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Non Ruminant Nutrition

The effect of supplementation of essential amino acid combinations in a low crude protein diet on growth performance in weanling pigs

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ABSTRACT

The present study investigated the impact of providing different supplemental essential amino acids (**EAA**) in a low crude protein (**CP**) diet on growth performance in weanling pigs. A total of 324 mixed-sex 24-d weaned piglets (initial BW 6.9 ± 0.34 kg) were used in a 27-d growth trial with six dietary treatments immediately post-weaning. The first two treatments were a control standard CP (19%) diet (positive control; **PC**) and a negative control (**NC**) diet with low CP (16%) and reduced Ile, Leu, and histidine levels. The rest of the treatments had low CP with varied EAA types and levels; **T1** had similar Ile, Leu, and His levels as **PC** but with low CP (16%), while **T2** had low CP and 10% higher His, Thr, Trp, and Met+Cys compared to PC. The **T3** was a low CP diet with 10% supplemental Leu, Ile, and Val compared to PC, while **T4** was a low CP diet with 10% supplementation with all the EAA except Lys compared to PC. The initial body weight (**BW**) was not statistically different ($P > 0.05$) among the treatments. Also, on d 6, no statistical differences in BW were observed among the treatments. The average BW recorded on d 13, 20, and 27 showed significant treatment differences where the PC had consistently higher BW than all the other treatments ($P < 0.05$). The average daily gain (**ADG**) of the PC was higher than the rest of the treatments. Between d 13 and 20, the average daily feed intake (**ADFI**) for PC was not different from NC and T1 ($P > 0.05$), but compared to T2, T3, and T4, the PC treatment showed a high ADFI ($P < 0.05$). Overall (d 0–27), the ADFI for PC was not different from T1 and was significantly higher than all other treatments. Overall, results showed that the gain to feed (**G:F**) ratio was higher ($P < 0.05$) for PC compared to other dietary treatments. In summary, although the treatments (T1–T4) consisted of varying levels of EAA above the recommended requirement levels for optimal performance, we did not see a significant impact on growth performance improvement, which may indicate that the targeted EAA (His, Val, Thr, Ile, Leu, Trp, and Met) may not have been limiting in these diets. On the other hand, the phenylalanine (**Phe**) requirement may be limited in the current formulations, or perhaps the **EAA**: total N ratio in T1, T2, T3, and T4 may have been too high, resulting in the inefficiency of EAA utilization for growth.

Key words: essential amino acids, growth performance, low crude protein, piglets

INTRODUCTION

In swine, post-weaning diarrhea (PWD) contributes to reduced production efficiency, increased piglet mortality, and reduced profitability (Bikker et al., 2006; de Lange et al., 2010). It has been reported that a significant contributing factor to PWD is the high levels of crude protein (**CP**) inclusion in weanling pig diets which may end up as substrates for microbial fermentation, exacerbating PWD and negatively impacting gut health (Pluske et al., 2002; Houdijk et al., 2007). The need for dietary CP is to meet the AA requirement of pigs for optimal growth performance. Reducing the total dietary CP levels in swine diets while providing essential AA (**EAA**) up to the required level is an effective nutritional strategy to address the issue of PWD caused by high CP diets, as reported extensively (Bikker et al., 2006; Nyachoti et al., 2006; Heo et al., 2008). There is evidence that reducing CP levels in diets (4% lower CP) while supplementing with crystalline EAA has no adverse effect on growth performance (Kerr et al., 1995). Feeding high CP diets result in excess nitrogen excretion in urine and feces, leading to environmental pollution (Bikker et al., 2006; Nyachoti et al., 2006). More recently, studies have suggested that supplementing EAA above recommended requirement for growth could be a way to improve overall intestinal health post-weaning (Yue and Qiao, 2008; Yi et al., 2018; Rodrigues et al., 2021) and reduce the incidence of PWD. This strategy has been reported to drive growth by supplying sufficient AA to support immune function due to post-weaning stress (Le Floc'h et al., 2009; Wellington et al., 2018, 2019). Therefore, supplementing EAA above requirement levels could be used as a nutritional strategy to alleviate the impacts of post-weaning stress on intestinal development and growth.

