









# Multi-objective optimization of methionine+cysteine levels in diets for growing pigs

*Nivel óptimo multiobjetivo de metionina+cisteína en dietas para cerdos en crecimiento*

*Otimização multiobjetivo do nível de metionina+cisteína em dietas para suínos em crescimento*

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## Abstract

**Background:** The addition of synthetic methionine to growing pig diets should consider the requirements of the methionine+cysteine complex, as most cysteine is obtained through dietary methionine. **Objective:** To determine an optimal level of methionine+cysteine (Met+Cys) in growing pig diets. **Methods:** Ninety-nine hybrid (Yorkshire×Landrace×Duroc) barrows were used in a completely randomized design (initial body weight: 25.90±3.99 kg). Nine levels of Met+Cys (0.500, 0.525, 0.550, 0.575, 0.600, 0.625, 0.650, 0.675, and 0.700%) were evaluated during 35 days. To determine a multi-objective optimal level of Met+Cys, growth performance and strongly related carcass traits were evaluated. **Results:** Average daily feed intake (ADFI) and feed:gain ratio (FGR) showed a linear response to the dietary Met+Cys level ( $p<0.05$ ), with estimated optimal concentration at 0.700%. Average daily gain (ADG) did not show any effect on the Met+Cys level ( $p>0.05$ ). Carcass characteristics were not affected by the Met+Cys concentration ( $p>0.05$ ). The multi-objective optimal level for ADG, ADFI, and FGR was 0.667% Met+Cys. **Conclusions:** According to our results, 0.667% Met+Cys is the best concentration in diets for growing pigs.

**Keywords:** *carcass characteristics; cysteine; growth performance; methionine; nutritional requirements; protein; pigs; sulfur amino acids; swine nutrition.*

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## Resumen

**Antecedentes:** Las dietas para cerdos en crecimiento deben adicionarse con metionina sintética para suplir los requerimientos del complejo metionina+cisteína (Met+Cis), pues ellos producen gran parte de la cisteína que requieren a partir de la metionina dietaria. **Objetivo:** determinar el nivel óptimo multiobjetivo de Met+Cis en dietas para cerdos en crecimiento. **Métodos:** noventa y nueve machos castrados híbridos (Yorkshire×Landrace×Duroc) se utilizaron en un diseño completamente al azar (peso vivo inicial: 25,90±3,99 kg). Se evaluaron nueve niveles de Met+Cys (0,500, 0,525, 0,550, 0,575, 0,600, 0,625, 0,650, 0,675 y 0,700%) durante 35 d. Para determinar el nivel óptimo multiobjetivo de Met+Cis se consideraron las variables de rendimiento de crecimiento y características de la canal que mantienen una fuerte interrelación. **Resultados:** el promedio de consumo de alimento diario (ADFI) y la conversión alimenticia (FGR) mostraron una respuesta lineal al nivel de Met+Cis en la dieta ( $p<0,05$ ), con una concentración óptima estimada en 0,700%. El nivel de Met+Cis no afectó a la ganancia diaria de peso (ADG;  $p>0,05$ ). La concentración de Met+Cis tampoco afectó las características de la canal ( $p>0,05$ ). El nivel óptimo multiobjetivo de Met+Cis para ADFI, FGR y ADG fue 0,667%. **Conclusión:** los resultados indican que 0,667% de Met+Cis es la mejor concentración en la dieta para cerdos en crecimiento.

**Palabras clave:** *aminoácidos azufrados; características de la canal; cerdos; cisteína; comportamiento productivo; metionina; nutrición de cerdos; proteína; requerimientos nutricionales.*

