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Effects of low- and high-crude protein diets supplemented up to the fourth limiting amino acid for two commercial crosses of starter piglets

Efeitos de dietas com baixo e alto teor de proteína bruta suplementadas até o quarto aminoácido limitante para dois cruzamentos comerciais de leitões iniciantes

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Highlights

High-crude protein diets positively influence nutrient digestibility and nitrogen.
Diets without supplement or unbalance in valine compromise piglet's performance.
There was no effect of commercial crosses on performance and metabolism.

Abstract

This study was composed of two experiments conducted to assess the effects of low- and high-crude protein diets supplemented up to the fourth limiting amino acid and two commercial crosses on the growth performance, plasma urea concentration (PUC), economic feasibility, apparent nutrient digestibility, and nitrogen balance in starter nursery piglets. In Expt. I, a total of 128 piglets (14.02 ± 1.96

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kg initial BW and 48 d of age) were allotted based on initial BW in a randomized block design with a 2 × 2 factorial arrangement. Two commercial crosses (DB and PIC) and two crude protein diets (low-crude protein, LCP, and high-crude protein, HCP) were evaluated. A total of four treatments, eight replicates, and four piglets per experimental unit were used. Expt. II was conducted using 24 entire male piglets (20.00 ± 1.41 kg initial BW) housed in metabolic cages for 12 d and distributed in the same experimental design as in Expt. I (six replicates). The results of the Expt. I suggest piglets fed HCP showed better growth performance. An increase of 25.2% in PUC was observed in piglets fed HCP. There was an increase of 18.2% in the economic efficiency index piglets fed HCP, and a reduction in the cost per kg of BW gain was also observed. In Expt. II, piglets fed HCP showed higher apparent nutrient digestibility coefficients, digestible protein and energy, N intake and absorption. There was no effect of commercial crosses on growth performance and metabolism variables. In conclusion, HCP diets, regardless of genetics, promoted improvements in performance and economic feasibility index, but increased PUC in piglets. In addition, HCP diets positively influenced apparent nutrient digestibility and N intake and absorption.

Key words: Economic feasibility. Genetics. Nutrient digestibility. Pig performance. Urea concentration.

Resumo

Este estudo foi composto por dois experimentos conduzidos para avaliar os efeitos de dietas com baixo e alto teor de proteína bruta suplementadas até o quarto aminoácido limitante e dois cruzamentos comerciais sobre o desempenho zootécnico, concentração de ureia plasmática (CUP), viabilidade econômica, digestibilidade aparente de nutrientes, e balanço de nitrogênio em leitões iniciantes. No Exp. I, um total de 128 leitões (14,02 ± 1,96 kg de peso corporal inicial e 48 dias de idade) foram distribuídos baseado no peso corporal inicial em um delineamento de blocos casualizados com arranjo fatorial 2 × 2. Foram avaliados dois cruzamentos comerciais (DB e PIC) e duas dietas de proteína bruta (baixa proteína bruta, BPB, e alta proteína bruta, APB). Foram utilizados quatro tratamentos, oito repetições e quatro leitões por unidade experimental. Exp. II foi conduzido utilizando 24 leitões machos inteiros (20,00 ± 1,41 kg de peso corporal inicial) alojados em gaiolas metabólicas por 12 dias e distribuídos no mesmo desenho experimental do Expt. I (seis repetições). Os resultados do Exp. I sugerem que os leitões alimentados com APB apresentaram melhor desempenho zootécnico. Foi observado um aumento de 25,2% na CUP em leitões alimentados com APB. Houve aumento de 18,2% no índice de eficiência econômica quando os leitões foram alimentados com APB, e também foi observada redução no custo por kg de ganho de peso corporal. No Exp. II, os leitões alimentados com APB apresentaram maiores coeficientes de digestibilidade aparente dos nutrientes, proteína e energia digestíveis, consumo e absorção de N. Não houve efeito dos cruzamentos comerciais sobre as variáveis de desempenho e metabolismo. Conclui-se que as dietas APB, independente da genética, promoveram melhorias no desempenho e no índice de viabilidade econômica, mas aumentaram a CUP nos leitões. Além disso, as dietas APB influenciaram positivamente a digestibilidade aparente dos nutrientes e a ingestão e absorção de N.

Palavras-chave: Viabilidade econômica. Genética. Digestibilidade de nutrientes. Desempenho suíno. Concentração de ureia.

Introduction

Pork production depends on key factors such as genetics and nutrition. Growth performance improvements promoted by breeding led to the necessity of nutrient requirements adjustments, especially relative to digestible ingredients, and CP levels in diets. National Research Council [NRC] (2012) has been used as one of the main references of pig nutritional requirements for diet formulation (Santos et al., 2019). However, studies have shown that it is necessary to adapt diets according to production systems and tropical conditions, considering genetics and environmental and health conditions (Paul et al., 2007; Santos et al., 2019).

Thus, evaluating different ingredients and diet formulations to meet the requirements of modern pig genetics (Rostagno et al., 2017), such as formulating low-crude protein diets (Castilha et al., 2013; Toledo et al., 2014; Souza et al., 2023), have shown positive effects mainly in reduced environmental impact, but starter pigs fed low CP-based diets grew more slowly (Ruusunen et al., 2007), and increased feed intake due to lower essential amino acid content in the diet (Schiavon et al., 2018).