In the present study, we aimed to explore different combinations of EAA to ascertain their impact on growth when supplemented at 10% above the recommended requirement level during the immediate post-weaning period. The first four limiting EAA [lysine (Lys)], methionine (Met), threonine (Thr), and tryptophan (Trp) are usually balanced via synthetic sources in a low CP diet as recommended (NRC, 2012). At this stage, the research focuses on identifying the next limiting AA and its impact on driving growth in the immediate post-weaning period. The branched-chain AA (**BCAA**) leucine (Leu), isoleucine (Ile), and valine (Val) have been reported to

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play an important role in driving protein synthesis (growth), mainly through the stimulation of the mTOR pathway primarily by the activity of Leu (Suryawan et al., 2011). Since the BCAA are structurally similar and share some steps in their catabolic pathway, an imbalance in either will usually cause an imbalance in the other (Harper, 1984). There is evidence that Val deficiency reduced feed intake, which can be amplified by an oversupply of Leu, leading to even higher catabolism of Val due to the shared pathway (Wiltafsky et al., 2010).

Therefore, we explored the hypothesis that feeding BCAA at 10% above requirement in combination with different EAA will increase growth performance due to reduced antagonism effects among the BCAA. Overall, we aim to evaluate the impact of providing supplemental AA (10% above NRC requirement for growth) in a low CP diet on growth performance in weanling pigs.

MATERIALS AND METHODS

Our internal Animal Welfare Body reviewed the experiment. It was considered a non-animal experiment according to the Dutch Act on Animal experimentation (revision 2014 for implementation of EU Directive 2010/63/EU).

Animals and Housing

A total of 324 mixed-sex piglets (Hypor Libra × Maxter, Hendrix Genetics B.V, Boxmeer, The Netherlands) were used in a 27-d growth performance experiment at the Swine Research Center (Trouw Nutrition, St. Anthonis, The Netherlands). The piglets were weaned at 24 ± 2 d (6.9 ± 0.34 kg) and group-housed (3 pigs per pen) in fully slatted floor pens of 1.5 m × 1.0 m dimensions. The body weight (BW) at weaning was balanced in each pen, and sex was balanced across the treatments. The pens were allocated to the experimental treatments in a randomized complete block design in six rooms. Within each room, there were three replicate pens/treatment, totaling 18 replicate pens per treatment. Feed and water (nipple drinkers) were provided ad libitum throughout the experimental period.

Experimental Diets and Procedures

The present study formulated and produced six experimental diets at the Research Feed Plant (ForFarmers, Heijen, The Netherlands). A wheat-barley-soybean meal-based diet was formulated at a standard 19% CP (Positive Control; **PC**) balanced for all AA (NRC, 2012). A second diet was formulated at 16% CP (Negative Control; **NC**) and contained 10% lower Ile, Arg, His, and Leu. The remaining treatments (T1-T4) were at a low CP (16%) but met all the EAA requirements with supplementation of different EAA combinations. Specifically, **T1** had the same AA profile as the **PC** diet except for a lower CP level (16 vs. 19 %). For the rest of the diets, **T2** had 10% higher His, Thr, Trp, and Met+Cys, while **T3** had 10% higher BCAA (Leu, Ile, and Val), and **T4** had a 10% higher level of all EAA except Lys which remained the same in all diets. Diet compositions can be found in Table 1. The diets were fed as a one-phase diet for 27 days. Diet samples were collected from each treatment and stored until analysis for DM, CP, crude fat, crude fiber, ash, and total AA during the experiment.

Growth Performance Calculation

Individual BW and feed intake were recorded weekly (every 7 days), and feed efficiency was calculated by dividing BW gain by feed intake as recorded every seven days. In case of mortality or culling, the animal was weighed, and BW gain and feed intake were adjusted accordingly.

Analytical Procedures

The dry matter (**DM**) was measured according to method 930.15 (AOAC, 2007), and the Nitrogen (**N**) was determined by the combustion method (method 990.03; LECO FP 528 MI, USA) using the LECO Nitrogen analyzer and CP calculated as N × 6.25. The crude fat was determined as an extraction method 920.39 according to AOAC (AOAC, 2007). The ash content of the diets was measured according to method 942.05 (AOAC, 2007). The Ca and P analyses were completed according to NEN-EN 15550 specifications (2017). This method uses the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method to determine calcium and phosphorus in animal feed after dry ashing. The amino acid analyzer was used to determine the total AA content of the experimental diets according to an in-house method (Nutreco-MasterLabs, Boxmeer, Netherlands) based on NEN-EN-ISO 13903. Briefly, for the determination of AA, the samples are hydrolyzed with hydrochloric acid solution and then evaporated into a film evaporator before being taken up by a buffer solution. Samples are then separated on a cation exchanger with a gradient step and post-column derivatization with ninhydrin; the AA are then measured at 440 nm and 570 nm using a flow colorimeter. All samples were analyzed in duplicates.

