JORGE YAIR PÉREZ PALENCIA

PROTEIN AND LYSINE SOURCES IN NURSERY PIGLETS DIETS

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não Ruminantes, para a obtenção do título de Doutor.

Prof. Dr. Márvio Lobão Teixeira de Abreu
Orientador

Prof. Dr. Rony Antônio Ferreira
Coorientador

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PROTEIN AND LYSINE SOURCES IN NURSERY PIGLETS DIETS

FONTES DE PROTEÍNA E LISINA EM RAÇÕES PARA LEITÕES NA FASE DE CRECHE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não Ruminantes, para a obtenção do título de Doutor.

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2018

A Deus por estar comigo incondicionalmente, guiando e sustentando cada um dos meus passos. A Ele toda a glória sempre.

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“Em seu coração o homem planeja o seu caminho, mas o Senhor determina os seus passos”.

Provérbios 16:9

“A man’s heart plans his way, But the Lord directs his steps.”

Proverbs 16:9

RESUMO GERAL

No artigo 1, foram avaliados os efeitos da substituição de proteínas animais e de peixe por uma mistura proteica fortificada (PROPLEX MVP) sobre o desempenho, estado imunológico, metabólitos microbianos e escore fecal de leitões na creche. Os tratamentos foram: 1) dieta controle (CONT) com fontes de proteína animal e de peixe; 2) MVP PROPLEX (MVP): CONT com o MVP substituindo a farinha de peixe; 3) PROPLEX MVP 100 (MVP100): CONT com MVP substituindo 100% das fontes de proteína animal e de peixe. Considerando todo o período experimental, não houve diferenças significativas (P>0,05) no desempenho dos leitões. Durante a maior parte do período experimental, leitões CONT apresentaram maior escore fecal (P <0,05), fezes mais moles e aquosas e aumentaram a incidência de diarreia em comparação com os leitões MVP100. A concentração total de ácidos graxos voláteis em amostras fecais foi maior (P<0,05) no grupo CONT em comparação ao grupo MVP100. A resposta imune não diferiu significativamente entre os tratamentos. O uso de MVP reduziu (P<0,05) em US $ 1,00 o custo total de alimentação por suíno e 10% a 12% do custo de alimentação por kg de ganho de peso vivo. Assim, a mistura proteica MVP pode ser utilizada como uma alternativa de baixo custo para substituir fontes de proteína animal e de peixe em rações para leitões. No artigo 2, objetivou-se avaliar a biodisponibilidade relativa (RBV) de L-lisina sulfato em comparação com L-lisina HCl e seus efeitos no desempenho, parâmetros sanguíneos, funcionalidade intestinal e digestibilidade aparente das rações de leitões na creche. Os tratamentos foram uma ração basal (CON) formulada para atender 73% dos requerimentos de lisina e outras seis rações em que a ração CON foi suplementada com três níveis (80%, 90% e 100% dos requerimentos de lisina) de L-lisina sulfato (70% L-lisina) ou L-lisina HCl (79% L-lisina). Não houve diferenças significativas (P>0,05) no desempenho e concentrações de ureia plasmática entre as fontes de L-lisina. A RBV da L-lisina sulfato em relação à L-lisina HCl foi de 106%, 119% e 117% para os efeitos no GPD, eficiência alimentar e ureia plasmática, respectivamente. A deficiência de lisina resultou em uma incidência maior de diarreia (P<0,05), enquanto leitões suplementados com L-lisina sulfato ou L-lisina HCl apresentaram maior (P<0,05) altura de vilosidades no jejuno quando comparados àqueles que receberam a ração CON. Rações suplementadas com L-lisina sulfato apresentaram maior (P<0,05) digestibilidade aparente da matéria seca, energia bruta e proteína bruta. Em conclusão, a RBV da L-lisina sulfato é equivalente à de L-lisina HCl para efeitos sobre o GPD, eficiência alimentar e ureia plasmática de leitões na creche.

Palavras-chave: Programa alimentar. Aminoácido limitante. Leitões. Fontes proteicas.

ABSTRACT

Article 1, this experiment observed the effects of replacing animal and fish proteins with a fortified protein blend (PROPLEX MVP) on growth performance, immune status, microbial metabolites, and fecal scoring of nursery piglets. Dietary treatments were: 1) Control diet (CONT) with animal and fish protein sources; 2) PROPLEX MVP (MVP): CONT with MVP replacing Fish meal; 3) PROPLEX MVP 100 (MVP100): CONT with MVP replacing 100% of animal and fish proteins. For the overall nursery period, there were no significant differences in pig final BW, overall ADG and G:F. From the second week and during most of the experimental period, pigs fed CONT had greater ($P < 0.05$) fecal score, more soft and watery feces, and increased diarrhea incidence compared to MVP100 pigs. The total concentration of fecal volatile fatty acids was greater ($P < 0.05$) in CONT compared to MVP100 fed pigs. Plasma concentration of IgG, IgA, TNF-α, IL-4, and IL-6 did not differ significantly among dietary treatments. The use of MVP reduced ($P < 0.05$) by US$1.00 the total feed cost per pig and 10% to 12% of the feed cost per kg of live weight gain. Thus, the MVP protein blend can be used as cost effective alternative to animal and fish proteins in nursery pig diets. Article 2, the aim of this study was to evaluate the relative bioavailability (RBV) of L-Lys sulfate in comparison with L-Lys HCl and its effects on performance, blood parameters, intestinal functionality, and the apparent total tract digestibility in nursery piglets. The basal diet (CON) was lysine-deficient formulated to meet 73% of standardized ileal digestible Lys requirements. For the other diets, the CON was supplemented with three levels (80%, 90%, and 100% of Lys requirements) of L-Lys sulfate (70% L-Lys) or L-Lys HCl (79% L-Lys). There were no significant differences ($P > 0.05$) in the performance and concentrations of plasma urea between the L-Lys sources. The RBV of L-Lys sulfate relative to L-Lys HCl was 106%, 119% and 117% for effects on ADG, G:F and plasma urea, respectively. Lys deficiency resulted in a greater ($P < 0.05$) incidence of diarrhea, while pigs supplemented with Lys sulfate or Lys HCl showed greater ($P < 0.05$) villus height in the jejunum when compared to those receiving the CON. Diets supplemented with L-Lys sulfate had greater ($P < 0.05$) apparent total tract digestibility of dry matter, gross energy and crude protein. In conclusion, the RBV of L-Lys sulfate for effects on ADG, G:F and plasma urea is equivalent to that of L-Lys HCl for nursery piglets.

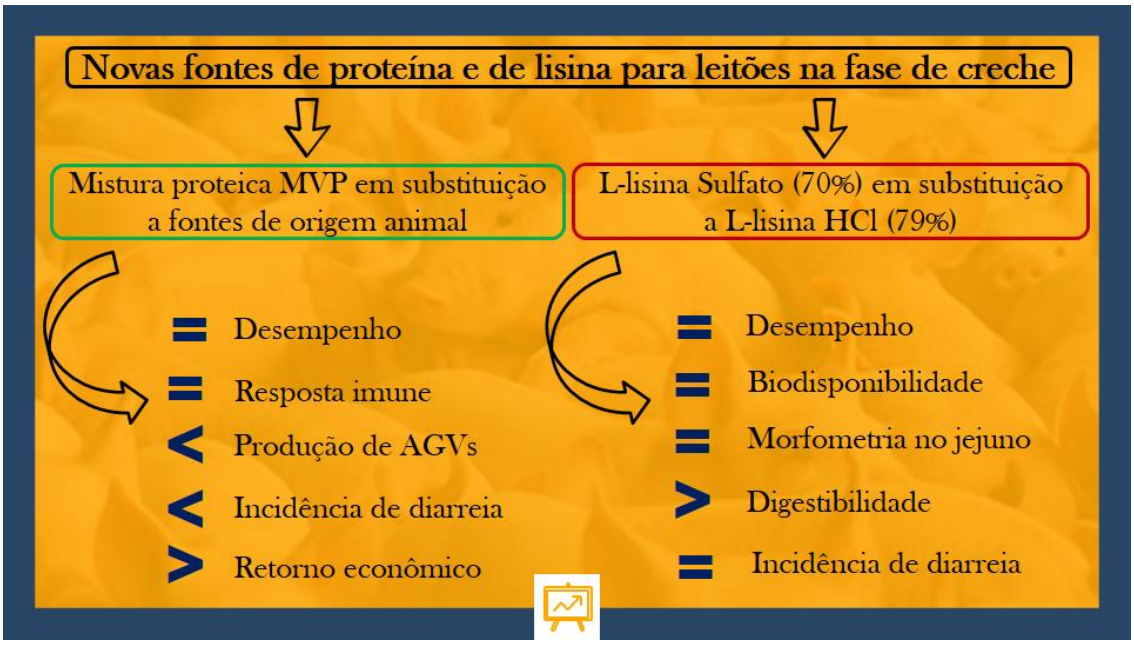
Keywords: Feeding program. Limiting amino acid. Piglet. Protein sources.

Fontes de proteína e lisina em rações para leitões na fase de creche

Elaborado por **Jorge Yair Pérez Palencia** e orientado por **Márvio Lobão Teixeira de Abreu**

Na produção de suínos, o desmame é uma fase de transição crítica para os leitões. Esta fase demanda cuidados especiais para garantir a saúde e o ótimo desempenho dos leitões. Ingredientes de alta digestibilidade e palatabilidade são comumente utilizados nessa fase, entretanto são caros e possuem disponibilidade limitada. Nessas rações, são também utilizados aminoácidos industriais para suprir os requerimentos nutricionais dos animais. Entre as fontes de L-lisina, encontra-se a L-lisina sulfato que, além de L-lisina, contém outros componentes que poderiam ter algum efeito benéfico sobre a saúde e desempenho dos leitões. No primeiro estudo, foi avaliada a substituição de proteínas animais e de peixe por uma mistura proteica fortificada (PROPLEX MVP). No segundo estudo, foi avaliada a biodisponibilidade relativa de L-lisina sulfato em comparação com L-lisina HCl.

