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Selection of an amino acid-deficient or -enriched diet by piglets under different sanitary conditions

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ABSTRACT

Sub-optimal sanitary conditions resulting from poor hygiene practices alter amino acid (**AA**) metabolism of pigs, thereby changing their quantitative requirements. Pigs can select a diet that meets their AA requirements over an AA-deficient one when given the choice, suggesting potential for self-supplementation of AA in conditions that affect AA requirements. The present study investigated whether the self-supplementation of AA to compensate for the provision of an AA-deficient diet in a choice-feeding setting differs for piglets kept under high or low sanitary conditions. To this end, an experiment (2 × 2 factorial design) was performed with 60 weaned female piglets (TN70 × Tempo) (two piglets per pen). Piglets were kept under high (**HSC**) or low (**LSC**) sanitary conditions and were offered either a diet deficient in eight indispensable AA (**IAA**) (**LP$^-$**) (No choice) or the choice between the LP$^-$ and a diet enriched with eight IAA (**LP$^+$**) (Choice) for 19 days. Average daily feed intake (**ADFI**) and average daily gain (**ADG**) were recorded, and blood samples were taken to determine immune parameters. Piglets under LSC had 22 and 38% higher blood leukocyte and monocyte counts ($P < 0.01$ for both), respectively, and a 129% higher haptoglobin concentration in serum ($P < 0.05$) than HSC piglets. Moreover, neutrophil (+53%) and eosinophil (+43%) counts were increased in LSC pigs (both $P < 0.001$). Low sanitary conditions piglets had 31, 44, and 21% lower ADFI, ADG, and gain-to-feed ratio, respectively, than HSC piglets during the experiment ($P < 0.01$), while no differences between No choice and Choice treatments on these parameters were observed. Both LSC and HSC choice piglets ingested more of the LP$^-$ than of the LP$^+$ diet ($P < 0.001$). The course of the consumption of the LP$^+$ diet in time differed between the two sanitary conditions ($P < 0.01$ for the interaction SC × Period): the percentage of LP$^+$ consumed out of the total feed intake from the start to the end of the experiment doubled (from 6.5 to 13.2%) for HSC piglets, while it decreased from 10.6 to 1.4% for LSC piglets. Low sanitary conditions, while decreasing performance and activating the immune system, did not increase the preference for an AA-enriched diet. Thus, offering piglets the choice to self-select between an AA-deficient diet and one enriched with AA above the assumed requirement did neither restore growth performance nor affect immune status when kept under low sanitary conditions.

Implications

Low sanitary conditions lead to a loss of performance and chronic low-grade inflammation in pigs, thereby modifying amino acid requirements. This study investigated whether piglets housed under low sanitary conditions would self-supplement amino acids in higher amounts compared to pigs under high sanitary conditions. Contrary to expectations, piglets decreased the preference for an amino acid-enriched diet under low compared to high sanitary conditions. This indicates that a decline in health status influences dietary preference by decreasing amino acid consumption despite increased amino acid requirements. Further research is needed to develop targeted amino acid supplementation strategies for piglets with increased amino acid requirements.

Introduction

Health status or stress can affect the amino acid (**AA**) requirements for pigs in terms of quantity and profile (Melchior et al., 2004; Le Floc'h et al., 2006; Le Floc'h et al., 2009; Kampman-van de Hoek et al., 2016; van der Meer et al., 2016). In addition, there

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is substantial between-animal variation in growth potential which also influences AA requirements of individual animals (Kampman-Van de Hoek et al., 2013; Remus et al., 2021). The effects of these factors on AA requirements, however, are generally not considered when feeding pigs in commercial farming systems. Amino acid requirements of pigs are commonly estimated based on obtaining maximal BW gain or feed efficiency of an average population. As AA are involved in many physiological functions other than growth, insufficient or imbalanced dietary AA intake has been shown not only to impair growth but also reduce resilience to immune challenges and increase the occurrence of damaging behaviours, especially in pigs kept in low sanitary conditions (van der Meer et al., 2016; van der Meer et al., 2017).

Low sanitary conditions due to poor hygiene measures modify the metabolism of AA, which are redirected from growth towards inflammatory and immunological processes, therefore changing the pattern of AA requirements (Le Floc'h et al., 2004), relative to energy. Low sanitary conditions, in addition, reduce total tract protein digestibility and protein efficiency, while also increasing energy expenditure for maintenance (Kampman-van de Hoek et al., 2015; van der Meer et al., 2016; van der Meer et al., 2020). This makes it important to further investigate strategies for dietary supplementation of AA for pigs housed under low sanitary conditions.

Pigs are known to show specific appetite for protein and AA, and optimise the ratio of protein to energy intake when given the choice between two diets (Kyriazakis et al., 1990; Dalby et al., 1995). Several studies have demonstrated that pigs are able to detect a dietary deficiency in a single AA (demonstrated for Lys, Thr (Ettle and Roth, 2005), Met (Roth et al., 2006), Trp (Ettle and Roth, 2004), and Val (Gloaguen et al., 2012; Suarez et al., 2012)) and consume greater amounts of a diet with a higher concentration of those specific AA when given the choice. In these studies, pigs showed a similar growth rate compared to the pigs only given a diet containing the specific AA at requirements for growth. Moreover, a recent study (Minussi et al., 2024) showed that pigs in a choice-feeding setting selected a diet with a higher Trp level than required for maximal growth. Possibly, pigs made this choice to fulfil functions other than growth, as a dietary Trp level higher than those recommended for growth has been shown to affect behaviour and stress (Martínez-Trejo et al., 2009; Poletto et al., 2014).

As most of the abovementioned studies were conducted under optimised experimental conditions, it remains unknown whether pigs can select an AA-enriched diet in a choice-feeding setting with increased AA requirements, as for instance demonstrated under low sanitary conditions (Le Floc'h et al., 2004). This choice-feeding approach could prevent issues arising from nutritional imbalances, such as growth retardation and the inability to cope with immune challenges, which may occur when individual AA requirements differ from what the animal is being offered via the diet.

The aim of the present study was to investigate whether self-supplementation of AA to compensate for offering an AA-deficient diet in a choice-feeding setting differs for piglets kept under high or low sanitary conditions. To this aim, piglets kept under either high or low sanitary conditions were either fed an AA-deficient diet or given the possibility to choose between an AA-deficient and an AA-enriched diet in a choice-feeding setting. We expected piglets to be able to supplement themselves with AA according to their requirement for maximal growth, and that selection for the AA-enriched diet would be higher in piglets kept under low sanitary conditions.