Statistical Analysis

Data residuals were tested for normality, and outliers were verified using the studentized residual analysis, with outliers determined as ± three standard deviations from the mean (PROC Univariate, SAS 9.4). The growth performance data were then analyzed as a randomized complete block design with fixed effects of treatments and random effects of the block (initial BW and sex) and room. The repeated measures variable (day) was included in the model to test the interactive impact of day × treatment. Since no significant day × treatment interactions were observed for any experimental parameters, the repeated variable was removed from the model. Significant differences in the model were determined at $P < 0.05$, and trends were identified at $P \leq 0.05$ but $P \leq 0.10$. When significant differences were observed, the Tukey–Kramer mean separation test was used to separate the means.

RESULTS

Analyzed Nutrient Content of Experimental Diets

The analyzed AA contents in the experimental diet are presented in Table 2. The diets were formulated to have different levels of EAA. As indicated in Table 2, although SID values were related to the total values (calculated), we see a consistency in the levels of the varied AA. For example, for NC, the levels of Leu, Ile, and His were formulated to be 10% lower, corresponding to the calculated values in both cases using the PC as the standard. The interpretation of the study results was consequently based on the analyzed AA values.

Growth Performance

The effect of dietary treatment on growth performance is shown in Table 3. Bodyweights, as measured during the

Table 1. The composition of the experimental diets

Ingredients, %	Positive Control (PC)	Negative Control (NC)	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4)
Wheat	33.2	38.04	38.6	38.7	38.8	39.1
Barley	20.00	20.00	20.00	20.00	20.00	20.00
Maize	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	10.00	10.00	10.00	10.00	10.00	9.64
Whey powder	7.00	7.00	7.00	7.00	7.00	7.00
Wheat gluten meal	4.23	0.00	0.00	0.00	0.00	0.00
Soycomil	2.83	1.99	0.71	0.24	0.23	0.00
Wheat bran	2.80	2.80	2.80	2.80	2.80	2.80
Soybean oil	2.61	2.46	2.20	2.18	2.04	2.01
Sugar	2.00	2.00	2.00	2.00	2.00	2.00
Multivitamin premix[1]	1.20	1.20	1.20	1.20	1.20	1.20
Monocalcium phosphate	0.82	0.83	0.84	0.85	0.85	0.86
Salt	0.23	0.24	0.13	0.08	0.10	0.06
L-Lysine HCl 98%	0.80	0.89	0.95	0.97	0.97	1.00
DL-Methionine 99%	0.16	0.28	0.30	0.39	0.31	0.39
L-Valine 96.5%	0.09	0.24	0.25	0.26	0.34	0.35
L-Threonine 98%	0.26	0.35	0.38	0.47	0.39	0.48
L-Histidine HCl 98%	0.07	0.00	0.19	0.26	0.20	0.27
L-Leucine 98.5%	0.05	0.00	0.36	0.39	0.52	0.54
L-Isoleucine 90%	0.04	0.00	0.24	0.25	0.33	0.35
L-Tryptophan 98%	0.04	0.07	0.08	0.11	0.09	0.12
Na Bicarbonate	0.55	0.54	0.70	0.76	0.74	0.80
Organic acid mix[2]	1.60	1.60	1.60	1.60	1.60	1.60
Calculated nutrient levels						
ME, kcal/kg	3365	3323	3312	3308	3309	3305
NE, kcal/kg	2450	2450	2450	2450	2450	2450
Dry matter, %	89.8	89.6	89.6	89.6	89.6	89.6
Crude protein, %	19.1	16.0	16.0	16.0	16.0	16.0
SID Lysine, %	1.30	1.30	1.30	1.30	1.30	1.30
SID Methionine+Cystine, %	0.75	0.75	0.75	0.83	0.75	0.83
SID Tryptophan, %	0.23	0.23	0.23	0.26	0.23	0.26
SID Threonine, %	0.82	0.82	0.82	0.90	0.82	0.90
SID Isoleucine, %	0.68	0.51	0.68	0.68	0.74	0.74
SID Leucine, %	1.30	1.01	1.30	1.30	1.43	1.43
SID Valine, %	0.85	0.85	0.85	0.85	0.93	0.93
SID Arginine, %	0.91	0.77	0.71	0.69	0.69	0.67
SID Histidine, %	0.44	0.32	0.44	0.49	0.44	0.49
SID Phenylalanine, %	0.79	0.61	0.55	0.57	0.56	0.56
Phosphorus, %	0.56	0.56	0.56	0.56	0.56	0.56
Calcium, %	0.59	0.59	0.59	0.59	0.59	0.59
Calcium: Phosphorus	1.06	1.05	1.06	1.06	1.06	1.06
Sum EAA[3], g/kg	83.2	74.8	79.5	81.3	81.9	83.7
Sum NEAA[4], g/kg	103.2	81.3	76.9	75.3	75.3	73.8
Total N, g/kg	186.4	156.1	156.4	156.6	157.2	157.5
EAA: Total N	0.45	0.48	0.51	0.52	0.52	0.53