A utilização da mistura proteica MVP reduziu o custo total de alimentação e diminuiu a incidência de diarreias nos leitões. Assim, a mistura proteica MVP pode ser utilizada como uma alternativa de baixo custo para substituir fontes de proteína animal e de peixe em rações para leitões. Ao comparar as fontes de L-lisina, não houve diferenças no desempenho, incidência de diarreias, concentrações de ureia plasmática e nem morfologia intestinal do jejuno. A biodisponibilidade da L-lisina sulfato foi equivalente à da L-lisina HCl. Assim, L-lisina sulfato pode ser utilizada em substituição à L-lisina HCl como fonte suplementar de lisina na ração de leitões na fase de creche.



Principais efeitos da utilização da mistura proteica MVP e da L-lisina sulfato como fontes alternativas de proteína e lisina nas rações de leitões na fase de creche.

Tese de Doutorado em Zootecnia na UFLA, defendida em 03/12/2018.

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PRIMEIRA PARTE

1 INTRODUÇÃO

Na produção de suínos, o desmame é considerado um dos eventos mais estressantes na vida dos leitões e que faz da creche uma fase de especial atenção a diversos fatores que podem influenciar a saúde e desempenho dos animais. Durante este período, os leitões são expostos a diversos desafios, que, associados a um sistema digestivo e imune imaturos, resultam em baixo consumo de ração, alterações da morfologia intestinal, predisposição a distúrbios gastrointestinais e, consequentemente, comprometimento do desempenho produtivo.

A inclusão de ingredientes palatáveis e de alta digestibilidade nas rações da creche permite a adaptação gradual às rações convencionais à base de milho e farelo de soja, promove o aumento do consumo de ração e melhora o desempenho dos leitões (BERROCOSO et al., 2012; SKINNER et al., 2014; TEIXEIRA et al., 2003). Soro de leite, leite em pó, plasma sanguíneo e farinha de peixe são ingredientes proteicos geralmente utilizados neste tipo de rações. Entretanto o alto preço e disponibilidade, muitas vezes limitada destas matérias-primas, aumenta os custos de produção.

A utilização de aminoácidos industriais e a redução da porcentagem de proteína bruta das rações têm sido também relacionadas com benefícios sobre a saúde intestinal e desempenho de leitões no pós-desmame (NYACHOTI et al., 2006; ZHAO et al., 2014; WU et al., 2015). Entre as fontes industriais de L-lisina, encontram-se a L-lisina HCl (78% de L-lisina) e a L-lisina sulfato (> 50% de L-lisina). A L-lisina sulfato contém, no mínimo, 50% de L-lisina na base e alguns componentes como outros aminoácidos, resíduos provenientes do processo fermentativo, macromoléculas, pigmentos e outras sustâncias orgânicas e inorgânicas que poderiam ter algum efeito benéfico sobre os processos digestivos e absortivos dos leitões, no período pós desmame e, ainda, promover um melhor desempenho.

Objetivou-se com o presente estudo, 1) avaliar os efeitos da substituição de proteínas animais por uma mistura proteica fortificada (PROPLEX MVP) sobre o desempenho, estado imunológico, metabólitos microbianos e escore fecal de leitões na creche com variação dos períodos de alimentação; 2) avaliar a biodisponibilidade relativa (RBV) de L-lisina sulfato em comparação com L-lisina HCl e seus efeitos no desempenho, parâmetros sanguíneos, funcionalidade intestinal e digestibilidade aparente das rações de leitões na creche.

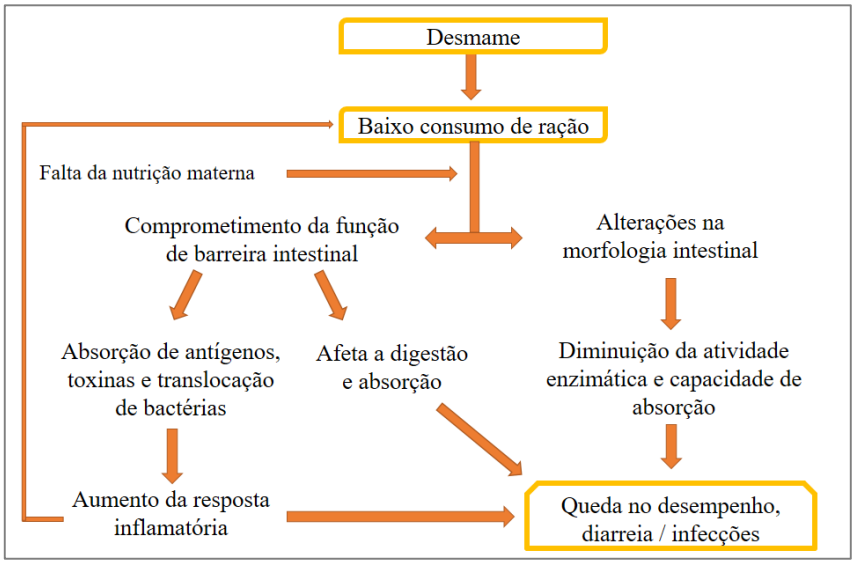
2 REFERENCIAL TEÓRICO

2.1 Implicações do desmame para o leitão

O desmame consiste na separação da fêmea e dos leitões, após o período de lactação e representa um período crítico na vida do leitões, sendo expostos a uma série de fatores que comprometem sua saúde e desempenho. Dentre esses fatores, podem ser citados a separação da mãe, o estresse da manipulação e transporte, um novo ambiente, a mistura de leitegadas e a alteração da dieta e hábito alimentar. Todos estes fatores associados a um sistema imune e digestivo imaturo, típico de leitões desmamados precocemente, desencadeiam baixo consumo de ração, alterações da morfologia intestinal, predisposição a distúrbios gastrointestinais e, consequentemente, comprometimento da saúde e desempenho produtivo dos animais (MCLAMB et al., 2013; POHL et al., 2017; SMITH et al., 2010).

Um dos principais fatores envolvidos na queda do desempenho, nas primeiras semanas após o desmame, é o baixo consumo de ração (DONG; PLUSKE, 2007; JAYARAMAN; NYACHOTI, 2017). Os mecanismos que explicam a queda no desempenho, saúde e bem-estar dos leitões, decorrentes do baixo consumo de ração, são apresentados na Figura 1. De acordo com Jayaraman e Nyachoti (2017), o baixo consumo de ração associado com a interrupção dos componentes nutricionais, advindos da mãe pelo leite, ocasionam alterações na morfologia e funcionalidade intestinal. Estas alterações incluem o encurtamento das vilosidades, hiperplasia das células da cripta e comprometimento da integridade da mucosa intestinal (DONG; PLUSKE, 2007; SPREEUWENBERG et al., 2001). Assim sendo, a fusão intestinal é comprometida pela diminuição da atividade enzimática, na borda em escova e diminuição da capacidade de absorção (VENTE-SPREEUWENBERG et al., 2004). Além disso, a queda da função de barreira intestinal torna o intestino suscetível à ação de antígenos luminais, toxinas e translocação de bactérias, resultando em ativação do sistema imune (SPREEUWENBERG et al., 2001).

Figura 1 - Consequências do baixo consumo de ração sobre a funcionalidade intestinal e desempenho de leitões no pós-desmame.



Fonte: Adaptado de Jayaraman e Nyachoti (2017).

2.2 Rações complexas no pós-desmame

Rações complexas é o termo adotado para designar aquelas rações que apresentam alta porcentagem de ingredientes de valor biológico elevado, oferecidas durante o período pós-desmame com o objetivo de aumentar o consumo de ração e promover uma maior digestibilidade dos componentes da dieta (DONG; PLUSKE, 2007; TEIXEIRA et al., 2003). A inclusão destes ingredientes permite uma adaptação gradual às rações convencionais à base de milho e farelo de soja e melhoram o desempenho dos leitões durante o período de creche (BERROCOSO et al., 2012; SKINNER et al., 2014; TEIXEIRA et al., 2003). Assim, as rações complexas no pós-desmame se caracterizam por altos níveis de lactose, utilização de grãos processados e pela inclusão de fontes proteicas de alta qualidade.

Dentre as fontes de lactose, encontram-se o soro de leite, leite em pó, lactose cristalina e o soro de leite desproteinizado. As Tabelas Brasileiras para Aves e Suínos (ROSTAGNO et al., 2017) demonstram que estas matérias-primas podem ser incluídas até 20% em rações para suínos na fase inicial. A lactose é um constituinte essencial das rações, para leitões nas primeiras fases de vida, principalmente, no período após o desmame. Este ingrediente estimula o consumo de ração e ajuda na transição de uma dieta líquida, à base de leite, para uma dieta solida, à base de matérias-primas de origem vegetal (MAHAN, 1993). Diversos estudos indicaram que a inclusão de altos níveis de lactose, em leitões desmamados, precocemente, melhoraram o desempenho, estimularam o apetite e, como resultado,

aumentaram o consumo de ração (CROMWELL; ALLEE; MAHAN, 2008; MAHAN; FASTINGER; PETERS, 2004; NESSMITH et al., 1997). Além disso, a lactose é o principal substrato que aumenta o crescimento de *Lactobacillus spp*., o que pode retardar a multiplicação de bactérias patogênicas e reduzir distúrbios digestivos nesta fase (KENWORTHY; CRABB, 1963; PARTRIDGE; GILL, 1993). Segundo Mahan, Fastinger e Peters (2004), os níveis recomendados de lactose variam ao longo do período da creche. Estes autores propõem, para a primeira semana pós-desmame, níveis de lactose em torno de 25%, seguidos por um nível aproximado de 15-20%, durante a segunda e terceira semana e, por último, 10-15% na quarta e quinta semanas pós-desmame.

A utilização de grãos processados, nas dietas pós-desmame, tem sido uma alternativa, para garantir uma adequada transição dos leitões, durante este período crítico. Os métodos de processamento alteram as características físico-químicas dos ingredientes, a fim de elevar o seu valor nutricional, aumentar a sua digestibilidade e eliminar fatores antinutricionais que possam comprometer a saúde e o desempenho dos animais. Dentre os métodos de processamento, encontram-se o cozimento, extrusão, micronização, irradiação e outros (HANCOCK; BEHNKE, 2001). Milho extrusado ou pré-genatilizado, soja integral extrusada, isolado proteico de soja e soja micronizada são algumas matérias-primas processadas utilizadas em rações durante o período de creche (BERROCOSO et al., 2014; MOREIRA; OLIVEIRA; FURLAN, 2001; WU et al., 2015).