## Resumo

**Antecedentes:** a adição de metionina em dietas para suínos em crescimento deve ser feita para atender às exigências do complexo metionina+cisteína (Met+Cis), porque grande parte da cisteína é obtida através da dieta com metionina. **Objetivo:** determinar o nível multiobjetivo ótimo de Met+Cis em dietas para suínos em crescimento. **Métodos:** noventa e nove suínos machos castrados híbridos (Yorkshire×Landrace×Duroc) foram utilizados em delineamento inteiramente casualizado, com peso inicial de 25,90±3,99 kg. Nove níveis de Met+Cis (0,500, 0,525, 0,550, 0,575, 0,600, 0,625, 0,650, 0,675 y 0,700%) foram avaliados em suínos em crescimento por 35 d. Para determinar o nível multiobjetivo ótimo de Met+Cis, foram consideradas as variáveis de comportamento de crescimento e características do canal que mantêm uma forte inter-relação. **Resultados:** a ingestão média diária de alimentos (ADFI) e a conversão alimentar (FGR) mostraram uma resposta linear ao nível de Met+Cis na dieta ( $p<0,05$ ), estimando a concentração ótima em 0,700%. O ganho de peso diário (ADG) não mostrou efeito devido ao nível de Met+Cis na dieta ( $p>0,05$ ). As características da carcaça não foram afetadas pela concentração de Met+Cis na dieta ( $p>0,05$ ). Com ADFI, FGR e ADG, o nível ótimo de Met+Cis foi obtido, encontrando este nível em 0,667%. **Conclusões:** os resultados indicam que 0,667% de Met+Cis é a melhor concentração na dieta para suínos em crescimento.

**Palavras-chave:** *aminoácidos sulfurados; características de carcaça; cisteína; comportamento produtivo; exigências nutricionais; metionina; nutrição suína; porcos; proteína.*

## Introduction

Methionine (Met) is the second or third most limiting amino acid (AA) in conventional diets for growing pigs (NRC, 1998; Chen *et al.*, 2014). This sulfur-containing AA plays a role in methylation processes, intestinal oxidative status, maintenance of intestinal mucosal growth and gut function, and is involved in the biosynthesis of creatine, epinephrine, carnitine, taurine, lecithin, choline, melatonin (Bauchart-Thevret *et al.*, 2009; Tesseraud *et al.*, 2009) and cysteine (NRC, 2012). Therefore, a Met deficiency could reduce its availability for protein synthesis and limit animal growth.

Cysteine (Cys) is included in the structure of many proteins, linking polypeptide chains as a precursor of taurine and glutathione (Baker, 1991). Most Cys must be obtained from dietary Met since many feed ingredients lack of adequate Cys levels (Santos *et al.*, 2011). Thus, Met should be added into pig diets to meet the methionine+cysteine (Met+Cys) complex requirement. If the Met level in the diet is insufficient, Cys requirements may not be fulfilled.

According to some reports, low dietary Met+Cys levels improve or have no effect on the productive performance and carcass characteristics of pigs (Santos *et al.*, 2011; Ying *et al.*, 2015). Methionine deficient diets could enhance adipose tissue lipid metabolism, decrease oxidative damage, alter antioxidant pathways, and alleviate the inflammatory responses in growing pigs (Ying *et al.*, 2015; Zhou *et al.*, 2016). Therefore, low levels of Met+Cys could improve growth performance (Santos *et al.*, 2011; Ying *et al.*, 2015; Zhou *et al.*, 2016). Nevertheless, Conde-Aguilera *et al.* (2010, 2014) observed that reduced Met+Cys levels in pig diets increased fat deposition, reduced protein synthesis and affected carcass characteristics, while Met supplementation seems to improve productive performance (Opapeju *et al.*, 2012; Chen *et al.*, 2014; de Oliveira *et al.*, 2015) when the sulfur-containing

AA requirements are met, in addition to producing extra benefits from Met acting as a functional AA (Wu *et al.*, 2013). Nevertheless, an excess of Met+Cys can also affect growth performance and carcass characteristics (Santos *et al.*, 2007; Pena *et al.*, 2008).

The appropriate dietary concentration of Met+Cys has not been established because the optimal level of AA depends on the characteristic to be optimized (Zhang *et al.*, 2013). Therefore, the objective of this study was to determine a multi-objective optimal level of Met+Cys in growing pig diets regarding growth performance and carcass characteristics.

