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Abstract

The aim of this experiment was to evaluate the effects of low crude protein (CP) level with essential amino acids (AA) addition on growth performance, nutrient digestibility, microbiota, and volatile fatty acid composition in growing pigs. A total of 160 growing pigs (LYD; average initial body weight 16.68 ± 0.12 kg) were randomly allotted to one of the four treatments on the basis of initial BW. A randomized complete block design was used to conduct this experiment in the Research Center of Animal Life Sciences at Kangwon National University. There were ten pigs/replicate with four replicates in each treatment. The treatments include; CON (Control, 17.2% dietary CP level), low protein (LP)-1.10 (15.7% dietary CP level + 1.10% lysine level), LP-1.15 (15.7% dietary CP level + 1.15% lysine level), LP1.2 (15.7% dietary CP level + 1.20% lysine level). The pigs fed CON and LP-1.2 diet showed greater final body weight than that of LP-1.1 diet ($p<0.05$). Although average daily gain, average daily feed intake, and feed efficiency did not show any difference in phase 2 and 3, average daily gain and feed efficiency was significantly greater in CON and LP-1.20 in phase 1. However, the average daily feed intake did not show any difference during the experimental period. Isobutyric acid and isovaleric acid composition of LP treatments were lower than CON treatment in phase 2. Total branched chain fatty acid composition was significantly lower in LP treatment in phases 1 and 2. However, there was no significant difference among treatments in phase 3. The results of this study underscore the importance of AA supplementation when implementing a low-protein diet during the early growth phase (16-50 kg) in pigs.

Keywords: pig, crude protein, amino acid, growth performance, volatile fatty acid

Introduction

Reducing crude protein (CP) might be effective to mitigate environmental and economic problems in swine production. Swine producers are able to lower the level of dietary CP when the diets satisfy the pig requirement for total nitrogen and essential amino acids (AA) [1]. Soybean meal is commonly added to corn-soybean meal feed to increase the lysine content, as corn contains lower levels of lysine [2]. However, excessive protein intake can lead to undigested AA and nitrogen being excreted in feces, resulting in decreased nitrogen utilization and protein fermentation in the hindgut, which can negatively impact intestinal health. Therefore, there is a growing trend towards reducing dietary CP levels and supplementing synthetic AA to meet the pig's nutritional needs [3-6].

Recent studies have shown that reducing dietary CP levels by 4% with limited amino acids, such as lysine, tryptophan, threonine, and methionine, does not affect the growth performance of growing and finishing pigs [7-9]. However, other studies have shown that reducing dietary CP levels by 4% with limited amino acid supplementation can have a negative impact on growth performance, particularly in younger pigs [10]. Additionally, reducing dietary CP levels by 5% can impair the growth performance of growing pigs due to essential AA deficits [11]. Dietary CP levels have also been found to affect microbial communities, with low levels decreasing pathogen activity in the intestine without affecting beneficial bacteria. Moreover, reducing dietary CP levels and supplementing synthetic AA may decrease odor emission by reducing branched-chain volatile fatty acid metabolism in the hindgut [14]. In light of these findings, this study aimed to evaluate the effects of low CP levels with essential AA supplementation on the growth performance, nutrient digestibility, microbiota, and volatile fatty acid (VFA) composition of growing pigs.

Materials and Methods

Animals and Experimental Design

A total of 160 growing pigs (LYD; average initial body weight 16.68 ± 0.12 kg) were randomly allotted to one of the four treatments on the basis of initial BW. A randomized complete block design was used to conduct this study in the Research Center of Animal Life Sciences at Kangwon National University. There were ten pigs per replicate, with four replicates for each treatment. The treatments

were CON (17.2% dietary CP level), LP-1.10 (15.7% dietary CP level + 1.10% Lys level), LP-1.15 (15.7% dietary CP level + 1.15% Lys level), LP-1.20 (15.7% dietary CP level + 1.20% Lys level). The experimental diets were supplemented for 52 days in three phases; phase 1 (day 0–14), phase 2 (day 15–28), phase 3 (day 29–42). The pigs were grouped in partially slatted concrete floor pens 2.80 m × 5.00 m in size. All pens contained a self-feeder and nipple drinker to allow *ad libitum* access to feed and water. The diets were formulated to provide all the nutrients that met or exceeded the nutrient requirements listed in the NRC [15], with the exception of Ca (Table 1).

