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ORIGINAL ARTICLE

Evaluation of reduced amino acids diets added with protected protease on productive performance in 25-100 kg barrows

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ABSTRACT. The objective of this research was to evaluate the effect of adding protected protease to low-amino acids (AA) diets on the growth performance of barrows. Three decreasing levels of AA (protein levels), with or without the addition of protease were fed to 48 hybrid barrows (27.42±3.48 kg initial body weight). The experimental design was a completely randomised with a factorial arrangement of treatments. An analysis of variance was performed with GLM of SAS and the means comparison was performed with Tukey test ($P \leq 0.05$). The productive performance was not affected by addition of proteases in the diet at the three stages ($P > 0.05$). Only in growing barrows, the interaction of standard protein diet and protease reduced backfat thickness ($P \leq 0.05$). Protein level in finishing I barrows did not affect ($P > 0.05$) growth performance variables. Low-protein diets increased ($P \leq 0.05$) average daily gain, final body weight and fat-free lean gain in growing and finishing II barrows. Concentration of urea in plasma decreased with the reduction of CP and increased with the addition of protease ($P \leq 0.05$) at the three stages. In conclusion, low protein diets improved or maintained growth performance variables and reduced the plasma urea nitrogen, whereas supplementation with protease did not show any effect on productive performance.

Key words: protein content, swine, growth performance, carcass characteristics.

INTRODUCTION

The use of low-protein diets (LPD) in fattening pigs is a viable option for reducing nitrogen emission into the environment, which reduces the amount of greenhouse gases and soil contamination (Osada *et al* 2011). In addition, LPD enable to obtain a similar growth performance compared to standard diets when supplemented with crystalline AA (He *et al* 2016, Jiao *et al* 2016, Peng *et al* 2016). However, the reduction of more than 4-6% of crude protein (CP) in the diet affects growth performance and digestive enzymatic production (He *et al* 2016). Therefore, it is necessary to find alternative additives that compensate for the reduction of protein ingredients and the amount of CP in pig diets, increasing AA digestibility.

Addition of protease might improve availability of protein in pig diets through an increment on protein digestibility and amino acid (AA) availability in the gastrointestinal tract of pigs (Wang *et al* 2011, Guggenbuhl *et al* 2012). Nevertheless, in some cases, using protease in LPD for weaning, growing and finishing pigs did not improve growth performance or carcass characteristics variables (Reyna *et al* 2006, Zamora *et al* 2011). The variability in protease effectiveness was attributed to the age of pigs, the use of different types of ingredients, the source of protease (Adeola and Cowieson 2011), and the high degradation and inactivation of these enzymes in the gastrointestinal tract (Pan *et al* 2016). To surpass this variability of results using protease for pig diets, recent technological advances have developed coated (protected) protease (Xu *et al* 2017), which works under different conditions in the gastrointestinal tract (Pan *et al* 2016). Thus, using protected protease in pig diets may improve digestibility of AA which, in turn, is reflected in a better growth performance (Zuo *et al* 2015).

This improved protein and AA digestibility and availability in the gastrointestinal tract of pigs (Wang *et al* 2011, Guggenbuhl *et al* 2012) means that the supplementation of protected protease could compensate for the reduction of AA in diets for pigs when fed LPD. For these reasons, the objective of this research was to evaluate the addition of protected protease to low AA concentration (low-crude protein) diets in 25-100 kg barrows in terms of growth performance, carcass characteristics, and plasma urea nitrogen concentration.

MATERIAL AND METHODS

The experimental procedures were performed accordingly to the recommendations of the International Guiding Principles for Biomedical Research Involving Animals[1], and observing the standards for ethics, biosafety, and animal

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[1] CIOMS, Council for International Organizations of Medical Sciences. 2012. International Guiding Principles for Biomedical Research Involving Animals. http://www. cioms (Accessed January 23, 2018).

well-being of the Colegio de Postgraduados, Mexico, under the Official Mexican Regulation (Norma Oficial Mexicana, 1999) for the use of animals in experimentation.