## Materials and Methods

### *Ethical considerations*

The experimental procedures followed the recommendations of the Council for International Organizations of Medical Sciences (CIOMS, 2012) and followed the standards for ethics, biosafety and animal well-being of the Official Mexican Standard (Norma Oficial Mexicana; NOM-062-ZOO, 1999) for the use of experimental animals.

### *Location*

The experiment was conducted in the Swine Unit at the Experimental Farm of Colegio de Postgraduados, located in Montecillo, State of Mexico (98° 48' 27" W and 19° 48' 23" N). The climate is temperate, semi-arid, with 15.9 °C average annual temperature, infrequent frosts, 686 mm average annual rainfall, and 2,250 m of altitude (García, 1988).

### *Animals and experimental design*

Ninety-nine (Yorkshire×Landrace×Duroc) hybrid growing barrows were used in a completely randomized design, with 25.90±3.99 kg average body weight (BW<sub>i</sub>). Nine Met+Cys levels (0.500, 0.525, 0.550, 0.575, 0.600, 0.625, 0.650, 0.675, and 0.700%) were evaluated during 35 days. These levels were obtained by adding synthetic methionine to the control diet.

### Diets and animal handling

Diets were formulated based on sorghum-soybean meal, supplemented with crystalline amino acids, and formulated with digestible amino acids (NRC, 2012) to meet or exceed the nu-

tritional requirements for this stage of growth (Table 1). Pigs were individually housed in 1.2×1.5 m pens with concrete floors, equipped with a single feeder and a nipple drinker. Feed and water were provided *ad libitum*.

**Table 1.** Composition of experimental diets for growing pigs (25–50 kg) fed different Methionine+Cysteine levels.

	T1	T2	T3	T4	T5	T6	T7	T8	T9
<b>Ingredient, %</b>									
Sorghum grain	77.27	77.25	77.23	77.21	77.19	77.17	77.14	77.12	77.10
Soybean meal	18.65	18.66	18.66	18.66	18.66	18.67	18.67	18.67	18.68
Soybean oil	1.13	1.12	1.11	1.10	1.10	1.09	1.08	1.07	1.06
Biolys *	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	0.73
DL-Methionine	0.09	0.11	0.14	0.16	0.19	0.21	0.24	0.26	0.29
L-Threonine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins **	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Minerals ***	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Orthophosphate	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Estimated content (%)								
Metabolizable Energy (Mcal kg <sup>-1</sup> )	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30	3.30
Crude protein	16.79	16.81	16.82	16.83	16.85	16.86	16.88	16.89	16.90
Lysine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.31	0.34	0.36	0.39	0.41	0.44	0.46	0.49	0.51
Met+Cys	0.500	0.525	0.550	0.575	0.600	0.625	0.650	0.675	0.700
Threonine	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Tryptophan	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Phenialanine	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Arginine	0.86	0.86	0.86	0.87	0.87	0.87	0.87	0.87	0.87
Histidine	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Isoleucine	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Leucine	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Valine	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Calcium	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

T, Treatment. \*Biolys, 50.7%; lysine. \*\*Supplied by kg of feed: 5.0×10<sup>6</sup> IU vitamin A; 1.0×10<sup>6</sup> IU vitamin D3; 2.0×10<sup>4</sup> IU vitamin E; 2 g vitamin K3; 1 g thiamine; 5 g riboflavin; 2 g pyridoxine; 25 g niacin; 15 g D-calcium pantothenate; 3 g folic acid; 225 g choline chloride; 0.3 g antioxidant; 15 mg vitamin B12; 180 mg vitamin H-biotin (REKA® Lapisa Animal Nutrition). \*\*\* Supplied by kg of feed: 0.2 g Se; 0.1 g Co; 0.3 g I; 10 g Cu; 100 g Zn; 100 g Fe; 100 g Mn (REKA® Lapisa Animal Nutrition).