Por último, as rações complexas incluem diversas fontes proteicas de alta qualidade, dentre as quais se destacam as farinhas de origem animal, como a farinha de peixe e o plasma sanguíneo. As farinhas de origem animal possuem alta porcentagem de proteína bruta, por exemplo, o plasma sanguíneo apresenta em torno de 70% de proteína bruta, enquanto a farinha de peixe alcança valores superiores a 50% (ROSTAGNO et al., 2017). A inclusão de plasma sanguíneo e farinha de peixe, nas rações de leitões no pós-desmame, foi relacionada com melhoras no desempenho, estado imunológico e saúde intestinal de leitões durante as primeiras semanas pós-desmame (BLASCO; FONDEVILA; GUADA, 2005; BOYER et al., 2015; CHE et al., 2012; PÉREZ-BOSQUE; POLO; TORRALLARDONA, 2016; SOLÀ-ORIOL; ROURA; TORRALLARDONA, 2011). Entretanto o alto custo e a disponibilidade destas matérias-primas limitam o seu uso, ao mesmo tempo em que aumentam os custos de produção. Além disso, o uso destes ingredientes e o conceito de rações complexas como um todo têm sido questionados recentemente, em alguns trabalhos, nos quais se evidencia ausência de efeitos deste tipo de manejos alimentares no desempenho até o final do ciclo produtivo dos suínos (COLLINS et al., 2013, 2017; SKINNER et al., 2014).

Nesse contexto, as novas abordagens do uso de rações complexas e manejo alimentar, durante o período de creche, propõem a diminuição da complexidade das rações (COLLINS et al., 2017; SKINNER et al., 2014), a redução da duração das fases de alimentação (LEE et al., 2014) e a substituição das matérias-primas comumente utilizadas por outras de menor custo, sem comprometer o desempenho dos leitões. Foi realizado um levantamento de artigos científicos que avaliaram fontes proteicas alternativas durante o pós-desmame de leitões. O levantamento consistiu numa busca nas principais bases de dados, empregando as palavras "protein sources", "nutrition", "piglets", "nusery", "weaning" e suas combinações. Após a seleção dos artigos, foram tabuladas as principais informações dos estudos e os resultados relacionados com o desempenho, incidência de diarreia, resposta imune, morfometria intestinal e produção de ácidos graxos voláteis (Tabelas 1 e 2). Com base neste levantamento, foi possível identificar algumas matérias-primas com alto potencial, para substituir as convencionais farinhas de origem animal, utilizadas nas rações da creche. Dentre elas, o concentrado proteico de soja, o concentrado proteico de soro de leite, farelo de soja fermentado, produtos da biomassa de fermentação, ovo inteiro seco por pulverização, utilização de um perfil ideal de aminoácidos industriais, entre outros (TABELA 1 e 2).

Tabela 1 - Resumo dos dados gerais dos trabalhos que avaliaram fontes alternativas de proteína em rações para leitões no pós-desmame.

(Continua)

N	Autor/Ano	Idade ao desmame (dias)	Peso ao desmame (Kg)	Repetições	# Animais/ repetição	Período experimental (dias)	Número de fases	Fonte de proteína[1]	% de inclusão por fase[2]
1	Che et al., 2012	22	7.07 ± 0.19	5	16	32	2	Plasma spray dried	4
								CPSoja	4,88
								CPSoro	9,18
								Ovos inteiros spray dried	6,5
2	Noh et al., 2014	NA	NA	6	10	28	2	Polpa cítrica, subproduto de peixe e biomassa de fermentação (PCPBF)	0
								Polpa cítrica, subproduto de peixe e biomassa de fermentação (PCPBF)	2,5
								Polpa cítrica, subproduto de peixe e biomassa de fermentação (PCPBF)	2,5

Tabela 1 - Resumo dos dados gerais dos trabalhos que avaliaram fontes alternativas de proteína em rações para leitões no pós-desmame.

(Continuação)

N	Autor/Ano	Idade ao desmame (dias)	Peso ao desmame (Kg)	Repetições	# Animais/ repetição	Período experimental (dias)	Número de fases	Fonte de proteína[1]	% de inclusão por fase[2]
3	Zhao et al., 2014	21	6.49 ± 0.57	8	5	28	2	FP + CPSoro	3; 5 / 5,6; 9.35
								FP + AA Industriais	3; 5 / L-Lys (0,50;0,30), L-Thr(0,18; 0,11), L-Trp(0,05; 0,04), DL-Met(0,11; 0,07), L-Val(0,15; 0,09), L-Ile (0,15; 0,09)
								CPSoro + AA Industriais	5,6; 9.35 / (0,40; 0,30), L-Thr(0,10; 0,06), L-Trp(0,02; 0,02), DL-Met(0,08; 0,05), L-Val(0,11; 0,07), L-Ile (0,10; 0,06)
								AA Industriais	L-Lys (0,90; 0,55), L-Thr(0,28; 0,17), L-Trp(0,08; 0,05), DL-Met(0,19; 0,12), L-Val(0,26; 0,16), L-Ile (0,26; 0,15)
4	Wu et al., 2015	21	5.99 ± 0.14	6	5	14	1	Controle FP (17% PB)	3
								CPSoja (19% PB)	4,37
								FP (19% PB)	7,37
								CPSoja (23.7% PB)	13,41
								FP (23.7% PB)	16,20

Tabela 1 - Resumo dos dados gerais dos trabalhos que avaliaram fontes alternativas de proteína em rações para leitões no pós-desmame.

(Conclusão)

N	Autor/Ano	Idade ao desmame (dias)	Peso ao desmame (Kg)	Repetições	# Animais/ repetição	Período experimental (dias)	Número de fases	Fonte de proteína[1]	% de inclusão por fase[2]
5	Zhang et al., 2015	21	7.06 ± 1.56	5	6	21	1	Ovos inteiros spray dried Albúmen em pó Plasma spray dried Solúveis de suínos secos FP	8 4,67 4,56 6,84 5,26
6	Sinn et al., 2017	21	6.1 ± 0.8	8	7	35	2	Farelo de soja + soro de leite FP MEPRO	37,3; 40,7 / 25;9,98 7,49 /4,99 7,48/4,99
7	Zhong et al., 2017	35	17.38 ± 0.26	24	1	28	1	Farelo de soja Caseína DDGS	18 5,93 17,67
8	Wen et al., 2018	21	5.99 ± 0.14	6	5	14	1	Controle FP (17% PB) CPSoja (19% PB) FP (19% PB) CPSoja (23.7% PB) FP (23.7% PB)	3 4,37 7,37 13,41 16,20

Fonte: Dados do autor (2018).

[1] **FP**= Farinha de peixe; **CPsoro**= Concentrado proteico de soro de leite; **CPsoja**= Concentrado proteico de Soja; **AA**= Aminoácidos; **DDGS**= grãos de destilaria; **MEPRO**= produto de soja melhorado microbianamente.

[2] Números separados por ponto e vírgula indicam mudança de fase com porcentagem de inclusão diferente e número ou conjunto de números separados por / indica que seguem as informações para outra matéria-prima.

[3] Não apresentado.

Tabela 2 - Efeito da utilização de diferentes fontes alternativas de proteína em rações para leitões no pós-desmame sobre o desempenho, incidência de diarreia (**ID**), resposta imune (**RI**), morfometria intestinal (**MI**) e produção de ácidos graxos voláteis (**AGVs**).[1]

(Continua)

Autor/Ano	Fonte de proteína[2]	Desempenho 1 semana				Desempenho geral				ID	Dia/Par[3]	RI	Dia/Par	MI	Dia/ Par	AGVs
		CDR	GPD	F:G	G:F	CDR	GPD	F:G	G:F							
Che et al., 2012	Plasma spray dried	a	a	a	NA	a	a	a	NA	NA	10 dias / IgG; IgA; C3; IL-1B; IL-10	a; a; a; a; a	10 dias /Altura de Vilosidades; profundidade de cripta; relação no jejuno	a; a; a	NA	NA
	CPSoja	a	b	b	NA	a	a	a	NA	NA		b; b; b; a; a		b; b; b		NA
	CPSoro	a	ab	ab	NA	a	a	a	NA	NA		ab; ab; ab; a; a		b; ab; a		NA
	Ovos inteiros spray dried	a	b	b	NA	a	a	a	NA	NA		a; a; a; b; a		ab; ab; ab		NA
Noh et al., 2014	PCPBF	NA	NA	NA	NA	a	a	a	b	NA	NA	NA	NA	NA	NA	NA
	PCPBF	NA	NA	NA	NA	a	a	a	ab	NA		NA		NA		NA
	PCPBF	NA	NA	NA	NA	a	a	a	a	NA		NA		NA		NA
Zhao et al., 2014	FP + CPSoro	a	b	NA	b	a	a	a	a	NA	7 dias /IG; IM; TNF-α	ab; a; a	7 dias /Altura de vilosidades	a	7 dias /Produção total de AGVs em Cécum e colón digesta	a
	FP + AA Industriais	a	a	NA	a	a	a	a	a	NA		bc; a; a		a		a
	CPSoro + AA Industriais	a	a	NA	a	a	a	a	a	NA		a; a; a		a		a
	AA Industriais	a	a	NA	a	a	a	a	a	NA		c; a; a		a		a

Tabela 2 - Efeito da utilização de diferentes fontes alternativas de proteína em rações para leitões no pós-desmame sobre o desempenho, incidência de diarreia (**ID**), resposta imune (**RI**), morfometria intestinal (**MI**) e produção de ácidos graxos voláteis (**AGVs**).[1]

(Continuação)

