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Standardized ileal digestible (SID) isoleucine requirement of barrows (15- to 30- kg) fed low crude protein diets[1]

Exigência de isoleucina digestível ileal estandardizada para suínos machos castrados (15-30 kg) alimentados com dietas de baixa proteína bruta

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Abstract

This study aimed to determine the SID isoleucine (Ile) requirement of starting barrows fed low crude protein. Two experiments were carried out. Experiment 1: Ten crossbred barrows were used in order to determine the SID AA of the basal diet (treatment with the lowest SID Ile level used in the growth performance experiment), averaging 15.00 ± 0.27 kg of initial weight, individually housed in metabolic cages and allotted in a complete randomized design, with two treatments, five replicates and one animal per experimental unit. Treatments consisted of a basal (14.13% CP and 0.450% of SID Ile) and a free protein diet. Experiment 2: A performance experiment was carried out to determine the SID Ile requirement when using low crude protein diets. Forty crossbred barrows were used, averaging 15.00 ± 0.87 kg of initial weight and distributed in a randomized block design with five treatments (0.450, 0.520, 0.590, 0.660 and 0.730% of SID Ile) and two animals per experimental unit. The average daily gain (ADG) (P=0.049) and protein deposition (P=0.01) were affected by the studied SID Ile levels. The daily need of SID Ile was estimated at 5.9 g when considering 0.61% as the optimum level of SID Ile in the diet for an improved ADG and protein deposition.

Key words: Branched chain amino acids. Protein deposition. Standardized ileal digestibility.

Resumo

Este estudo teve como objetivo determinar a exigência de isoleucina digestível (Ile Dig) para leitões machos castrados, em fase inicial, alimentados com dietas de baixa proteína bruta. Foram realizados dois experimentos. Experimento 1: Dez leitões mestiços foram utilizados, a fim de determinar os aminoácidos digestíveis da dieta basal (tratamento com o nível mais baixo Ile Dig utilizado no experimento de desempenho), sendo a média de peso inicial dos suínos de 15,00 ± 0,27 kg, alojados individualmente em gaiolas metabólicas e distribuídos em delineamento inteiramente casualizado, com dois tratamentos, cinco repetições e um animal por unidade experimental.Os tratamentos consistiram de uma dieta basal (14,13% PB e 0,450% de Ile Dig) e uma dieta isenta de proteína. Experimento 2: Foi realizado um experimento de desempenho, para determinar a exigência de Ile Dig em dietas de baixa proteína bruta. Foram utilizados 40 suínos mestiços, machos castrados, com média de 15,00 ± 0,87 kg

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de peso inicial, os quais foram distribuídos em delineamento de blocos ao acaso, com cinco tratamentos (0,450; 0,520; 0,590; 0,660 e 0,730% de Ile Dig) e dois animais por unidade experimental. O ganho de peso diário (P = 0,049) e a deposição proteica (P = 0,010) foram afetados pelos níveis de Ile Dig estudados. As necessidades diárias de Ile Dig foram estimadas em 5,9 g quando considerado 0,61% como o nível ótimo de Ile Dig na dieta para um melhor ganho de peso diário e deposição proteica.
Palavras-chave: Aminoácidos de cadeia ramificada. Deposição de proteína. Digestibilidade ileal estandarizada.

Introduction

With the availability of different industrial amino acids (AA), it is theoretically possible to formulate low-protein diets for growing pigs in which seven AA are co-limiting for performance (VAN MILGEN et al., 2012). It is a way to reduce the crude protein (CP) to some extent without reducing growth performance, due to a balanced AA profile in the diet (LORDELO et al., 2008; NØRGAARD; FERNÁNDEZ, 2009).

In the same way, Mavromichalis et al. (1998, 2001) suggested that valine is one of the five limiting AA for 10 kg pigs when using a low CP diet based on corn and soybean meal, but not isoleucine (Ile). However, Liu et al. (2000 a,b) reported that Ile may be a limiting AA in low CP diets based on corn and soybean meal for high protein deposition pigs.