In addition to nutrients requirements available in the world for swine nutrition, there are several pig genetics companies producing different hybrid strains. DanBred and Agroceres-PIC are breeding companies that operate worldwide. For this reason, it is necessary to know the performance of these genetics (Torres et al., 2005) when fed different nutritional regimes (e.g. low- and high-protein diets). Genetic strains selection for high performance and lean growth has been investigated (Schiavon et al., 2018; Abeni et al., 2018). However, little attention has been focused on different levels of CP in formulations to maximize muscle development (Souza et al., 2023), nutrient assimilation, and performance in commercial crosses.

Therefore, this study was conducted to assess the effects of two low- and high-crude protein diets supplemented up to the fourth amino acid and two commercial crosses on the growth performance, plasma urea concentration, economic feasibility, apparent nutrient digestibility, and nitrogen balance in starter nursery piglets.

Material and Methods

Experiments I and II were conducted in a pig unit (24°31′52″ S and 54°01′03″ W) located at an altitude of ≥ 387 m in Marechal Cândido Rondon, PR, Brazil. Research on animals was conducted according to the institutional committee on animal use (protocol no. 32/2017). Animals came from modern commercial crosses focused on production of hybrids with superior meat and carcass quality both considered to be the top elite (top 20% sires) (Rostagno et al., 2017).

Experiment I (growth performance)

Experimental design, animals, housing, and diets

Fathers of piglets from this study were DanBred-DB (LI7600) and Agroceres-PIC (AGPIC 337). Mothers of the pigs were all

PIC (Camborough 22). In experiment I (Expt. I), a total of 128 piglets (14.02 ± 1.96 kg BW and 48 d of age) were allotted in a randomized block design based on initial BW, with a 2 × 2 factorial arrangement. Two commercial crosses (DB or PIC) and two crude protein diets (LCP or HCP) were evaluated. A total of four treatments, eight replicates, and four piglets per experimental unit were used.

At the beginning of the experiment, animals were weighed using a digital scale (Digi-tron, modelo 50 kg, Curitiba, PR, Brazil), tagged for identification purposes (Allflex, Joinville, SC, Brazil), and housed in a masonry nursery room with ceramic roof. The facility had a central aisle with slatted plastic floor pens (1.32 m^2) equipped with gutter feeders and nipple drinkers on both sides. Pens were equipped with chains to reduce undesirable behavior in piglets.

The nursery room ventilation was provided with the aid of fans, exhaust fans, and tilt and turn glass windows. The heating of the experimental pens was controlled using individual infrared heat lamps. Room temperature and relative humidity were recorded by a datalogger with a digital display (UNI-T UT 330B digital USB; Beijing, China), which was installed in the middle of the nursery room. The room temperature and relative humidity averaged 24 ± 3.6°C and 67 ± 18.13%, respectively.

Diets containing LCP or HCP (Table 1) were formulated for starter piglets, close to the nutritional and energy values contained to the NRC (2012) or Brazilian Tables (Rostagno et al., 2017), respectively. Both diets met the requirements up to the fourth limiting amino acid.

Table 1
Centesimal and calculated composition of the experimental diets fed to entire male piglets in the starter phase of nursery (as fed basis)

Ingredients (%)	Experimental diets	
	Low-crude protein	High-crude protein
Ground corn, 7.86% CP	74.35	59.84
Soybean meal, 45.4% CP	13.38	27.11
Micronized soybean, 38% CP	3.00	3.00
Fish meal, 53% CP	3.00	3.00
Soybean oil	1.66	2.61
Calcitic limestone	0.80	1.00
Dicalcium phosphate	0.79	1.28
Common salt	0.63	0.44
Mineral-vitamin premix[1]	0.50	0.50
Lysine sulphate, 54%	1.122	0.632
DL-methionine, 99%	0.270	0.202
L-threonine, 98%	0.315	0.237
L-tryptophan, 99%	0.082	0.052
Amoxicillin	0.052	0.052
Neomycin sulphate	0.011	0.011
Calculated composition		
Crude protein, %	16.00	20.55
Metabolizable energy, kcal kg^{-1}	3,350	3,350
Digestible lysine, %	1.230	1.281
Digestible methionine + cysteine, %	0.680	0.730
Digestible threonine, %	0.730	0.833
Digestible tryptophan, %	0.200	0.243
Digestible valine, %	0.610	0.829
Total calcium, %	0.700	0.910
Available phosphorus, %	0.330	0.450
Total sodium, %	0.280	0.205
Dietary fiber, %	2.032	2.448

[1] Guarantee levels per kg (5 g of premix kg^{-1} of diet): folic acid (103.12 mg); pantothenic acid (2,249 mg); biotin (16.88 mg); chlorohydroxyquinoline (15.00 g); copper (22.07 g); ethoxyquin (206 mg); iron (6,733 mg); phytase (62,500 IU); glucanase (19,000 IU); iodine (37.51 mg); lysine (123.76 g); manganese (1,866 mg); methionine (110.25 g); niacin (4,687 mg); selenium (43.75 mg); threonine (46.64 g); vitamin A (1,437,500 IU); vitamin B_1 (224.96 mg); vitamin B_{12} (2,537 mg); vitamin B_2 (537.50 mg); vitamin B_6 (437.50 mg); vitamin D_3 (262,500 IU); vitamin E (4,250 IU); vitamin K_3 (375 mg); xylanase (152,500 UI); zinc (1,000 mg).