Growth Performance

The body weights of all the pigs were measured at the end of each phase. The amount of feed supplemented was measured throughout the experimental period o calculate the average daily feed intake. The average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G/F) were calculated at the end of each phase.

Nutrient Digestibility

The effects of dietary CP and AA supplementation on nutrient digestibility were determined as follows: pigs were fed a diet containing 2.5 g Cr_2O_3/kg for seven days before sampling, and fecal samples were collected for four days before sampling. In this trial, we evaluated dry matter (DM), gross energy (GE), and CP digestibility. Prior to fecal sample collection, the floor was cleaned to avoid contamination, and fecal samples were retrieved and placed in vacuum-sealed plastic bags. Fecal samples were stored in a freezer at −20℃ to preserve the state until analyzed. Samples were thawed, dried at 60 ℃ for 72 h in a forced-air oven, grounded in a 1-mm screen Wiley mill (Thomas Model 4 Wiley Mill; Thomas Scientific, Swedesboro, NJ, USA), and analyzed to calculate digestibility. Each fecal sample was analyzed in quadruplicate for DM (Method 930.15), CP (Method 990.03) according to AOAC methodology [16]. A bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL, USA) was used to analyze gross energy.

Fecal Microflora DNA

At the end of each phase, pigs were selected based on their average body weight for each treatment, and samples were collected via gentle rectal massage. The samples were immediately kept in liquid nitrogen and moved to a deep freezer at −80℃ until analysis. DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (cat. No. 51604 2016; QIAGEN, Hilden, Germany). The fecal sample (200 mg) was weighed in a 2 mL centrifuge tube and kept on ice. To ensure the highest possible DNA concentration in the final eluate, 1 mL of InhibitEX Buffer was added to each sample and vortexed continuously for 1 min until the sample was thoroughly homogenized. The samples were incubated in a 70℃ water bath for 5 min and vortexed for 15 s to achieve consistent lysis. The samples were then centrifuged at 20,000 × *g* and 14,000 rpm for 1 min to pellet the feces. Secondly, 25 µL of proteinase K and 600 µL of the first sample`s supernatant were combined in a fresh 2 mL centrifuge tube. Next, 600 µL of Buffer AL was added and vortexed for 15 s to create a homogeneous solution that was incubated for 10 min at 70℃ and centrifuged briefly to eliminate drops inside the tube lid. The lysate was mixed with 600 µL of ethanol (96%) and vortexed. In the QIAamp spin column, the lysate (600 µL) was added to a QIAamp spin column and centrifuged at 20,000 × g" and 14,000 rpm for 1 min. The QIAamp spin column was moved to a new collection tube, and the former tube was removed. QIAamp spin column was carefully opened, and 500 µL of Buffer AW1 was added and centrifuged at 20,000 × *g* and 14,000 rpm for 1 min. The QIAamp spin column was stored in a new collection tube. Subsequently, 500 µL of Buffer AW1 was added to the QIAamp spin column and centrifuged for 3 min at 20,000 × *g* and 14,000 rpm. Finally, the QIAamp spin column was moved into a new 2 mL centrifuge tube, and 200 µL of Buffer ATE was mixed directly onto the QIAamp membrane, kept for 1 min at room temperature, then centrifuged at 20,000 × *g* and 14,000 rpm for 1 min to elute the DNA, which was then later quantified using a spectrophotometer. The levels of fecal microflora, such as *Lactobacillus* spp., *Bifidobacterium spp*., *Clostridium spp*., and *E. col.*, were estimated using the methodology of Habeeb et al. [17].

Volatile fatty acids

Samples from pigs that were chosen based on the average body weight of each pen to minimize errors were collected (d 14, 28, and 42) directly through rectal massage to estimate VFA concentrations in feces. Fecal samples were immediately stored in collection tubes and placed on ice.

VFA concentrations in the feces were estimated using gas chromatography (HP 6890 Plus; Hewlett Packard, Houston, TX, USA) according to the method of Jeon et al. [18].