The experiment was conducted in the Swine Unit of the Experimental Farm at the Colegio de Postgraduados, located in Montecillo, Estado de Mexico (98º 48' 27'' W and 19º 48' 23" N). The climate is temperate, semi-arid, with an average annual temperature of 15.9 °C, infrequent frosts, average annual rainfall of 686 mm and an altitude of 2241 m (García 1988).

Forty eight hybrid (Landrace × Yorkshire × Duroc; 27.42 ± 3.48 kg initial body weight and 60 days-old) barrows were used, housed in individual pens, each equipped with a single feeder and nipple drinker. Water and feed were provided *ad libitum*.

The experiment consisted in the evaluation of six treatments (T). Three decreasing levels of protein (standardized ileal digestible (SID) basis AA levels; control, medium, and low), with or without the addition of protease were evaluated (0.03% of protease was added, according to the amount indicated by the JEFO Company; Poultry Grow 250 ®, *Streptomyces griseus*, Type XIV, Puebla, Mexico) in pigs diet. Three stages were evaluated (growing, 25-50 kg body weight; finishing I, 50-75 kg; finishing II, 75-100 kg; tables 1-3 respectively), changing the feed for each stage accordingly to the average body weight of the pigs, trying to follow the recommendations of the NRC (2012) for each stage. Forty-eight barrows were distributed (each pig was considered a replicate) in a completely randomised design with a factorial arrangement of treatments; there were eight replicates (barrows) per treatment. The AA or CP (low-protein diets; LPD) reduction was achieved by decreasing the lysine content by 0.05% and 0.10% relative to control diet, with a proportionate reduction in concentrations of the remaining AA in the diet, trying to maintain the ratio relative to Lys (ensuring that all AA were supplied, at least the Lys proportion).

The nutritional values of the control treatment were established as recommended by the NRC (2012) to cover or exceed the requirements for each stage of growth. The diets for each stage were formulated with the Solver command[2] using the least-cost feed formulation method.

CHEMICAL ANALYSIS

Crude protein content of diets for each stage was determined by the Macrokjeldahl method (AOAC 2005), calcium and phosphorus content by atomic absorption spectrophotometry (Karl *et al* 1979) using a Perkin Elmer 4000 Model (Series Lambda 2, Perkin Elmer Inc., Norwalk, CT, USA). On the last day of the experiment, blood samples (5 mL; pre-prandial 08:00 h) were collected by vena cava puncture in live pigs, using Vacutainer tubes without anticoagulant (BD Vacutainer®), and stored at 4 °C. The blood samples were centrifuged (SIGMA 2-16 k, Germany) at 3500 *g* for 20 min to obtain blood serum. Serum samples were stored in Eppendorf tubes at –20 °C in a freezer (SANYO MDF-436, USA) until determination of plasma urea (Chaney and Marbach 1962).

VARIABLES

Growth performance variables were: average daily feed intake (ADFI), average daily gain (ADG), feed:gain ratio (FGR), fat-free lean gain (FFLG) and final body weight (BW). Carcass characteristics determined were: backfat thickness (BT), *longissimus* muscle area (LMA), lean meat percentage (LMP); and plasma urea nitrogen (PUN) concentration. The BT and LMA were measured using real-time ultrasound (SonoVet 600, MEDISON: Medison, Inc., Cypress, CA, USA) at the 10th rib on the first and last day of the experiment. The BT, LMA, initial and final BW data were used to determine FFLG and LMP, following the procedure indicated by Burson and Berg (2001): Lb. lean=5.7769+(0.401 x warm carcass wt., lbs)–(18.838 x 10th rib fat depth, in.)+(4.357 x 10th rib loin muscle area, sq. in.)+(1.006 x sex of pig) (barrow=1, gilt=2)).

STATISTICAL ANALYSIS

The experimental design was completely randomised with a factorial arrangement of treatments, where the factors were three CP levels and two protected protease levels (with and without). Shapiro-Wilk and Levene's test were used to check normal distribution and homogeneity of variance for all variables. Data were analyzed with the GLM procedure, and Tukey's test ($P \leq 0.05$) was used to compare treatment means (Statistical Analysis System 2010. Inc. Cary, NC, USA). The initial body weight was used as a covariate ($P \leq 0.05$).

