.10	0.11	0.08	0.10	
Dartamox	0.10	0.10	0.12	0.10			
premix <sup>®a</sup>	0.10	0.10	0.10	0.10			
Denagard					0.20	0.20	
CTC <sup>®b</sup>					0.20	0.20	
L-Valine	0.10		0.10				
Trace minerals,	0.10	0.10	0.08	0.08	0.09	0.10	
premix <sup>c</sup>	0.10	0.10	0.00	0.00	0.07	0.10	
Vitamins,	0.08	0.08	0.08	0.08	0.08	0.08	
premix <sup>d</sup>	0.00	0.00	0.00	0.00	0.00	0.00	
L-Isoleucine	0.08	0.08	0.08				
Choline-HCl,	0.07	0.07	0.07	0.07	0.10	0.10	
60 %							
L-Tryptophan	0.06	0.04	0.05	0.01	0.01		
Total	100.0	100.0	100.0	100.0	100.0	100.0	
Calculated analys			matter)				
ME, Mcal/kg	3.43	3.48	3.42	3.42	3.35	3.35	
NE, Mcal/kg	2.55	2.55	2.54	2.46	2.48	2.43	
Crude protein,	18.69	19.33	20.02	21.99	21.43	22.16	
%							
Total lysine, %	1.55	1.58	1.51	1.56	1.49	1.52	
Digestible	1.38	1.38	1.32	1.32	1.28	1.28	
lysine, %							
Digestible	0.87	0.87	0.83	0.83	0.82	0.82	
threonine, %							
Total calcium,	0.68	0.68	0.65	0.65	0.60	0.60	
%							
Total	0.61	0.61	0.60	0.60	0.53	0.53	
phosphorus, %							
Digestible	0.40	0.40	0.37	0.37	0.27	0.27	
phosphorus, %							
<u> </u>	* All diete in	cluded 0.201	kg/t of Ronozyn	ne® HiPhos (	TT)		

<sup>\*</sup> All diets included 0.20 kg/t of Ronozyme® HiPhos (GT).

<sup>&</sup>lt;sup>a</sup> Amoxicillin trihydrate (200 ppm).

<sup>&</sup>lt;sup>b</sup> Tiamulin hydrogen fumarate (100 ppm) and chlortetracycline (300 ppm).

 <sup>&</sup>lt;sup>c</sup> The mineral premix provided the following elements, in mg/kg of premix: Co (carbonate), 0.60; Cu (sulfate), 12; Fe (sulfate), 100; I (EDDI), 0.80; Mn (sulfate), 33; Se, 0.25 (sodium selenite); Zn, 120 (sulfate).
<sup>d</sup> The vitamin premix provided the following concentrations per kilogram of premix: vitamin A 13,300 IU; vitamin D<sup>3</sup> 3,700 IU; vitamin E 160 mg; vitamin K 9.38 mg; biotin 0.67 mg; cyanocobalamin 0.07 mg; folic acid 5.30 mg; niacin 66.70 mg; pantothenic acid 46.70 mg; pyridoxine 6.67 mg; riboflavin 12.00 mg; thiamine 4.00 mg; ascorbic acid 266.70 mg.

All treatments included 200 g/t of a 6-phytase (Ronozyme<sup>®</sup> HiPhos, DSM Nutritional products Mexico) with an activity of 10,000 phytase units (FYT) per gram of product, from *Citrobacter braakii* and expressed in *Aspergillus oryzae*. The enzymes that were additionally used to make up the treatments were: 300 g/t of a multienzyme complex (Ronozyme<sup>®</sup> VP, DSM Nutritional products Mexico) comprising the following activities per gram of product: β-glucanase, with an activity of 5,000 units; pectinase, with an activity of 50 units, and one hemicellulase, all from *Aspergillus aculeatus*; 150 g/t of an α-amylase (Ronozyme<sup>®</sup> HiStarch, DSM Nutritional products Mexico) that provided an activity of 90 units per gram of product, from *Bacillus licheniformis*, and 200 g/t of a serine protease (Ronozyme<sup>®</sup> Proact, DSM Nutritional products Mexico) with an activity of 70,000 units per gram, produced from *Nocardiopsis prasina* and expressed in *Bacillus licheniformis*.