Sampling, procedures, and evaluations

The experiment lasted 14 d. Piglets were allowed *ad libitum* access to diet and water throughout the experimental period. Animals were individually weighed on a digital scale (Digi-tron, 50 kg model, Curitiba, PR, Brazil) at the beginning and the end of the study. Average daily gain (ADG) was determined by the difference between initial (IBW) and final BW (FBW) divided by the number of days of housing. To determine the average daily feed intake (ADFI) and feed conversion ratio (FCR), diet offered and leftovers were recorded daily. Average daily feed intake was calculated by subtracting leftovers from the amount of diet offered. Feed conversion ratio was calculated by dividing feed intake by weight gain.

Blood sampling

To assess plasma urea concentrations, piglets fasted for 8h on d 14 of experimental period. Then, 10 mL blood samples were withdrawn (from 16 animals per treatment at 08:30h) from the jugular vein using 1.2 × 40 mm (18 G × 1 1/2") needles. The choice of animal was based on the one with the BW closest to the average BW of their respective pen. Blood was collected in identified vacutainer tubes containing ethylenediamine tetraacetic acid and then send to the lab on ice (4°C). Plasma was obtained by centrifuging blood samples at 3,000 g for 15 min (Model 80-2B, Centrilab). Plasma samples (duplicates) were stored in microtubes (2 mL) at -18°C until urea analysis. Plasma urea concentration was analyzed by spectrophotometry (Bel SPECTRO S05) using a commercial kit (Gold Analisa Diagnostic).

Economic feasibility analysis

The prices of each ingredient were based on values registered by the local market (Marechal Cândido Rondon, PR, Brazil) in the year 2017, but which represent the quotation prices practiced and used for the composition of the pig production cost in Brazil. To determine the economic feasibility, the prices of the ingredients used in the elaboration of the experimental diets were as follows: ground corn, \$ 0.09 kg^{-1}; soybean meal, \$ 0.37 kg^{-1}; micronized soybean, \$ 0.43 kg^{-1}; fish meal, \$ 0.79 kg^{-1}; soybean oil, \$ 0.63 kg^{-1}; calcitic limestone, \$ 0.06 kg^{-1}; monocalcium phosphate, \$ 0.50 kg^{-1}; common salt, \$ 0.11 kg^{-1}; mineral-vitamin supplement, \$ 4.81 kg^{-1}; lysine sulphate, \$ 0.91 kg^{-1}; DL-methionine, \$ 2.55 kg^{-1}; L-threonine, \$ 2.00 kg^{-1}; L-tryptophan, \$ 18.28 kg^{-1}; amoxicillin, \$ 80.17 kg^{-1}; neomycin sulphate \$ 80.17 kg^{-1}. The dollar closed the month of July 2017 quoted at R\$ 3.1176. To perform economic feasibility analysis, the cost of each diet per kg of BW gain was determined as previously reported by Bellaver et al. (1985). Then, the economic efficiency index (EEI) was calculated as previously reported by Fialho et al. (1992).

Experiment II (metabolism)

Experimental design, animals, housing, and diets

Fathers and mothers of the pigs in this study were as mentioned in Expt. I. The animals used in Experiment II (Expt. II) was not the same subset tested in Expt. I. Experiment II was conducted using 24 entire male piglets (20.00 ± 1.41 kg BW) distributed in a randomized block design based on initial BW, with a 2 × 2 factorial arrangement [two commercial crosses (DB or PIC) × two crude protein diets (LCP or HCP)]. The experiment had a total of four dietary treatments, six replicates per treatment, and one pig per experimental unit. Diets were formulated as described in Expt. I (Table 1). All diets were provided as mash.

Animals were housed in metabolic cages (0.80 m^2) as previously described by Pekas (1968). Cages were equipped with a drinker, a feeder, and a plastic screen which was used to collect leftovers and avoid fecal samples losses. Cages were also equipped with a urine funnel and feces collection box. Environmental enrichment was provided by placing 500 mL bottles in the cages. The experimental facility had an aluzinc roof, side curtains, and fans, which allowed airflow control and welfare. A digital thermohygrometer (model 1566-1, J.Prolab, São Jose dos Pinhais, PR, Brazil) was placed in the middle of the facility at piglets' dorsal height. Room temperature and relative humidity averaged 23 ± 4.4°C and 78 ± 23.38%, respectively.

Procedures and analyses

Experiment II procedures were performed as previously described by Sakomura and Rostagno (2016). Piglets were fed experimental diets for 12 d. The initial 7 d were considered an adaptation period to the diet and cage. Urine and feces were sampled for 5 d. During the adaptation period, piglets had free access to water and feed (08:00 and 15:00 h).