RESULTS

LOW-PROTEIN DIETS

No differences ($P > 0.05$) were detected between treatments for FGR, LMP, BT and LMA due to the protein level in the diet for growing (table 4) and finishing II barrows (table 6). An improved response was observed in ADG, final BW, ADFI and FFLG in growing barrows fed 16.17 % and 15.49 % CP compared to 16.86 % CP ($P \leq 0.05$) (table 4). In finishing II barrows, values for ADG and final BW improved ($P \leq 0.05$) when using 12.08 % CP in the diet compared to 12.86 % and 13.63 % CP. The FFLG was greater ($P \leq 0.05$) when using 12.86 % and 12.08 % CP compared to control diet (13.63 % CP) (table 6). There were no effects ($P > 0.05$) of protein level on any growth performance variables in finishing I (50-75 kg) barrows (table 5).

[2] Microsoft Excel 2007

Table 1. Composition of diets for growing barrows (25-50 kg BW) fed low-protein diets supplemented with protease.

Ingredient, %	T1[¶]	T2	T3	T4	T5	T6
Sorghum grain (*Sorghum bicolor*)	77.55	77.51	79.35	79.31	81.11	81.06
Soybean (*Glycine max*) meal	18.66	18.66	16.90	16.91	15.19	15.20
Soybean oil	0.97	0.99	0.94	0.96	0.91	0.92
Biolys[†]	0.73	0.73	0.72	0.72	0.71	0.71
DL-Methionine	0.16	0.16	0.14	0.14	0.13	0.13
L-Threonine	0.10	0.10	0.09	0.09	0.09	0.09
Vitamins[§]	0.20	0.20	0.20	0.20	0.20	0.20
Minerals[Þ]	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	0.91	0.91	0.89	0.89	0.87	0.87
Calcium orthophosphate	0.26	0.26	0.29	0.30	0.33	0.33
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Protease	0.00	0.03	0.00	0.03	0.00	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition calculated (SID AA)						
Metabolizable Energy (Mcal kg^{-1})	3.30	3.30	3.30	3.30	3.30	3.30
Crude Protein (%)	16.86	16.86	16.17	16.17	15.49	15.49
Lysine (%)	1.00	1.00	0.95	0.95	0.90	0.90
Threonine (%)	0.60	0.60	0.57	0.57	0.54	0.54
Tryptophan (%)	0.17	0.17	0.16	0.16	0.15	0.15
Phenialanine (%)	0.73	0.73	0.70	0.70	0.67	0.67
Arginine (%)	0.87	0.87	0.81	0.81	0.76	0.76
Histidine (%)	0.36	0.36	0.34	0.34	0.32	0.32
Isoleucine (%)	0.61	0.61	0.58	0.58	0.55	0.55
Leucine (%)	1.45	1.45	1.41	1.41	1.38	1.38
Valine (%)	0.76	0.76	0.73	0.73	0.69	0.69
Methionine + Cysteine (%)	0.57	0.57	0.54	0.54	0.51	0.51
Calcium (%)	0.67	0.67	0.67	0.67	0.67	0.67
Phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40
Determined nutrient composition						
CP (%)	16.76	16.74	16.09	16.07	15.39	15.42
Calcium (%)	0.72	0.73	0.71	0.75	0.74	0.73
Phosphorus (%)	0.38	0.37	0.39	0.38	0.37	0.36

[†] Biolys, 50.7%; lysine. [¶]T, Treatment. [§]Supplied by kg: 5.0×106 IU vitamin A, 1.0×106 IU vitamin D3, 2.0×104 IU vitamin E; 2 g vitamin K3, 1 g tiamine, 5 g rivoflavin, 2 g pyridoxine, 25 g niacin, 15 g D-calcium panthotenate, 3 g folic acid, 225 g choline chloride, 0.3 g antioxidant, 15 mg vitamin B12 and 180 mg vitamin H-biotin. REKA® Lapisa Animal Nutrition. [Þ]Supplied by kg: 0.2 g Se, 0.1 g Co, 0.3 g I, 10 g Cu, 100 g Zn, 100 g Fe and 100 g Mn. REKA® Lapisa Animal Nutrition.