[1]Supplied per kilogram of complete diet: Vitamin A, 8000 IU; Vitamin D3, 2000 IU; Vitamin E, 30 IU; Vitamin K3-menadione, 1.5 mg; Vitamin B12, 0.03 mg; Thiamine, 1.00 mg; Niacin, 20 mg; Riboflavin, 4 mg; Pantothenate, 13 mg; Folic acid, 0.30 mg; Pyridoxine, 1.0 mg; Iron sulphate, 100 mg; Zinc sulphate, 100 mg; Magnesium oxide, 30 mg; Copper sulphate, 20 mg; Copper chelate, 70 mg; Sodium selenite, 0.30 mg; Iodine, 1 mg.
[2]Organic acid mix: sorbic acid, 0.50%; Citric acid, 0.50%; and formic acid, 0.60%.
[3]EAA: essential amino acid.
[4]NEAA; non-essential amino acids.

Table 2. Analyzed nutrient content of the experimental diets

Nutrients, %	Positive Control (PC)	Negative Control (NC)	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4)
Dry matter	88.4	88.5	88.8	89.1	88.5	88.7
Crude protein	18.9	15.9	16.1	15.9	15.9	16.1
Crude ash	4.5	4.5	4.5	4.4	4.5	4.5
Crude fiber	2.7	2.9	2.7	2.7	2.6	2.4
Crude fat	4.3	4.1	3.9	4.0	4.0	4.1
Arginine	0.93 (0.98)*	0.80 (0.84)	0.75 (0.78)	0.76 (0.76)	0.76 (0.76)	0.72 (0.74)
Cysteine	0.30 (0.34)	0.25 (0.27)	0.24 (0.26)	0.24 (0.26)	0.24 (0.26)	0.24 (0.25)
Phenylalanine	0.82 (0.87)	0.65 (0.69)	0.63 (0.65)	0.63 (0.64)	0.63 (0.63)	0.61 (0.62)
Glycine	0.66 (0.69)	0.55 (0.58)	0.53 (0.55)	0.53 (0.54)	0.53 (0.54)	0.52 (0.53)
Histidine	0.45 (0.48)	0.35 (0.36)	0.46 (0.48)	0.52 (0.53)	0.46 (0.48)	0.50 (0.52)
Isoleucine	0.71 (0.75)	0.55 (0.58)	0.72 (0.74)	0.72 (0.74)	0.78 (0.81)	0.77 (0.81
Leucine	1.31 (1.37)	1.01 (1.08)	1.31 (1.37)	1.33 (1.37)	1.45 (1.50)	1.45 (1.49)
Lysine	1.28 (1.37)	1.33 (1.37)	1.35 (1.37)	1.28 (1.36)	1.30 (1.37)	1.30 (1.36)
Methionine	0.39 (0.45)	0.47 (0.52)	0.47 (0.53)	0.50 (0.61)	0.45 (0.54)	0.52 (0.62)
Met + Cys[1]	0.69 (0.79)	0.72 (0.79)	0.71 (0.79)	0.74 (0.84)	0.69 (0.79)	0.76 (0.87)
Proline	1.39 (1.49)	1.01 (1.10)	0.98 (1.06)	1.02 (1.05)	1.02 (1.05)	1.03 (1.04)
Serine	0.83 (0.86)	0.67 (0.69)	0.64 (0.65)	0.63 (0.63)	0.63 (0.63)	0.62 (0.62)
Threonine	0.84 (0.87)	0.83 (0.86)	0.83 (0.86)	0.88 (0.94)	0.83 (0.86)	0.89 (0.94)
Tyrosine	0.56 (0.59)	0.46 (0.47)	0.44 (0.44)	0.44 (0.43)	0.45 (0.43)	0.43 (0.42)
Valine	0.86 (0.91)	0.83 (0.91)	0.88 (0.90)	0.83 (0.90)	0.95 (0.98)	0.92 (0.96)
Alanine	0.66 (0.71)	0.57 (0.62)	0.55 (0.59)	0.56 (0.57)	0.55 (0.57)	0.55 (0.56)
Aspartic	1.27 (1.37)	1.12 (1.21)	1.05 (1.11)	1.05 (1.07)	1.04 (1.07)	1.00 (1.04)
Calcium	0.58 (0.59)	0.58 (0.59)	0.56 (0.59)	0.57 (0.59)	0.59 (0.59)	0.60 (0.59)
Phosphorus	0.50 (0.56)	0.55 (0.56)	0.50 (0.56)	0.50 (0.56)	0.52 (0.56)	0.51 (0.56)