To control endemic infectious problems at the experimental site (*Haemophilus parasuis* and *Pasteurella* sp.), amoxicillin trihydrate (400 ppm, Dartamox® premix, Animal Care Products) was included in Feeding Phases 1 and 2, and tiamulin hydrogen fumarate (100 ppm) plus chlortetracycline (300 ppm) in Phase 3 (Denagard CTC®, Elanco Animal Health).

The feed offered was recorded daily and at the end of the week, the remnants of the feeder were weighed to estimate the daily feed intake (DFI) by difference; at the beginning of the experimental period and at the end of each week, the piglets were weighed individually to estimate the daily weight gain (DWG) and the feed efficiency was calculated as the gain as a function of intake ( $G\times I$ ). Daily, after the first feed offer, the average consistency of the feces per EU was rated, using a subjective scale from 1 to  $5^{(25)}$ , where: 1= dry and hard feces, 2= normal feces, 3= pasty feces, 4= semi-liquid feces, and 5= liquid feces.

The diets were formulated based on the analysis of the raw material through linear programming at minimum cost (Nutrion®) and were prepared in the form of meal. The main ingredients were cereal grains (corn and sorghum), oilseed meals (soybeans and canola), whey, and fishmeal. Table 1 details the experimental diets and some of their nutrients. For quality control and verification purposes, total dry matter (DM), crude protein (CP), calcium (Ca), and phosphorus (P) were determined in the laboratory in accordance with procedures 934.01, 976.05, 927.02, and 964.07 of the AOAC<sup>(26)</sup>. The calculated levels of metabolizable energy (ME) were: Phase 1= 3.45, Phase 2= 3.42, and Phase 3= 3.35 Mcal/kg of feed standardized to 90 % dry matter. Digestible lysine levels (standardized ileal digestibility) were established in Phase 1, 1.38 %; Phase 2, 1.32 %, and Phase 3, 1.28 % (adjusted to 90 % dry matter). Due to the addition of phytase, the concentration of digestible phosphorus for the formulation was reduced by 0.1 percentage units, and calcium concentrations were adjusted for a Ca:P ratio between 1 and 1.15. The rest of the nutrients complied with the recommendations of the NRC 2012<sup>(27)</sup>.

Data were analyzed according to a randomized complete block design (where each block consisted of a consecutive group of monthly births) with a factorial arrangement  $(2\times2)$ , distinguishing the major effects of the inclusion strategy of soybean meal (SOY), exogenous enzymes (EXE), and their possible interaction (SOY $\times$ EXE). The response variables were: DFI, DWG, G $\times$ I, and fecal consistency.

The statistical model used was as follows:

$$\mathbf{Y_{ijk}} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$$

Where:

 $Y_{ijk}$  = response variable.

 $\mu$  = overall mean.

 $A_i$  = effect of the addition of SOY at level i.

 $\mathbf{B_j}$  = effect of the addition of EXE at the level j.

 $(AB)_{ij}$  = effect of the exogenous SOY×EXE interaction at level i, j.

 $\mathbf{E}_{ijk} = \text{random error}.$ 

At the end of the evaluation, in order to distinguish the response of weaning weight to the treatments, the EU were classified into two categories (CAT): Lightweight, with the EU weighing 1 standard deviation below the mean, and Heavy, the rest of the EU.

The routines of the SAS (v. 9.3)<sup>(28)</sup> statistical package were used. Through the following procedures: UNIVARIATE to study the normality and homogeneity of variances; GLM for the analysis of variance of production parameters (DFI, DWG, G×I, and fecal consistency); MIXED<sup>(29)</sup> for the study of the responses in time of body weight by EU and its standard deviation, and REG to subject body weight to a regression analysis, in which, previously with orthogonal polynomial contrasts, the most interesting linear, quadratic, and cubic effects were distinguished in order to predict body weight as a function of days of age by CAT. The results are presented as the least squares means of the SOY×EXE interaction and of the categories of piglet weaning weight.