PROTEASE

The addition of protease to diets during three growth phases (growing, finishing I and finishing II) did not change (P>0.05) the growth performance and carcass characteristics of barrows (tables 4, 5 and 6, respectively).

In the growing stage, the control diet (16.86 % CP) with added protease reduced BT (P≤0.05); the protease interaction with the CP level had no effect (P>0.05) on other variables (table 4). There were no effects (P>0.05) of the interaction between protein level and protease addition on growth performance and carcass characteristics in finishing I and finishing II barrows (tables 5 and 6).

PLASMA UREA NITROGEN

The addition of dietary protease in the three phases of growth increased PUN concentration (P≤0.05) of barrows; while protein reduction reduced PUN concentration in all three phases (P≤0.05). In growing barrows, the lowest

Table 2. Composition of diets for finishing I barrows (50-75 kg BW) fed low-protein diets supplemented with protease.

Ingredient, %	T1[¶]	T2	T3	T4	T5	T6
Sorghum grain	81.80	81.80	83.88	83.83	85.91	85.86
Soybean meal	14.58	14.58	12.57	12.58	10.56	10.57
Soybean oil	0.91	0.91	0.86	0.87	0.82	0.84
Biolys[†]	0.64	0.64	0.64	0.64	0.65	0.65
DL-Methionine	0.10	0.10	0.09	0.09	0.08	0.08
L-Threonine	0.07	0.07	0.07	0.07	0.07	0.07
Vitamins[§]	0.20	0.20	0.20	0.20	0.20	0.20
Minerals[Þ]	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	0.87	0.87	0.84	0.84	0.82	0.82
Calcium orthophosphate	0.34	0.34	0.39	0.39	0.43	0.43
Phytases	0.01	0.01	0.01	0.01	0.01	0.01
Protease	0.00	0.03	0.00	0.03	0.00	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
	Nutrient composition calculated (SID AA)					
Metabolizable Energy (Mcal kg^{-1})	3.30	3.30	3.30	3.30	3.30	3.30
Crude Protein (%)	15.18	15.18	14.40	14.40	13.63	13.63
Lysine (%)	0.85	0.85	0.80	0.80	0.75	0.75
Threonine (%)	0.52	0.52	0.49	0.49	0.46	0.46
Tryptophan (%)	0.15	0.15	0.14	0.14	0.13	0.13
Phenialanine (%)	0.66	0.66	0.63	0.63	0.59	0.59
Arginine (%)	0.75	0.75	0.69	0.69	0.63	0.63
Histidine (%)	0.32	0.32	0.30	0.30	0.28	0.28
Isoleucine (%)	0.54	0.54	0.51	0.51	0.48	0.48
Leucine (%)	1.36	1.36	1.32	1.32	1.28	1.28
Valine (%)	0.68	0.68	0.64	0.64	0.60	0.60
Methionine+Cysteine (%)	0.48	0.48	0.45	0.45	0.42	0.42
Calcium (%)	0.62	0.62	0.62	0.62	0.62	0.62
Phosphorus (%)	0.33	0.33	0.33	0.33	0.33	0.33
	Nutrient composition (evaluated in laboratory)					
Crude Protein (%)	15.12	15.16	14.24	14.32	13.57	13.52
Calcium (%)	0.71	0.72	0.75	0.73	0.70	0.73
Phosphorus (%)	0.36	0.37	0.38	0.37	0.34	0.39