Material and methods

The experiment was conducted at Wageningen University and Research from September to November 2023. Piglets were exposed to one of four experimental treatments for a period of 19 days. In a 2 × 2 factorial design, pairs of piglets were allocated to either high sanitary conditions (**HSC**) or low sanitary conditions (**LSC**), and were offered either a diet deficient in AA (No choice) or the choice between an AA-deficient and an AA-enriched diet (Choice). Further details are provided below.

Animals, housing, and management

The experiment was carried out using three batches of animals. A total of 60 female piglets (Topigs Norsvin, TN70xTempo), offspring of 15 sows, were used over an experimental period of 19 days. Piglets were weaned at 4 weeks of age (8.38 ± 0.73 kg BW) on a commercial farm. On the weaning day, four female piglets closest to their litter's mean BW were selected from each litter. After selection, piglets were transported to the experimental facilities and housed in one of four climate respiration chambers containing three pens each. The experiment started on the day following arrival. Piglets were housed with one non-littermate (n = 2 piglets per pen). The four piglets from each litter were distributed over the four treatments, whereby both between-treatment BW differences and BW variation within pairs among treatments were minimised. Initial BW did not differ per treatment (8.10 ± 0.72 kg). Two chambers were dedicated to each sanitary status treatment and within each chamber, the three pens were assigned to either the No-choice or the Choice treatment (always two pens to one dietary treatment, and the third one to the other treatment within one chamber). In the first batch of animals, intake of the AA-enriched diet by the piglets in the Choice treatments was very low, possibly due to the high concentration of free AA in the diet leading to taste aversion. Therefore, in the second and third batch of animals, the AA-enriched diet was diluted (see details below). Data related to the Choice treatments in the first batch of animals were not further used in the study. Subsequently, it was decided to allocate two more pens to each Choice treatment at the expense of No choice pens in the batches 2 and 3, leading to a total number of seven replicates for the HSC-No choice and LSC-No choice treatments and eight replicates for the HSC-Choice and LSC-Choice treatments.

Pens (0.88 × 2.88 m) had a partially slatted floor and contained a nipple drinker and a feeder. Each feeder had two separate feeding spaces (25 x 25 cm). Pens contained a plastic toy (PorkyPlay, Ketchum Manufacturing, Lake Luzerne, NY, USA) and a metal chain (90 cm) as enrichment materials. Piglets had *ad libitum* access to the experimental diets and water. Lights were on between 0700 and 1900 h. Room temperature gradually decreased from 28 °C to 25 °C and relative humidity decreased from 65 to 62% from arrival to the end of the experimental period. Ventilation was set at 18 m^3/h. The experimental period started the day after arrival.

Sanitary conditions

The protocol used to impose contrasting sanitary conditions was adapted from Noorman et al. (2023). The LSC treatment was obtained by spreading twice a week 1.5 kg of faeces in each pen. The faeces were collected from the floor of the pens of weaned piglets of four commercial farms. Faeces from each farm were collected separately from multiple rooms, and per farm faeces from animals of the same age were homogenised and sampled for analysis on the presence of pathogens. After the sampling procedure, faeces from each age group within farm were mixed with a 0.9% NaCl solution (2:1 w/w) and glycerol (12% w/w) before storage at −20 °C. The samples were analysed for *Clostridium perfringens* toxin CPA, CPB, CPB2, *Escherichia coli* virulence factor F4, F42, F5, F6, and Rotavirus A with q-PCR (GD Diergezondheid, Deventer, The Netherlands). Faecal subsamples with two or more of the analysed

pathogens exceeding defined critical levels (4 out of 13 samples) were excluded to minimise the risk of introducing diseases (see Supplementary Table S1 for results of the analysis). The remaining faecal samples were pooled into one large batch, homogenised, and divided over buckets containing 1.5 kg of diluted pooled faeces each, and stored at −20 °C. Six buckets with pooled faeces were thawed overnight at room temperature per timepoint before spreading the faeces into the LSC pens (days 0, 4, 7, 11, 14, and 18). Manure was removed daily from the pens only in the HSC treatment. To avoid contamination, the rooms of HSC and LSC had each their own entrance, manure pit, and ventilation system. Prior to the arrival of the piglets, chambers of both treatments were cleaned with high-pressure washing, but only the HSC chambers were treated with disinfectants (Desbest400, Veugen Technology B.V., Nederweert, The Netherlands; Virocid, CID LINES N.V., Ieper, Belgium). In addition, a strict hygiene protocol, which included showering, change of clothes, and the use of a hairnet and face mask, was applied when entering the HSC rooms. These procedures were not applied to the LSC chambers.

Diets

From experimental day 0, piglets were assigned to one of the two dietary treatments. In the No choice treatment, piglets were fed a diet deficient in indispensable (**IAA**) (**LP$^-$**), while in the Choice treatment, piglets could choose between the LP$^-$ diet and a diet enriched with IAA above requirements for maximal BW gain (**LP$^+$**). The LP$^-$ diet was formulated to be deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His by 20% compared to requirements for maximal BW gain (CVB, 2020). The LP$^+$ diet was supplemented with L-Lys, DL-Met, L-Thr, L-Trp, L-Val, L-Leu, L-Ile, and L-His at +20% above requirements for maximal BW gain (CVB, 2020).

In the Choice treatments, diets were formulated so that piglets could meet their AA requirements for maximal BW gain by ingesting both diets in a certain ratio (50/50 ratio) according to their free choice. In the No Choice treatments, in which only one diet was provided, the diet was presented in both feeding spaces per feeder, while for the Choice treatment, each of the two diets was given in one of the two feeding spaces. The position of the two diets of the Choice treatment within the feeders was changed on day 4 and day 11 to avoid an effect of feeding place preference on diet choice.

A theoretical low protein diet (**LP**) was formulated by reducing the CP level of a standard practical diet for weaned piglets in the Netherlands by relatively 20% (from 191 till 153 g/kg). Then, the LP$^-$ diet was formulated by reducing the concentration of standardised ileal digestible (**SID**) Lys, SID Thr, SID Met, SID Trp, SID Val, SID Ile, SID Leu, and SID His by 20% compared to the LP diet, resulting in a dietary CP content of 141 g/kg. The diet was based on barley, wheat, maize, and supplemented with L-Lys, DL-Met, L-Thr, L-Trp, L-Val, L-Ile, and L-His. The LP$^+$ diet was formulated by increasing the SID Lys, SID Thr, SID Met, SID Trp, SID Val, SID Ile, SID Leu, and SID His by 20% compared to LP diet through further supplementation of L-Lys, DL-Met, L-Thr, L-Trp, L-Val, L-Ile, L-Leu, and L-His at the expense of maize starch. The SID Lys concentration in the LP diet (1.13 g/MJ net energy) was based on the requirements values for piglets for maximal average daily gain and gain-to-feed ratio (**G:F**) (CVB, 2020). The profile of IAA in ratio to Lys was based on recommended dietary AA levels for piglets (CVB, 2020), and for Trp was based on a recommendation from a published meta-analysis (Simongiovanni et al., 2012). The calculated net energy and other nutrient values were based on the Centraal Veevoeder Bureau Feed Table (CVB, 2022). The diets were isocaloric on a net energy basis. Diets were provided *ad libitum* in pelleted form. The ingredients and nutrient composition of the diets are shown in Table 1. In the first batch, the LP$^+$ diet was supplemented with eight IAA at 60% above requirements. Because of the extremely low consumption of the LP$^+$ diet in batch one, the LP$^+$ and LP$^-$ diets were mixed in a ratio of 1 to 1 to obtain a new LP$^+$ diet with IAA levels at 20% above assumed requirements in batch 2 and batch 3.