Autor/Ano	Fonte de proteína[2]	Desempenho 1 semana				Desempenho geral				ID	Dia/Par[3]	RI	Dia/Par	MI	Dia/ Par	AGVs
		CDR	GPD	F:G	G:F	CDR	GPD	F:G	G:F							
Wu et al., 2015	Controle FP (17% PB)	NA	NA	NA	NA	a	a	NA	a	c	14 dias / IL-1β; IFN-γ no jejuno	b; c	14 dias /Altura de Vilosidades; profundidade de cripta; relação no jejuno	a; c; a	NA	NA
	CPSoja (19% PB)	NA	NA	NA	NA	a	b	NA	b	ab		ab; ab		bc; abc; bc		NA
	FP (19% PB)	NA	NA	NA	NA	a	b	NA	b	b		ab; b		ab; bc; b		NA
	CPSoja (23.7% PB)	NA	NA	NA	NA	a	b	NA	b	a		a; a		c; ab; c		NA
	FP (23.7% PB)	NA	NA	NA	NA	a	b	NA	b	a		a; ab		bc; a; c		NA
Zhang et al., 2015	Ovos inteiros spray dried	NA	NA	NA	NA	a	a	a	a	NA	IGG; IGM	a; a	NA	NA	NA	NA
	Albúmen em pó	NA	NA	NA	NA	a	a	a	a	NA		a; a		NA		NA
	Plasma spray dried	NA	NA	NA	NA	a	a	a	a	NA		a; a		NA		NA
	Solúveis de suínos secos	NA	NA	NA	NA	a	a	a	a	NA		a; a		NA		NA
	FP	NA	NA	NA	NA	a	a	a	a	NA		a; a		NA		NA
Sinn et al., 2017	Farelo de soja + soro de leite	a	a	a	a	a	a	a	a	a	NA	NA	NA	NA	NA	NA
	FP	a	a	a	a	a	a	a	a	b		NA		NA		NA
	MEPRO	a	a	a	a	a	a	a	a	b		NA		NA		NA

Tabela 2 - Efeito da utilização de diferentes fontes alternativas de proteína em rações para leitões no pós-desmame sobre o desempenho, incidência de diarreia (**ID**), resposta imune (**RI**), morfometria intestinal (**MI**) e produção de ácidos graxos voláteis (**AGVs**).[1]

(Conclusão)

Autor/Ano	Fonte de proteína[2]	Desempenho 1 semana				Desempenho geral				ID	Dia/Par[3]	RI	Dia/Par	MI	Dia/ Par	AGVs
		CDR	GPD	F:G	G:F	CDR	GPD	F:G	G:F							
Zhong et al., 2017	Farelo de soja	NA	NA	NA	NA	a	a	b	NA	NA	NA	NA	NA	NA	NA	NA
	Caseína	NA	NA	NA	NA	a	a	b	NA	NA						
	DDGS	NA	NA	NA	NA	b	b	a	NA	NA						
Wen et al., 2018	Controle FP (17% PB)	NA	NA	NA	NA	NA	NA	NA	NA	b	NA	NA	NA	NA	14 dias /Produção de AGVs na digesta do colón	b
	CPSoja (19% PB)	NA	NA	NA	NA	NA	NA	NA	NA	a						b
	FP (19% PB)	NA	NA	NA	NA	NA	NA	NA	NA	a						b
	CPSoja (23.7% PB)	NA	NA	NA	NA	NA	NA	NA	NA	a						b
	FP (23.7% PB)	NA	NA	NA	NA	NA	NA	NA	NA	a						a

Fonte: Dados do autor (2018).

[1abcd]Letras diferentes na coluna indicam diferenças significativas entre as fortes de proteína para cada trabalho; **NA**= Não avaliado; letras separadas por ponto e coma na linha indicam diferentes parâmetros na sequência na coluna anterior.

[2] **FP**= Farinha de peixe; **CPsoro**= Concentrado proteico de soro de leite; **CPsoja**= Concentrado proteico de Soja; **PCPBF**= Polpa cítrica, subproduto de peixe e biomassa de fermentação **AA**= Aminoácidos; **DDGS**= grãos de destilaria; **MEPRO**= produto de soja melhorado microbianamente.

[3] Dia de coleta/parâmetros avaliados.

2.3 Lisina na nutrição de suínos em crescimento

O crescimento em espécies animais, incluídas as destinadas à produção, é o resultado de uma série de processos biológicos, sendo o genótipo o fator que determina a expressão máxima desses processos. O crescimento é o resultado de um processo metabólico que inclui como evento principal a retenção de nitrogênio, representada pela deposição de proteína na carcaça. Nesse sentido, quando se consideram os animais de produção de carne, como a espécie suína, o principal objetivo é o aumento do tecido magro, o qual representa o fator que mais influencia as exigências de aminoácidos, principalmente, de lisina (WU et al., 2013). Essas exigências são influenciadas por fatores como genética, peso corporal, sexo, status sanitário e ambiente, que devem ser considerados em sua estimação (DU et al., 2015). Ao atentar a esses fatores, as dietas calculadas irão atender as necessidades de mantença e produção, possibilitando que o suíno expresse o seu potencial genético máximo para a deposição de carne.

Os aminoácidos são a parte dos nutrientes mais importante na nutrição animal. Responsáveis pela formação das proteínas e precursores de substâncias biologicamente ativas, a resposta animal pode ser limitada pela sua deficiência ou melhorada por sua correta estimação e fornecimento. Os aminoácidos (AA) são definidos como compostos orgânicos simples, que contêm tanto um grupo carboxila quanto um grupo amino, que podem estar ligados aos diferentes átomos de carbono da molécula. Existem mais de 700 AA na natureza. Dentre eles, 20 servem como blocos de construção das proteínas (WU, 2013). Estes AA são classificados como essenciais (AAE), não essenciais (AANE) ou condicionalmente essenciais para os animais. Os AA essenciais não podem ser sintetizados pelo organismo animal, o que faz necessária sua suplementação na dieta. Na nutrição de suínos, existem 9 AA classificados como essenciais: lisina (Lis), histidina (His), isoleucina (Ile), leucina (Leu), metionina (Met), fenilalanina (Fen), treonina (Tre), triptofano (Tri) e valina (Val). O AA não essenciais são a alanina (Ala), a asparagina (Asn), o aspartato (Asp), o glutamato (Glu), a glicina (Gli) e a serina (Ser). Já os AA condicionalmente essenciais são a arginina (Arg), a cisteína (Cis), a glutamina (Gln), a prolina (Pro) e a tirosina (Tir) (NATIONAL RESEARCH COUNCI - NRC, 2012; WU, 2010).

Os aminoácidos essenciais e limitantes às rações dos animais de produção devem ser balanceados, com o objetivo de atender as exigências nutricionais para que os animais tenham um adequado desempenho. Entre eles, a lisina é o primeiro aminoácido limitante em rações para suínos e se considera o aminoácido de maior relevância para a deposição de carne magra

na carcaça dos suínos em crescimento. Isto se deve à sua constância na proteína corporal e à sua destinação metabólica preferencial para deposição de carne. Além disso, a lisina tem sido utilizada como aminoácido de referência, para a determinação dos demais aminoácidos, os quais são calculados em relação ao conteúdo de lisina, para não haver excesso ou deficiência, segundo um perfil proteico ideal (NRC, 2012; ROSTAGNO et al., 2017).

A deficiência de lisina dietética influencia no turnover proteico corporal de suínos em crescimento com inibição no acréscimo de proteína (FULLER et al., 1987; SALTER et al., 1990), reduz a massa de proteína muscular, prejudica a taxa de crescimento de suínos no periodo após o desmame (CHANG; WEI, 2005) e diminui o ganho de peso e eficiência alimentar em suínos na fase de crescimento (ZHANG et al., 2008). A deficiência de lisina foi também relacionada à inibição da ingestão de alimentos e ao compormetimento da absorção intestinal e ao metabolismo de aminoácidos em leitões (HE et al., 2013). Han et al. (2018) reportaram que a deficiência de lisina comprometia a resposta inflamatoria em leitões, visto que leitões submetidos à deficiencia deste aminoácido (ração atendendo a 70% das exigências) apresentaram menores concentrações de anticorpos sericos e citocinas inflamatorias.

Em relação às exigências de lisina para suínos, os avanços, no estudo da nutrição para esta espécie, têm gerado ferramentas que auxiliam aos nutricionistas e produtores no conhecimento das exigências nutricionais dos animais e na formulação de rações com maior precisão. Atualmente existem duas principais referências de exigências nutricionais para suínos: as Tabelas Brasileiras para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais (ROSTAGNO et al., 2017) e o Nutrient Requirements of Swine (NRC, 2012). As exigências de lisina, para suínos em crescimento, propostas por estas referências, apresentam algumas diferenças consideráveis (Tabela 3; Figura 2). As exigências de lisina, propostas pelas Tabelas Brasileiras 2011 e 2017, são semelhantes para suínos até 15 kg de peso vivo, enquanto o NRC apresenta valores inferiores. Considerando animais de 15 kg até o abate, as exigências de lisina diminuíram na versão 2017, continuando sendo maiores do que as propostas pelo NRC (Figura 2).

As diferenças encontradas na nova edição das Tabelas Brasileiras obedecem a um padrão de contínua evolução da suinocultura brasileira, principalmente, com o aprimoramento das genéticas suínas trabalhadas nos sistemas de produção. O melhoramento genético tem procurado por animais cada vez mais eficientes, que, associados com os avanços em sanidade, manejo, ambiência e bem-estar animal, podem explicar a diminuição nas recomendações de consumo e as menores exigências de lisina para animais em crescimento. Além disso, as

atuais Tabelas Brasileiras (ROSTAGNO et al., 2017), também, dispõem recomendações nutricionais, para suínos em crescimento, em ambientes acima da faixa de conforto térmico, que permitirá o melhor atendimento das exigências nutricionais destes animas sobre essas condições.

Tabela 3 - Exigências nutricionais de suínos machos de alto potencial genético com desempenho regular - médio segundo as Tabelas Brasileiras 2011 e 2017 e NRC 2012.