### Variables measured

The following variables were analyzed: Average daily gain (ADG), average daily feed intake (ADFI), and feed: gain ratio (FGR). On the first and last days of experimentation, backfat thickness (BT) and *longissimus* muscle area (LMA) were measured using a real-time ultrasound Sonovet 600 with a 3.5 MHz transducer (Medison, Inc., Cypress, California, USA). These data, along with the initial and final body weights, were used to determine the fat-free lean gain (FFLG) and the lean meat percentage (LMP) using the NPPC (Burson and Berg, 2001) equation.

### Statistical analysis

The animals were assigned to one of the nine treatments, using a completely randomized design with nine Met+Cys levels. Each pig was considered an experimental unit. The Shapiro–Wilk and Levene's tests were used to check for normal distribution and variance homogeneity assumptions for the analysis of variance. Analysis of variance was performed with the GLM procedure (MINITAB, 2007) to estimate regression coefficients, which included linear and/or quadratic effects, using the p-value ( $p \leq 0.05$ ), and the coefficient of determination ( $R^2$ ) as criteria to determine the best model. Initial body weight (BW<sub>i</sub>) was used as a covariate.

To obtain the optimal concentrations of Met, a response surface model was built using the RSREG command (MINITAB, 2007), with an optimization technique based on factorial planning to obtain the Met+Cys levels for the different productive and carcass characteristics. Then, maximization of ADG, ADFI, and minimization of FGR were considered to achieve the optimal multi-objective level of the variables affected by the Met+Cys level in the diet. The regression model was adjusted for each variable using the adjusted treatments means by BW<sub>i</sub>. Subsequently, the Response Optimizer was used to identify the combined configuration values of the input variables to optimize the overall response and determine the individual and

composite desirability of the multiple response system (MINITAB, 2007).

### Results

Growth performance variables are presented in Table 2. The ADFI and FGR showed a linear response to the Met+Cys levels in the diet ( $p < 0.05$ ). The optimal concentration of Met+Cys was estimated at 0.500% to maximize ADFI, and 0.700% to minimize ADFI ( $ADFI = 1.68 - 1.01 * Met + Cys + 0.032 * BW_i$ ;  $R^2 = 0.45$ ). The optimal concentration of Met+Cys to minimize FGR was 0.700% ( $FGR = 1.94 - 0.27 * Met + Cys + 0.05 * BW_i$ ;  $R^2 = 0.41$ ). The ADG did not show any effect ( $p > 0.05$ ) of the dietary level of Met+Cys. However, to determine the optimum biological level of Met+Cys with multi-objective optimization we thought necessary to consider that ADG showed a quadratic tendency ( $p = 0.14$ ) because ADG has an important interrelationship with ADFI and FGR, besides, it improves the adjustment of the model. The optimum biological level of Met+Cys that optimized the multi-objective solution was 0.677%. The FFLG, LMP, BT, and LMA were not affected ( $p > 0.05$ ) by the dietary concentration of Met+Cys.

### Discussion

Methionine requirements can be altered by the protein level of the diet, feeding system, growth potential of the animals, interrelationships with other limiting amino acids, health status of the animal (Rakhshandeh *et al.*, 2014) and interaction with other sulfur-containing amino acids such as cysteine since part of the supplemented Met is converted to Cys (NRC, 2012). Thus, the addition of Met into diets is an alternative to meet the requirements of the Met+Cys complex. Digestible Met+Cys requirements were established based on a minimum of 50% of sulfur-containing amino acids being supplied by Met (Rostagno *et al.*, 2011).

There is no agreement on the digestible Met+Cys requirements of pigs. In the present experiment, we found that the optimal level of Met+Cys for ADFI and FGR was 0.70%; however, when considering the multi-



**Table 2.** Treatment means for the productive performance of growing pigs fed with different levels of methionine+cysteine (Met+Cys).