Spray dried blood cells can be used in experimental diets to determine the Ile requirement to ensure a high CP level, but can overestimate its requirement due to the imbalanced supply of branched chain AA (DEAN et al., 2005), including leucine, the of concentration which is very high in spray dried blood cells (NØRGAARD et al., 2013), justifying studies using low CP diets without blood products as a protein source, in order to determine the Ile requirements for pigs.

Another question is that studies performed 20 to 50 years ago showed some difficulties in the interpretation of results; the inability to determine Ile digestibility in the basal diet was indicated as one of these problems (PARR et al., 2003). The standardized ileal digestible (SID) AA of the ingredients used in experimental diets are found in feed composition tables; nevertheless, these values do not accurately represent the content of these AA in the feedstuffs used in experiments, since variability in the chemical composition of the ingredients affects the AA composition of these feedstuffs.

Additionally, reducing the CP content of experimental diets, in order to determine the requirement of the sixth limiting AA for pigs (i.e. isoleucine), has led to low levels of the other five limiting amino acids (below the requirements) when the SID AA are not accurately known, thus impairing the precise determination of the requirements. Therefore, adequate knowledge of SID Ile of the basal diet is fundamentally important to accurately estimate its requirements for pigs, similar to knowledge of the other limiting amino acids. In this way, using a low-protein diet and determining the SID Ile of the basal diet are important approaches to determining the Ile requirements for pigs.

This study aimed to determine the SID isoleucine requirement of starting barrows (15- to 30- kg) fed low crude protein diets.

Material and Methods

The experiment was conducted at the Swine Experimental Station, State University of Paraná West – UNIOESTE. All experimental procedures were previously submitted to the Animal Care and Use Committee of UNIOESTE, and were approved for execution (Protocol number 204/2007).

Experiment 1 – Standardized ileal digestibility of the amino acids of the basal diet

This experiment was carried out in order to determine the SID AA of the basal diet, corresponding to the treatment with the lowest SID

Ile level used in the growth performance experiment (Experiment 2).

Ten crossbred barrows (Landrace x Large White x Piétrain) averaging 15.00 ± 0.27 kg of initial body weight were distributed in a complete randomized design with two treatments and five replicates. Treatments consisted of a basal diet (0.450% SID Ile) and a free protein diet (FPD).

The basal diet (Table 1) was based on corn and soybean meal to achieve the nutritional requirements proposed by Rostagno et al. (2005), except for crude protein and SID Ile. The free protein diet was used to determine the endogenous losses of amino acids. The experimental diets contained 0.5% chromic oxide (Cr_2O_3) as an external marker to determine the digestibility and rice hull was used as the fiber source in the FPD.

Table 1. Composition of the basal and free protein diets, as-fed basis.

Ingredients	Basal diet (%)	Free protein diet (%)
Corn	78.50	-
Soybean meal	14.50	-
Oil	0.81	4.00
Starch	-	22.88
Rice hulls	-	18.88
Sugar	-	50.00
Dicalcium phosphate	1.680	2.210
Limestone	0.651	0.460
Sodium bicarbonate	0.619	0.690
Antioxidant[A]	0.010	0.010
Sodium Chloride	0.045	0.100
Vitamin premix[B]	0.100	0.100
Trace mineral premix[C]	0.050	0.050
L – Lysine HCl	0.611	-
DL – Methionine	0.141	-
L – Threonine	0.211	-
L – Tryptophan	0.045	-
L – Valine	0.153	-
L – Isoleucine	-	-
L – Glutamic acid	0.874	-
Chromic oxide	0.500	0.500
Inert[D]	0.500	0.120
Composition		
ME (Mcal/kg)	3.23	3.01
Crude Protein (%)	14.12	-
NDF (%)	11.21	11.21
ADF (%)	9.20	10.15
Calcium (%)	0.720	0.733
Available Phosphorus (%)	0.400	0.409
Sodium (%)	0.204	0.226
Potassium (%)	0.487	-
Chlorine (%)	0.191	0.060
SID Lysine (%)	0.991	-