[†] Biolys, 50.7%; lysine. [¶]T, Treatment. [§]Supplied by kg: 5.0×106 IU vitamin A, 1.0×106 IU vitamin D3, 2.0×104 IU vitamin E; 2 g vitamin K3, 1 g tiamine, 5 g rivoflavin, 2 g pyridoxine, 25 g niacin, 15 g D-calcium panthotenate, 3 g folic acid, 225 g choline chloride, 0.3 g antioxidant, 15 mg vitamin B12 and 180 mg vitamin H-biotin. REKA® Lapisa Animal Nutrition. [Þ]Supplied by kg: 0.2 g Se, 0.1 g Co, 0.3 g I, 10 g Cu, 100 g Zn, 100 g Fe and 100 g Mn. REKA® Lapisa Animal Nutrition.

concentration of this metabolite occurred with the lowest CP level (15.49 %) and the addition of protease ($P \leq 0.05$). In finishing I barrows, the lowest PUN concentration was also detected with the lowest CP concentration (13.63 %) regardless the addition or not of protease ($P \leq 0.05$). Finishing II barrows showed lower PUN ($P \leq 0.05$) with the lowest PC concentration (12.08 %) as in the previous growth stages.

DISCUSSION

The NRC (2012) does not establish a CP value for diets formulation, so, its concentration in the diet is the result of the AA level. The concentrations of lysine, methionine, threonine, tryptophan and other AA were reduced in the diets used in this study, trying to maintain the proportion of AA with respect to lysine (tables 1-3), which reduced the CP level of the diet. However, under the concept of minimum cost formulation of diets for pigs, in economic terms it is more feasible to exceed the recommendation

Table 3. Composition of diets for finishing II barrows (75-100 kg BW) fed low-protein diets supplemented with protease.

Ingredient, %	T1[¶]	T2	T3	T4	T5	T6
Sorghum grain	87.03	86.98	89.10	89.05	91.16	91.11
Soybean meal	10.41	10.42	8.40	8.41	6.39	6.40
Soybean oil	0.45	0.47	0.40	0.42	0.35	0.37
Biolys[†]	0.61	0.61	0.61	0.61	0.61	0.61
DL-Methionine	0.08	0.08	0.07	0.07	0.06	0.06
L-Threonine	0.07	0.07	0.06	0.06	0.06	0.06
Vitamins[§]	0.20	0.20	0.20	0.20	0.20	0.20
Minerals[Þ]	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	0.69	0.69	0.70	0.70	0.70	0.70
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Protease	0.00	0.03	0.00	0.03	0.00	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition calculated (SID AA)						
Metabolizable Energy (Mcal kg^{-1})	3.30	3.30	3.30	3.3	3.30	3.3
Crude Protein (%)	13.63	13.63	12.86	12.85	12.08	12.08
Lysine (%)	0.73	0.73	0.68	0.68	0.63	0.63
Threonine (%)	0.46	0.46	0.43	0.43	0.40	0.40
Tryptophan (%)	0.13	0.13	0.12	0.12	0.11	0.11
Phenialanine (%)	0.59	0.59	0.56	0.56	0.53	0.53
Arginine (%)	0.63	0.63	0.57	0.57	0.51	0.51
Histidine (%)	0.28	0.28	0.26	0.26	0.24	0.24
Isoleucine (%)	0.48	0.48	0.45	0.45	0.41	0.41
Leucine (%)	1.29	1.29	1.24	1.24	1.20	1.20
Valine (%)	0.60	0.60	0.56	0.56	0.52	0.52
Methionine + Cysteine (%)	0.42	0.42	0.39	0.39	0.36	0.36
Calcium (%)	0.52	0.52	0.52	0.52	0.52	0.52
Phosphorus (%)	0.32	0.32	0.32	0.32	0.31	0.31
Nutrient composition (evaluated in laboratory)						
Crude Protein (%)	13.43	13.41	12.92	12.85	12.01	12.10
Calcium (%)	0.52	0.54	0.51	0.53	0.52	0.53
Phosphorus (%)	0.37	0.36	0.39	0.33	0.34	0.36