Feed analyses

Diets were analysed for DM according to ISO 6496 (ISO, 1999), starch according to ISO 15914 (ISO, 2004), and CP according to ISO

Table 1
Ingredient composition and analysed nutrient levels of the experimental diets fed to piglets over a period of 19 days after weaning.

	Diets	
Item	LP$^-$[1]	LP$^+$[2]
Ingredients (g/kg as fed)		
Barley	300.0	300.0
Wheat	279.7	279.7
Maize	200.0	200.0
Soybean meal	68.0	68.0
Wheat middlings	25.0	25.0
Cane molasses	20.0	20.0
Maize starch	50.0	25.1
Soybean oil	10.0	11.5
Limestone fine	10.0	10.0
Monocalcium phosphate	8.6	8.6
Sodium propionate	5.0	5.0
Potato protein	5.0	5.0
Sodium chloride	3.0	3.0
Vitamin + mineral mix[3]	2.0	2.0
Potassium	2.4	2.4
Axtra PHY 20 000 FTU/g	0.1	0.1
Sodium bicarbonate	0.001	0.001
L-Lysine HCl	5.6	11.2
L-Threonine	1.9	4.4
DL-Methionine	1.9	4.4
L-Valine	0.9	4.1
L-Leucine	–	4.4
L-Isoleucine	0.3	2.6
L-Tryptophan	0.6	1.6
L-Histidine	0.2	1.6
Nutrient composition (g/kg)		
DM	881	884
NE_{2015}[4]	10.4	10.4
Analysed CP	123	142
Analysed starch	493	469
SID amino acids[5] (total analysed)		
Lys	8.8 (9.3)	13.2 (13.6)
Met + Cys	5.7 (5.4)	8.1 (8.0)
Thr	5.7 (5.7)	8.6 (8.5)
Trp	1.9 (2.0)	2.9 (2.9)
Val	6.2 (6.1)	9.2 (12.2)
Ile	4.7 (4.5)	7.0 (8.8)
Arg	6.1 (6.2)	6.1 (6.3)
Phe	5.5 (5.4)	5.5 (5.5)
His	2.8 (2.8)	4.2 (3.8)
Leu	8.9 (8.7)	13.2 (13.1)
Tyr	3.6 (3.5)	3.6 (3.6)

Abbreviations: LP = Low protein; SID = Standardised ileal digestible.
[1] LP$^-$: A low protein diet formulated to be deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His by 20% compared to requirements for maximal BW gain (CVB, 2020).
[2] LP$^+$: A low protein diet formulated with Lys, Met, Thr, Trp, Val, Leu, Ile, and His at 20% above requirements for maximal BW gain (CVB, 2020).
[3] Vitamin + mineral mix composition (/kg diet): vitamin A (10 000 IU), vitamin D_3 (2 000 IU), vitamin E (40 mg), vitamin K_3 (1.5 mg), vitamin B_1 (1.0 mg), vitamin B_2 (4.0 mg), vitamin B_6 (1.5 mg), vitamin B_{12} (20 µg), niacin (30 mg), D-pantothenic acid (15 mg), choline chloride (150 mg), folic acid (0.4 mg), biotin (0.05 mg), iron (100 mg), copper (20 mg), manganese (30 mg), zinc (70 mg), iodate (0.7 mg), selenium (0.25 mg).
[4] NE = net energy, MJ/kg (CVB, 2022).
[5] Standardised ileal digestible (SID) amino acids based on table values for pigs (CVB, 2020), in brackets the total amino acid concentration analysed with NF EN ISO13903 (ISO, 2009) and ISO13904 (ISO, 2005b).

5983-1 (ISO, 2005a). The AA composition of the diets was analysed with a JLC-500/V AminoTac Amino Acid Analyzer (Jeol, Croissy-sur-Seine, France) according to NF EN ISO 13903 (ISO, 2009). For Cys and Met analysis, the samples were oxidised with performic acid prior to hydrolysis. The AA were separated by ion exchange chromatography and quantified after reaction with ninhydrin. Tryptophan was analysed by reversed-phase C18 high–performance liquid chromatography according to NF EN ISO13904, MOD.0094 (ISO, 2005b). These chemical analyses were executed in duplicate, and when the CV of both values was >5%, analyses were repeated.

Performance measurements

Feed intake per pen and individual BW were determined on experimental days 0, 4, 7, 11, 14, and 18. Feed intake was determined by weighing the provided feed and the unconsumed feed. In case the feed was contaminated with water or urine, wet feed was collected, weighed, and analysed for DM to correct feed intake. The individual average daily feed intake (**ADFI**) was calculated as the amount of feed consumed per pen per period divided by the number of piglets in the respective pen. The average daily gain of individual piglets was calculated from the difference in BW between the start and end of each period. The G:F was calculated per piglet per week as BW gain divided by the calculated feed intake of the respective week.

Blood sampling

A blood sample was taken from all piglets on experimental day 18 from the jugular vein. Per sampling moment, blood was collected in a 1-mL EDTA tube (Vacuette; Greiner Bio-One, Kremsmünster, Austria) for blood cell counts in whole blood, and in a 9-mL serum tube (Vacuette; Greiner Bio-One, Kremsmünster, Austria) for haptoglobin analysis. Blood samples collected in EDTA tubes were immediately stored on ice and transported to the lab. Whole blood cell count was determined with a cell counter (ADVIA® 2120i Hematology System with Autoslide, Siemens Healthineers AG, Forchheim, Germany). Blood from the 9-ml serum tube clotted for 1 h at room temperature, then, it was centrifuged for 10 min at 5 251 × g at room temperature, after which serum was collected and stored at –20 °C pending analysis of haptoglobin (Tridelta Phase Haptoglobin Assay, catalogue number TP-801; Tridelta Development, Ltd., Maynooth, Ireland).

Calculations and statistical analysis

Lysine intake (g/pig/d) was calculated by multiplying the feed intake of the diets with the analysed AA content of the diets. Individual BWs were averaged per pen as the experimental unit.