(Continua)

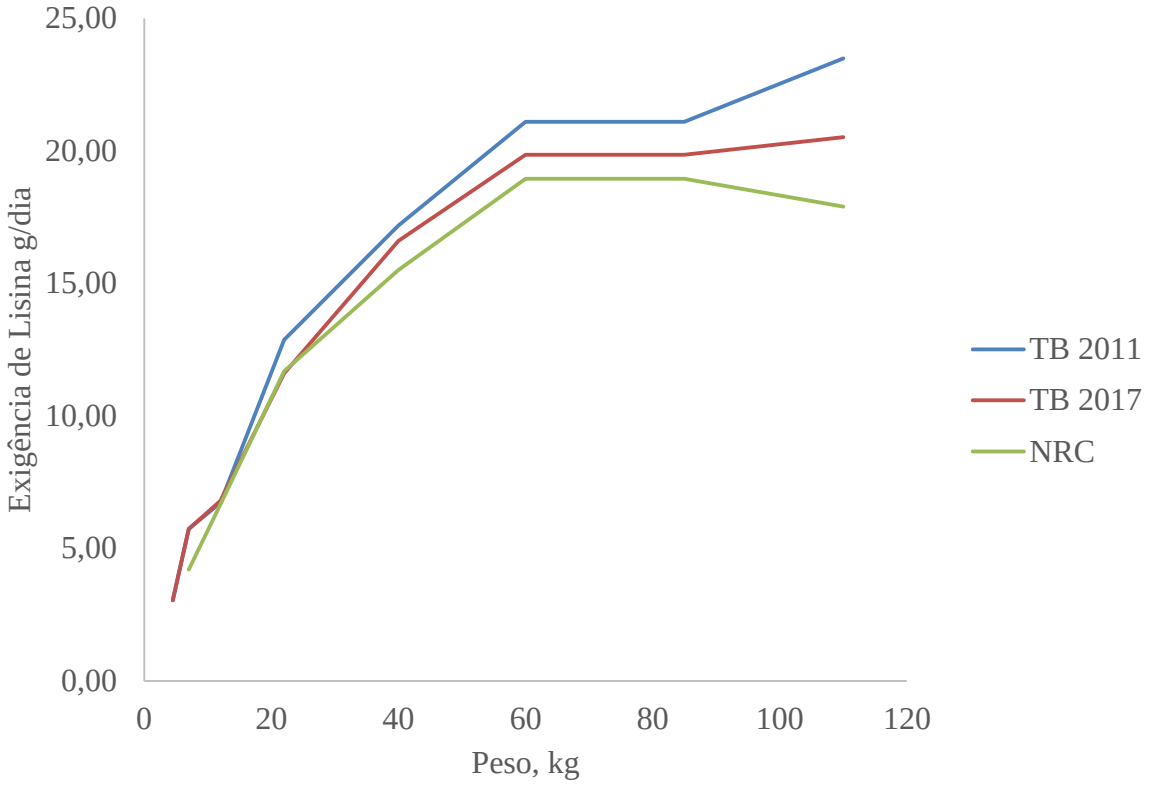
Parâmetros	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012
Peso vivo, kg	3,5-5,3	3,5-5,3	NA	5,5-9	5,5-9	5-7	9,3-15	9,3-15	7-11	15 - 30	15 - 30	11 - 25
Ganho de peso, kg	NA	NA	NA	NA	0,324	0,21	NA	0,368	0,335	0,693	0,618	0,585
Consumo, kg/dia	***0,2***	***0,2***	NA	0,395	0,395	0,28	***0,505***	0,505	0,493	1,241	1,036	0,95
Energia Met, kcal/dia	690,00	691,80	NA	1343	1343	952	1704,375	1704,375	1676,2	4008,43	3367	3182,5
Minerais												
Fósforo Digestível, g/dia	1,00	1,00	NA	1,78	2,02	1,15	2,07	2,35	1,77	4,36	3,94	2,76
Cálcio, g/dia	1,78	1,78	NA	3,36	4,22	2,38	4,17	4,91	3,94	9,10	8,23	6,65
Potássio, g/dia	1,04	1,04	NA	2,05	2,05	0,84	2,53	2,60	1,38	5,83	4,90	2,47
Sódio, g/dia	0,56	0,56	NA	1,11	0,88	1,12	1,16	1,11	1,73	2,48	2,06	2,66
Cloro, g/dia	0,50	0,50	NA	0,99	0,85	1,40	1,11	1,06	2,22	2,36	1,97	3,04
Aminoácidos (Dig)												
Lisina, g/dia	3,04	3,04	NA	5,73	5,73	4,20	6,72	6,80	6,66	12,87	11,60	11,69
Metionina, g/dia	0,85	0,85	NA	1,60	1,60	1,20	1,88	1,90	1,92	3,60	3,37	3,42
Metionina + Cistina, g/dia	1,70	1,70	NA	3,21	3,21	2,30	3,76	3,81	3,65	7,21	6,61	6,46
Treonina, g/dia	1,92	2,04	NA	3,61	3,84	2,46	4,23	4,56	3,89	8,10	7,54	6,94
Triptofano, g/dia	0,55	0,58	NA	1,03	1,09	0,70	1,21	1,29	1,08	2,32	2,21	1,90
Arginina, g/dia	2,58	3,04	NA	4,87	5,73	1,90	5,71	6,80	3,01	5,41	5,22	5,32
Valina, g/dia	2,10	2,10	NA	3,95	3,95	2,66	4,64	4,69	4,24	8,89	8,01	7,41
Isoleucina, g/dia	1,67	1,67	NA	3,15	3,15	2,16	3,70	3,74	3,40	7,07	6,38	5,99
Leucina, g/dia	3,04	3,04	NA	5,73	5,73	4,20	6,72	6,80	6,66	12,87	11,60	11,69
Histidina, g/dia	1,00	1,00	NA	1,89	1,89	1,46	2,22	2,24	2,27	4,24	3,83	3,99
Fenilalanina, g/dia	1,52	1,52	NA	2,86	2,87	2,46	3,36	3,40	3,89	6,44	5,80	6,84
Fenilal. + Tirosina, g/dia	3,04	3,04	NA	5,73	5,73	3,86	6,72	6,80	6,16	12,87	11,60	10,83

Tabela 3 - Exigências nutricionais de suínos machos de alto potencial genético com desempenho regular - médio segundo as Tabelas Brasileiras 2011 e 2017 e NRC 2012. (Conclusão)

Parâmetros	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012	TB 2011	TB 2017	NRC 2012
Peso vivo, kg	30 - 50	30 - 50	25 - 50	50 - 70	50 - 70	50 - 75	70 - 100	70 - 100	75 - 100	100 - 120	100 - 120	100 - 135
Ganho de peso, kg	0,868	0,838	0,758	1,014	0,954	0,9	1,071	0,99	0,917	1,084	0,912	0,867
Consumo, kg/dia	1,854	1,729	1,582	2,563	2,378	2,229	3,027	2,967	2,636	3,399	3,257	2,933
Energia Met, kcal/ dia	5988,42	5619,25	5220,6	8278,49	7728,5	7355,7	9777,21	9642,75	8698,8	10978,77	10585,25	9678,9
Minerais												
Fósforo Digestível, g/dia	5,60	5,43	4,11	6,36	6,04	5,13	6,96	6,53	5,54	7,31	6,42	5,28
Cálcio, g/dia	11,68	11,32	10,44	13,12	12,46	13,15	14,35	13,47	13,71	15,06	13,22	13,49
Potássio, g/dia	8,31	7,80	3,64	10,89	10,15	4,24	12,11	11,87	4,48	12,64	12,12	4,99
Sódio, g/dia	3,34	3,20	1,58	4,36	4,07	2,23	4,84	4,75	2,64	5,10	4,98	2,93
Cloro, g/dia	3,15	3,03	1,27	4,10	3,83	1,78	4,54	4,45	2,11	4,76	4,69	2,35
Aminoácidos (Dig)												
Lisina, g/dia	17,19	16,60	15,50	21,09	19,86	18,95	21,09	19,86	18,95	23,49	20,52	17,89
Metionina, g/dia	5,15	4,98	4,43	6,33	5,97	5,35	7,17	6,47	5,54	7,27	6,16	5,28
Metionina+Cistina, g/dia	10,14	9,82	8,70	12,46	11,72	10,70	13,86	12,94	11,07	14,11	12,31	10,56
Treonina, g/dia	11,18	10,79	9,33	13,71	12,91	11,59	15,47	14,00	12,13	15,74	13,35	11,73
Triptofano, g/dia	3,10	3,32	2,69	3,79	3,97	3,34	4,15	4,30	3,43	4,21	4,10	3,23
Arginina, g/dia	7,05	6,97	7,12	8,64	8,35	8,69	7,39	8,60	8,70	7,51	8,21	8,21
Valina, g/dia	11,87	11,45	10,12	14,56	13,70	12,26	15,92	14,86	12,65	16,21	14,17	12,03
Isoleucina, g/dia	9,46	9,13	8,07	11,61	10,92	10,03	12,71	11,84	10,28	12,92	11,30	9,68
Leucina, g/dia	17,19	16,60	15,66	21,09	19,86	18,95	23,10	21,54	19,51	23,49	20,52	18,18
Histidina, g/dia	5,67	5,48	5,38	6,97	6,56	6,46	7,63	7,12	6,59	7,75	6,77	6,16
Fenilalanina, g/dia	8,60	8,30	9,33	10,56	9,94	11,37	11,56	10,77	11,60	11,76	10,26	10,85
Fenilal. + Tirosina, g/dia	17,19	16,60	14,55	21,09	19,86	17,83	23,10	21,54	18,19	23,49	20,52	17,01

Fonte: Dados do autor (2018).

Figura 2 - Exigências de Lisina (g/ dia), para suínos machos em crescimento, de alto potencial genético com desempenho regular - médio segundo as Tabelas Brasileiras 2011 e 2017 e NRC 2012.



Fonte: Dados do autor (2018).

As rações convencionais, à base de milho e soja, geralmente, não atendem as exigências nutricionais de suínos em crescimento, porque as matérias-primas utilizadas, em maior proporção dentro da ração, apresentam déficit deste aminoácido. Este é o caso do milho, o qual apresenta 0,2 a 0,4 de lisina. O farelo de soja, utilizado com ingrediente proteico, possui entre 2,7% e 3,0% de lisina, porém aspectos econômicos e zootécnicos limitam sua utilização em rações. Nesse contexto, surgem os aminoácidos industriais, com o objetivo de suprir essa demanda.