Statistical Analysis

The collected data from this experiment were analyzed using the Analysis of Variance (ANOVA), implemented through the General Linear Model (GLM) procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). For assessing growth performance, the initial BW was employed as a covariate, but was omitted from the model if it proved insignificant. Each pig served as an experimental unit for parameters such as growth performance, feed consumption, nutrient digestibility, blood electrolyte equilibrium, and bone measurements. The Tukey mean comparison test was utilized for treatment mean separation, with a significance level set at $p < 0.05$. Any probability below 0.1 was recognized25 as a trend.

Results

Growth performance

The effects of dietary CP and AA levels on growth performance are shown in Table 2. Pigs fed the control and LP-1.20 diet showed greater final body weight than those fed the LP-1.1 diet. Although ADG, ADFI, and G/F did not differ in phases 2 and 3, ADG and G/F were significantly greater in the CON and LP-1.20 in phase 1. However, ADFI showed no difference during the experimental period.

Nutrient digestibility

The effects of dietary CP and AA levels on nutrient digestibility are presented in Table 3**.** DM, ME, and CP digestibility were calculated to evaluate the effects of crude protein and amino acid level in the diet. In phase 1, CP digestibility was higher in the LP-1.2 than LP-1.1. However, there was no significant difference between treatments. In this study, DM, ME, and CP digestibility showed no significant differences among the treatments in the phase 2 and 3.

Microflora

The effects of dietary CP and AA levels on the microflora quantity are shown in Table 4. The content of *lactobacillus* spp., *bifidobacterium* spp., *E.coli*, *Clostridium* in the feces were analyzed to evaluate the effects of crude protein and amino acid level in the diet. Although *lactobacillus* spp. was increased and *clostridium* was decreased in the LP-1.2 than LP-1.1 in phase 1, there was no significant difference among the treatments. In the phase 2 and 3, the content of microflora didn`t show difference among the various treatments.

Volatile fatty acids

The effect of dietary CP and AA level on VFA is shown in Table 5. Isobutyric acid and isovaleric acid compositions of LP treatments were lower than those of CON in phase 2. The total branched-chain fatty acid composition was significantly lower in the LP treatment in phases 1 and 2. However, there were no significant differences among the treatments in phase 3.

Discussion

The present study investigated the effects of varying levels of dietary CP and AA supplementation on the growth performance, nutrient digestibility, microbiota, and VFA concentrations in growing pigs. The results indicate that reducing the CP level by 1.5% with a balanced supply of essential AA did not significantly affect the growth performance of pigs, but it did lead to a significant reduction in some branched-chain fatty acids. According to Kerr et al. [3], a low-CP diet, reduced by 2-4%, with the addition of a limited amount of AA, such as lysine, threonine, methionine, and tryptophan, is a viable option for pigs. However, an excessive decrease in dietary CP may hinder pig growth performance [19-21]. In our study, pigs that consumed low-protein diets and received additional essential AA, such as Lys, Thr, and Met, demonstrated growth performance comparable to that of the CON group during the trial period.

This study found that pigs fed the LP diet had poorer ADG and feed efficiency in phase 1 compared to those fed the CON diet, but no significant differences were observed in phases 2 and 3. These results suggest that pigs require a higher nitrogen intake for protein deposition, and the requirements for the first five essential AA are less well-defined in the early growth phase compared to the later phases. This finding is consistent with previous studies that have shown pigs to be more sensitive to dietary CP levels during the growing phase than the finishing phase [22,23]. However, nitrogen retention was observed during the finishing phase [24]. The LP diet contained a higher proportion of corn than the CON diet, resulting in an increased availability of starch, which may explain the compensatory growth observed in the finishing phase. Starch is known to be more efficient for fat deposition than protein [25]. No significant differences in growth performance were observed between the dietary treatments in each growth phase. However, it is possible that the nutrient supply, particularly non-essential AA in LP diets, may be insufficient for protein deposition in rapidly growing pigs, resulting in a lower growth rate compared to pigs fed high-protein diets [26].