Feed intake, average daily gain, G:F, and Lys intake averaged per period were analysed in a linear mixed model (with 'lmer' function from the R package 'lme4′) including a fixed effect of sanitary conditions, choice, period (d 0–7, 7–14, 14–18, or d 0–4, 4–7, 7–11, 11–14, 14–18), and their interactions, and batch, and a random effect of pen. If the interaction effect of sanitary conditions or choice × period was significant ($P < 0.05$), to further explore the interaction, data were analysed per period with a linear model (with 'lm' function from the R package 'lme4′) including the fixed effect of sanitary conditions, choice, sanitary conditions × choice, and batch. Performance data of the overall experiment were analysed with the same model.

Average daily feed intake and Lys intake per diet type in the choice treatments were analysed in a linear mixed model for each sanitary condition separately (with 'lmer' function from the R package 'lme4′) including a fixed effect of diet, period (d 0–4, 4–7, 7–11, 11–14, 14–18), interaction diet × period, and batch, and a random effect of pen. If the interaction diet × period was significant ($P < 0.05$), data were further analysed for each period separately with a linear model (with 'lm' function from the R package 'lme4′) including the fixed effect of diet and batch. Average daily feed intake and Lys intake per diet type of the overall experiment were analysed with the same model.

Blood parameters of individual piglets were analysed in a linear mixed model (with 'lmer' function from the R package 'lme4′) including a fixed effect of sanitary conditions, choice, interaction sanitary conditions × choice, batch, and a random effect of pen.

Residual normality and variance homogeneity were evaluated using the Shapiro-Wilk tests and visual inspection of the data. When residuals did not meet normality assumptions, data were square root- or log-transformed. *P*-values below 0.05 were considered statistically significant, and *P*-values between 0.05 and 0.10 as tendencies. In case of significant fixed effects, pairwise comparisons were done using differences in least square means with a Tukey's HSD correction on the transformed data. Data are presented as means ± SD unless stated otherwise.

Results

On d 8 of batch 2, one piglet allocated to the HSC-Choice treatment died from intestinal torsion and its pen mate was removed for welfare reasons (i.e. to avoid social isolation). Therefore, data from these two piglets were included until d 8 of the experiment only. All other animals remained clinically healthy during the entire experiment. The three-way interaction of sanitary conditions × choice × period was not significant in either case, and related results were therefore not reported below or in the tables.

Blood parameters

Piglets under LSC had 22 and 38% higher leukocyte and monocyte counts in whole blood ($P < 0.01$ for both), respectively, and a 129% higher haptoglobin concentration in serum ($P < 0.05$) compared to HSC piglets. Piglets under LSC had 53 and 43% higher neutrophil and eosinophil counts in whole blood ($P < 0.001$ for both), respectively, compared to HSC piglets. The remaining blood parameters were not affected by sanitary conditions, neither by choice, nor by their interaction ($P > 0.05$) (Table 2).

Performance

Effect of sanitary conditions and choice on feed intake, BW gain, and feed efficiency

The BW of piglets under LSC at d 18 was 16% lower compared to piglets under HSC ($P < 0.001$), while providing either or not a diet choice did not affect BW ($P = 0.70$). The interaction of sanitary conditions × choice was not significant ($P = 0.73$). Piglets under LSC had 31, 44, and 21% lower ADFI, average daily gain, and G:F, respectively, compared to piglets under HSC in the overall experimental period ($P < 0.01$). Choice and the interaction of sanitary conditions × choice did not affect overall performance ($P > 0.05$). Average daily feed intake was affected by the interaction of sanitary conditions × period ($P < 0.05$). When analysed per period, ADFI was higher for HSC piglets than for LSC piglets ($P < 0.01$ in 0–7 d, and $P < 0.001$ in 7–14 d and 14–18 d), and no effects of choice nor sanitary conditions × choice were found ($P > 0.05$). Average daily gain was higher for HSC piglets compared to LSC piglets ($P < 0.001$) and increased with period ($P < 0.001$). Choice and the other interactions did not affect average daily gain ($P > 0.05$). Within each period, average daily gain of the HSC piglets was higher than the LSC piglets ($P < 0.001$ in 0–7 d and 7–14 d, and

Table 2
Blood parameters of piglets kept under different sanitary conditions and given a choice or not between an amino acid-deficient and an amino acid-enriched diet at 19 days postweaning[1].

	HSC		LSC		*P*-value		
Item	No choice[2]	Choice[3]	No choice[2]	Choice[3]	SC	Choice	SC × Choice
n	7	7	7	8			
Leukocytes, 10^9/L	17.0 ± 3.2	17.1 ± 4.4	20.3 ± 4.1	21.2 ± 6.5	<0.01	0.967	0.836
Lymphocytes, 10^9/L	9.3 ± 1.8	9.5 ± 3.7	9.2 ± 2.3	9.2 ± 2.5	0.87	0.717	0.903
Monocytes, 10^9/L	0.6 ± 0.3	0.7 ± 0.4	0.9 ± 0.4	0.9 ± 0.5	<0.01	0.609	0.727
Granulocytes, 10^9/L							
Neutrophils	6.71 ± 2.88	6.51 ± 1.94	9.62 ± 3.83	10.66 ± 5.01	<0.001	0.681	0.676
Eosinophils	0.36 ± 0.18	0.34 ± 0.12	0.54 ± 0.16	0.46 ± 0.25	<0.001	0.426	0.139
Basophils	0.04 ± 0.07	0.04 ± 0.08	0.06 ± 0.05	0.02 ± 0.04	0.807	0.811	0.459
Erythrocytes, 10^{12}/L	6.6 ± 0.5	6.2 ± 0.6	6.2 ± 0.5	6.1 ± 0.6	0.153	0.569	0.398
Hb, mmol/L	7.4 ± 0.7	6.9 ± 0.8	7.1 ± 0.5	7.1 ± 0.6	0.881	0.861	0.272
Ht, %	39.5 ± 4.0	36.7 ± 4.6	37.6 ± 3.2	36.2 ± 4.0	0.297	0.704	0.561
MCV, 10^{-15}L	59.9 ± 3.1	58.9 ± 3.8	60.5 ± 3.7	59.2 ± 2.8	0.655	0.737	0.719
PTL, 10^9/L	334 ± 113	281 ± 90	334 ± 144	430 ± 508	0.551	0.776	0.371
Haptoglobin, mg/ml serum	0.3 ± 0.2	0.4 ± 0.5	0.7 ± 0.7	0.9 ± 0.9	<0.05	0.537	0.364

Abbreviations: HSC = High sanitary conditions; LSC = Low sanitary conditions; SC = Sanitary conditions; Hb = Haemoglobin; Ht = Haematocrit; MCV = Mean cell volume; PTL = Platelets.
[1] Data are presented as means ± SD.
[2] No-Choice: piglets could consume only the LP⁻ diet, a low protein diet formulated to be deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His by 20% compared to requirements for maximal BW gain (CVB, 2020).
[3] Choice: piglets could choose to consume a low protein diet with Lys, Met, Thr, Trp, Val, Leu, Ile, His either 20% deficient (LP⁻) or 20% above (LP⁺) requirements for maximal BW gain (CVB, 2020).