[1]Met + Cys; Methionine + Cysteine.
*Calculate amino acids (% total) are included in the parenthesis.

experimental period, show that the initial BW (d 0; $P > 0.05$) was not statistically different among the treatments. On day 6, no treatment differences were observed either ($P > 0.05$). However, on d 13, 20, and 27, significant treatment differences were observed ($P < 0.05$), where the PC showed a higher BW compared to all the other treatments ($P < 0.05$) at the specified time points. There were no differences among the treatments at any time ($P > 0.05$). The average daily gain (**ADG**) was not different between d 0 and 6 post-weaning ($P > 0.05$) but showed significant differences ($P < 0.05$) among treatments in the rest of the periods. Between d 6 and 13, the PC showed a higher ADG than NC, T2, T3, and T4, but it was not different from T1. This observation was different between d 13 and 20, where the PC showed a higher ADG than the rest of the treatments (NC, T1, T2, T3, and T4), amongst which there were no significant differences. Between d 20 and 27, the PC was once again higher when compared to the rest of the treatments, but also T1 was more elevated than NC ($P < 0.05$) but not different from T2, T3, and T4 ($P > 0.05$). Overall (d 0–27), the ADG of the PC was higher compared to the other treatments, while there were no differences among the other treatments. The average daily feed intake (**ADFI**) was not different between d 0–6 and d 6–13 ($P > 0.05$). Between d 13 and 20, ADFI for the PC was not different from the NC or T1 ($P > 0.05$) but significantly higher when compared to T2, T3, and T4 ($P < 0.05$). Overall (d 0–27), the ADFI for the PC was not different from T1 but was significantly higher than the other treatments (Table 3). We observed significant treatment effects between periods for gain:feed (G:F). Within the periods (d 6–13, d 13–20, d 20–27, and d 0–27), the data show that the G:F was higher ($P < 0.05$) in the PC compared to the rest of the treatments, while there were no differences among the other treatments ($P > 0.05$).

DISCUSSION

The concept of feeding weanling piglets with low CP diets supplemented with crystalline EAA has been previously investigated, mainly because of the associated adverse effects of high CP diets on diarrhea incidence and the subsequent impact on intestinal health and performance (Pluske et al., 2002; Yue and Qiao, 2008; He et al., 2016). Reducing CP levels in diets by up to 4% while supplementing with EAA has been reported to have no adverse effect on growth performance (Kerr et al., 1995). The present study evaluated the impact of supplemental AA (10% above requirement) in a low CP diet (16 % CP vs. 19% for standard CP) on growth performance and diarrhea incidence in weanling pigs. As shown in Table 2, the analyzed experimental diets showed varying levels of the EAA under investigation; these EAA were included in the diets to meet and exceed NRC dietary requirements at 110% (NRC, 2012). We formulated a low CP diet in experimental treatment 1 (T1) while meeting the recommended EAA requirement equal to the standard CP diet (**PC**). Although the PC and T1 differed by three percentage points in their CP level, both treatments met the

Table 3. Growth performance data[1]