[†] Biolys, 50.7%; lysine. [¶]T, Treatment. [§]Supplied by kg: 5.0×106 IU vitamin A, 1.0×106 IU vitamin D3, 2.0×104 IU vitamin E; 2 g vitamin K3, 1 g tiamine, 5 g rivoflavin, 2 g pyridoxine, 25 g niacin, 15 g D-calcium panthotenate, 3 g folic acid, 225 g choline chloride, 0.3 g antioxidant, 15 mg vitamin B12 and 180 mg vitamin H-biotin. REKA® Lapisa Animal Nutrition. [Þ]Supplied by kg: 0.2 g Se, 0.1 g Co, 0.3 g I, 10 g Cu, 100 g Zn, 100 g Fe and 100 g Mn. REKA® Lapisa Animal Nutrition.

of some AA (NRC 2012) than trying to accomplish the lower (goal) values (Dubeau *et al* 2011). In our study, the reduction of phenylalanine, arginine, isoleucine, leucine and valine was limited because the basal ingredients (sorghum grain-soybean meal) have a high concentration of these AA.

LOW-PROTEIN DIETS

The results indicate that it is feasible to improve or maintain growth performance variables when the amount of AA in barrow diets is reduced, as long as the lysine:AA ratio is maintained. Results of other studies showed that finishing pigs fed LPD, with an unchanged AA ratio per CP unit, had similar and even improved growth variables (Gallo *et al* 2014, Tous *et al* 2014).

In contrast, Qin *et al* (2015), Jiao *et al* (2016) and Peng *et al* (2016) reported that these growth variables were unaffected when CP content was reduced, but the requirement for the more limiting AA (Lys, Met, Trp, Thr) must be reached in the diet. The use of lower levels of AA in our experiment with a performance similar to that of the control protein diet, leads us to determine that

Table 4. Productive performance, carcass characteristics and plasma urea nitrogen concentration of growing barrows (25-50 kg) fed three levels of crude protein and two levels of protease.

% CP	Protease	ADFI ($kg\ d^{-1}$)	ADG ($kg\ d^{-1}$)	FGR	BWi (kg)	BWf (kg)	FFLG ($kg\ d^{-1}$)	LMP (%)	BT (mm)	LMA (cm^2)	PUN ($mg\ dL^{-1}$)
16.86	–	1.67	0.70	2.44	26.62	49.03	0.251	30.07	6.22^{a}	19.36	21.26^{b}
16.86	+	1.53	0.64	2.42	27.76	47.20	0.236	30.65	5.20^{b}	18.86	27.93^{a}
16.17	–	1.74	0.77	2.26	27.58	51.51	0.287	30.07	5.84^{ab}	19.86	18.73^{bc}
16.17	+	1.79	0.75	2.43	27.27	50.56	0.274	30.17	6.16^{a}	19.77	21.10^{b}
15.49	–	1.87	0.72	2.63	27.75	49.82	0.267	29.97	6.05^{ab}	19.37	12.43^{d}
15.49	+	1.85	0.76	2.44	27.47	50.93	0.279	29.90	5.87^{ab}	19.35	16.54^{c}
SEM		0.07	0.03	0.11	1.30	0.91	0.01	0.39	0.26	0.77	0.80
P value											
% CP × Protease		0.39	0.26	0.26	–	0.26	0.51	0.68	0.04	0.94	0.03
% CP		0.002	0.005	0.22	–	0.005	0.01	0.54	0.48	0.65	0.001
Protease		0.51	0.45	0.87	–	0.452	0.61	0.53	0.18	0.75	0.001

CP= Crude Protein; ADFI= average daily feed intake; ADG= average daily gain; FGR= feed:gain ratio; BWi= initial body weight; BWf= final body weight; FFLG= Fat free lean gain; LMP= lean meat percentage; BT= backfat thickness; LMA= *Longissimus* muscle area; PUN= plasma urea nitrogen concentration, SEM= standard error of the mean. [a,b,c,d] Means with different superscript differ ($P\leq0.05$).

Table 5. Productive performance, carcass characteristics and plasma urea nitrogen concentration of finishing I barrows (50-75 kg) fed three levels of protein and two levels of protease.