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SID Met+Cys (%)	0.555	-
SID Methionine (%)	0.336	-
SID Threonine (%)	0.624	-
SID Tryptophan (%)	0.168	-
SID Arginine (%)	0.718	-
SID Valine (%)	0.684	
SID Leucine (%)	1.173	-
SID Isoleucine (%)	0.450	

[A] BHT. [B] Content/kg: vit. A, 10,000,000 I.U.; vit. D3, 1,500,000 I.U.; vit B1, 2 g; vit B2, 5.0 g; vit. B6, 3.0 g; vit B12, 30,000 mcg; nicotinic acid, 30,000 mcg; pantothenic acid, 12,000 mcg; vit K3, 2,000 mg; folic acid, 800 mg; biotin, 100 mg; and QSAD vehicle (1,000 g). [C] Content/kg: iron, 100 g; copper, 10 g; cobalt, 1 g; manganese, 40 g; zinc, 100 g; iodine, 1.5 g; and QSAD vehicle (1,000 g). [D] Fine and cleaned sand.

The daily provided feed during the experimental period was estimated according on the metabolic body weight ($BW^{0.75}$). The animals were fed at 7:00 and 19:00.

Barrows were kept in metabolic cages and submitted to a five-day adaptation period. On the sixth day, digesta was collected in the terminal ileum, three hours after ingestion of the experimental diets. The animals were held for intramuscular application of 1.5 mL of a tranquilizer with a xylazine chloride base, associated with 1 mL of atropine sulfate. Five minutes after this procedure, 3 mL of general anesthetic (ketamine) was applied via intramuscular injection. After anesthesia and having already performed hair removal and asepsis of the surgical area of the abdominal cavity, laparotomy was carried out through a ventral incision in the cephalocaudal direction.

An ileum segment was exteriorized and the digesta passage was obstructed with a hemostatic forceps between the ileocecal valve and the end of the ileocecal fold. A 20 cm segment was removed, washed with distilled water and dried with towel paper for digesta occlusion.

Dry matter and chromium contents of the digesta, basal diet and FPD were determined according to the techniques described by the AOAC (2005). The amino acid composition of the basal diet and digesta were determined by high performance liquid chromatography at Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda.

Experiment 2 – Performance experiment

This experiment was carried out to determine the SID Ile requirement of starting barrows receiving low crude protein diets and considering the SID AA determined in Experiment 1. Forty crossbreed barrows of high performance (Landrace x Large White x Piétrain) averaging 15.00 ± 0.87 kg of initial weight and 30.46 ± 1.24 kg of final weight were distributed in a randomized block design with five treatments (0.450, 0.520 0.590, 0.660 and 0.730% of SID Ile) and two animals per experimental unit. The animals were kept in suspended metallic pens, with polypropylene flooring and screened sides, provided with semi-automatic feeders and nipple drinkers.

The SID of the amino acids obtained in the digestibility assay (Experiment 1) were used to correct the SID Ile content and also other essential amino acids of the experimental diets, in which synthetic amino acids replaced the inert material to, at least, meet the requirements proposed by Rostagno et al. (2005), except for CP (reduced from 19.24 to 14.13%) and SID Ile levels. L-isoleucine replaced the inert material of the basal diet in order to meet the studied SID Ile levels. Glutamic acid was used to keep diets with the same nitrogen amount for all experimental diets (Table 2).

Table 2. Composition of experimental diets fed to barrows from 15- to 30- Kg (as-fed basis).