## **Results and discussion**

Table 2 shows the cumulative productive behavior of the piglets during the 42 d of the evaluation. No SOY×EXE interaction was observed for any of the variables assessed (P>1.00). In the piglets' response at 7 d postweaning, the DFI was the same (P>0.66) for animals that consumed the high or low SOY level and the addition or not of EXE; however, animals that consumed the EXE diet gained more weight (P<0.02; SEM, 0.007; 30 vs 10

g/d), which resulted in pigs receiving EXE having better feed efficiency (P<0.07; SEM, 0.074; 0.25 vs 0.11 kg). Nonetheless, the effects associated with EXE were temporary as they were observed only during the first week after weaning, when the DFI was low (0.11 kg  $\pm$  0.019 kg). Poor feed intake within the first 24 to 48 h after weaning causes degeneration of the intestinal mucosa (enterocytes), which induces a decrease in the digestive enzymes of the brush border and the activity of pancreatic enzymes ( $^{(30,31,32)}$ ); thus, the presence of exogenous enzymes (EXX) may have acted to complement the digestive incapacity of the piglet, which would explain the improvements in DWG and G×I at the same consumption during the first 7 d after weaning.

Table 2: Productive behavior of weaned piglets in response to treatments<sup>a,b</sup>

Soybean meal inclusion <sup>c</sup>	Low		High		CEN 4d	SOY	EXE	SOY×EXE	
<b>Enzyme inclusion</b>	No	Yes	No	Yes	SEM <sup>d</sup>	<i>P</i> <	<b>P</b> <	<b>P</b> <	
Number of observations	23	23	23	23					
Initial weight, kg	6.25	6.22	6.24	6.24	0.246	0.99	0.94	0.94	
Response at 7 days after	er weani	ng (day	0 to day	7)					
Feed intake, kg/day	0.11	0.11	0.11	0.11	0.003	0.19	0.43	1.00	
Weight gain, kg/day	0.01	0.03	0.01	0.03	0.007	0.71	0.02	0.60	
Feed efficiency, kg	0.11	0.22	0.11	0.28	0.074	0.68	0.07	0.69	
Feces consistency <sup>e</sup>	2.68	2.90	3.02	3.07	0.108	0.83	0.04	0.26	
Cumulative response at 21 days after weaning (day 0 to day 21)									
Feed intake, kg/day	0.24	0.24	0.24	0.25	0.006	0.45	0.56	0.94	
Weight gain, kg/day	0.15	0.17	0.15	0.17	0.011	0.40	0.38	0.45	
Feed efficiency, kg	0.66	0.73	0.63	0.63	0.056	0.27	0.52	0.50	
Feces consistency	3.78	3.54	3.89	3.78	0.154	0.79	0.31	0.31	
Cumulative response a	t 42 day	s after v	veaning	(day 0 1	to day 42	()			
Feed intake, kg/day	0.60	0.57	0.59	0.59	0.013	0.82	0.17	0.46	
Weight gain, kg/day	0.34	0.33	0.32	0.33	0.011	0.46	0.78	0.45	
Feed efficiency, kg	0.57	0.58	0.55	0.57	0.018	0.32	0.59	0.66	
Feces consistency	2.40	2.40	2.36	2.42	0.076	0.25	0.91	0.76	

 $<sup>^{\</sup>rm a}$  Least squares means from male and female piglets (50 % males and 50 % females).

The cumulative responses of the productive variables at 21 and 42 days after weaning were the same regardless of the level of EXE or SOY (P>0.91); likewise, no SOY×EXE interaction was found (P>0.94). This suggests a rapid adaptation to the consumption of soybean meal

<sup>&</sup>lt;sup>b</sup> All treatments included phytase (0.20 kg/t).