% CP	Protease	ADFI ($kg\ d^{-1}$)	ADG ($kg\ d^{-1}$)	FGR	BWi (kg)	BWf (kg)	FFLG ($kg\ d^{-1}$)	LMP (%)	BT (mm)	LMA (cm^2)	PUN ($mg\ dL^{-1}$)
15.18	–	2.63	0.94	2.81	47.77	76.44	0.311	28.12	10.34	25.74	23.50^{b}
15.18	+	2.57	0.93	2.78	49.08	76.02	0.307	28.33	9.56	25.52	30.66^{a}
14.40	–	2.46	0.93	2.67	51.77	76.03	0.307	28.21	9.64	25.33	22.57^{b}
14.40	+	2.42	0.87	2.83	50.31	74.35	0.290	28.34	10.09	25.59	28.42^{a}
13.63	–	2.67	0.90	3.00	50.33	75.17	0.298	28.34	10.08	25.45	17.45^{c}
13.63	+	2.51	0.89	2.84	50.98	75.07	0.297	28.19	9.74	25.04	17.90^{c}
SEM		0.09	0.04	0.11	2.25	1.20	0.011	0.23	0.25	0.75	0.93
P value											
% CP × Protease		0.52	0.94	0.41	–	0.95	0.94	0.92	0.08	0.93	0.001
% CP		0.19	0.90	0.60	–	0.91	0.90	0.98	0.95	0.94	0.001
Protease		0.43	0.67	0.76	–	0.66	0.67	0.73	0.31	0.96	0.001

CP= Crude Protein; ADFI= average daily feed intake; ADG= average daily gain; FGR= feed:gain ratio; BWi= initial body weight; BWf= final body weight; FFLG= Fat free lean gain; LMP= lean meat percentage; BT= backfat thickness; LMA= *Longissimus* muscle area; PUN= plasma urea nitrogen concentration, SEM= standard error of the mean. [a,b,c,d] Means with different superscript differ ($P\leq0.05$).

the nutritional requirement values coincide with the recommendations for pigs with low genetic potential for lean growth, when comparing CP and lysine concentrations evaluated in our study with the NRC (2012) and Brazilian tables (Rostagno *et al* 2017).

In the present research, the decrease in CP as a result of AA reduction showed beneficial or null effects on the growth performance. Gloaguen *et al* (2014) and González *et al* (2016) reported that a drastic reduction in dietary CP should not be made, because lowering CP by more than three percentage units affected ADG, ADFI, FGR, final BW and FFLG. The negative response to LPD may be attributed to AA deficiency, because CP reduction limits the concentration of AA and the amount of nitrogen necessary for the synthesis of non-essential AA (Gloaguen *et al* 2014).

Consistent with results of our study, Zamora *et al* (2011) and Qin *et al* (2015) confirmed that decreasing CP by less than two percentage units in diets for fattening pigs did not affect carcass characteristics. This may be a result of better balance of AA for protein synthesis and, therefore, AA are not required as an energy source, reflected by similar LMP values with different CP concentrations (Orlando *et al* 2007). However, González *et al* (2016) and Figueroa *et al* (2012) observed that in nursery and finishing pigs, decreasing dietary CP by more than three

Table 6. Productive performance, carcass characteristics and plasma urea nitrogen concentration of finishing II barrows (75-100 kg) fed with three levels of protein and two levels of protease.

% CP	Protease	ADFI (kg d^{-1})	ADG (kg d^{-1})	FGR	BWi (kg)	BWf (kg)	FFLG (kg d^{-1})	LMP (%)	BT (mm)	LMA (cm^2)	PUN (mg dL^{-1})
13.63	–	3.19	0.85	3.87	76.44	102.77	0.321	28.10	16.70	36.83	17.64[a]
13.63	+	3.59	0.96	3.74	76.02	106.32	0.357	28.12	14.52	36.02	19.47[a]
12.86	–	3.16	0.92	3.44	76.03	104.97	0.358	28.41	13.96	36.58	16.69[a]
12.86	+	3.39	0.88	3.91	74.35	103.72	0.336	28.32	15.85	37.30	18.97[a]
12.08	–	3.55	1.01	3.53	75.17	108.00	0.380	28.19	15.18	36.92	13.36[b]
12.08	+	3.51	1.02	3.44	75.07	108.20	0.365	27.69	16.33	36.26	17.51[a]
SEM		0.15	0.04	0.17	1.20	1.38	0.015	0.23	0.07	0.80	0.80
P value											
% CP × Protease		0.22	0.20	0.15	–	0.20	0.14	0.51	0.10	0.57	0.05
% CP		0.22	0.01	0.17	–	0.01	0.05	0.19	0.46	0.80	0.001
Protease		0.10	0.46	0.54	–	0.45	0.97	0.31	0.62	0.69	0.001