During the sampling period, piglets were fed based on metabolic BW ($kg^{0.75}$). The daily amount of feed was divided into 2 uneven meals (55% at 08:00 h and 45% at 15:00 h). Water was offered after feeding based on the amount of diet supplied (3 mL g^{-1} diet). Prior to each meal, water was added to diet (20%) to stimulate intake, reduce dust, and hence reduce waste. Offered diet and leftovers were weighed (stainless steel digital scale, model UL50i; Beijing, China) and recorded at each meal.

To mark the beginning and the end of feces collection, 1.5% iron oxide was used in diets to indicate the sampling period. Feces were sampled twice a day (morning and afternoon), weighed on a digital scale (stainless steel digital scale, model UL50i; Beijing, China), placed in identified plastic bags, and stored at -18°C until the end of the sampling period. Then, samples were thawed, pooled by pig, and dried in a forced-air oven (Technal brand, SF-325 NM model; Piracicaba, SP, Brazil) at 55°C for 72 h. All samples were then ground through a 1 mm screen in a Wiley mill for further analyses.

Urine excreted over a 24-h period was collected in polyethylene buckets, with 20 mL of a 1:1 solution (distilled water: HCL 0.01 mol/L) previously added to avoid bacterial proliferation and N losses by volatilization. The collected urine had its volume measured and an aliquot of 10% of the total volume was placed in identified bottles and stored in a freezer (-18°C) until the end of the collection period. Afterwards, they were homogenized, filtered, and sent to the laboratory for CP analysis (Association of Official Analytical Chemists [AOAC], 1990).

To determine the apparent digestibility coefficients (ADC) and digestible nutrient (DN) and energy values, dry matter (DM, method 930.15), organic matter (OM) by the difference between the value of DM and mineral matter (MM, method 942.05), CP by multiplying the total N by 6.25 (method 990.03) and gross energy (GE) in diet and feces samples were analyzed according to the methodologies described by AOAC (1990). To determine the GE of the feces and diet, the samples were weighed on an analytical balance (Marte, model AY220; Uberaba, MG, Brazil) and pressed using a pelleting press (IKA - Werke, model C 21; Campinas, SP, Brazil). Sample combustion was performed using isoperibol bomb calorimetry (Parr Instrument Company, 6200 model; Moline, IL, USA).

Data on physical-chemical analyzes were used in the equations proposed by Matterson et al. (1965) to determine ADC of DM (ADCDM), OM (ADCOM), CP (ADCCP), and GE (ADCGE). From digestibility coefficients, DN and digestible energy were calculated. Nitrogen intake, absorption, excretion (feces and urine), retention, and retention to absorption ratio were determined. Crude protein intake and excretion (feces and urine) were obtained by multiplying protein concentrations by feed intake or fecal and urine output, respectively (Sakomura & Rostagno, 2016).

Blood sampling

On d 12, piglets were fasted for 8 h to assess PUC. Then, ± 10 mL of blood was collected via jugular vein puncture from 6 animals per treatment (8:30 am). All sampling and collection methods were similar to those conducted in Expt. I.

Statistical analysis

All results were analyzed using the MIXED procedure of SAS University Edition (SAS Inst. Inc., Cary, NC, USA) within a 2 × 2 factorial arrangement of treatments with the following general model: $Y_{ijkl} = \mu + D_i + G_j + DG_{ij} + \beta (X_{ijkl} - \bar{X} \ldots) + b_l + \varepsilon_{ijkl}$, in which Y_{ijkl} = average observation of the dependent variable in each plot measured in the i-th diet class, in the j-th genetic class, in the k-th replication, and at the l-th block; μ = effect of the overall average. Treatments were compared to provide factorial contrasts: (1) LCP *vs* HCP, (2) DB vs PIC, and (3) the interaction between diets and commercial crosses. Whenever significant, the interaction between diets and commercial crosses was decomposed into simple comparisons using the Tukey-Kramer test of SLICEDIFF option of the LSMEANS statement. Initial BW was used as a covariate $[\beta (X_{ijkl} - \bar{X} \ldots)]$ whenever significant. The block (bl) represented by initial BW was used as a random effect. Heterogeneity of variances was tested using the REPEATE command and GROUP option and a variance were used for each treatment class whenever significant. Significant differences were set at $p < 0.05$.

Results and Discussion

Piglets fed HCP had higher ($p < 0.01$) FBW, ADG, ADFI, and better FCR compared to those fed LCP diet (Table 2). An interaction between diets and commercial crosses for FCR ($p = 0.008$) was observed, in which DB piglets fed HCP had lower FCR than those fed LCP from both commercial crosses. An increase in PUC was observed ($p = 0.003$) in piglets fed HCP compared to LCP diet. Piglets from PIC strain had a lower ($p = 0.034$) PUC compared to those from DB strain. There was an interaction (p = 0.001) between diets and commercial crosses for PUC, in which piglets from both DB and PIC strain fed LCP showed lower PUC than those from PIC strain fed HCP.