BW, kg	Positive Control (PC)	Negative Control (NC)	Treatment (T1)	Treatment (T2)	Treatment (T3)	Treatment (T4)	SEM	*P*-value
Day 0	6.89	6.86	6.87	6.93	6.90	6.87	0.34	0.4251
Day 6	8.18	8.11	7.99	7.96	7.97	8.03	0.38	0.3381
Day 13	9.94[a]	9.57[b]	9.57[b]	9.34[b]	9.51[b]	9.51[b]	0.42	0.0194
Day 20	12.85[a]	11.93[b]	11.97[b]	11.63[b]	11.97[b]	11.74[b]	0.50	<0.0001
Day 27	16.52[a]	14.76[b]	15.17[b]	14.62[b]	15.01[b]	14.64[b]	0.61	<0.0001
ADG, g								
Day 0–6	214.5	208.6	187.1	170.7	179.1	194.1	14.9	0.1089
Day 6–13	251.6[a]	208.9[b]	220.8[ab]	197.4[b]	219.6[b]	211.9[b]	12.1	0.0290
Day 13–20	419.2[a]	337.3[b]	342.5[b]	328.1[b]	350.3[b]	318.8[b]	17.7	<0.0001
Day 20–27	523.4[a]	404.5[c]	457.0[b]	427.3[bc]	434.8[bc]	410.9[c]	18.9	<0.0001
Overall (0–27)	356.6[a]	292.9[b]	307.2[b]	284.9[b]	300.4[b]	287.8[b]	12.3	<0.0001
ADFI, g								
Day 0–6	159.5	168.4	164.7	143.9	148.9	162.7	10.6	0.3126
Day 6–13	274.8	261.1	271.1	242.9	266.4	270.1	12.4	0.2013
Day 13–20	468.7[a]	427.5[ab]	444.1[ab]	408.8[b]	409.5[b]	409.7[b]	21.9	0.0399
Day 20–27	614.9[a]	518.2[c]	579.4[ab]	545.9[bc]	552.9[bc]	530.3[c]	22.6	0.0008
Overall (d 0–27)	387.6[a]	350.3[b]	372.3[ab]	342.5[b]	344.6[b]	349.9[b]	15.8	0.0238
Gain:feed, g/g								
Day 0–6	1.34[a]	1.22[b]	1.11[c]	1.17[bc]	1.20[b]	1.20[b]	0.03	<0.0001
Day 6–13	0.91[a]	0.81[b]	0.82[b]	0.81[b]	0.83[b]	0.78[b]	0.03	0.0044
Day 13–20	0.89[a]	0.79[b]	0.77[b]	0.80[b]	0.76[b]	0.78[b]	0.02	0.0029
Day 20–27	0.86[a]	0.78[b]	0.79[b]	0.78[b]	0.74[b]	0.78[b]	0.02	0.0356
Overall (d 0–27)	0.92[a]	0.84[b]	0.83[b]	0.83[b]	0.79[b]	0.83[b]	0.02	0.0012

BW, bodyweight; ADG, average daily gain; ADFI, average daily feed intake; SEM, standard error of means.
[1]Data represents LSMEANS of 18 replicate pens per treatment.
[a–c]LSMeans within a row without a common superscript differ at ($P < 0.05$).

recommended SID EAA requirement levels (NRC, 2012) we expected a similar growth performance. The other experimental diets were varied, so specific EAA were increased by 10% above recommended requirement levels. The focus was on the branched-chained AA (Ile, Leu, and Val), as some have suggested that they may be the next limiting AA for growth in the piglet diet (Opapeju et al., 2008). We can confirm that the experimental diets were appropriate and adequately reflected the intended experimental concept based on the analyzed diets compared to the formulated diets. The piglets fed the PC showed a higher growth performance relative to T1. However, both treatments had the same EAA content (as total analyzed and formulated SID levels), except that the PC had a standard CP level and, therefore, higher total nitrogen. This observation contradicts previous studies that reported similar growth performance effects of low CP diets supplemented with EAA compared with a relatively high CP diet (Kerr et al., 1995; Le Bellego et al., 2001; Yue and Qiao, 2008). However, considering that some authors have questioned the possibility of nitrogen becoming limiting in such low CP diets (Gloaguen et al., 2014; Millet et al., 2018; Liu et al., 2019), this observation might confirm that. In the metabolism of AA, the de novo synthesis of non-essential AA (**NEAA**) requires a nitrogen source, such that in cases where there is insufficient dietary free nitrogen; EAA are catabolized to release N, thereby reducing the efficiency of EAA utilization for protein synthesis and growth (Mansilla et al., 2017, 2018). An earlier study reported that at an EAA: total N ratio of 0.48, N retention was not reduced, but when EAA: total N ratio increased to 0.66, N utilization decreased (Heger et al., 1998; Lenis et al., 1999; Wu et al., 2014). Hence, the higher the ratio of EAA: total N, the lower the efficiency of N utilization, particularly in low CP diets (Heger et al., 1998; Lenis et al., 1999; Wu et al., 2014). In the present study, the EAA: total N ratio; was higher than optimal levels in T1–T4, based on the amounts of crystalline AA added to formulate those diets, hence the ratio of EAA: total N was higher than the optimal 0.48 level to ensure efficient N utilization (Heger et al., 1998; Lenis et al., 1999; Wu et al., 2014). Therefore, the efficiency of EAA utilization may have been compromised, possibly explaining the lack of response to the EAA-balanced low CP diets. This is important because when there is optimal protein utilization, EAA will be catabolized to release N necessary for synthesizing non-essential AA de novo (Heger et al., 1998; Lenis et al., 1999). In the other treatments (T2, T3, and T4), no differences were observed for any performance parameters compared to the control T1 with low CP. Although these treatments consisted of varying levels of AA, each at 10% above the requirement for optimal growth, we did not observe any significant impact on the growth performance results, indicating that these targeted EAA (His, Val, Thr, lle, Leu, Trp, and Met) were not limiting in the low CP diets and that the current EAA ratio to Lys as per NRC recommendations, appears sufficient to maintain similar performance among the treatments T1–T4 under unchallenged health conditions. A careful evaluation