Although this study reduced the CP concentration in pig feed by decreasing the soybean meal content, nutrient digestibility was not affected when dietary CP was decreased to 1.5%. The main factors affecting protein digestibility are the levels and balance of essential AA and animal requirements [8]. In this study, the levels (%) of Lys, Met, and Thr were similar for different CP treatments, indicating that the levels of limited AAs were not affected by reducing CP levels. The

digestibility of CP remained unchanged despite the reduction in dietary CP levels. When the limiting AA levels are constant, a decrease in CP levels can potentially lead to a more balanced AA composition compared to elevated CP levels. Ball [27] discovered a reduction in energy digestibility as CP levels decreased in diets featuring 6.9 g/kg of readily available lysine. Zervas and Zijlstra [28] echoed this finding, attributing it to the diminished fiber content in diets rich in protein [29]. The influence of CP level on nutrient digestibility warrants consideration since a decrease in digestibility could lower the slurry DM concentration, subsequently resulting in a surge in slurry output [30].

Portune et al. [31] showed a significant correlation between gut microbiota and the metabolism of dietary proteins. Undigested proteins in the gut provide nitrogen for saccharolytic bacterial growth and AA for fermentation by asaccharolytic species. The mammalian intestine harbors a multitude of microbial strains, numbering over 10^{14} microbial cells. These microorganisms play pivotal roles in the host's physiology and metabolism. The fermentation process of undigested dietary proteins can foster the growth of protein-fermenting bacteria, thereby suggesting that the origin, quality, and volume of dietary protein can have a bearing on microbial communities. Research indicates that the level of dietary CP exerts a more profound effect on the composition of gut microbiota compared to its origin [32]. In the case of weaned piglets, a decrease in dietary CP led to a reduction in the *Clostridium* count, however, it didn't affect the total bacteria, *Lactobacilli*, *Enterobacteria*, and *Bacteroides*. Nonetheless, alterations in the dietary CP content didn't significantly impact bacterial communities in any part of the intestine under normal physiological circumstances, as the microbiota possesses a certain degree of adaptability. The outcomes of studies examining the influence of dietary CP levels on the microbiota composition are inconsistent, possibly due to the limitations of conventional culture-dependent or low-throughput culture-independent methodologies.

Canh et al. [33] stated that fermentable non-starch polysaccharides are the primary dietary components that affect the VFA concentrations in manure. Most VFAs in manure consist of short straight-chain VFAs such as acetic, propionic, and butyric acids, which account for 91% of the total VFA content. This was consistent with the findings of Otto et al. [34] and Le et al. [35]. They proposed that branched-chain VFAs are only produced during protein metabolism, which could explain the slight increase in isobutyric and isopentanoic acid concentrations in manure as dietary CP levels increased from 12% to 18%, although these changes were not statistically significant.

In summary, a reduction of 1.5% in protein levels by 1.5% with a balance of essential AA did not significantly affect on the growth of pigs; however, it did result in a significant reduction in some branched-chain fatty acids. In the case of lower protein diets, supplementation with AA balance led to increased body weight gain during the 16–50 kg phase, whereas not supplementing with AA during the same phases led to reduced growth performance. In this study, the nutrient digestibility and microbiota of pigs fed diets with different levels of CP were not affected. These results imply that a low-protein diet may be a viable choice when the AA composition is well-balanced.

Acknowledgments

References

1. Gloaguen M, Le Floc'H N, Corrent E, Primot Y, van Milgen J. The use of free amino acids allows formulating very low crude protein diets for piglets. J Anim Sci. 2014;92:637–44. https://doi.org/10.2527/jas.2013-6514

2. Houmard NM, Mainville JL, Bonin CP, Huang S, Luethy MH, Malvar TM. Highlysine corn generated by endosperm-specific suppression of lysine catabolism using RNAi. Plant Biotechnol J. 2007;5:605–14. https://doi.org/10.1111/j.1467-7652.2007.00265.x

3. Kerr BJ, Southern LL, Bidner TD, Friesen KG, Easter RA. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. J Anim Sci. 2003;81:3075–87. https://doi.org/10.2527/2003.81123075x

4. Nyachoti CM, Omogbenigun FO, Rademacher M, Blank G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J Anim Sci. 2006;84:125–34. https://doi.org/10.2527/2006.841125x

5. Yue L, Qiao S. 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. 2008;115:144–52. https://doi.org/10.1016/j.livsci.2007.06.018

6. Fan P, Liu P, Song P, Chen X, Ma X. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. Sci Rep-Uk. 2017;7:43412. https://doi.org/10.1038/srep43412

7. Kerr BJ, Mckeith FK, Easter RA. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J Anim Sci. 1995;73(2):433–40. https://doi.org/1 0.2527/1995.732433x.