Table 2

Growth performance and plasma urea concentration (PUC) of piglets in the starter phase from two commercial crosses fed diets with low- and high-crude protein (Expt. I)

Variables[1]	DB		PIC		SEM[2]	p-value		
	LCP	HCP	LCP	HCP		Diet	Gen[3]	Int[4]
IBW, kg	14.43	14.17	13.55	13.93	0.444	0.892	0.215	0.483
FBW, kg	19.36	22.81	19.40	22.53	0.317	<0.001	0.720	0.612
ADG, kg day^{-1}	0.420	0.685	0.423	0.660	0.052	<0.001	0.647	0.539
ADFI, kg day^{-1}	0.773	0.871	0.687	0.885	0.107	0.001	0.412	0.242
FCR, kg kg^{-1}	1.83^{a}	1.29^{c}	1.60^{ab}	1.35^{bc}	0.052	<0.001	0.093	0.008
Plasma urea concentration, mg dL^{-1}								
PUC	20.76^{b}	21.84^{ab}	18.19^{b}	26.93^{a}	1.021	0.003	0.034	0.001

^{abc}Differ by the Tukey-Kramer test using the SLICEDIFF option of the LSMEANS statement at 5% probability level.

[1] IBW = initial body weight; FBW = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

[2] SEM = standard error of the mean.

[3] Gen = effect of the commercial crosses factor.

[4] Int = interaction between the factors (diets × commercial crosses); LCP = diet containing 16% CP; HCP = diet containing 20.55% CP.

The present study showed that reducing the CP content of the diet was detrimental to the growth performance of pigs fed LCP, even when the diet is balanced for the four essential amino acids. However, the differences between the diets for starter pigs such as the contents of CP, essential amino acids, and minerals were determinant. Although LCP diet was greater for total sodium (0.280 vs. 0.205), and the metabolizable energy (ME) content was analogous to the HCP diet for starter pigs, this was not able to support animal performance.

This is corroborated by He et al. (2016), who reported that LCP diets (16% CP, 13% CP, and 10 % CP) supplemented with essential amino acids may not maintain weight gain or feed efficiency during the growing phase. This shows the importance of adequate amounts of industrial amino acids in animal nutrition. Consequently, the different ADFI between diets leads to an unbalanced intake of energy content and essential amino acids. In the current study, piglets on LCP diets were not able to self-regulate amino acid reduction (e.g. valine, and isoleucine) by increasing feed intake, as previously reported by Schiavon et al. (2018). In addition, reduced feed intake in piglets fed LCP diets compromised energy and nutrient intake, negatively affecting growth rate.

The growth performance of piglets fed HCP suggests these diets were met and they are more adequate to animals in tropical conditions (e.g. Brazil). Nutritional requirements under tropical conditions [e.g. (Rostagno et al., 2017)] differ from those in temperate countries [e.g. (NRC, 2012)] due to differences in genetic strains, body size, growth rate, quality of ingredients, and variations in climatic elements (Paul et al., 2007). In tropical countries, feeding standards and nutritional recommendations from NRC for piglets need to be updated to meet climate requirements (Paul et al., 2007; Santos et al., 2019). That piglets fed HCP showed higher ADFI could be explained by different dietary ingredient concentrations for nutritional and energy supply. In fact, HCP diet had lower corn and higher soybean meal concentrations to meet CP requirements. Hence, a higher oil concentration was used in HCP diet, which may have improved the palatability and increased feed intake (Solà-Oriol et al., 2011).

Luo et al. (2015) studied starter diets formulated close to meeting NRC (1998) requirements (20% CP) or to have LCP (14%) supplemented with industrial amino acids fed to 36 crossbred piglets [Duroc × (Landrace × Large White)]. The authors reported that piglets fed 14% CP diets with industrial amino acids added showed a lower ADFI and ADG. Furthermore, the authors observed lower microbial population and diversity, which may have compromised the health of animals. Although the diets in the study of Luo et al. (2015) have been balanced according to NRC (1998), the effects obtained for growth performance in the present study are corroborated by those previously reported.

The CP level for piglets weighing 10 kg to 20 kg is 20.9% (NRC, 1998), which is similar to the requirement used in the present study on HCP diets (20.5%) (Rostagno et al., 2017), differing from the LCP diets (NRC, 2012). This may explain the similarity between studies regarding growth performance when diets are formulated using the nutritional recommendations of the NRC (1998). Indeed, Luo et al. (2015) reported ADFI of 838 g and ADG of 505 g, while in the present study an average ADFI of 878 g and ADG of 672.5 g were observed. These results suggest, regardless of commercial crosses, minimum CP and essential amino acids requirements should be met in order to obtain optimal growth performance.

Lean tissue growth is the main purpose of pig production which shows the importance of meeting dietary amino acid requirements (Cameron et al., 2003; Hulsegge et al., 2019). According to Cameron et al. (2003), amino acids supply (e.g. lysine), makes it possible to meet requirements for maintenance, protein deposition, and hence for optimal growth performance. As breeding involves the selection of genes encoding to performance traits, the interaction between commercial crosses and diets formulated using different CP contents may indicate modulation in metabolism driven by specific genes of each strain. Genetically improved piglets, classified as medium to high performance, possibly have genes that are involved with greater metabolic response for protein synthesis, which is linked to tissue synthesis (Hulsegge et al., 2019).