of the dietary AA profiles may suggest that phenylalanine (Phe) could be limiting. Because based on the analyzed Phe levels in the diet (Table 2), the PC diet had 0.82% total Phe compared to 0.61–0.65 % total Phe for the rest of the low CP treatments. Phenylalanine, an understudied essential AA, has previously been suggested to become limiting in low-CP diets and was recommended to be investigated together with Tyrosine in low-CP diets (Heger et al., 2003).

CONCLUSION

In summary, the current study presents data on the dynamics of low CP and the supplemental EAA concept in weanling pig diets. It concludes that the lack of treatment effects may be due to either a limiting EAA (Phenylalanine) or the high EAA: total N ratio in the experimental treatments, which compromises the efficient use of EAA for protein synthesis and growth. Further studies are warranted to evaluate these concepts to ensure optimal dietary EAA supply in weanling pigs when fed low CP diets.

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Conflict of Interest

All authors are Trouw Nutrition R&D Swine employees, and we declare no actual or potential conflict of interest, financial, or otherwise.

LITERATURE CITED

AOAC International. 2007. *Official methods of analysis of AOAC International*. 18th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. le Huërou-Luron, J. P. Lallës, and A. Awati. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. *J. Anim. Sci.* 84:3337–3345. doi:10.2527/jas.2006-076.

de Lange, C. F. M., J. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livest. Sci.* 134:124–134. doi:10.1016/j.livsci.2010.06.117.

Gloaguen, M., N. le Floc', E. Corrent, Y. Primot, and J. van Milgen. 2014. The use of free amino acids allows formulating very low crude protein diets for piglets. *J. Anim. Sci.* 92:637–644. doi:10.2527/jas.2013-6514.

Harper, A. E., R. H. Miller, K. P. Block, R. H. Miller, and K. P. Block. 1984. Branched-chain amino acid metabolism. *Annu. Rev. Nutr.* 4:409–454. doi:10.1146/annurev.nu.04.070184.002205.

He, L., L. Wu, Z. Xu, T. Li, K. Yao, Z. Cui, Y. Yin, and G. Wu. 2016. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino Acids*. 48:21–30. doi:10.1007/s00726-015-2059-1.

Heger, J., S. Mengesha, and D. Vodehnal. 1998. Effect of essential:total nitrogen ratio on protein utilization in the growing pig. *Br. J. Nutr.* 80:537–544. doi:10.1017/s0007114598001639.

Heger, J., T. Van Phung, L. Krízová, M. Sustala, and K. Simecek. 2003. Efficiency of amino acid utilization in the growing pig at suboptimal levels of intake: branched-chain amino acids, histidine and phenylalanine + tyrosine. *J. Anim. Physiol. Anim. Nutr.* 87:52–65. doi:10.1046/j.1439-0396.2003.00406.x.

Heo, J.-M., J.-C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. *Arch. Anim. Nutr.* 62:343–358. doi:10.1080/17450390802327811.

Houdijk, J. G. M., F. M. Campbell, P. D. Fortomaris, P. D. Eckersall, and I. Kyriazakis. 2007. Effects of sub-clinical post-weaning colibacillosis and dietary protein on acute phase proteins in weaner pigs. *Livest. Sci.* 108:182–185. doi:10.1016/j.livsci.2007.01.048.

Kerr, B. J., F. K. McKeith, and R. A. Easter. 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. *J. Anim. Sci.* 73:433–440. doi:10.2527/1995.732433x.

Le Bellego, L., J. van Milgen, S. Dubois, and J. Noblet. 2001. Energy utilization of low-protein diets in growing pigs. *J. Anim. Sci.* 79:1259–1271. doi:10.2527/2001.7951259x.

Le Floc'h, N., L. LeBellego, J. J. Matte, D. Melchior, and B. Sève. 2009. The effect of sanitary status degradation and dietary tryptophan content on growth rate and tryptophan metabolism in weaning pigs. *J. Anim. Sci.* 87:1686–1694. doi:10.2527/jas.2008-1348.