Met+Cys (%)	ADFI (kg d <sup>-1</sup> )	ADG (kg d <sup>-1</sup> )	FGR	BWi (kg)	BWf (kg)	FFLG (kg d <sup>-1</sup> )	LMP (%)	BT (mm)	LMA (cm <sup>2</sup> )
0.500	1.82	0.74	2.46	28.28	51.89	0.28	30.09	7.71	21.35
0.525	1.68	0.75	2.25	27.80	52.28	0.27	29.44	7.25	20.15
0.550	1.72	0.80	2.17	23.05	53.88	0.30	29.23	8.16	20.69
0.575	1.81	0.75	2.40	27.73	52.33	0.27	29.37	7.50	19.97
0.600	1.83	0.77	2.45	24.13	53.01	0.30	29.61	8.28	21.51
0.625	1.84	0.79	2.32	28.63	53.72	0.29	29.92	8.04	21.69
0.650	1.54	0.76	2.04	23.43	52.63	0.28	29.47	7.94	21.04
0.675	1.63	0.72	2.25	26.91	51.32	0.29	30.16	9.27	22.43
0.700	1.54	0.75	2.06	23.64	52.09	0.27	29.41	7.55	20.12
SEM	0.07	0.03	0.09	1.41	1.20	0.02	0.41	0.42	0.84
<b>Effect</b>					<b>p-value</b>				
Linear	0.001	0.74	0.001	-	0.74	0.99	0.90	0.73	0.63
Quadratic	0.01	0.14	0.16	-	0.18	0.16	0.71	0.31	0.46
	R <sup>2</sup>								
Linear	0.45	0.03	0.41	-	0.04	0.04	0.09	0.10	0.23
Quadratic	0.48	0.04	0.42	-	0.01	0.06	0.08	0.11	0.23

ADFI, average daily feed intake; ADG, average daily gain; FGR, feed:gain ratio; BWi, initial body weight; BWf, final body weight; FFLG, fat-free lean gain; LMP, lean meat percentage; BT, backfat thickness; LMA, *longissimus* muscle area; SEM, standard error of the mean.

objective optimal level, it was 0.677%. At this concentration, the best FGR is obtained with a lower ADFI, without affecting ADG. According to the NRC (1998, 2012), the requirements for digestible Met+Cys for growing pigs (20–50 kg) are estimated to be 0.47 and 0.55%, respectively, whereas the Brazilian tables (Rostagno *et al.*, 2017) have established their requirements based on productive performance (standard and high production) at 0.566 and 0.631%, respectively.

The optimal Met+Cys requirement in our study was higher than that recommended by the NRC (1998, 2012), and very close to the one determined by Rostagno *et al.* (2017). A higher level could lead to extra benefits in the productive performance of the animal. Oral supplementation of Met at 0.02, 0.04, and 0.06% in growing pigs (20 kg), improves daily gain, feed intake, and feed: gain ratio, regardless of whether or not there was a deficiency

(Opapeju *et al.*, 2012). Other studies evaluating various dietary Met levels have shown better feed conversion and daily protein deposition in pigs as Met concentrations increase (de Oliveira *et al.*, 2015). It is also reported that an extra 0.12% of Met supplementation increased ADG and improved feed efficiency during post-weaning (Chen *et al.*, 2014). All these benefits are probably due to the functional role of dietary Met for intestinal growth and function, in addition to its role as a precursor for protein synthesis (Wu *et al.*, 2013).

Our study confirms that reduction of Met+Cys in growing pig diets affects productive variables, as protein synthesis and accretion are affected by insufficient Met levels. Conde-Aguilera *et al.* (2010) found that pigs fed a Met deficient diet had lower body weight gain than pigs fed a control diet. Similarly, Kiefer *et al.* (2005) observed that productive performance and carcass characteristics had a