Another study showed the effects of dietary nutrients on expression of genes linked to metabolism that can improve performance (Bižienė et al., 2018). The performance attributed to modern commercial crosses in response to different dietary nutrients can be enlightened via nutrigenomics (Sales et al., 2014) to find and explain possible reciprocal interactions between genes and nutrients at molecular level.

In the present study, a higher PUC was observed in PIC piglets fed HCP diets, that is, higher content of CP and amino acid requirement (lysine, methionine + cysteine, threonine, and tryptophan), which possibly contributed to such effect. Our results are corroborated by those reported by Fang et al. (2019), who observed lower PUC in pigs fed LCP diets, even when the same levels of amino acids were kept. In addition, PUC is directly affected by the quantity as well as the quality of the protein consumed, supported by the proper balance of essential amino acids. Toledo et al. (2014) reported a linear decrease on PUC in piglets (15 to 30 kg BW) fed decreased dietary CP (16.24%, 14.74%, and 13.24%). In this aforementioned study, diets were formulated to meet essential amino acid requirements and authors suggested that reducing CP promoted optimal amino acid utilization.

The cost per kg of LCP and HCP diets was $ 0.23 and 0.26, respectively. There was an increase of 18.2% in the EEI when piglets were fed HCP compared to LCP diet, and a reduction ($p < 0.0001$) in the cost per kg of BW gain was also observed (Table 3). There was an interaction between diets and commercial crosses for cost per kg of BW gain ($p = 0.006$), in which piglets from both DB and PIC fed HCP and piglets from PIC fed LCP showed the optimal results.

Table 3
Cost per kg body weight gain (Cost), and economic efficiency index (EEI) of piglets in the starter phase from two commercial crosses fed diets with low- and high-crude protein

Variables	DB		PIC		SEM[1]	p-value		
	LCP	HCP	LCP	HCP		Diet	Gen[2]	Int[3]
Cost, $	0.44^{a}	0.34^{c}	0.38^{b}	0.35^{bc}	0.024	<0.001	0.139	0.006
EEI, %	77.49	100.00	89.00	95.90	2.815	-	-	-

[abc]Differ by the Tukey-Kramer test using the SLICEDIFF option of the LSMEANS statement at 5% probability level.
[1]SEM = standard error of the mean.
[2]Gen = effect of the commercial crosses factor.
[3]Int= interaction between the factors (diets × commercial crosses); LCP = diet containing 16% CP; HCP = diet containing 20.55% CP.

The LCP diet had a higher cost per kg of produced BW (CPBW). Although the LCP cost per kg diet was $ 0.03 lower than HCP, CPBW was not reduced and HCP showed the best results. It shows the importance of evaluating not only the cost of diets within a production system, but also its effects on growth performance which can directly affect the cost per kg of produced BW (Bellaver et al., 1985). Hamill et al. (2013) reported that nutritional restriction (e.g. CP) can negatively affect protein synthesis bringing potential consequences for growth and economic losses. The LCP diet of the present study contained 16% CP, while HCP diet had 20.5% CP, which directly influenced the economic indexes. Therefore, the optimal performance observed in HCP contributed to more favorable economic indexes. The interaction between diets and commercial crosses may suggest better associations regarding strains and diet formulations. However, these results could change depending upon variable economic conditions related to changing diets ingredient prices or value received for piglets.

Piglets fed HCP showed higher ADCOM ($p = 0.008$), ADCCP ($p = 0.046$), and ADCGE ($p = 0.002$) compared to LCP diet (Table 4). The same effect was observed for digestible protein ($p < 0.001$) and digestible energy ($p = 0.046$). There was a significant interaction between diets and commercial crosses for ADCDM ($p = 0.021$), ADCCP ($p = 0.026$), digestible dry matter (DDM, $p = 0.018$), and digestible protein ($p = 0.031$). DanBred piglets fed HCP showed higher ADCDM, ADCCP, and DDM. Piglets fed HCP also showed higher digestible protein, regardless of commercial crosses. There was no effect ($p > 0.05$) of commercial crosses on the evaluated variables. No effect ($p > 0.05$) on PUC was observed either.

Table 4
Apparent digestibility coefficients, digestible nutrients and energy, and plasma urea concentration (PUC) evaluated in piglets in the starter phase from two commercial crosses fed diets with low- and high-crude protein (Expt. II)

Variables[1]	DB		PIC		SEM[2]	p-value		
	LCP	HCP	LCP	HCP		Diet	Gen[3]	Int[4]
ADCDM, %	85.15^{b}	89.08^{a}	88.05^{ab}	86.53^{ab}	0.615	0.308	0.139	0.021
ADCOM, %	86.74	89.26	86.54	87.56	0.592	0.008	0.124	0.219
ADCCP, %	81.83^{b}	87.85^{a}	84.40^{ab}	84.08^{ab}	1.315	0.046	0.654	0.026
ADCGE, %	85.30	88.40	85.23	86.64	0.630	0.002	0.162	0.195
Digestible nutrients and energy[5]								
DDM, %	77.12^{b}	80.61^{a}	79.74^{ab}	78.33^{ab}	0.631	0.289	0.315	0.018
DOM, %	83.18	84.56	83.05	83.14	0.695	0.310	0.278	0.362
DP, %	12.91^{b}	17.39^{a}	13.69^{b}	16.62^{a}	0.223	<0.001	0.341	0.031
DE, kcal kg^{-1}	3,317	3,417	3,325	3,354	29.599	0.046	0.358	0.245
Plasma urea concentration, mg dL^{-1}								
PUC	13.51	16.18	14.70	17.04	1.444	0.100	0.489	0.910

