

# **Improving the estimation of amino acid requirements to maximize nitrogen retention in precision feeding for growing-finishing pigs**

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## **SUPPLEMENTARY MATERIAL S1**

### ***Digestibility trial detailed information***

Before the performance trial, the experimental feeds were evaluated for their standardized ileal digestible (SID) amino acids (AA) digestibility coefficients (S1). Ten barrows of approximately  $32.3 \pm 3.73$  kg body weight (BW) were surgically fitted with silicone rubber postvalvular T-cecum cannulas at the ileocecal junction, as described by van Leeuwen *et al.* (1991). The barrows were then individually penned and offered a commercial 19% crude protein (CP) starter diet. After a recovery period of at least 10 d, the pigs received the corresponding 100% experimental feed until the beginning of the first digestibility trial. The feeds were evaluated for standardized ileal digestibilities of Lys, methionine (Met), threonine (Thr), histidine (His), isoleucine (Ile), leucine (Leu), cysteine (Cys), phenylalanine (Phe), tyrosine (Tyr), and valine (Val) in two separate trials. Feeds A1 and A2 were evaluated in the first digestibility trial (five pigs per feed; initial BW of  $48.8 \pm 3.18$  kg), and feeds B1 and B2 were evaluated in the second digestibility trial (five pigs per feed; initial BW of  $75.5 \pm 5.80$  kg BW). A 7-d interval was allowed between the two digestibility trials.

Each digestibility trial lasted 18 d. The pigs were housed individually in large pens in a temperature-controlled room (20°C) for a 5-d feed adaptation period during which feed was offered *ad libitum* and refusals were collected daily before feeding. From d 6 until the end of the trial, the pigs were housed in metabolic crates and were offered 90% of the previously measured *ad libitum* intake in two

equal meals at 0800 h and 1600 h. Total collection of feces and urine was carried out from d 8 until d 11. On d 12, chromic oxide was added at 0.5% of the feed. Ileal chyme was collected from d 15 to d 18 for 8 h following the morning meal. The collection was performed in sterile polyethylene bags attached to the external part of the cannula. The bags were removed when full or once every 30 min and were then frozen immediately. The collected chyme was thawed, pooled on an individual-animal basis, and mixed. At the end of the collection periods, feces, urine, and chyme were pooled separately to produce one sample of each material for each pig. At the end of the experiment, the pigs were slaughtered for *post mortem* examination. Apparent ileal AA (AID) digestibility was calculated as follows (Fan and Sauer, 1995):

$$AID = \left\{ 100 - \left[ \left( \frac{AA_d}{AA_{feed}} \right) \times \left( \frac{Cr_f}{Cr_d} \right) \right] \right\} \times 100$$

where *AID* is the apparent ileal AA digestibility coefficient for each AA (%), *AA<sub>d</sub>* is the AA content in the ileal digesta (mg/kg DM), *AA<sub>feed</sub>* is the AA content in the feed (mg/kg DM), *Cr<sub>f</sub>* is the chromium in the feed DM, and *Cr<sub>d</sub>* is the chromium in the feces DM. Values for the basal endogenous losses of AA (ileal endogenous losses of AA; *IELA<sub>AA</sub>*) used in this experiment were the average value of 16 trials using N-free diets, as described by Cervantes-Pahm *et al.* (2014). The SID of each AA was calculated as follows (Stein *et al.*, 2007):

$$SID = AID + \left( \frac{IELA_{AA}}{AA_{feed}} \right) \times 100\% .$$

### ***Analytical procedures: digestibility and performance trials***

In the digestibility trial, the chyme samples were freeze-dried and ground to pass through a 1-mm screen before analysis. Dry matter was determined by freeze-drying and corrected by oven-drying to constant weight at 100°C. Chromic oxide concentrations were determined using the method of Fenton and Fenton (1979).

## **RESULTS**

### ***Digestibility trial***

The barrows remained healthy and consumed their meal allowances throughout the trial. *Post mortem* examinations at the end of the trial revealed no intestinal adhesions or other abnormalities. The average daily feed intake was  $2.2 \pm 0.35$  and  $2.6 \pm 0.48$  kg/d during the first and second collection periods, respectively. During the first collection period, average daily gain was  $0.8 \pm 0.10$  kg/d, and it was  $0.6 \pm 0.25$  kg/d during the second period. The fecal digestibilities of CP for feeds A1, A2, B1, and B2 were  $85.4\% \pm 1.6\%$ ,  $85.3\% \pm 3.8\%$ ,  $82.5\% \pm 3.6\%$ , and  $82.5\% \pm 1.9\%$ , respectively. The standardized ileal digestibilities of CP for feeds A1, A2, B1, and B2 were  $97.7\% \pm 0.2\%$ ,  $97.4\% \pm 0.30\%$ ,  $97\% \pm 0.12\%$ , and  $96.8\% \pm 0.4\%$ , respectively. Amino acid digestibility values were close to the expected values. Still, SID Lys digestibilities for feeds A1, A2, B1, and B2 were 6%, -3%, 7%, and -8%, respectively, different from the values calculated from the tables. Given that feeds A1, A2, B1, and B2 had, respectively, analyzed SID Lys concentrations that were 2%, 7%, 12%, and 12% higher than the calculated

concentrations, the amounts of SID Lys served to the pigs were also slightly different from the calculated amounts. Thus, the calculated provisions of 60%, 70%, 80%, 90%, 100%, and 110% of the estimated SID Lys requirements were in fact, respectively, 82%, 84%, 89%, 93%, 95%, and 107% during the first growing period and 72%, 76%, 82%, 89%, 94%, and 100% during the second growth period. Actual values were used in the calculations.