<sup>&</sup>lt;sup>c</sup> The inclusion of soybean meal was: Phase 1, 12 %; Phase 2, 16 %, Phase 3, 20 % for low-soybean diets and Phase 1, 24 %; Phase 2, 31.7 %; Phase 3, 32 % for high-soybean diets.

<sup>&</sup>lt;sup>d</sup> SEM= standard error of the mean.

<sup>&</sup>lt;sup>e</sup> 1= dry and hard, 2= normal, 3= pasty, 4= semi-liquid, 5= liquid.

without the effect of enzymes<sup>(33,34)</sup>. Fecal consistency during the first week after weaning was better for piglets that did not consume EXE (P<0.04; Without, 2.85 vs With, 2.99; SEM, 0.108) regardless of the SOY level (P>0.83); this parameter made it possible to subjectively rate digestive processes caused by the excess of nutrients that could be used by bacterial fermentation in the colon, giving rise to an increase in osmotic pressure and secretion (mechanical diarrhea)<sup>(35,36)</sup>; nevertheless, as it is a subjective rating per EU, both results indicate that the feces had a consistency between normal and pasty (2.92  $\pm$  0.518) and in no way does it suggest the presence of diarrhea, which would be classified as semi-liquid or liquid feces (rating 4-5). From d 8 to d 14 after weaning, an increase in the presence of liquid feces was noted in all EUs, so that in the cumulative response at 21 d after weaning, no significant differences were found in the feces rating due to the addition of SOY (P>0.79), EXE (P>0.31), or in the SOY×EXE interaction (P>0.31), observing pasty and semi-liquid feces  $(3.75 \pm 0.739)$  in all treatments; the disappearance of liquid feces after d 14 was noted until the end of the experimental period when all treatments showed a normal consistency  $(2.40 \pm 0.364)$ . In summary, the results show a benefit of enzymatic activities (amylase, protease, glucanase, hemicellulase, and pectinase) additional to the phytase action, and the effects were noticed only during the first week, time in which the animals learned to consume feed(10,11).

As described in the material and methods section, at the end of the experiment, it was investigated whether weaning weight influenced the response to treatments, so the EU were classified according to weaning weight into two categories (CAT): Lightweight vs Heavy, resulting in an average weight of the lightweight=  $4.44 \pm 0.600$  kg vs heavy=  $6.72 \pm 0.738$  kg; having 20 total EU (5 EU/Treatment) of lightweight piglets (22 % of the population) and 72 total EU (18 EU/treatment) of heavy piglets (78 % of the population). Table 3 shows the results of the analysis of CAT and its interaction with treatments. In the first week after weaning, piglets that were rated as lightweight consumed approximately 9 % less feed than heavy pigs (P<0.01) and showed better DWG (P<0.01; SE, 0.037; lightweight= 40 vs heavy= 20 g/d) and higher GxI (P<0.01; SE 0.353; lightweight= 0.44 vs heavy= 0.12 kg), perhaps because they proportionally consumed more feed (depending on their body weight) than heavy piglets (2.30 vs 1.60 % of weaning weight in lightweight and heavy piglets, respectively). CAT did not influence the strategy of using SOY in the diet (P>0.15), nor was any interaction found between treatments and CAT (P>0.47).