^{abc}Differ by the Tukey-Kramer test using the SLICEDIFF option of the LSMEANS statement at 5% probability level.
[1] ADCDM = apparent digestibility coefficient of dry matter; ADCOM = apparent digestibility coefficient of organic matter; ADCCP = apparent digestibility coefficient of crude protein; ADCGE = apparent digestibility coefficient of gross energy.
[2] SEM = standard error of the mean.
[3] Gen = effect of the commercial crosses factor.
[4] Int = interaction between the factors (diets × commercial crosses); LCP = diet containing 16% CP; HCP = diet containing 20.55% CP.
[5] DDM = digestible dry matter; DOM = digestible organic matter; DP = digestible protein; DE = digestible energy.

As with most variables evaluated in Expt. I, there was also no effect of commercial crosses on nutrient digestibility in Expt. II. Several factors are responsible for digestibility variation in animal trials. Silva and Silva (2015) reported that genetics, age, diet composition, and room temperature have a direct effect on animal metabolism. Sartor et al. (2006) reported that studies with pig commercial crosses, besides presenting variable results, do not consider differences among commercial crosses. In addition, the aforementioned authors did not observe any effect of commercial crosses on apparent digestibility of DM and CP in commercial crosses of grower pigs (Agroceres, Dalland, and Embrapa), which agrees with our findings.

Nutrient digestibility in piglets fed reduced CP diets was reported to depend on CP reduction, industrial amino acid supplementation, and dietary energy concentration (Zhao et al., 2019). In addition to animal-related factors, diet composition, fiber content, and quality of ingredients can also affect nutrient digestibility (Le Sciellour et al., 2018). In the present study, animals fed HCP showed higher ADCOM, ADCCP, and ADCCE, which hence improved DP and DE, regardless of commercial crosses. This is supported by dietary energy levels as energy intake affects protein deposition (Zhao et al., 2019). Diets were isoenergetic, but total energy intake depends on feed intake which can affect several nutritional and digestibility responses (Beaulieu et al., 2006; Zhao et al., 2019).

It is well established on literature that amino acid requirements depend on genetic capacity for protein retention, environmental influence on dietary nutrients, gastrointestinal health due to gut mucosal desquamation degree, and dietary chemical composition (Paul et al., 2007). Although studies have reported an effect of dietary oil (Kerr et al., 2015), and crude fiber concentrations on nutrient digestibility (Le Sciellour et al., 2018), these factors did not affect piglets fed HCP.

Fang et al. (2019) studied the effect of low dietary ME and CP on growth performance, blood profile, and nutrient digestibility in weaned piglets. These authors did not observe an effect on DM digestibility and reported a digestibility coefficient of 89.1% when piglets fed a diet containing 3,300 kcal kg^{-1} and 19.7% CP. However, protein digestibility linearly increased with dietary CP reduction. These effects differ from those we observed in the present study, where DB strain showed higher protein digestibility when fed HCP.

Jin et al. (1998) reported dietary concentration and balance of essential amino acids, and animal requirements as the main factors affecting dietary protein digestibility. Fang et al. (2019) fed piglets with diets containing reduced CP but with similar amino acid concentration and observed higher digestibility. These authors suggested reducing dietary CP without changing limiting dietary amino acid concentrations could result in better amino acid balance than high dietary CP concentration. This agrees with the study of Zhao et al. (2019), who observed no effect on nutrient digestibility when dietary CP was reduced.

Plasma urea concentration at the end of the experimental period was not influenced by diets, unlike what was observed in Expt. I. Piglets were fed for ad libitum intake throughout growth performance experiment while in metabolism trial piglets were fed based on metabolic BW. This probably affected protein intake based on N consumed of each diet. Fang et al. (2019) reported that a 4% CP reduction improved protein digestibility and, similarly to the present study, decreased PUC. According to Coma et al. (1995), through PUC analysis is possible to evaluate N concentrations in short-term experiments. These authors reported that N metabolism has a fast response, according to different dietary amino acids concentration, which agrees with our findings. The increased activity of urea cycle enzymes is essential to convert ammonia, generated during oxidative deamination of protein metabolites, into urea. Therefore, higher PUC could be a result of increased dietary protein concentration (Genova et al., 2019).