CP= Crude Protein; ADFI= average daily feed intake; ADG= average daily gain; FGR= feed:gain ratio; BWi= initial body weight; BWf= final body weight; FFLG= Fat free lean gain; LMP= lean meat percentage; BT= backfat thickness; LMA= *Longissimus* muscle area; PUN= plasma urea nitrogen concentration, SEM= standard error of the mean. [a,b,c,d] Means with different superscript differ ($P \leq 0.05$).

percentage units had a negative effect on BT, LMP and LMA. Reduction in FFLG and increase in BT in pigs fed LPD were attributed to the greater availability of energy for adipose tissue accretion, due to an imbalance between a greater amount of available energy and a deficiency of AA (Gómez *et al* 2002).

PROTEASE

Various reports (Zuo *et al* 2015, Pan *et al* 2016, Yu *et al* 2016) have demonstrated the efficacy of adding protected protease to pig diets, increasing CP digestibility and AA availability, improvements in the plasma concentration of total protein, increased pepsin enzyme activity in the stomach, as well as increased pancreatic amylase and trypsin. The combination of an increase in CP digestibility and lower consumption could reflect improvements in the growth performance of pigs fed protease (Mc Alpine *et al* 2012, O'Shea *et al* 2014). These changes resulted in a better productive performance in both weaning and nursery pigs, which does not have adequate protease production (Zuo *et al* 2015, Greiner *et al* 2016).

Previous research showed that supplementation of LPD with protease did not improve growth performance in fattening pigs (Morales *et al* 2002, Reyna *et al* 2006, Zamora *et al* 2011). Besides, Mc Alpine *et al* (2012) and O'Shea *et al* (2014) observed that the use of protected protease in pig diets could reduce ADG in response to a reduction of ADFI.

This phenomenon could be explained because the protease hydrolyses the protein in the small intestine, which releases components that can be absorbed, and increases the ileal digestibility of protein, increasing the amount of available N from the diet (Mc Alpine *et al* 2012, O'Shea *et al* 2014). Additional nutrients released could trigger a feedback mechanism to reduce feed intake because of a glucostatic and/or aminostatic response that could create nutrient imbalance in the gastrointestinal tract of pig (Nortey *et al* 2007).

PLASMA UREA NITROGEN

Decreasing dietary protein in pigs reduced the concentration of PUN (Qin *et al* 2015, Peng *et al* 2016). This lower PUN concentration is associated with a decrease in metabolic heat production associated with pig metabolism derived from lower amount of total nitrogen and hence, lower synthesis and excretion of urea originated by the AA excess when fed a standard CP diet, indicating better utilization of nitrogen by pigs fed LPD (Qin *et al* 2015). The increase in PUN concentration, which was observed in the present research when protease was added to the diet, may be due to greater digestibility of the CP diet and increased AA availability (Reyna *et al* 2006). The effect of the protease cannot be determined because reduction of protein never decreased growth performance of pigs. Thus, the extra amino acid available from the inclusion of the protease will be catabolized without further effect.

Based on the results of this study, we conclude that the reduction of the concentration of AA in the diet (low CP) improves or maintains growth performance and reduce the plasma urea nitrogen in 25-100 kg hybrid barrows compared to standard CP diets, however, the lysine:AA ratio in the diet must be maintained. The addition of protected protease to pig diets does not show any effect on productive performance, because reduction of protein never decreased growth performance of pigs.

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