<b>Table 3:</b> Productive behavior of piglets at weaning depending on the weight category
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Cotogowy (CAT)h	Tiabteresials4	Haarer	CIEC	CAT	<b>EXE</b> <sup>d</sup>	SOYe	INT*		
Category (CAT) <sup>b</sup>	Lightweight	Heavy	SEc	<b>P</b> <	<b>P</b> <	<b>P</b> <	<b>P</b> <		
Number of observations	20	72							
Initial weight, kg	4.44	6.72	0.733	0.01	0.94	0.07	0.95		
Response at 7 days after weaning (day 0 a day 7)									
Feed intake, kg/day	0.10	0.11	0.018	0.01	0.29	0.40	0.86		
Weight gain, kg/day	0.04	0.02	0.037	0.01	0.03	0.95	0.88		
Feed efficiency, kg	0.44	0.12	0.353	0.01	0.06	0.83	0.81		
Cumulative response at 21 days after weaning (day 0 a day 21)									
Feed intake, kg/day	0.21	0.28	0.037	0.01	0.34	0.83	0.99		
Weight gain, kg/day	0.16	0.17	0.030	0.46	0.40	0.23	0.78		
Feed efficiency, kg	0.79	0.64	0.170	0.01	0.97	0.25	0.98		
Cumulative response at 42 days after weaning (day 0 a day 42)									
Feed intake, kg/day	0.55	0.57	0.073	0.34	0.27	0.98	0.91		
Weight gain, kg/day	0.33	0.34	0.034	0.20	0.96	0.16	0.02		
Feed efficiency, kg	0.60	0.61	0.085	0.76	0.37	0.34	0.47		

<sup>&</sup>lt;sup>a</sup> Least squares means from male and female piglets (50 % castrated males and 50 % females).

\* INT= SOY×EXE×CAT interaction.

When analyzing the growth responses due to the use of EXE, they were independent of the CAT of pigs and are observed only during the first week, when transient digestive failure is more noticeable in piglets<sup>(12,37)</sup>. Having reduced the variation in weaning weight of the EU (by randomization procedures) made the consequences of environmental and social stress smaller in lightweight piglets. Pig growth by CAT is shown in Figure 1A as the average weight per EU as a function of time  $\pm$  1 standard deviation (error bars). The trend lines represent the increase in body weight over time and show the convergent growth of lightweight piglets compared to heavy piglets, where, at weaning, lightweight piglets weighed 66 % of heavy piglets and 42 d later, 88 %. The effect that best describes the behavior of weight was the quadratic one (P<0.01), which is shown in Figure 1A. This response is relevant and is explained by the fact that, in both CATs, the change in body weight as a function of time was slow until day 35 of life, growth accelerated as time increased. The following equation describes the behavioral trend of the body weight of lightweight piglets:

$$Y = (8.211 \pm 1.096) - (0.356X \pm 0.058) + (0.008X^2 \pm 0.001), P < 0.001, R^2 = 0.94.$$

<sup>&</sup>lt;sup>b</sup> CAT= weaning weight categories: lightweight, less than 5.05 kg at weaning; heavy, more than 5.051 kg at weaning.

<sup>&</sup>lt;sup>c</sup> SE= square root of the mean square of the error.

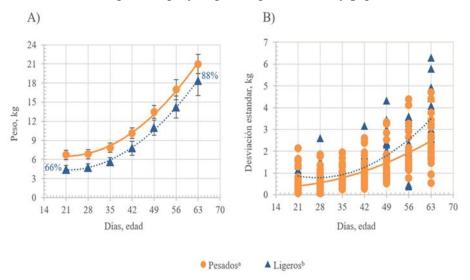
<sup>&</sup>lt;sup>d</sup> EXE= enzymes.

<sup>&</sup>lt;sup>e</sup> SOY= soybean: Phase 1, 12 %; Phase 2, 16 %, Phase 3, 20 % for low-soybean diets and Phase 1, 24 %; Phase 2, 31.7 %; Phase 3, 32 % for high-soybean diets.

For the heavy piglets, the equation that describes the growth was:  $Y = (10.844 \pm 0.484) - (0.383X \pm 0.026) + (0.009X^2 \pm 0.001), P < 0.001, R^2 = 0.96.$ 

Where, for both equations: Y= body weight, kg. X= days of age. ±= standard deviation of the parameter.