quadratic effect in response to the Met+Cys levels (0.44–0.61%) in the diets for growing pigs, with the best level between 0.527 and 0.551%. Met and Cys deficiency have been observed to significantly suppress intestinal mucosal growth, reduced intestinal epithelial cell proliferation, and increased intestinal oxidative stress in piglets (Bauchart-Thevret *et al.*, 2009; Chen *et al.*, 2014). Methionine deficiency alters lipid metabolism, decreases oxidative damage, alters antioxidant pathways, and alleviates inflammatory responses in growing pigs (Ying *et al.*, 2015; Zhou *et al.*, 2016). Lower Met+Cys levels in pig diets, compared with NRC (1998) requirements, increase lipid content in subcutaneous adipose tissue and *Longissimus dorsi* muscle (Conde-Aguilera *et al.*, 2014; Castellano *et al.*, 2015). Pigs fed a Met deficient diet have a higher lipid composition than pigs fed a basal diet (Batterham *et al.*, 1993; Martinez-Ramirez *et al.*, 2008; Conde-Aguilera *et al.*, 2014). In addition, pigs fed a Met-restricted diet had increased triglyceride content in subcutaneous adipose tissue with unchanged plasma triglyceride contents (Castellano *et al.*, 2015).

Theoretically, a Met deficiency could affect the amounts of Cys and glutathione, consequently increasing oxidative stress, causing damage to the synthesis of important cellular components (fat, protein, DNA; Metayer *et al.*, 2008). Oxidative stress may result in poor performance, disturbed health, and even death (Zheng *et al.*, 2013). When pigs show a stressed-activated immune system, Met requirements are higher since Cys synthesis from Met tends to increase (Li *et al.*, 2014; de Oliveira *et al.*, 2015). A recent study (Conde-Aguilera *et al.*, 2016) suggests that insufficient intake of sulfur AA during early growth may reduce glutathione content in the muscle of growing pigs. Moreover, Met supplementation (0.12%) is required for optimal protein synthesis, so, it could increase cysteine and glutathione concentrations in the plasma and tissues, leading to reductions in plasma and tissue redox potential and the maintenance of mucosal integrity in the small intestine of weaned piglets (Chen *et al.*, 2014).

Some studies have shown that excess of Met+Cys can affect growth performance, carcass weight, amount of meat, backfat thickness and daily meat deposition in finishing pigs (Santos *et al.*, 2007; Pena *et al.*, 2008). Recent studies reported that dietary Met restriction in pig diets improved or did not influence growth performance and carcass characteristics (Santos *et al.*, 2011; Ying *et al.*, 2015). In contrast, Caldara *et al.* (2003) did not find differences in productive variables when using 0.63 to 0.74% Met+Cys. In addition, Castellano *et al.* (2015) observed that a dietary Met deficiency resulted in increased lipid content in subcutaneous adipose tissue and *Longissimus dorsi* muscle, which was associated with an increase in enzyme activity and expression levels of critical genes involved in de novo lipogenesis in these tissues. The activity of antioxidant enzymes increased in adipose and muscle tissues and altered the cellular redox status of young pigs fed a Met-deficient diet. Total antioxidant capacity in the plasma and glutathione levels in the liver were lower in pigs fed Met-deficient diets. These authors (Castellano *et al.*, 2015) concluded that tissue metabolism adapts differently when facing an insufficient supply of dietary Met.

In conclusion, the optimal level of Met+Cys is 0.677% in growing pig diets to obtain the best feed:gain ratio, average daily gain, and daily feed intake. Our results indicate that below 0.667% Met+Cys daily feed intake increases to compensate for the lower intake of Met+Cys.

## Declarations

### *Funding*

Economic support to conduct this research was obtained from "Colegio de Postgraduados".

### *Conflicts of interest*

The authors declare they have no conflicts of interest with regard to the work presented in this report.

*Author contribution*

José L. Figueroa-Velasco participated in the experimental design, supervision of experimental protocol and wrote the manuscript. José A. Martínez-Aispuro did the experimental work, laboratory analysis, statistical analysis of data and wrote the manuscript. María T. Sánchez-Torres-Esqueda designed and supervised the research and revised final manuscript. Humberto Vaquera-Huerta revised the research proposal, statistical analysis and final manuscript. Jose L. Cordero-Mora collaborated with the proposal set, experimental work, supervised health status of pigs, obtained samples and data from pigs and revised the final version of the manuscript. Rafael Nieto-Aquino collaborated with data collection, revised the research proposal and the final manuscript.

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