Ingredient (%)	SID Isoleucine (%)				
	0.450	0.520	0.590	0.660	0.730
Corn	78.50	78.50	78.50	78.50	78.50
Soybean meal	14.50	14.50	14.50	14.50	14.50
Oil	0.810	0.810	0.810	0.810	0.810
Dicalcium phosphate	1.680	1.680	1.680	1.680	1.680
Limestone	0.651	0.651	0.651	0.651	0.651
Sodium bicarbonate	0.619	0.619	0.619	0.619	0.619
Antioxidant[A]	0.010	0.010	0.010	0.010	0.010
Sodium Chloride	0.045	0.045	0.045	0.045	0.045
Vitamin premix[B]	0.100	0.100	0.100	0.100	0.100
Trace mineral premix[C]	0.050	0.050	0.050	0.050	0.050
L – Lysine HCl	0.611	0.611	0.611	0.611	0.611
DL – Methionine	0.205	0.205	0.205	0.205	0.205
L – Theonine	0.211	0.211	0.211	0.211	0.211
L – Tryptophan	0.045	0.045	0.045	0.045	0.045
L- Valine	0.161	0.161	0.161	0.161	0.161
L – Isoleucine	0.012	0.084	0.156	0.228	0.300
L – Glutamic acid	0.874	0.782	0.697	0.611	0.525
Inert[D]	0.622	0.642	0.655	0.669	0.683
	Composition				
ME (Mcal/kg)	3.23	3.23	3.23	3.23	3.23
Crude Protein (%)	14.13	14.13	14.13	14.13	14.13
Calcium (%)	0.720	0.720	0.720	0.720	0.720
Available Phosphorus (%)	0.400	0.400	0.400	0.400	0.400
Sodium (%)	0.204	0.204	0.204	0.204	0.204
Potassium (%)	0.487	0.487	0.487	0.487	0.487
Chlorine (%)	0.191	0.191	0.191	0.191	0.191
SID Lysine (%)	1.057	1.057	1.057	1.057	1.057
SID Met+Cys (%)	0.555	0.555	0.555	0.555	0.555
SID Methionine (%)	0.429	0.429	0.429	0.429	0.429
SID Threonine (%)	0.646	0.646	0.646	0.646	0.646
SID Tryptophan (%)	0.168	0.168	0.168	0.168	0.168
SID Arginine (%)	0.534	0.534	0.534	0.534	0.534
SID Valine (%)	0.684	0.684	0.684	0.684	0.684
SID Leucine (%)	1.042	1.042	1.042	1.042	1.042
SID Isoleucine (%)	0.450	0.520	0.590	0.660	0.730
DEB[E] (mEq/kg)	159	159	159	159	159

[A] BHT. [B] Content/kg: vit. A, 10,000,000 I.U.; vit. D3, 1,500,000 I.U.; vit B1, 2 g; vit B2, 5.0 g; vit. B6, 3.0 g; vit B12, 30,000 mcg; nicotinic acid, 30,000 mcg; pantothenic acid, 12,000 mcg; vit K3, 2,000 mg; folic acid, 800 mg; biotin, 100 mg; and QSAD vehicle (1,000 g). [C] Content/kg: iron, 100 g; copper, 10 g; cobalt, 1 g; manganese, 40 g; zinc, 100 g; iodine, 1.5 g; and QSAD vehicle (1,000 g). [D] Fine clean sand. [E] Dietary electrolyte balance.

One animal per experimental unit was slaughtered at the end of the experiment, after 24 hours of fasting. The carcasses were weighed and cooled for 24 hours. Afterwards, the carcasses were processed to determine the moisture, CP and fat content, according to techniques described by the AOAC (2005).

At the beginning of the experiment, an additional group of four animals, averaging 15.00 ± 1.52 kg of body weight were slaughtered to determine the carcass composition. The protein and fat deposition were determined by using the compositions of the animal carcasses at the beginning and end of the experiment. Furthermore, protein and fat deposition were determined, indicative of the energy retained in the carcass, in addition to assessing the relative weights of the liver, kidneys and pancreas.

The UNIVARIATE procedure was applied to test for the presence of outliers. Data on performance, tissue deposition and relative weight of organs were submitted to variance analysis (ANOVA). The degrees of freedom relating to SID Ile levels were deployed in orthogonal polynomials to obtain the regression equations. Linear and quadratic models were used to demonstrate the effect of SID Ile on the studied parameters and the significance was declared at $P < 0.05$. All statistical tests were performed using the SAS(2002) procedures (SAS Institute Inc. 9.2, Cary, North Carolina, USA).