8. Jin CF, Kim JH, Han IK, Bea SH. Effects of supplemental synthetic amino acids to the low protein diets on the performance of growing pigs. Asian Australas J Anim Sci. 1998;11(1):1–7. https://doi.org/10.5713/ajas.1998.1.

9. Qin CF, Huang P, Qiu K, Sun WJ, Xu L, Zhang X, et al. Influences of dietary protein sources and crude protein levels on intracellular free amino acid profile in the longissimus dorsi muscle of finishing gilts. J Anim Sci Biotechnol. 2015;6(1):52. https://doi.org/10.1186/s40104-015-0052-x.

10. Yi XW, Zhang SR, Yang Q, Yin HH, Qiao SY. Influence of dietary net energy content on performance of growing pigs fed low crude protein diets supplemented with crystalline amino acids. J Swine Health Prod. 2010;18(6): 294–300. https://www.aasv.org/shap/issues/v18n6/v18n6p294.pdf

11. Figueroa JL, Lewis AJ, Miller PS, Fischer RL, Gómez RS, Diedrichsen RM. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. J Anim Sci. 2002;80(11):2911–9. https://doi.org/10.2527/2002.80112911x.

12. Libao-Mercado AJO, Zhu CL, Cant JP, Lapierre H, Thibault J, Sève B, et al. Dietary and endogenous amino acids are the main contributors to microbial protein in the upper gut of normally nourished pigs. J Nutr. 2009; 139:1088–94. https://doi.org/10.3945/jn.108.103267

13. Pieper R, Kröger S, Richter JF, Wang J, Martin L, Bindelle J, Htoo JK, Smolinski D, Vahjen W, Zentek J, Kessel AGV. Fermentable fiber ameliorates fermentable protein-induced changes in microbial ecology, but not the mucosal response, in the colon of piglets. J Nutr. 2012;142:661–7. https://doi.org/10.3945/jn.111.156190

14. Le PD, Aarnink AJA, Jongbloed AW, Vander Peet-Schwering CMC, Ogink NWM, Verstegen MWA. Effects of dietary crude protein level on odour from pig manure. Animal. 2007;1(5):734-744. https://doi.org/10.1017/S1751731107710303

15. National Research Council. Nutrient requirements of swine. 2012.

16. AOAC. Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC; 2012. 121-130.

17. Tajudeen H, Mun J, Ha S, Hosseindoust A, Lee S, Kim J. Effect of wild ginseng on the laying performance, egg quality, cytokine expression, ginsenoside concentration, and microflora quantity of laying hens. J Anim Sci Technol. 2023;65(2):351-364. https://doi.org/10.5187/jast.2022.e108

18. Jeon SM, Hosseindoust A, Choi YH, Kim MJ, Kim KY, Lee JH, Kil DY, Kim BG, Chae BJ. Comparative standardized ileal amino acid digestibility and metabolizable energy contents of main feed ingredients for growing pigs when adding dietary β-mannanase. Anim Nutr. 2019;5(4):359-365. https://doi.org/10.1016/j.aninu.2019.07.001

19. Morales A, Buenabad L, Castillo G, Arce N, Araiza BA, Htoo JK, Crevantes M. Low protein amino acid-supplemented diets for growing pigs: effect on expression of amino acid transporters, serum concentration, performance, and carcass composition. J Anim Sci. 2015;93:2154–64. https://doi.org/10.2527/jas.2014-8834

20. Mansilla WD, Htoo JK, de Lange CFM. Nitrogen from ammonia is as efficient as that from free amino acids or protein for improving growth performance of pigs fed diets deficient in nonessential amino acid nitrogen. J Anim Sci. 2017;95:3093–102. https://doi.org/10.2527/jas.2016.0959

21. Nemechek JE. Evaluation of compensatory gain, standardized ileal digestible lysine requirement, and replacing specialty protein sources with crystalline amino acids on growth performance of nursery pigs [Ph.D. dissertation]. Manhattan, MS. Kansas State Univ; 2011.