$P < 0.05$ in 14–18 d), and no effects of choice nor sanitary conditions × choice were found ($P > 0.05$). The G:F was higher for HSC than for LSC piglets ($P < 0.05$) and decreased with period ($P < 0.01$). Choice and the other interactions did not affect G:F ($P > 0.05$). The G:F tended to be higher in HSC compared to LSC piglets in 0–7 d ($P < 0.1$), while the effect was not significant in 7–14 and 14–18 d ($P > 0.05$). Choice and sanitary conditions × choice did not affect G:F in any period ($P > 0.05$) (Table 3).

Effect of sanitary conditions and choice on amino acid intake

As eight IAA were reduced by 20% in the LP⁻ diet, Lys was taken as an example for these IAA. Lysine intake was affected by sanitary conditions ($P < 0.001$), period ($P < 0.001$), and their interaction ($P < 0.01$). Lysine intake increased with period to a larger extent for piglets under LSC compared to piglets under HSC ($P < 0.01$). In all periods, piglets under HSC had a higher Lys intake compared to piglets under LSC ($P < 0.05$). Choice and its interaction with sanitary conditions or period did not affect Lys intake ($P > 0.05$). Piglets under HSC had 31% higher Lys intake compared to piglets under LSC in the overall experimental period ($P < 0.001$). Choice and interaction of sanitary conditions × choice did not have an effect on overall Lys intake ($P > 0.05$) (Table 4).

Feed and amino acid intake per diet in the choice treatments

Average daily feed intake of the HSC-Choice treatment was affected by diet ($P < 0.001$), period ($P < 0.001$), and their interaction ($P < 0.001$). The intake of the LP⁺ diet initially decreased and then increased with period ($P < 0.001$). In each period, the LP⁻ diet was consumed more than the LP⁺ diet ($P < 0.001$ in all periods, and $P < 0.01$ in the 0–4 d period). Average daily feed intake of the LSC-Choice treatment was affected by diet ($P < 0.001$), period ($P < 0.001$), and their interaction ($P < 0.001$). The intake of the LP⁺ diet initially increased and then decreased with period ($P < 0.001$). In each period, the LP⁻ diet was consumed more than the LP⁺ diet ($P < 0.001$ in all periods, and $P < 0.01$ in the 0–3 d period). For the overall period, the ADFI of the LP⁻ diet was higher than the LP⁺ diet, representing 92 and 95% of the total ADFI respectively in the HSC and LSC ($P < 0.001$ for both). The effects of diet, period, and interaction diet × period on Lys intake were comparable to the ones on the ADFI. For the overall period, Lys intake from the LP⁻ diet was higher than from the LP⁺ diet, representing 89 and 92% of the total Lys intake in the HSC and LSC, respectively ($P < 0.001$ for both) (Table 5).

Discussion and conclusions

The aim of this study was to investigate whether piglets in a choice-feeding setting consume higher amounts of an AA-enriched diet to compensate for ingesting an AA-deficient diet when kept under LSC compared to HSC. Performance parameters and immune status were observed for 18 d following weaning. While sanitary conditions affected the performance and immune status of piglets, no effects of providing piglets with a choice between an AA-deficient and an AA-enriched diet per se were found. The time course of the dietary choice was, however, affected by sanitary conditions, with an increasing selection of the overall less preferred AA-enriched diet in pigs under high sanitary conditions only.

The contrast in sanitary conditions was achieved by spreading faeces from four commercial pig farms in the LSC, and by removing faeces daily from pens in the HSC. In addition, this contrast was maintained by imposing a different hygiene protocol for the two sanitary treatments. In line with previous studies (Le Floc'h et al., 2006; Le Floc'h et al., 2009; Le Floc'h et al., 2014; van der Meer et al., 2016), piglets kept under LSC had higher counts of leukocytes, monocytes, neutrophil and eosinophil granulocytes in blood, and a higher concentration of haptoglobin in serum compared to piglets kept under HSC, without showing clinical signs of sickness such as fever. This indicates that the poor hygienic conditions induced mild inflammation and immune stimulation, as intended.

Low sanitary conditions also rapidly and largely impacted piglet performance, causing a reduced feed intake, BW gain, and feed efficiency compared to piglets kept under HSC. Most other studies found similar effects of sanitary conditions on ADFI, average daily gain, and G:F (Le Floc'h et al., 2006; Le Floc'h et al., 2009; Le Floc'h et al., 2014; van der Meer et al., 2020; Noorman et al., 2023). Reduced growth observed in low sanitary conditions is caused by a combination of decreased feed intake and lower feed efficiency, as shown by a meta-analysis (Pastorelli et al., 2012). Appetite reduction in low sanitary conditions likely reflects a subclinical

Table 3
Body weight, average daily feed intake, average daily gain, and gain-to-feed ratio of piglets kept under different sanitary conditions and given a choice or not between an amino acid-deficient and an amino acid-enriched diet over the experiment[1].

	HSC		LSC		*P*-value					
Item	No choice[2]	Choice[3]	No choice[2]	Choice[3]	SC	Choice	SC × Choice	Period	SC × Period	Choice × Period
n	7	8	7	8						
BW (kg)										
0 d	8.15 ± 0.54	8.03 ± 0.86	8.10 ± 0.61	8.14 ± 0.86	0.533	0.855	0.426			
18 d	12.63[a] ± 1.38	12.65[a] ± 1.36	10.79[b] ± 1.30	10.51[b] ± 1.52	<0.001	0.701	0.730			
ADFI (g/d)					<0.001	0.590	0.723	<0.001	<0.05	0.735
0–7 d	221 ± 105	190 ± 88	130 ± 57	106 ± 63	<0.01	0.337	0.879			
7–14 d	463 ± 117	402 ± 93	318 ± 89	304 ± 91	<0.001	0.241	0.490			
14–18 d	628 ± 55	615 ± 48	466 ± 94	440 ± 84	<0.001	0.466	0.810			
Overall, 0–18 d	406 ± 92	371 ± 77	278 ± 68	257 ± 71	<0.001	0.284	0.806			
ADG (g/d)					<0.001	0.579	0.696	<0.001	0.690	0.481
0–7 d	181 ± 91	152 ± 87	60 ± 68	21 ± 66	<0.001	0.195	0.845			
7–14 d	294 ± 60	309 ± 39	208 ± 57	198 ± 61	<0.001	0.921	0.535			
14–18 d	290 ± 155	309 ± 52	202 ± 88	212 ± 95	<0.05	0.726	0.910			
Overall, 0–18 d	249 ± 49	252 ± 44	149 ± 53	132 ± 52	<0.001	0.681	0.569			
G:F					<0.05	0.814	0.743	<0.01	0.280	0.414
0–7 d	0.81 ± 0.07	0.72 ± 0.32	0.19 ± 0.94	−0.23 ± 1.00	0.082	0.390	0.524			
7–14 d	0.65 ± 0.12	0.79 ± 0.13	0.66 ± 0.10	0.67 ± 0.21	0.301	0.192	0.204			
14–18 d	0.46 ± 0.25	0.50 ± 0.09	0.43 ± 0.18	0.47 ± 0.16	0.197	0.850	0.530			
Overall, 0–18 d	0.62 ± 0.05	0.69 ± 0.04	0.52 ± 0.11	0.51 ± 0.15	<0.01	0.623	0.377			