**Figure 1:** Weight behavior and standard deviation of pigs as a function of age and weaning weight category (lightweight and heavy piglets)



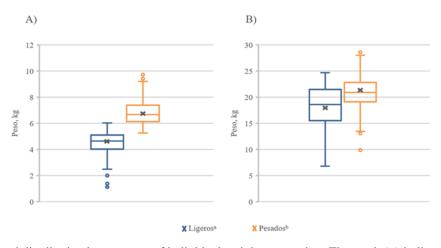
A) Average body weight per EU as a function of age and by weight categories at the beginning of the experiment  $\pm$  one standard deviation (error bars); heavy,  $6.72 \pm 0.738$  kg and lightweight,  $4.44 \pm 0.600$  kg. At weaning ( $20 \pm 0.83$  d), lightweight piglets weighed 66 % of the heavy piglets and, at d 42 after weaning, 88 %. B) Standard deviation per EU as a function of age in days per weight category.

In the case of the variation represented in Figure 1B as the standard deviation of each of the EUs over time, it was observed that both CAT showed a quadratic response trend (P<0.01). It should be noted that the deviations are not very different at d 35 of age and it is from d 42 of life, when the piglets were changed from the weaning room to the open-front type building, that the trend lines show the divergence more clearly; EUs of lightweight piglets did not have the ability to recover from their small piglet status when facing the new stress events, whereas a large part of the animals in this group were able to reach the body weight of heavy piglets. This has been discussed by Paredes *et al*<sup>(38)</sup>, where piglets up to 2.5 standard deviations below the population mean at birth have the ability to overcome their status as small piglets, and more than 40 % of these piglets can compensate for the growth they did not previously achieve, which affects weaning weight.

To show this more clearly, Figures 2A and 2B are presented. The box diagrams represent the central tendency parameters, such as the mean and mode, and exemplifies the form and distribution of the individual weight of the piglets of both CAT at the time of weaning (2A)

and at day 42 of life (2B). At weaning, the intervention in distribution by randomization practices is notorious, where even lightweight piglets showed a negative asymmetry with 2.5 % of abnormally lightweight piglets. On the other hand, heavy piglets had a positive asymmetry, with 0.46 % of abnormally heavy piglets, which is explained by having a greater number of piglets of lower weight forming the EUs than heavy piglets, and it can see it in graph 2A, where they are represented as atypical observations. At d 42 after weaning, negative asymmetry prevailed in lightweight piglets with an increase in the proportion of piglets that reached some of the heaviest piglets, whereas heavy piglets showed a slight negative asymmetry (-0.397). The compensation of the lower weight piglets could be the result of the decrease in competition due to the removal of heavy piglets, which was manifested in the recovery capacity in stages after weaning<sup>(39)</sup>. In this regard, it should be noted that lightweight piglets were the first to consume feed, which was manifested with a better efficiency (P<0.01; SE, 0.353; 0.44 vs 0.12, Table 3). In addition, the DWG of lightweight piglets that consumed the diet with the addition of enzymes was 50 % higher than that of heavy piglets (P<0.01; SE, 0.037; 40 vs 20 g/d, Table 2) during the first week after weaning.

**Figure 2:** Distribution of body weight at weaning (d 0) and at the end of experiment (d 42 post-weaning) of the pig population according to the weaning weight category (lightweight and heavy piglets)



- A) Segregated distribution by category of individual weight at weaning. The mark ( $\times$ ) indicates the mean of each weight category; lightweight,  $4.77 \pm 0.788$  kg and heavy,  $6.77 \pm 0.906$  kg.
- B) Distribution by category of body weight at d 42 after weaning. The mark (×) indicates the mean of each weight category; lightweight,  $18.14 \pm 3.926$  kg and heavy,  $21.30 \pm 2.985$  kg.

# **Conclusions and implications**

The increase in the concentration of soybean meal in the diet did not affect the productive behavior of piglets after weaning. Regardless of the level of soybean meal in the diet and the weight of the animals at weaning, the benefit in the productive variables (daily weight gain and feed efficiency) was clear in piglets that consumed the diet with the addition of exogenous enzymes only during the first week; this suggests the complementation of the transitory digestive incapacity. Besides, by forming the experimental units in a more homogeneous way in terms of weight, there was a better weight gain and feed efficiency in lightweight piglets due to the reduction of competition with heavier piglets.

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