22. Hinson RB, Schinckel AP, Radcliffe JS, Allee GL, Sutton AL, Richert BT. Effect of feeding reduced crude protein and phosphorus diets on weaning-finishing pig growth performance, carcass characteristics, and bone characteristics. J. Anim. Sci. 2009;87:1502–1517. https://doi.org/10.252

23. Prandini A, Sigolo S, Morlacchini M, Grilli E, Fiorentini L. Microencapsulated lysine and low-protein diets: effects on performance, carcass characteristics and nitrogen excretion in heavy growing–finishing pigs. J. Anim. Sci. 2013;91:4226–4234. https://doi.org/10.2527/j

24. Fabian J, Chiba LI, Frobish LT, McElhenney WH, Kuhlers DL, Nadarajah K. Compensatory growth and nitrogen balance in grower-finisher pigs. J. Anim. Sci. 2004:82:2579–2587. https://doi.org/10.2527/2004.829

25. van Milgen J, Noblet J, Dubois S. Energetic efficiency of starch, protein and lipid utilization in growing pigs. J. Nutr. 2001;131(4):1309-1318. https://doi.org/10.1093/jn/131.4.1309

26. Ruusunen M, Partanen K, Pösö R, Puolanne E. The effect of dietary protein supply on carcass composition, size of organs, muscle properties and meat quality of pigs. Livest. Sci. 2007;107(2-3):170. https://doi.org/10.1016/j.livsci.2006.09.021

27. Ball MEE, Magowan E, McCracken KJ, Beattie VE, Bradford R, Gordon FJ, Robinson MJ, Smyth S, Henry W. The effect of level of crude protein and available lysine on finishing pig performance, nitrogen balance and nutrient digestibility. Asian-Australas J Anim Sci. 2013;26(4):564. 10.5713/ajas.2012.12177

28. Zervas S, Zijlstra RT. Effects of dietary protein and fermentable fibre on nitrogen excretion patterns and plasma urea in grower pigs. J Anim Sci. 2002;80(12):3247–3256. https://doi.org/10.2527/2002.80123247x

29. Just A, Fernandez JA, Jorgensen H. The net energy value of diets for growth in pigs in relation to the fermentative processes in the digestive tract and the site of absorption of the nutrients. Livest Prod Sci. 1983;10:171–186. https://doi.org/10.1016/0301-6226(83)90033-7

30. McCann MEE, Magowan E, Beattie VE, McCracken KJ, Bradford R, Gordon FJ, Robinson MJ, Mayne CS. The effect of crude protein and lysine level in diets for finishing pigs on nutrient digestibility and nitrogen balance. Proc Br Soc Anim Sci. 2007;82. https://doi.org/10.1017/S1752756200019852

31. Portune KJ, Beaumont M, Davila AM, Tomé D, Blachier F, Sanz Y. Gut microbiota role in dietary protein metabolism and health-related outcomes: the two sides of the coin. Trends Food Sci Technol. 2016;57:213-232. https://doi.org/10.1016/j.tifs.2016.08.011

32. Rist VTS, Weiss E, Eklund M, Mosenthin R. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 2013;7(7):1067–1078. https://doi.org/10.1017/S1751731113000062

33. Canh TT, Sutton AL, Aarnink AJA, Verstegen MWA, Schrama JW, Bakker GCM. Dietary carbohydrates alter faecal composition and pH and ammonia emission from slurry of growing pigs. J Anim Sci. 1998;76:1887-1895. https://edepot.wur.nl/201622#page=47

34. Otto ER, Yokoyama M, Von Bermuth RD, Van Kempen T, Trottier NL. Ammonia, volatile fatty acids, phenolics and odour offensiveness in manure from growing pigs fed diets reduced in

protein concentration. J Anim Sci. 2003;81(7):1754-1763. https://doi.org/10.2527/2003.8171754x

35. Le PD, Aarnink AJA, Ogink NWM, Verstegen MWA. Effects of environmental factors on odor emission from pig manure. Trans ASABE. 2005;48(2):757-765. https://elibrary.asabe.org/abstract.asp?aid=18318

348 # Tables and Figures

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Table 1. Ingredient and calculated composition of experimental diets (as-fed diets).