Results and Discussion

The endogenous losses of the essential AA (Table 3) did not show the same profile compared to those obtained by Pozza et al. (2003), who observed lower methionine loss and higher loss of valine (0.065 and 0.400 mg/g of FPD, respectively). The endogenous loss of isoleucine observed by Pozza et al. (2003) and Costa et al. (2008) were lower (0.265 and 0.268 mg/g of FPD intake, respectively) than the obtained in this study (0.041 mg/g FPD intake).

Table 3. Ileal endogenous amino acids (IEAA) determined using a free protein diet (FPD), apparent ileal digestibility coefficient (AIDC), standardized ileal digestibility coefficient (SIDC), standardized ileal digestible amino acids (SIDAA) and calculated ileal digestible amino acids (CIDAA) of the basal diet.

Essential amino acids	IEAA (mg/g FPD intake)	AIDC (%)	SIDC (%)	SIDAA (%)	CIDAA (%)[A]
Arginine	0.049	84.90	92.55	0.534	0.718
Histidine	0.022	84.25	89.86	0.327	0.335
Isoleucine	0.041	84.82	92.67	0.438	0.450
Leucine	0.070	84.50	89.90	1.042	1.173
Lysine	0.036	89.89	92.73	1.057	0.991
Methionine	0.008	92.21	94.45	0.315	0.336
Meth.+ Cys.	0.046	86.78	94.80	0.492	0.555
Phenylalanine	0.045	84.51	91.11	0.553	0.590
Threonine	0.101	85.62	99.64	0.646	0.624
Valine	0.071	85.82	94.79	0.676	0.684

[A] Values obtained using data proposed by Rostagno et al. (2005).

As previously reported, the endogenous losses (Table 3) differ from the aforementioned studies, mainly related to differences in the concentrations of the various endogenous secretions of the digestive tract (FAN et al., 1995).

The SIDC obtained for isoleucine (Table 3) is higher than those observed by Pozza et al. (2003) and Costa et al. (2008), corresponding to 88.46 and 85.98%, respectively; however, Parr et al. (2003) using pigs submitted to simple "T" cannula surgery reported a coefficient of 97.00%. This variation may be due to some differences observed in feed intake, diets composition and environmental temperatures as factors affecting amino acid digestibility (COSTA et al., 2008).

The standardized ileal digestibility of arginine, histidine, isoleucine, leucine, methionine, met + cys, phenylalanine and valine were below the standardized ileal digestibility values calculated upon using the SID AA values of the ingredients proposed by Rostagno et al. (2005). Nevertheless, methionine + cysteine and valine were the amino acids that did not met the requirements proposed by the authors. Thus, the supplementation of DL-methionine and L-valine were necessary in the experimental diets for carrying out the performance experiment (Experiment 2), because the intake of a diet containing amino acids below metabolic needs may lead to physiological alterations, with metabolic changes affecting feed intake (LE BELLEGO;NOBLET, 2002).

The antagonism of branched-chain amino acids (BCAAs) may cause an increase and/or decrease on the activity of specific enzymes of AA metabolism (LIMA; SILVA, 2007). A consequence of AA antagonism belonging to the same structural group (i.e. one amino acid exceeding another) is decreased feed intake (D'MELLO, 2003). Nørgaard et al. (2013) observed a decreased average daily feed intake (ADFI) when SID Ile was fed in excess to starting pigs, compared to its requirement, explaining that the branched-chain keto acid dehydrogenase (BCKDH) activity may be stimulated when Ile is fed in a higher concentration than that required for pigs (8 to 18 kg) and submitted to low CP diets. However, a decreased ADFI was not observed (P>0.05) in the present study when SID Ile increased in the diet (Table 4), indicating that the studied levels of SID Ile did not affect the ADFI when using low CP diets. A BCAA imbalance may result in negative responses on ADFI, but most of it is associated with Leu excess in the diet, as shown for Val deficiency in diets containing Leu excess (GLAGUEN et al., 2012). More recently, Wessels et al. (2016) corroborated the aforementioned finding, reporting that high levels of Leu in the liver can stimulate the BCKDH complex that catalyzes the irreversible degradation of all BCAAs, including valine (Val) and Ile. The result for ADFI (P>0.05) agrees with this explanation, since no effects were observed for increased SID Ile and because the SID Leu level was low in the experimental diets, due to the reduced CP content and not using spray dried blood cells.