Piglets fed HCP showed higher N intake and absorption when expressed as g d^{-1} (p = 0.021 and p = 0.021, respectively) or as g kg^{-1} BW$^{0.75}$ d^{-1} (p = 0.048 and p = 0.038, respectively) compared to LCP diet (Table 5). There was a significant interaction between diets and commercial crosses for fecal N (p

= 0.055), N retained intake^{-1} (p = 0.035), and total N excretion expressed as g d^{-1} (p = 0.047) or g kg^{-1} BW$^{0.75}$ d^{-1} (p = 0.035). PIC piglets fed HCP showed higher fecal N compared to PIC piglets fed LCP, and DB piglets fed HCP and LCP diets. A lower N retained intake^{-1} was observed in DB piglets fed LCP compared to those fed HCP. A similar effect was observed in PIC piglets fed LCP. An increase of 46.7% was observed for total N excretion in PIC piglets fed HCP compared to those fed LCP diet.

Table 5
Nitrogen (N) and protein balance in piglets in the starter phase from two commercial crosses fed diets with low- and high-crude protein (Expt. II)

Variables	DB		PIC		SEM[1]	p-value		
	LCP	HCP	LCP	HCP		Diet	Gen[2]	Int[3]
N intake, g d^{-1}	16.63	21.13	18.39	21.50	0.857	0.021	0.492	0.655
N intake, g kg^{-1} BW$^{0.75}$ d^{-1}	1.71	2.07	1.88	2.14	0.076	0.048	0.421	0.715
N fecal, g d^{-1}	3.59^{ab}	3.25^{b}	3.28^{b}	4.04^{a}	0.150	0.438	0.385	0.055
N fecal, g kg^{-1} BW$^{0.75}$ d^{-1}	0.36	0.31	0.34	0.40	0.014	0.808	0.282	0.061
N urine, g d^{-1}	5.04	4.96	3.57	6.00	0.447	0.162	0.794	0.137
N urine, g kg^{-1} BW$^{0.75}$ d^{-1}	0.50	0.48	0.36	0.59	0.041	0.185	0.847	0.116
N absorbed, g d^{-1}	13.04	17.87	15.10	17.45	0.798	0.021	0.569	0.396
N absorbed, g kg^{-1} BW$^{0.75}$ d^{-1}	1.34	1.75	1.54	1.73	0.072	0.038	0.521	0.427
N retained, g d^{-1}	7.99	12.91	11.53	11.45	0.783	0.117	0.487	0.105
N retained, g kg^{-1} BW$^{0.75}$ d^{-1}	0.83	1.27	1.18	1.13	0.075	0.198	0.481	0.114
N retained intake^{-1} (%)	44.92^{b}	60.87^{a}	61.85^{a}	53.34^{ab}	2.943	0.498	0.394	0.035
N retained absorbed^{-1} (%)	57.33	71.81	75.57	66.13	3.285	0.684	0.318	0.065
Total N excretion, g d^{-1}	8.63^{ab}	8.22^{ab}	6.85^{b}	10.05^{a}	0.510	0.118	0.977	0.047
Total N excretion, g kg^{-1} BW$^{0.75}$ d^{-1}	0.87^{ab}	0.80^{ab}	0.70^{b}	1.00^{a}	0.045	0.177	0.848	0.035

[abc] Differ by the Tukey-Kramer test using the SLICEDIFF option of the LSMEANS statement at 5% probability level.
[1] SEM = standard error of the mean.
[2] Gen = effect of the commercial crosses factor.
[3] Int = interaction between the factors (diets × commercial crosses); LCP = diet containing 16% CP; HCP = diet containing 20.55% CP.

In the metabolism trial, metabolic intake favored minor alterations in N balance due to energy and nutrient imbalance in starter piglets, differing from the outcomes for growth performance and apparent nutrient digestibility. In the present study, we observed that decreasing CP concentration in LCP diet reduced N intake, but it did not change N excretion in urine and total N excretion (TNE), probably due to the low content or differences in dietary amino acid balance, as well as the CP:ME or digestible lysine:CP ratio. According to Moreira et al. (2004), feeding programs focus on increasing the use of amino acids and reducing N excretion. Piglets fed HCP showed higher absorbed N, which may be explained by better usage of protein compounds with reduced urea synthesis (Genova et al., 2019). Even though N retention was not increased in these animals. Retained N suggests proteins usage and a balance between synthesis and degradation of metabolism proteins (Zhao et al., 2019).

A higher catabolism of amino acids, observed only in fecal N in PIC piglets, promoted an effect on TNE since fecal and urine N are used to determine absorbed and retained N. The highest TNE did not change urine N concentration. This could cause lower N intestinal absorption and greater N usage (Castilha et al., 2013). Additionally, when energy intake is lower than maximum N deposition requirement, urine N excretion would increase (Zhao et al., 2019). A lower dietary CP along with industrial amino acid supplementation can reduce TNE, even though this was not observed in this study in animals fed LCP diet.

Therefore, the emergence of new commercial high-performance pig strains (with high lean tissue production capacity in a short time), suggests that diets should be formulated to meet requirements in conditions where these animals are bred.

Conclusions

Diets HCP improved growth performance and economic feasibility index, but increased PUC in piglets. The HCP diets positively affected apparent nutrient digestibility and N intake and absorption, without promoting alterations on PUC in piglets in the metabolism experiment. In addition, the study also suggested that diets without supplementation or unbalanced in the amino acid valine compromise piglet's production performance, regardless of commercial crosses.

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