Abbreviations: HSC = High sanitary conditions; LSC = Low sanitary conditions; SC = Sanitary conditions; ADFI = Average daily feed intake; ADG = Average daily gain; G:F = Gain-to-feed ratio. For the HSC-Choice treatment after 0–7 d period: n = 7.
[1] Data are presented as means ± SD. The three-way interaction SC × Choice × Period was not significant and was not included in the table.
[2] No-Choice: piglets could consume only the LP$^-$ diet, a low protein diet formulated to be deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His by 20% compared to requirements for maximal BW gain (CVB, 2020).
[3] Choice: piglets could choose to consume a low protein diet with Lys, Met, Thr, Trp, Val, Leu, Ile, His either 20% deficient (LP$^-$) or 20% above (LP$^+$) requirements for maximal BW gain (CVB, 2020).
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4
Lysine intake of piglets kept under different sanitary conditions and given a choice or not between an amino acid-deficient and an amino acid-enriched diet over the experiment[1].

	HSC		LSC		*P*-value					
Item	No choice[2]	Choice[3]	No choice[2]	Choice[3]	SC	Choice	SC × Choice	Period	SC × Period	Choice × Period
n	7	8	7	8						
Lys intake (g/d)					<0.001	0.413	0.762	<0.001	<0.01	0.677
0–4 d	1.4 ± 1.01	1.2 ± 0.84	0.8 ± 0.56	0.6 ± 0.55	<0.05	0.404	0.917			
4–7 d	2.9 ± 1.06	2.7 ± 1.09	1.7 ± 0.51	1.6 ± 0.77	<0.01	0.556	0.857			
7–11 d	4.0 ± 1.13	3.2 ± 0.90	2.5 ± 0.91	2.5 ± 0.90	<0.01	0.239	0.231			
11–14 d	4.8 ± 1.19	4.6 ± 0.94	3.6 ± 0.85	3.5 ± 0.77	<0.01	0.695	0.984			
14–18 d	5.8 ± 0.51	6.1 ± 0.50	4.3 ± 0.87	4.1 ± 0.76	<0.001	0.990	0.366			
0–18 d	3.8 ± 0.86	3.6 ± 0.75	2.6 ± 0.63	2.4 ± 0.64	<0.001	0.490	0.910			

Abbreviations: HSC = High sanitary conditions; LSC = Low sanitary conditions; SC = Sanitary conditions. For the HSC-Choice treatment after 0–7 d period: n = 7.
[1] Data are presented as means ± SD. The three-way interaction SC × Choice × Period was not significant and was not included in the table.
[2] No-Choice: piglets could consume only the LP$^-$ diet, a low protein diet formulated to be deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His by 20% compared to requirements for maximal BW gain (CVB, 2020).
[3] Choice: piglets could choose to consume a low protein diet with Lys, Met, Thr, Trp, Val, Leu, Ile, His either 20% deficient (LP$^-$) or 20% above (LP$^+$) requirements for maximal BW gain (CVB, 2020).

sickness response due to the mild inflammatory status caused by poor environmental hygiene. This response results from the release of inflammatory mediators like cytokines, which modulate the sites in the hypothalamus associated with the feeding response (Plata-Salamán, 1996). Lower feed efficiency is caused by the redirection of AA from use for protein deposition for growth to use for synthesis of e.g. acute-phase proteins and immunoglobulins, and for gluconeogenesis in the liver (Le Floc'h et al., 2004). As a result, low sanitary conditions increase energy expenditure and decrease incremental protein efficiency in growing pigs (van der Meer et al., 2020). The size of the effects of sanitary conditions on performance parameters observed in the present study is larger than reported in a meta-analysis on the effects of a sanitary challenge on feed intake and growth (Pastorelli et al., 2012). The meta-analysis showed an average reduction of ADFI and average daily gain due to poor hygiene conditions of 4 and 10%, respectively, while in the current study, it was 31 and 44%, respectively. The level and variation of the response to poor hygienic conditions could be due to different factors, the first being the protocol to obtain the contrast in sanitary conditions: some studies imposed low sanitary conditions by omitting cleaning and disinfection of pens (Le Floc'h et al., 2006; Le Floc'h et al., 2014; van der Meer et al., 2016), while others enhanced high sanitary conditions by implementing strict hygiene measures and applying additional vaccinations against specific pathogens, and a preventive antibiotic treatment at the start of the study (van der Meer et al., 2016; van der Meer et al., 2020). In the current study, a large contrast between high and low sanitary conditions was obtained by also spreading faeces from commercial pig farms. Studies also differ from the current one in the age of the pigs and the duration of the trial, focusing on longer time

Table 5
Average daily feed intake and Lys intake per diet of piglets kept under different sanitary conditions and given the choice between an amino acid-deficient and an amino acid-enriched diet over the experiment[1].