Table 4. Growth performance and nitrogen utilization of starting barrows (15- to 30- kg) fed low crude protein diets containing different levels of SID isoleucine.

Item[A]	SID isoleucine levels (%)					Pooled SEM	*P* value	
	0.450	0.520	0.590	0.660	0.730		Linear	Quadratic
IBW (kg)	14.92	15.02	14.95	14.96	15.11	0.21	-	-
FBW (kg)	29.84	31.94	31.76	30.52	31.24	0.51	0.216	0.410
ADFI (g/day)	982	1008	980	967	951	54.22	0.377	0.623
ADG (g/day)	507	578	578	563	554	28.28	0.271	0.049[a]
F:G (g/g)	1.96	1.75	1.69	1.72	1.72	0.13	0.065	0.113
NI (g/day)	22.19	22.77	22.13	21.84	21.49	1.22	0.377	0.623
NEWG (g/g)	22.85	25.44	26.22	25.99	25.77	1.78	0.091	0.132
ADIleI (g/day)	4.42	5.24	5.78	6.38	6.94	0.84	0.001[b]	0.578

[A] IBW: initial body weight; FBW: final body weight; ADFI: average daily feed intake; ADG: average daily gain; F:G: feed:gain ratio; NI: nitrogen intake; NEWG: nitrogen efficiency for weight gain; ADIleI: average daily SID isoleucine intake; [a] $Y = -371.421 + 3116.15X - 2546.17X^2$ (R^2=0.82); [b] $Y = -9.7912 + 10.1677X$ (R^2=0.99).

Nitrogen intake (NI) was not affected (P>0.05) by SID Ile levels (Table 4) due to a similar ADFI (P>0.05) associated to the same nitrogen content of the experimental diets (Table 2), achieved by using glutamic acid. However, the average daily SID Ile intake (ADIleI) was expected to increase linearly (P= 0.001) since the ADFI was not affected, making the ADIleI as high as the SID Ile levels increased in the diets (Table 4).

The efficiency of nitrogen utilization will be highest when the amino acid supply approaches the requirement of the animal (VAN MILGEN et al., 2012). However, nitrogen efficiency for weight gain (NEWG) only showed a tendency toward a linear increase (P = 0.091) as the SID Ile was supplied in the diets (Table 4).

The average daily gain (ADG) was affected by SID Ile levels (Table 2), fitting the equation $Y = -371.421 + 3116.15X - 2546.17X^2$ (R^2=0.82) and estimating the maximum ADG at 0.61% SID Ile, that is close to 0.63% suggested by NRC (2012) for 11 to 25kg pigs.

The response obtained for ADG may be associated with the results obtained for protein deposition (Table 5) SINCE it was affected by the SID Ile levels (P = 0.001), providing the adjustment of a quadratic model expressed by the equation $Y = -198.023 + 996.63 - 820.27X^2$ (R^2=0.82). The estimated value of THE SID Ile level for high protein deposition was the same as that estimated for ADG (0.61%). Conversely, Szabo et al. (2001) evaluated SID Ile levels for barrows from 30 to 105 kg and observed the best protein deposition at 0.95% SID Ile, i.e. higher than the level obtained in our study. However, differences in live weight must be considered.

The optimum SID Ile level obtained for ADG and protein deposition (0.61%) was a SID Ile:Lys ratio of 0.57. Nørgaard et al. (2013) also obtained a quadratic effect for ADG according to the increasing SID Ile:Lys in diets for 8 to 18 kg pigs, estimating the best ratio at 0.52 and the NRC (2012) suggested 0.55 as the optimum SID Ile:Lys for 11 to 25 kg pigs.