No mercado mundial de produtos fermentados, os aminoácidos figuram entre os mais importantes. São produzidos industrialmente por quatro vias: fermentação, síntese química, extração por hidrólises ácidas e método enzimático. Dentre elas, a via de fermentação é a mais utilizada, produzindo aminoácidos de forma L-isômero (SAKOMURA et al., 2014). Com o crescimento contínuo da produção animal, a demanda de aminoácidos tem aumentado, significativamente, ao mesmo tempo em que as tecnologias, para a sua produção, têm

evoluído favoravelmente. Por isso, existe grande variedade de aminoácidos, na forma de produtos comerciais, que são oferecidos ao mercado.

Entre as fontes suplementares de L-lisina, encontram-se a L-lisina HCl (78% de L-lisina) e a L-lisina sulfato (> 50% de L-lisina). A L-lisina pura, por ser altamente higroscópica, tem seu uso limitado na fábricação de alimentos para animais, sendo necessária a utilização de alternativas. A forma mais usada, para empregar a L-lisina, é de mono-hidrocloreto (L-lisina HCl), tendo 98% de pureza, com cerca de 78% de lisina base e 19 – 20% de cloro (SAKOMURA et al., 2014). Também a lisina se encontra disponível como sulfato de lisina, nas formas líquida ou sólida, por meio da qual obtém um processor semelhante à L-lisina HCl, porém as formas de processamento diferem, tendo, ao final, um produto que contém, no mínimo, 50% de L-lisina na base. Este produto contém outros nutrientes além da lisina, como outros aminoácidos e impurezas provenientes do processo fermentativo, macromoléculas, pigmentos e outras sustâncias orgânicas e inorgânicas. Estas diferenças se originam no processamento e, para a obtenção de L-lisina sulfato, o produto final da fermentação não passa pelas etapas de separação e purificação (SAKOMURA et al., 2014). Estas sustâncias a mais, presentes na L-lisina sulfato, poderiam ter algum efeito benéfico sobre os processos digestivos e absortivos dos leitões, no período pós desmame e, ainda, promover um melhor desempenho.

Pelos estudos de biodisponibilidade, tem sido avaliada a efetividade e viabilidade da utilização de fontes de L-lisina na ração de suínos. A biodisponibilidade relativa (RBV) é definida como “O grau ao qual um nutriente ingerido numa determinada fonte é absorvido de uma forma que pode ser utilizada no metabolismo pelo animal" (BAKER, 1986). Smiricky-Tjardes et al. (2004) avaliaram a RBV de uma fonte de L-lisina sulfato (47,3% de L-lisina) em comparação com a L-lisina HCl (78,5% de L-lisina), em dietas para leitões de 9,5 Kg, durante 21 dias. Estes autores relataram que a RBV de L-lisina sulfato não foi significativamente diferente da RBV de L-lisina HCl com base nas variáveis ganho de peso diário e eficiência alimentar.

Em um estudo semelhante, Liu et al. (2007) avaliaram a RBV de duas fontes suplementares de lisina em rações para suínos dos 10 até 20 kg, a L-lisina sulfato (50% de L-lisina) e a L-lisina HCl (78% de L-lisina). Nesta pesquisa, a RBV da L-lisina sulfato em comparação à L-lisina HCl foi de 101%, 105%, 104% e 95%, para as variáveis GPD, CA, nitrogênio da ureia plasmática e nitrogênio retido, respetivamente. Em um estudo mais recente, a RBV da L-Lisina sulfato (54,5% de L-lisina) em comparação com a L-lisina HCl (78% de L-lisina), foi estimada em 104% e 112% para as variáveis GPD e eficiência

alimentar de suínos em crescimento, dos 57 kg a 87kg (HTOO et al., 2016). Em conjunto, os resultados destes autores evidenciam que não há diferenças marcantes entre a RBV da L-lisina sulfato e a L-lisina HCl, quando suplementadas para suínos em crescimento.

3 CONSIDERAÇÕES GERAIS

O desmame é uma fase de transição crítica para os leitões, caraterizada por diversos eventos e mudanças físicas e fisiológicas que comprometem a saúde e desempenho dos animais no período pós-desmame. O manejo nessa fase torna-se então um grande desafio e demanda de especiais cuidados, a fim de minimizar os fatores estressantes, garantindo adequados índices produtivos ao longo do período de creche e até o abate.

Ingredientes palatáveis e de alta digestibilidade têm sido amplamente utilizados, nas rações pós-desmame, facilitando a transição para as rações convencionais e mostrando efeitos positivos sobre o consumo de ração e o desempenho dos leitões. Todavia esses ingredientes são caros e possuem disponibilidade limitada. Fontes proteicas alternativas que possam substituir as fontes de proteína animal de alto preço sem comprometer o desempenho, têm sido estudadas. O concentrado proteico de soro e os ovos inteiros, entre elas, na forma spray dried, têm apresentado um grande potencial de utilização, mantendo um adequado desempenho e, ainda, trazendo benefícios sobre parâmetros de saúde intestinal dos leitões durante o período de creche.

A diminuição da porcentagem da proteína bruta da ração com o emprego de aminoácidos industriais tem sido outra estratégia nutricional durante o período pós-desmame. Valores de proteína bruta entre 17% e 19% mostram melhores resultados quando comparados com valores acima de 23% de proteína bruta na ração. Ao considerar fontes industriais de lisina, os primeiros aminoácidos limitantes, para suínos em crescimento, são a L-lisina HCl e a L-lisina sulfato. Além de L-lisina, a L-lisina sulfato contém outros componentes que poderiam ter algum efeito benéfico sobre a saúde intestinal dos leitões, no período pós-desmame, proporcionar um adequado desempenho e ainda diminuir o custo da ração.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - A FORTIFIED PROTEIN BLEND AS A REPLACEMENT FOR ANIMAL AND FISH PROTEINS IN NURSERY PIG DIETS

JORGE YAIR PÉREZ PALENCIA

ARTIGO FORMATADO DE ACORDO COM AS NORMAS DA REVISTA CIENTIFICA JOURNAL ANIMAL SCIENCE (JAS).

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A fortified protein blend as a replacement for animal and fish proteins in nursery pig diets[1]

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ABSTRACT: These experiments observed the effects of replacing animal and fish proteins with a fortified protein blend (PROPLEX MVP) on growth performance, immune status, microbial metabolites, and fecal scoring of nursery piglets. In Exp. 1, 306 barrows and gilts

[(Duroc x (Landrace x Yorkshire), 22 d of age and BW 6.37 ± 1.24 kg)] were used in a randomized complete block design with three dietary treatments. Dietary treatments were: 1) Control diet (CONT) with animal and fish protein sources (blood meal, spray-dried plasma, and fish meal); 2) PROPLEX MVP (MVP): CONT with MVP replacing fish meal; 3) PROPLEX MVP 100 (MVP100): CONT with MVP replacing 100% of animal and fish proteins. In Exp. 2, 244 barrows and gilts [Duroc x (Landrace x Yorkshire), 19 d of age and BW 5.88 ± 1.38 kg] were used in a 2 × 2 factorial design (2 dietary treatments × 2 feeding programs). The dietary treatments used corresponded to the CONT and MVP100 treatments as described in Exp. 1. The feeding programs for each feeding phase were: high budget (H): Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig and low budget (L): Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig. For both experiments, pigs fed CONT had greater performance ($P < 0.05$) compared to MVP100 pigs d 0-7 post-weaning. However, from d 7 to 14, pigs fed MVP and MVP100 had a greater ADG, and G:F ($P < 0.05$) than pigs fed CONT diet. For the remainder of the experimental period (Exp. 1 and Exp. 2), there were no differences in pig final BW, overall ADG and G:F. In Exp. 2, from the second week and during most of the experimental period, pigs fed CONT had a greater ($P < 0.05$) fecal score, more soft and watery feces, and increased diarrhea incidence compared to MVP100 pigs. The total concentration of d 13 fecal volatile fatty acids was greater ($P < 0.05$) in CONT compared to MVP100 fed pigs. Plasma concentration of IgG, IgA, TNF-α, IL-4, and IL-6 on d 13 did not differ among pigs from different dietary treatments. The use of MVP reduced ($P < 0.05$) by US$1.00 the total feed cost per pig and 12% of the feed cost per kg of live weight gain in Exp. 1 and when combined with a low feed budget farther reduced feed costs by US$1.88 per pig in Exp. 2. In conclusion, the MVP protein blend can be used as a cost effective alternative to animal and fish proteins in nursery pig diets without compromising overall growth performance, immune status, and post-weaning diarrhea.

Keywords: cytokines, diarrhea, feeding program, piglet, protein sources.

INTRODUCTION

Piglet management post-weaning is one of the most challenging aspects of swine production. During this period, piglets are usually exposed to environmental, social, and psychological stressors which have direct or indirect effects on gut health and overall growth performance (Khafipour et al., 2014; McLamb et al., 2013). These factors predispose piglets to reduced feed intake which, in concert with immature digestive and immune systems, results in increased incidences of diarrhea, decreased performance, mortality and (or) morbidity, and subsequent economic losses (Lallés et al., 2004; Smith et al., 2010; Pohl et al., 2017).

In pork production, the diets for weanling pigs are expensive due to the inclusion of ingredients with high digestibility and palatability in order to meet the nutritional requirements of pigs, reduce the negative effects of weaning, and consequently improve growth performance (Berrocoso et al., 2012; Berrocoso et al., 2013). Protein is normally the most expensive component in diets for production animals. Fish meal, spray-dried plasma, and blood meal are usually used as high quality animal protein sources in diets for nursery pigs. However, the high price and low availability of these ingredients increase the overall cost of pork production. Thus, it is important to find alternative protein sources that are cost-effective and can replace the high price animal protein sources without compromising pig performance and health.

Several studies have evaluated individually some possible alternatives to replace animal protein sources in nursery diets. Within them, soy protein concentrate, fermented soybean meal, fermented biomass product, spray-dried whole egg, cottonseed meal, supplemental amino acids, and microbially enhanced soybean products have been evaluated

as potential replacements (Sulabo et al., 2013; Che et al., 2014; Noh et al., 2014; Zhao et al., 2014; Wu et al., 2015; Cao et al., 2016; Sinn et al., 2017; Wen et al., 2018). However, there is little information available in the literature on the evaluation of protein mixtures to substitute all animal protein sources in diets for weaned piglets. Furthermore, the varying nursery phase-feeding programs seem to be related to pig growth performance and production cost (Lee et al., 2014). Therefore, the objective of this study was to evaluate the effects of replacing animal and fish proteins with a fortified protein blend (PROPLEX MVP) on the growth performance, immune status, microbial metabolites, and fecal scoring of weaned piglets by the duration of feeding periods.