**Supplementary material Table S1** *Standardized ileal digestibility coefficients (%) of amino acids for growing-finishing pigs in the four experimental feeds (A1, A1, B1 and B2) (Means  $\pm$  SD)<sup>1,2</sup>*

Amino acid <sup>3</sup>	Feeds			
	A1	A2	B1	B2
Lys	93.3 $\pm$ 1.8	87.5 $\pm$ 2.0	91.9 $\pm$ 1.7	87.9 $\pm$ 4.3
Met	91.6 $\pm$ 1.1	90.4 $\pm$ 1.3	85.6 $\pm$ 2.2	87.1 $\pm$ 2.3
Thr	87.0 $\pm$ 3.0	84.2 $\pm$ 3.0	84.6 $\pm$ 2.7	83.7 $\pm$ 5.3
His	86.7 $\pm$ 2.0	86.8 $\pm$ 2.4	87.4 $\pm$ 1.6	87.9 $\pm$ 4.1
Ile	90.5 $\pm$ 3.6	86.7 $\pm$ 3.3	91.0 $\pm$ 5.0	93.9 $\pm$ 4.2
Leu	89.7 $\pm$ 2.6	87.9 $\pm$ 2.0	90.8 $\pm$ 2.1	91.5 $\pm$ 3.0
Cys	81.9 $\pm$ 3.3	84.4 $\pm$ 2.2	90.8 $\pm$ 2.3	84.9 $\pm$ 5.4
Phe	90.0 $\pm$ 2.3	88.1 $\pm$ 2.1	91.2 $\pm$ 2.2	92.0 $\pm$ 3.1
Tyr	90.1 $\pm$ 2.3	87.9 $\pm$ 2.2	89.8 $\pm$ 2.3	90.4 $\pm$ 3.1
Val	89.4 $\pm$ 4.1	85.5 $\pm$ 3.8	90.5 $\pm$ 4.9	93.2 $\pm$ 4.6

<sup>1</sup>Means and SD of five observations per feed.

<sup>2</sup>Initial BW of pigs used to estimate standard ileal digestibility values were 48.3  $\pm$  3.59 and 49.2  $\pm$  3.07 kg for feeds A1 and A2, respectively, and 74.4  $\pm$  5.71 and 76.6  $\pm$  6.32 kg for feeds B1 and B2, respectively.

<sup>3</sup>Lys = lysine; Met = methionine; Thr = threonine; His = histidine; Ile = isoleucine; Leu = leucine; Cys = cysteine; Phe = phenylalanine; Tyr = tyrosine; Val = valine.

## **SUPPLEMENTARY MATERIAL S2**

### ***Calculations detailed information***

Total average daily gain (ADG) was calculated as the difference between body weight measured at the beginning of the experimental phases and body weight measured the end of the experimental phases. Intake inclusion rates of standardized ileal digestible (SID) Lysine (Lys) and crude protein were measured for each pig by tallying the daily amount of nutrients provided by each of the served feeds. The Dual X-Ray (DXA) body lean and fat masses were converted to their respective protein and lipid chemical equivalents as proposed by (Pomar and Rivest, 1996). Total body phosphorus (P) was estimated assuming that 18% of bone mineral content is P and that DXA bone mineral content represents 80% of total body P (Merkatoris *et al.*, 2012). Nitrogen excretion and P excretion values were obtained by subtracting the respective nutrient retention values from the nutrient intake values. Total body protein deposition (PD), lipid deposition, and P deposition were calculated as the difference between the respective body constituents estimated from DXA readings at the beginning and end of the experimental periods. Protein deposition in body weight gain was calculated by dividing PD by ADG. Lysine and nitrogen efficiency were calculated by dividing the corresponding retained values by available nutrients. Lysine retention was estimated assuming that 7% of body protein is Lys (van Milgen *et al.*, 2008). Lysine availability was estimated by subtracting the amount used for maintenance

from the SID Lys pool. Maintenance requirements for Lys were estimated as described in the main text for the individual precision feeding model.



SUPPLEMENTARY MATERIAL S3

**Supplementary Table S2** Non-linear model estimates of the independent response variables (protein deposition, average daily gain, N efficiency) in relation to the standardized ileal digestible lysine inclusion rate (%) predicted by the individual precision feeding mathematical model (Hauschild et al., 2012), estimated with a linear-plateau model<sup>1</sup> for growing-finishing pigs.

Response	U	SEe	R	SEe	L	SEe	P-value	RSE
First Growth Period								
PD	-3.6828	0.8349	94.5050	2.4029	214.9	4.9087	<0.0001	21.4
ADG	-0.0160	0.00408	93.5231	2.5301	0.988	0.0240	<0.0001	0.11
Second Growth Period								
PD	-2.6672	0.9601	87.6203	4.5017	170.7	3.8292	<0.0001	20.97
ADG	-0.0104	0.00182	94.4246	4.0347	1.188	0.033	<0.0001	0.10
N efficiency (%)	-0.4901	0.2817	87.5964	7.1755	49.78	1.1235	<0.01	6.15

<sup>1</sup>Abbreviations used: U = fit intercept; SEe = standard error of the estimation; R = parameter corresponding to the standard ileal digestible inclusion rate of lysine required to reach the plateau; L = average response estimated by the model; RSE = residual standard error; PD = protein deposition (g/d); ADG = average daily gain (kg/d); N efficiency = efficiency of nitrogen (N) utilization (%) calculated as N intake minus N retained in the whole body.

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