Lenis, N. P., H. T. van Diepen, P. Bikker, A. W. Jongbloed, and J. van der Meulen. 1999. Effect of the ratio between essential and nonessential amino acids in the diet on utilization of nitrogen and amino acids by growing pigs. *J. Anim. Sci.* 77:1777–1787. doi:10.2527/1999.7771777x.

Liu, H., L. Wu, H. Han, Y. Li, L. Wang, J. Yin, W. Fan, M. Bai, J. Yao, X. Huang, et al. 2019. Reduced dietary nitrogen with a high Lys:CP ratio restricted dietary N excretion without negatively affecting weaned piglets. *Anim. Nutr.* 5:115–123. doi:10.1016/j.aninu.2019.01.001.

Mansilla, W. D., K. E. Silva, C. L. Zhu, C. M. Nyachoti, J. K. Htoo, J. P. Cant, and C. F. M. de Lange. 2017. Ammonia nitrogen added to diets deficient in dispensable amino acid nitrogen is poorly utilized for urea production in growing pigs. *J. Nutr.* 147:2228–2234. doi:10.3945/jn.117.251314.

Mansilla, W. D., K. E. Silva, C. Zhu, C. M. Nyachoti, J. K. Htoo, J. P. Cant, and C. F. M. de Lange. 2018. Ammonia-nitrogen added to low-crude-protein diets deficient in dispensable amino acid-nitrogen increases the net release of alanine, citrulline, and glutamate post-splanchnic organ metabolism in growing pigs. *J. Nutr.* 148:1081–1087. doi:10.1093/jn/nxy076.

Millet, S., M. Aluwé, J. De Boever, B. De Witte, L. Douidah, A. V. Broeke, F. Leen, C. De Cuyper, B. Ampe, and S. De Campeneere. 2018. The effect of crude protein reduction on performance and nitrogen metabolism in piglets (four to nine weeks of age) fed two dietary lysine levels. *J. Anim. Sci.* 96:3824–3836. doi:10.1093/jas/sky254.

NRC. 2012. *Nutrient requirements of swine*. Washington, DC: The National Academies Press.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* 84:125–134. doi:10.2527/2006.841125x.

Opapeju, F. O., M. Rademacher, G. Blank, and C. M. Nyachoti. 2008. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. *Animal*. 2:1457–1464. doi:10.1017/S175173110800270X.

Pluske, J. R., D. W. Pethick, D. E. Hopwood, and D. J. Hampson. 2002. Nutritional influences on some major enteric bacterial diseases of pig. *Nutr. Res. Rev.* 15:333–371. doi:10.1079/nrr200242.

Rodrigues, L. A., M. O. Wellington, J. C. González-Vega, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2021. Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella typhimurium. *J. Anim. Sci.* 99:skaa365. doi:10.1093/jas/skaa365.

Suryawan, A., R. A. Orellana, M. L. Fiorotto, and T. A. Davis. 2011. Triennial growth symposium: Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs. *J. Anim. Sci.* 89:2004–2016. doi:10.2527/jas.2010-3400.

Wellington, M. O., A. K. Agyekum, K. Hamonic, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2019. Effect of supplemental threonine above requirement on growth performance of Salmonella typhimurium challenged pigs fed high-fiber diets. *J. Anim. Sci.* 97:3636–3647. doi:10.1093/jas/skz225.

Wellington, M. O., J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2018. Impact of dietary fiber and immune system stimulation on threonine requirement for protein deposition in growing pigs. *J. Anim. Sci.* 96:5222–5232. doi:10.1093/jas/sky381.

Wiltafsky, M. K., M. W. Pfaffl, and F. X. Roth. 2010. The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. *Br. J. Nutr.* 103:964–976. doi:10.1017/s0007114509992212.

Wu, G., F. W. Bazer, Z. Dai, D. Li, J. Wang, and Z. Wu. 2014. Amino acid nutrition in animals: protein synthesis and beyond. *Annu. Rev. Anim. Biosci.* 2:387–417. doi:10.1146/annurev-animal-022513-114113.

Yi, D., B. Li, Y. Hou, L. Wang, D. Zhao, H. Chen, T. Wu, Y. Zhou, B. Ding, and G. Wu. 2018. Dietary supplementation with an amino acid blend enhances intestinal function in piglets. *Amino Acids.* 50:1089–1100. doi:10.1007/s00726-018-2586-7.

Yue, L. Y., and S. Y. Qiao. 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. *Livest. Sci.* 115:144–152. doi:10.1016/j.livsci.2007.06.018.