	HSC-choice[2]		*P*-value			LSC-choice[2]		*P*-value		
Item	LP^{-3}	LP^{+3}	Diet	Period	Diet × Period	LP^{-3}	LP^{+3}	Diet	Period	Diet × Period
n	8					8				
ADFI (g/d)			<0.001	<0.001	<0.001			<0.001	<0.001	<0.001
0–4 d	115 ± 83	8 ± 14	<0.01			59 ± 50	7 ± 11	<0.01		
4–7 d	265 ± 108	14 ± 9	<0.001			133 ± 81	25 ± 27	<0.01		
7–11 d	336 ± 93	6 ± 7	<0.001			236 ± 102	20 ± 30	<0.001		
11–14 d	448 ± 109	34 ± 42	<0.001			353 ± 115	17 ± 28	<0.001		
14–18 d	534 ± 112	81 ± 89	<0.001			434 ± 90	6 ± 12	<0.001		
0–18 d	341 ± 78	30 ± 29	<0.001			243 ± 78	14 ± 16	<0.001		
Lys intake (g/d)			<0.001	<0.001	<0.001			<0.001	<0.001	<0.001
0–4 d	1.1 ± 0.77	0.1 ± 0.18	<0.01			0.5 ± 0.46	0.1 ± 0.14	<0.05		
4–7 d	2.5 ± 1.00	0.2 ± 0.12	<0.001			1.2 ± 0.75	0.3 ± 0.37	<0.05		
7–11 d	3.1 ± 0.87	0.1 ± 0.09	<0.001			2.2 ± 0.95	0.3 ± 0.40	<0.001		
11–14 d	4.2 ± 1.02	0.5 ± 0.57	<0.001			3.3 ± 1.07	0.2 ± 0.37	<0.001		
14–18 d	5.0 ± 1.04	1.1 ± 1.20	<0.001			4.0 ± 0.84	0.1 ± 0.16	<0.001		
0–18 d	3.2 ± 0.72	0.4 ± 0.40	<0.001			2.3 ± 0.73	0.2 ± 0.21	<0.001		

Abbreviations: HSC = High sanitary conditions; LP^{-} = Low protein diet deficient in Lys, Met, Thr, Trp, Val, Leu, Ile, His; LP^{+} = Low protein diet supplemented with Lys, Met, Thr, Trp, Val, Leu, Ile, His; LSC = Low sanitary conditions; ADFI = Average daily feed intake. For the HSC-Choice treatment after 0–7 d period: n = 7.
[1] Data are presented as means ± SD.
[2] Choice: piglets could choose to consume a low protein diet with Lys, Met, Thr, Trp, Val, Leu, Ile, His either 20% deficient (LP^{-}) or 20% above (LP^{+}) requirements for maximal BW gain (CVB, 2020).

periods and therefore older pigs (Le Floc'h et al., 2006; Le Floc'h et al., 2009; van der Meer et al., 2016; van der Meer et al., 2020). Furthermore, the absence of an adaptation period immediately following the weaning transition, which is inherently stressful for piglets, may have further increased the size of the response to the contrast in sanitary conditions.

Having the dietary choice between an AA-deficient and an AA-enriched diet, as opposed to an AA-deficient diet only, did not have any effect on performance parameters or the blood parameters measured. We expected piglets to supplement themselves with AA according to their requirement for maximal growth, and that piglets kept under LSC would show a stronger preference and hence a relatively higher selection of the AA-enriched diet. Piglets, however, did not consume higher amounts of the AA-enriched diet compared to the AA-deficient diet in either sanitary condition. In a previous choice-feeding study, it was also observed that pigs had an unexpected preference for a diet deficient by 20% in Thr, Trp, and Val compared to a diet with Thr, Trp, and Val at requirements for maximal growth (Minussi et al., 2024). A first possible explanation, both in the previous study and in the current one, is related to the uncertainty of the extent of the AA deficiency. Although diets were formulated to be 20% deficient, the actual AA requirements of pigs under these specific experimental conditions may have differed from the ones used as reference, making the LP^{-} diet not deficient enough to cause a rejection in the pigs.

Another explanation for the low consumption of the AA-enriched diet in the current study could be a potential aversive taste of the diet. In the first batch of animals in the current study, in fact, the observed intake of the LP^{+} diet supplemented with eight IAA at 60% above requirements was very low, pointing towards an adverse taste of this diet. For the next two batches of animals, the LP^{+} and LP^{-} diet were mixed to obtain a modified LP^{+} diet with lower IAA concentrations, leading to a higher intake of the AA-enriched diet, albeit still small relative to the total feed intake. While there is no literature evaluating the combination of all first eight limiting IAA in excess of requirements, there are some indications of aversion to diets with high concentrations of free IAA (Edmonds et al., 1987; Harper and Peters, 1989). Limited evidence reports that some AA in their L-isomer form, among which L-Trp, L-His, L-Ile, L-Leu, L-Val, have an aversive taste for pigs (Tinti et al., 2000). Nevertheless, when being in a nutrient-deficient status, animals may prioritise intake of specific nutrients over taste preference, as shown in rats (Yamamoto et al., 1985; Leung et al., 1986). In a study from Minussi et al. (2024), pigs consumed a diet supplemented with L-Thr at 60% above requirements and strongly preferred a diet supplemented with L-Trp at 60% above requirements, while rejecting a diet supplemented with L-Val at 60% above requirements or a diet with each L-Thr, L-Trp, and L-Val supplemented at 60% levels above assumed reference requirements in the same diet (Minussi et al., 2024). Regarding the other supplemented IAA, apart from L-Thr, L-Trp, and L-Val, female growing pigs consumed a diet with L-Lys of 40% above requirement more than a Lys-deficient diet, while males did not (Henry, 1993). Wessels et al. (2016) tested L-Leu and L-His at 20% above requirements without a drop in feed intake in piglets, and Parr et al. (2003) tested L-Ile at 10% above requirement without a drop in feed intake in growing pigs. Since these trials tested combined supplementations of up to three individual AA at higher concentrations, the adverse taste could potentially be attributed to the inclusion of one of the AA with adverse taste or to the combination of the free IAA used to enrich the diet. In addition, piglets in the current study may have had increased requirements for only some specific IAA, for instance, related to the immune system activation, and avoided the diet supplemented with a mixture of eight IAA in free form. However, even though several studies have shown no clear aversion to diets high in specific (combinations of) AA compared to deficient diets (Henry, 1993; Parr et al., 2003; Wessels et al., 2016; Minussi et al., 2024), we cannot exclude that a potentially aversive taste significantly contributed to the low selection of the LP^{+} diet.

Finally, there is evidence in humans and laboratory animals that under stressful conditions, high-carbohydrate, low-protein diets are preferred over low-carbohydrate, high-protein diets due to serotonin (5-HT) mediated effects on mood (see Leigh Gibson 2006 for review). Meals rich in carbohydrates and low in protein raise blood Trp and lower serum large neutral AA concentration via insulin secretion, thereby favouring the Trp transport into the brain and 5-HT synthesis (Fernstrom and Fernstrom, 1995). Possibly, such a shift in preference for different macronutrients also played a role in the piglets in the current study that were exposed to a stressful weaning transition.