CP, %	17.2		15.7	
Lysine, %	1.10	1.10	1.15	1.20
Ingredients composition, %				
Corn	56.72	60.55	60.52	60.51
Bakery by product	5.00	5.00	5.00	5.00
Molasses	2.00	2.00	2.00	2.00
Soybean meal	22.75	18.40	18.09	17.81
DDGS	7.00	7.00	7.00	7.00
Animal fat	3.65	3.68	3.71	3.74
Salt	0.40	0.40	0.40	0.40
TCP	0.90	0.94	0.95	0.95
Limestone	0.62	0.60	0.60	0.60
Lysine (78%)	0.34	0.49	0.56	0.64
Tryptophan (100%)	0.15	0.37	0.50	0.61
Threonine (98.5%)	0.09	0.15	0.20	0.24
Methionine (99.5%)	0.08	0.12	0.16	0.20
Choline chloride	0.05	0.05	0.05	0.05
Mineral premix[1]	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.10	0.10	0.10
Phytase	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00
Calculated composition, %				
ME, kcal/kg	3,400	3,400	3,400	3,400
CP	17.20	15.70	15.70	15.70
EE	7.01	7.13	7.16	7.19
Lysine	1.10	1.10	1.15	1.20
Methionine + cysteine	0.59	0.59	0.63	0.66
Threonine	0.63	0.63	0.67	0.70
Tryptophan	0.18	0.18	0.19	0.20
Valine	0.73	0.66	0.65	0.65
Ca	0.63	0.62	0.62	0.62
P	0.59	0.58	0.58	0.58

[1]Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

[2]Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin D3, 40 IU vitamin E, 5.0 mg vitamin K3, 5.0 mg vitamin B_1, 20 mg vitamin B_2, 4 mg vitamin B_6, 0.08 mg vitamin B_{12}, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.

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Table 2. The effects of CP and AA level on growth performance in growing pigs.

CP, %	17.2	15.7			SEM	p-value
Lysine, %	1.10	1.10	1.15	1.20		
BW, kg						
Initial	16.78	16.71	16.69	16.69	0.11	0.827
Final	51.43[a]	48.72[c]	49.84[bc]	50.75[ab]	0.51	0.001
Phase 1 (d 0-14)						
ADG, kg	812[a]	638[b]	707[ab]	763[a]	38.31	0.004
ADFI, kg	1,552	1,433	1,488	1,452	64.75	0.316
G/F	0.524[a]	0.445[b]	0.475[ab]	0.526[a]	0.02	0.012
Phase 2 (d 15-28)						
ADG, kg	827	816	825	831	47.53	0.991
ADFI, kg	1,656	1,653	1,665	1,657	50.28	0.995
G/F	0.499	0.493	0.494	0.502	0.02	0.953
Phase 3 (d 28-42)						
ADG, kg	837	832	836	0.839	23.48	0.993
ADFI, kg	1,634	1,611	1,616	1,631	15.83	0.414
G/F	0.512	0.517	0.517	0.514	0.02	0.987
Overall (d 0-42)						
ADG, kg	825[a]	762[c]	789[bc]	811[ab]	10.64	<0.001
ADFI, kg	1,614	1,566	1,590	1,580	23.06	0.251
G/F	0.511[a]	0.487[b]	0.497[ab]	0.513[a]	0.01	0.010

CP, crude protein; AA, amino acid; SEM, standard error of means; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, feed efficiency.

[a-b]means different superscript letters indicate significant differences ($p<0.05$).

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Table 3. The effects of CP and AA level on nutrient digestibility in growing pigs.

CP, %	17.2	15.7			SEM	p-value
Lysine, %	1.10	1.10	1.15	1.20		
Phase 1 (d 14)						
DM	84.50	85.00	83.98	84.85	0.85	0.652
ME	80.22	79.71	79.12	79.50	0.71	0.494
CP	80.07	79.51	78.44	80.48	1.41	0.524
Phase 2 (d 28)						
DM	84.36	84.24	83.39	84.21	0.65	0.443
ME	79.24	79.52	78.47	79.36	1.12	0.794
CP	79.48	78.21	79.17	79.29	1.64	0.867
Phase 3 (d 42)						
DM	83.98	83.52	83.07	83.79	0.69	0.589
ME	78.78	78.30	78.04	79.01	1.08	0.801
CP	78.42	78.39	78.10	78.40	1.54	0.996

CP, crude protein; AA, amino acid; SEM, standard error of means; DM, dry matter; ME, metabolizable energy; CP, crude protein.