MATERIALS AND METHODS

The experimental protocols used in this study were approved by the Purdue University Animal Care and Use Committee, and animal care and use standards were based upon the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010). The experiments were performed in the weaning facilities of the Animal Sciences Research and Education Center (ASREC) at Purdue University, in West Lafayette, IN 47906, USA.

Exp 1.

A total of 306 newly weaned pigs [(Duroc x (Landrace x Yorkshire), barrows and gilts, 22 d of age, initial BW 6.37 ± 1.24 kg)] were used in a randomized complete block design and assigned to one of three dietary treatments in BW blocks, with 19 replicates (pens) of 5 piglets and sex ratios maintained within BW blocks. The experiment was conducted in a wean-to-finish barn containing 12 rooms, each with 12 pens (1.83 m × 2.44 m) that provided initially approximately 0.84 m^2 per pig. All pens contained one dry self-feeder (0.28 m^2) and a

nipple waterer to allow for *ad libitum* access to feed and water. The rooms had a deep pit for manure storage and completely slotted concrete floors. The rooms operated on mechanical ventilation using a 6-stage digital controller (Fancom Panningen, The Netherlands), with the temperatures set at 30, 29, 28, 26.5, 25, 24 ºC for weeks 1-6, respectively.

Diets were corn-soybean meal-based provided to meet or exceed nutrient requirements (NRC, 2012) in meal form in 4 phases during the nursery period (42 d). Pigs were fed the assigned experimental diets for the first three feeding phases (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig, Table 1). In Phase 1, the dietary treatments were 1) Control diet (**CONT**): diet with animal and fish protein sources [blood meal (**BM**) at 1%, spray-dried plasma (**SDP**) at 5%, and fish meal menhaden (**FM**) at 4.5%.]; 2) PROPLEX MVP (**MVP**): CONT diet with replacement of FM with MVP at 4.5%; 3) PROPLEX MVP (**MVP100**): CONT diet with replacement of all the animal and fish proteins with MVP at 10%. In Phase 2, the dietary treatments were 1) CONT: diet with BM at 1% and FM at 4.5%; 2) MVP: CONT diet with replacement of FM with MVP at 4.5%; 3) MVP100: CONT diet with replacement of all the animal and fish proteins with MVP at 8%. In Phase 3, the dietary treatments were 1) CONT: diet with FM at 4% and Proplex-DY at 2%; 2) MVP: CONT diet with the replacement of FM with MVP at 2.5%; 3) MVP100: CONT diet with the replacement of all the animal and fish proteins sources with MVP at 5%. The MVP protein source is a blend of two distinct fermentation biomass streams, refined soybean products, select amino acids, and a non-starch polysaccharide (**NSP**) enzyme complex. The PROPLEX MVP was obtained from a commercial company (Daniels Midland Company, NYSE: ADM, 77 West Wacker Drive, Suite 4600, Chicago, Illinois 60601, USA).

The common diet was provided *ad libitum* in meal form to meet or exceed nutrient requirements (NRC, 2012) in Phase 4 (Table 1). This diet did not contain the experimental

MVP nor any animal protein sources. The feeding of the Phase 4 diet began on an individual pen basis whenever a pen had consumed the feed budget for Phase 3.

Exp 2.

A total of 244 newly weaned pigs [(Duroc x (Landrace x Yorkshire)), barrows and gilts, 19 d of age, initial BW 5.88 ± 1.38 kg] were used in a randomized complete block design. The pigs were assigned to 1 of 4 dietary treatments in a 2 × 2 factorial (2 dietary treatments × 2 feeding programs). Each of the dietary treatments had 7 replicates (pens), 2 with 8 pigs per pen and 5 with 9 pigs per pen. Pig sex ratios in pens were constant within BW blocks.

The nursery facility where the trial was conducted contained 32 pens (1.37 m × 1.52 m) that provided initially approximately 0.23 m^2 of floor space per pig. All pens contained one 5-hole dry self-feeder and a cup waterer to allow for *ad libitum* access to feed and water. The nursery barn had a shallow pit for manure storage and completely slotted plastic floors. The nursery room operated on mechanical ventilation using a 4-stage digital controller (Airstream TC5-2V25A, Automated Production Systems, Assumption, IL, USA). The room temperatures were set at 30, 29, 28, 26.5, 25 °C for weeks 1-5, respectively.

Diets were corn-soybean meal-based diets provided to meet or exceed nutrient requirements (NRC, 2012) in meal form in 4 phases during the nursery period (35 d). Pigs were fed the assigned experimental diets for the first three feeding phases. The dietary treatments used corresponded to the CONT and MVP100 treatments as described in Exp 1 but with a different formulation (Table 2). In phase 4, a common diet was provided *ad libitum* in meal form to meet or exceed nutrient requirements (NRC, 2012) whenever a pen had consumed all of its Phase 3 budget (Table 2). The feeding programs were two different budgets for each feeding phase, high budget (H): Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig;

Phase 3: 6.8 kg/pig and low budget (L): Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig.

General experimental procedures

In both experiments, the twice-daily check consisted of poking feed down for the pigs, checking the waterers, observing the pigs, checking the temperature in each room, filling feeders and treating pigs if needed. The therapeutic antibiotic administration was recorded for the duration of the trial. The researchers and research farm staff were trained to identify pigs needing therapeutic injectable antibiotic treatment and were blinded to the study treatments. Pigs were treated when exhibiting clinical signs of illness. Treatment dose, the given product, the date given, pig and pen identification, and reason administered were recorded.

Growth performance and cost-benefit analyses

In Exp. 1, pigs were weighed on d 0, 7, 14, 21, 28, 35, and 42 of the nursery period. In Exp. 2, pigs were weighed on d 0, 7, 14, 21, 28, and 35. In both experiments, the same digital scale was used for all weighing. Feed intake was measured in order to calculate ADG, ADFI, and G:F.

In both Exp. 1 and Exp. 2, cost-benefit analyses (American dollars; current as of October 2018) were undertaken to compare the financial outcomes associated with the protein sources in nursery diets and feeding programs. The analysis was performed using the ADFI and ADG data for each pen of pigs and the raw material costs of each nursery phase diets. Thus, the total feed cost per pig and the feed cost per kg of live weight gain for the entire nursery period were calculated.

Fecal scores

In Exp. 2, daily fecal scoring of the pen was assessed visually using a fecal consistency scale with 4 categories (Pedersen and Toft, 2011). The four consistency categories were score one = firm and shaped, score two = soft and shaped, score three = loose and score four = watery, where scores of 1 and 2 represented normal feces and scores of 3 and 4 represented diarrhea. The incidence of diarrhea (%) was calculated as the sum of the total number of daily diarrheal piglet observations for the period divided by the number of piglet days in the period, and the quotient multiplied by 100. Diarrheal piglets were identified when they presented scores of 3 and 4.

Sampling procedure

On d 13 of Exp. 2, two piglets (a barrow and gilt) with BW closest to the pen average weight were chosen from each pen to collect fecal samples of at least 10 g. The barrow from each pen was also used to collect a blood sample (approximately 8 mL) from the jugular into heparinized vacutainers. In the laboratory, plasma was then collected by centrifuging at 2,000 × g, 15 min, 4°C, allocated into 1.5 mL microcentrifuge tubes, and stored at -20°C until analysis. Plasma from blood was later analyzed for the contents of immunoglobulin G (IgG), immunoglobulin A (IgA), tumor necrosis factor α (TNF-α), and cytokines IL-4 and IL-6.

Volatile fatty acid analysis

Volatile fatty acid (**VFA**) concentrations in fecal samples were determined by a gas chromatographic method (Erwin et al., 1961). Briefly, fecal samples were thawed and 4 ± 0.1 g samples were taken, diluted with 4 mL distilled water and 2 mL of 25% metaphosphoric acid, mixed (VWR Mini Vortexer MV1, IKA Works, Inc., Wilmington, NC 28405), and centrifuged at 15,000 × g, 4°C, for 10 min (Beckman J-21C, Beckman Instruments, Inc., Palo Alto, CA 94304). After centrifugation, the supernatant was transferred into a 2-dram vial. The sample was re-centrifuged (15,000 × g, 4°C, for 15 min) and the supernatant was filtered

through a polyethersulphone membrane filter (0.25 mm, Whatman, UK) and 1.5 mL transferred into a DP I-D vial. The concentrations of VFA were determined by gas chromatography (Varian 3900, Varian, Inc., Walnut Creek, CA 94598). The least detectable limit for all VFA was 0.1 mmol/L.

Immune responses

Total concentrations of IgG and IgA in plasma of pigs were measured according to the method described by Chaytor et al. (2011) using commercially available ELISA kits (Bethyl Laboratories, Inc. Montgomery, TX 77356). Each sample was analyzed in duplicates. The optical density (OD) value was read at 450 nm within 30 min by an ELISA plate reader (Tecan Spark 10M, Tecan Group Ltd. Seestrasse 103, 8708 Männedorf, Switzerland). A standard curve of OD value versus IgG or IgA concentration was generated, and the plasma IgG or IgA concentration was then determined according to the standard curve.

Plasma TNF-α concentrations were determined using a solid-phase sandwich ELISA kits (Chaytor et al., 2011). All the recommendations of the manufacturing company were followed (Bender MedSystems GmbH, Campus Vienna Biocenter 2, 1030 Vienna, Austria). The OD value was read at 450 nm within 2 h by an ELISA plate reader (Tecan Spark 10M, Tecan Group Ltd. Seestrasse 103, 8708 Männedorf, Switzerland). A standard curve of OD value versus TNF-α concentration was generated, and the plasma TNF-α concentration was then determined according to the standard curve. The cytokines IL-4 and IL-6 in plasma were determined using commercially available ELISA kits (Sigma-Aldrich inc. St. Louis, MO 63103 USA; R&D Systems, Inc. Minneapolis, MN 55413, USA) and ELISA plate reader at 450 nm (Tecan Spark 10M, Tecan Group Ltd. Seestrasse 103, 8708 Männedorf, Switzerland).