While all of the factors described above may potentially have played a role in the overall low consumption of the LP$^+$ diet, they do not explain the differences in the intake of the LP$^+$ diet observed between animals under the high and low sanitary conditions, as illustrated in Fig. 1. The development of the consumption of the LP$^+$ diet in time differed strongly between the two sanitary conditions (Fig. 1, $P < 0.01$ for the interaction SC × Period). Despite the absence of significant interactions between sanitary conditions and choice in any of the response parameters, the percentage of LP$^+$ consumed out of the total intake of LP$^-$ and LP$^+$ doubled from days 1–4 to days 14–18 for the piglets kept under HSC (from 6.5 to 13.2%). For piglets kept under LSC, instead, the percentage decreased by more than seven times from days 1–4 to days 14–18 (from 10.6 to 1.4%). The same patterns were observed for the proportion of intake of Lys from the LP$^+$ diet out of the total Lys intake via the diet. The higher relative intake of the LP$^+$ diet at the start of the experiment for piglets under low compared to high sanitary conditions can be related to their lower absolute feed intake in the first part of the experimental period starting immediately upon arrival at the experimental site.

This result was opposite to our expectations, as we assumed that piglets under LSC would select a diet supplemented with AA in higher amounts compared to piglets under HSC because of their higher AA requirements. Previous studies have found evidence that AA are needed in higher quantities when undergoing a challenge, and in a profile that differs from the one required for maximal growth (Kampman-van de Hoek et al., 2015; Le Floc'h et al., 2004; van der Meer et al., 2016). Supplementing specific AA (Met, Thr, and Trp) to the diet improved performance and resistance to disease after an immune or pathogen challenge or when kept under LSC (van der Meer et al., 2016; Alves da Cunha Valini et al., 2023). Moreover, it has been shown that pigs can select for AA for maximal growth and also for a different AA profile than growth under non-challenged conditions (Minussi et al., 2024). Despite these findings, in the present study, piglets kept under LSC did not choose to consume a diet with higher AA concentrations. They even showed a decreased proportional intake of the AA-enriched diet over time as compared with piglets kept under HSC.

To explain this outcome, the feed intake response of pigs kept under LSC should be examined in the context of a subclinical immune response. There are indications, in fact, that taste and preference for macronutrients can also be affected by an altered immune status (Bernstein et al., 1984; Aubert et al., 1995; Aubert, 1999). An experiment in rats showed that lipopolysaccharide challenged rats displayed less ingestive responses to saccharin and showed an aversive response to bitter taste more rapidly compared to rats in a normal state, indicating that sickness and hedonic processes are related (Aubert, 1999). Another study showed that rats treated with lipopolysaccharide and IL-1b and given the choice between a fat, protein, or carbohydrate-rich food increased their relative intake of carbohydrates, decreased that of protein, while that of fat remained unchanged (Aubert et al., 1995). In line with this, rats allowed to self-select from separate protein and carbohydrate macronutrient sources during illness developed significant aversions to the protein but not the carbohydrate ones (Bernstein et al., 1984). The preference for carbohydrates could be due to the fact that starch and sugars are macronutrients that offer energy more efficiently compared to fat and protein in response to an increased energy demand caused by an immune challenge response (Plata-Salamán, 1996). As glucose requirements are increased during sickness, AA of dietary origin might be oxidised to obtain precursors for glucose formation, and even to a greater extent if AA are presented in free form compared to as slowly digestible protein (Ye et al., 2022). As in the current study, AA were supplemented mainly in their free form and at higher levels in the LP$^+$ diet, higher AA oxidation could have occurred in LSC piglets, making the supplemented AA in the LP$^+$ diet less available to synthetise immune system-related proteins. For these reasons, and by the fact that AA utilisation efficiency is lower in animals under LSC, it is not beneficial to offer piglets the choice to self-select between an AA-deficient diet and one enriched with IAA above requirements in an untargeted manner with the aim of restoring growth performance and support the immune status.

In conclusion, LSC, while decreasing performance and activating the immune system, did not increase the preference for an AA-enriched diet. Thus, offering piglets the choice to self-select between an AA-deficient diet and one supplemented with AA above the assumed requirement did neither restore growth performance nor affect immune status when kept under LSC. Contrary to expectations, piglets increased the preference for an AA-enriched diet under high compared to low sanitary conditions. This indicates that a decline in health status influences diet preference by decreasing AA consumption via the diet despite the assumed increased AA requirements of the animal. Further research should focus on identifying specific AA that target the immune system and on choice-feeding strategies in recovery periods after reduced health status.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101528) can be found at the foot of the online page, in the Appendix section.

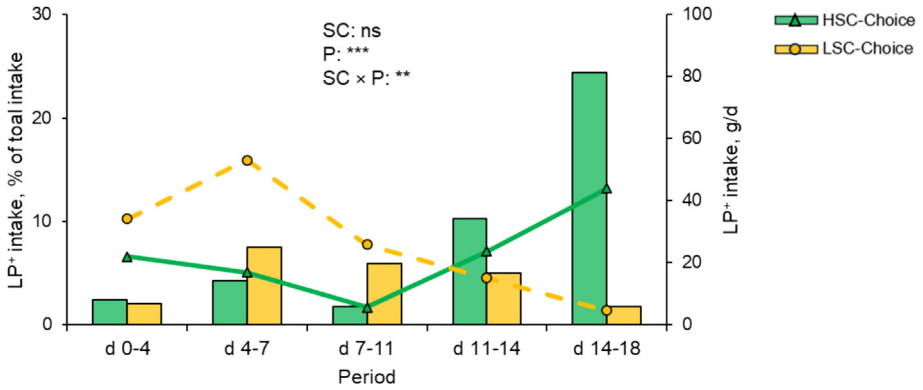

Fig. 1. Intake of an amino acid-enriched low protein diet by piglets offered a dietary choice (Choice) under high (HSC) and low (LSC) sanitary conditions. In the Choice treatment, piglets could choose to consume a low protein diet with Lys, Met, Thr, Trp, Val, Leu, Ile, His either 20% deficient (LP$^-$) or 20% above (LP$^+$) requirements for maximal BW gain (CVB, 2020). The intake of the LP$^+$ diet is shown both as a percentage of the total intake (sum of LP$^+$ and LP$^-$ diets, lines) and as absolute value (bars). The proportion of intake of the LP$^+$ diet was analysed with a linear model including the effect of sanitary conditions (SC), period (P), and interaction SC × P. *** when $P < 0.001$, ** when $P < 0.05$, ns when $P > 0.5$.

Ethics approval

The experiment followed the ARRIVE animal experimentation guidelines and the EU directive 2010/63/EU for animal experiments. The study was authorised by the Dutch Council on Animal Experiments (CCD), and experimental procedures were approved by the Animal Welfare Body of Wageningen University & Research (AVD10400202216386). The animal experiment was conducted at Wageningen University and Research from September to November 2023.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors have no relevant interests to disclose.

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