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Table 4. The effects of CP and AA level on microbiota in growing pigs.

CP, %	17.2	15.7			SEM	p-value
Lysine, %	1.10	1.10	1.15	1.20		
Phase 1 (d 14)						
Lactobacillus spp.	1.247	1.241	1.258	1.271	0.02	0.412
Bifidobacterium spp.	0.774	0.819	0.774	0.787	0.02	0.139
E.coli	0.320	0.283	0.292	0.302	0.02	0.217
Clostridium	0.539	0.505	0.498	0.482	0.03	0.204
Phase 2 (d 28)						
Lactobacillus spp.	1.236	1.271	1.291	1.301	0.03	0.244
Bifidobacterium spp.	0.795	0.806	0.826	0.817	0.01	0.212
E.coli	0.323	0.306	0.308	0.293	0.02	0.357
Clostridium	0.515	0.500	0.498	0.494	0.02	0.619
Phase 3 (d 42)						
Lactobacillus spp.	1.281	1.318	1.281	1.301	0.03	0.624
Bifidobacterium spp.	0.791	0.798	0.782	0.792	0.02	0.929
E.coli	0.278	0.288	0.306	0.298	0.02	0.361
Clostridium	0.508	0.504	0.510	0.490	0.03	0.884

CP, crude protein; AA, amino acid; SEM, standard error of means.

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Table 5. The effects of CP and AA level on volatile fatty acid in growing pigs (g/kg)

CP, %	17.2	15.7			SEM	p-value
Lysine, %	1.10	1.10	1.15	1.20		
Phase 1 (d 14)						
Acetic acid	4.15	4.26	4.29	4.26	0.14	0.799
Propionic acid	1.46	1.45	1.50	1.51	0.04	0.485
Butyric acid	1.14	1.15	1.11	1.16	0.03	0.336
Isobutyric acid	0.68	0.61	0.62	0.64	0.03	0.166
Isovaleric acid	0.87	0.76	0.77	0.80	0.04	0.059
Total SCFA	6.75	6.86	6.90	6.92	0.17	0.724
Total BCFA	1.55[a]	1.37[b]	1.39[b]	1.44[ab]	0.05	0.009
Total VFA	8.29	8.23	8.29	8.36	0.17	0.880
Phase 2 (d 28)						
Acetic acid	4.27	4.22	4.22	4.39	0.13	0.537
Propionic acid	1.54	1.47	1.50	1.49	0.04	0.430
Butyric acid	1.17	1.10	1.11	1.08	0.07	0.602
Isobutyric acid	0.54[a]	0.39[b]	0.42[b]	0.39[b]	0.04	<0.007
Isovaleric acid	0.84[a]	0.60[b]	0.61[b]	0.62[b]	0.04	<0.001
Total SCFA	6.98	6.79	6.84	6.96	0.16	0.584
Total BCFA	1.39[a]	0.99[b]	1.04[b]	1.00[b]	0.05	<0.001
Total VFA	8.37	7.78	7.88	7.97	0.17	0.880
Phase 3 (d 42)						
Acetic acid	4.10	4.33	4.18	4.12	0.11	0.175
Propionic acid	1.48	1.49	1.50	1.48	0.05	0.977
Butyric acid	1.10	1.13	1.08	1.13	0.05	0.703
Isobutyric acid	0.55	0.53	0.53	0.52	0.08	0.984
Isovaleric acid	0.70	0.74	0.70	0.73	0.06	0.900
Total SCFA	6.68	6.95	6.76	6.72	0.15	0.316
Total BCFA	1.25	1.27	1.23	1.24	0.13	0.994
Total VFA	7.93	8.22	7.99	7.97	0.19	0.434

CP, crude protein; AA, amino acid; SEM, standard error of means; SCFA, short chain fatty acid; BCFA, branched chain fatty acid; VFA, volatile fatty acid.

[a-b]means different superscript letters indicate significant differences ($p<0.05$).

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