Table 5. Carcass deposition and relative organs weight of starting barrows (15- to 30- Kg) fed low crude protein diets containing different levels of SID isoleucine.

Item	SID isoleucine levels (%)					Pooled SEM	*P* value	
	0.450	0.520	0.590	0.660	0.730		Linear	Quadratic
Protein deposition (g/day)	70.15	83.06	90.32	77.66	72.39	7.83	0.926	0.001[a]
Fat deposition (g/day)	86.53	84.38	91.16	91.38	89.70	8.96	0.443	0.389
Retained energy (Kcal)	1208	1261	1365	1296	1250	101.09	0.491	0.087
Liver (%)	3.76	3.77	3.61	4.29	3.71	0.43	0.657	0.767
Kidneys (%)	0.57	0.53	0.51	0.57	0.56	0.07	0.809	0.399
Pancreas (%)	0.34	0.35	0.31	0.30	0.34	0.04	0.523	0.273

[a] $Y = -198.023 + 996.63 - 820.27X^2$ (R^2=0.82).

In a previous study, no effects of SID Ile levels were observed on protein and fat deposition, nor for relative organ weight of starting gilts fed low crude protein diets (CASTILHA et al., 2012). These authors concluded that levels from 0.45 to 0.73% of SID Ile did not affect the carcass traits and relative organ weight of gilts from 15 to 30 kg. Nevertheless, the level of 0.506% SID Ile provided the best efficiency of Ile for weight gain, providing a SID isoleucine:lysine ratio of 0.51.

Many reasons are mentioned as possible causes of the differences in SID AA requirement estimates, like fitted models. In this study, a quadratic model was used to estimate the best SID Ile level. It is well-known that a drawback of this model is the parabolic design, but Nørgaard et al. (2013) evaluated alternative models and reported that the assumptions of the linear broken-line and curvilinear-plateau models of a plateau in animal performance after the breakpoint did not fit the data very well and forced the breakpoint to very low estimates of requirement. Additionally, a lack of response has also been observed in many studies performed to determine the Ile requirements for pigs. Evaluating SID Ile levels (0.60 to 0.88%) for barrows from 12 to 22 kg, Fu et al. (2006) did not observe any changes in growth performance, concluding that 0.60% met the requirements. In the same way, Barea et al. (2009), upon evaluating SID Ile levels (0.50 to 0.58%) for barrows and gilts from 11 to 23 kg, observed no effects on performance, reporting that the optimum SID Ile was the lowest evaluated level (0.50%). In the same way, Van Milgen et al. (2012), using a meta-analysis procedure, reported that 13 dose-response experiments (a total of 46) demonstrated no evidence ($P>0.25$) for, at least, a linear response in ADFI or ADG as a function of an increasing Ile concentration in the diet, suggesting that different factors may have contributed to the lack of response, and explaining that other AA besides Ile and Lys may have limited the growth performance in seven experiments, although the extent of this limitation was modest.

In our study, the limiting AA of the basal diet were corrected by adding crystalline AA to the experimental diets (Tables 1 and 2), except for the SID Ile concentration, because the determination of the SID AA of the basal diet was carried out in Experiment 1, corroborating the SID Ile requirement estimate (Experiment 2), i.e. the only limiting AA was Ile, and significant responses were observed when supplying industrial Ile in the diet.

The daily requirement of SID Ile was estimated at 5.9 g when considering 0.61% as the optimum level of SID Ile in the diet for improved ADG and protein deposition. This level is close to the 6.0 g/day proposed by the NRC (2012) for barrows and gilts from 11 to 25 kg.

Conclusion

Barrows from 15 to 30 kg fed diets with a low crude protein content require 0.61% SID isoleucine in the diet to maximize the average daily gain and protein deposition, corresponding to a daily requirement of 5.9 g/day.

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