Statistical analysis

In Exp. 1, data were analyzed as a randomized complete block design using the GLM procedure (SAS Inst., Inc., Cary, NC). In the model, the dietary treatment was considered as the main effect and BW as a block. The pen was the experimental unit. In Exp. 2, data were analyzed as a randomized complete block design using the PROC GLM procedure in SAS 9.4 (SAS Institute INC., Cary, NC), with the pen as the experimental unit. In the 2 × 2 factorial, main effects of dietary treatment, feeding programs, and their interactions were tested with initial BW as the blocking factor. Least squares means were calculated for each independent variable. Statistical significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$, respectively, for all statistical tests. Data were tested for outliers and any pig or pen with performance greater than 2.5 standard deviations from the study means was removed from the data.

RESULTS AND DISCUSSION

Growth performance and cost-benefit analyses

In experiment 1, pigs fed the CONT had greater ($P < 0.05$) ADG when compared to MVP100 pigs in the first week post-weaning (Table 3). Furthermore, BW and ADFI tended ($P < 0.10$) to increase for pigs fed CONT in relation to MVP100. From d 7 to 14 of the nursery period, pigs fed MVP and MVP100 had a greater ADG and G:F ($P < 0.05$) than pigs fed CONT diet. For the third week (d 14 to 21), pigs fed CONT and MVP100 had a greater ADG ($P < 0.05$) than pigs fed MVP diet and pigs fed CONT had a greater G:F ($P < 0.05$) than pigs fed MVP diet. Gain:feed ratio of pigs fed MVP100 was reduced ($P < 0.05$) compared to pigs fed CONT and MVP at d 28 to 35. Overall, there were no differences among dietary treatments for growth performance (ADG, ADFI, and G:F) from d 0 to 42 after weaning.

In experiment 2, no significant interactions ($P > 0.05$) were observed between dietary treatment and feeding programs during the nursery period (Table 4). In the first week, pigs fed CONT had greater ($P < 0.0001$) ADG, ADFI, G:F, and d 7 BW compared to MVP100 fed pigs. No differences were observed between high and low budget in this period, but ADFI tended to be greater ($P = 0.06$) for the low budget compared to high budget. From d 7 to 14 of the nursery period, pigs fed CONT were heavier on d 14 ($P < 0.003$). However, ADG was greater ($P = 0.033$) for pigs fed MVP100 compared to CONT pigs. Moreover, pigs fed MVP100 were more efficient ($P = 0.013$) when compared to CONT pigs. When considering the feeding programs, pigs fed the high budget had a greater BW ($P = 0.096$), ADG ($P = 0.021$), and G:F ($P = 0.014$) compared to pigs fed with the low budget during the d 7-14 period.

For the third week (d 14 to 21), no differences were observed between dietary treatment and feeding programs. Average daily gain from d 21 to 28 was greater ($P = 0.032$) for pigs fed CONT compared to the MVP100 treatment. No differences were observed between high and low budget during d 21 to 28. For the final week (d 28-35), ADFI was less ($P = 0.041$) among pigs fed MVP100 compared to CONT pigs, and d 35 BW tended to be greater ($P = 0.072$) in pigs fed CONT. Furthermore, ADG ($P = 0.034$) and ADFI ($P = 0.025$) were greater among pigs fed the low budget compared to the high budget.

Overall, from d 0 to 35 of the nursery period, there were no differences in piglet performance between feed budgeting programs. However, ADFI was greater ($P = 0.022$) for pigs fed CONT compared to MVP100 pigs, resulting in a tendency for ADG ($P = 0.065$) to be greater for pigs fed CONT compared to MVP100 treatment.

For the cost-benefit analyses of this current study (Exp. 1), the use of MVP as a replacement for animal and fish proteins in nursery pig diets reduced ($P < 0.05$) by US$1.00 the total feed cost per pig and 12% of the feed cost per kg of live weight gain for the entire

nursery period (Table 5). In Exp. 2, significant interactions (P = 0.003) were observed between dietary treatment and feeding budget for the feed cost per pig. When combined with a low feed budget, the use of MVP farther reduced feed costs by US$1.88 (24%) per pig and 20% of the feed cost per kg of live weight gain for the entire nursery period.

By substituting the animal and fish protein sources with MVP, the main negative effects are related with a slight drop in performance in the first week of the nursery period but then the pigs present a rapid recovery in the following week. When considering the overall nursery period, there are no differences in pig performance. Similar results were also reported in other studies, where the positive effect of high digestibility and palatability of animal protein sources during two weeks after weaning (spray-dried porcine plasma) was lost in the subsequent period or the entire nursery period (Bergstrom et al., 1997; Che et al., 2012). In this regard, it was described that pigs maintaining or losing weight in the first 7 to 10 d after weaning required an additional 10 d to reach market weight compared with pigs gaining 250 g/d during this period (Tokach et al., 1992). However, recent studies indicate the absence of impacts on lifetime growth or carcass quality of pigs with an initial slight drop in performance and fed a less complex diet during the post-weaning period (Collins et al., 2013; Skinner et al., 2014; Collins et al., 2017). Furthermore, by substituting the expensive animal protein sources with lower cost alternative sources, production costs linked to feeding could be reduced.

One of the main factors involved in falling performance in the first weeks after weaning is low feed intake (Jayaraman and Nyachoti, 2017). The mechanisms that explain the fall in performance by a low feed intake are related to adverse morphological and functional changes in the intestine, which include the shortening of the villi, hyperplasia of crypt cells, and reduced gut mucosal integrity (Spreeuwenberg et al., 2001; Dong and Pluske, 2007). Owing to these changes, there is a detriment to gut functions by the decrease of brush-border

enzyme activity, and fall of absorptive capacity (Vente Spreeuwenberg et al., 2004). Furthermore, the increase in paracellular permeability makes the gut susceptible to the action of luminal antigens, resulting in inflammation and potential disease (Spreeuwenberg et al., 2001).

In this study, we observed changes in voluntary feed intake during the period immediately after weaning indicating that high-quality protein ingredients from animal sources appear to be more palatable. It seems that pigs may choose to consume feed based the properties of the feed, such as odor, taste, and texture or even based on nutritional requirements of the pig (Kyriazakis et al., 1990; Figueroa et al., 2012). Guzmán-Pino et al. (2014) concluded that piglets may be able to select and prefer flavors conditioned by the post-ingestive consequences of a protein source. Thus, factors related to palatability may explain the results in the current study being that it has been demonstrated that animal protein sources, as fish meal and spray-dried porcine plasma, presented the greatest preference among protein ingredients. Hence, feed intake was improved when these ingredients were used. (Kyriazakis and Emmans, 1993; Makkink et al., 1994; Blasco et al., 2005; Solà-Oriol et al., 2011; Che et al., 2012; Pérez-Bosque et al., 2016).

In Exp. 2, there were no consistent differences in piglet performance during the nursery period due to feeding programs. Hence, the feeding duration on each of the nursery phase diets can be decreased with the use of lesser amounts of feed in each phase (low budget) without affecting the pigs' performance. This strategy shows to be a viable alternative to reduce the costs of nursery diets and the profitability in the pig industry, as found in other studies (Leliveld et al., 2013; Lee et al., 2014). In the cost-benefit analyses of this current study, the use of low budget reduced ($P < 0.05$) by 12.5% the total feed cost per pig and 11.8% of the feed cost per kg of live weight gain for the entire nursery period, when compared to high budget (Table 5).

When compared to Exp. 1, in our Exp. 2 the pigs' performance during the first week was more affected by substituting the animal and fish proteins sources with MVP. This can be associated with the age and weight of piglets at weaning. In the first experiment, pigs were weaned at 22 d with BW 6.37 ± 1.24 kg, while in the second experiment, pigs were weaned at 19 d with BW 5.88 ± 1.38 kg. According to Collins et al. (2017), the benefits associated with feeding complex diets may differ depending on the weight of the pig at weaning. In their study, a high complexity feeding program only benefited lightweight pigs at weaning (less than 6.5 kg at 27 d of age) when compared to pigs with medium or heavy weaning weights (> 6.5 kg). Thus, high-quality protein ingredients from animal sources seem to decrease the impact of weaning on low-weight piglets, allowing greater feed intake and consequently better performance post-weaning.

Fecal scores

The main effects of dietary treatment and feeding programs on fecal scores, diarrhea incidence, and treatment rate are presented in Table 6. From the second week post-weaning and during most of the experimental period, pigs fed CONT had greater ($P < 0.05$) fecal scores, more ($P < 0.05$) soft and watery feces, and consequently greater ($P < 0.05$) diarrhea incidence compared to MVP100 pigs. This can be clearly observed when considering the total experiment period (d 0 to 35). No differences were observed between high and low budget in fecal score and consistency. However, in the second week and from d 0 to 35 of the nursery period the diarrhea incidence was greater ($P < 0.05$) among pigs fed the low budget compared to the high budget. No difference ($P > 0.05$) was observed when considering dietary treatment and feeding programs for therapeutic treatment rates.

The stressful events associated with weaning pigs causes a high incidence of intestinal disturbances during the first weeks post-weaning. Within them, post-weaning diarrhea is the most critical problem of piglets, most often accompanied by decreased performance (Pluske et

al., 2003; Smith et al., 2010; McLamb et al., 2013; Pohl et al., 2017). The strategies used to prevent diarrhea is based on maintenance of intestinal micro-ecosystem, which in turn is closely related to dietary components (Park et al., 2014; Zimmermann et al., 2016). In Exp. 2 however, the increased incidence of diarrhea did not result in reduced growth performance, but actually had slightly greater growth rates and efficiencies.

Different dietary protein sources and levels affect the performance and gut health of piglets (Pluske et al., 2002; Che et al., 2012; Wen et al., 2018). It was proposed that variations in the use of dietary protein ingredients may beneficially affect microbial composition in the gastrointestinal tract of piglets (Rist et al., 2013). This is related with a greater or lesser protein fermentation in the small and large intestine of piglets (Rist et al., 2013; Cao et al., 2016).

High fermentation of protein was previously related to postweaning diarrhea and with the growth of potentially pathogenic bacteria (Ball and Aherne, 1987; Wellock et al., 2008). According to several studies, the reduction of the total dietary protein can avoid excessive protein fermentation and hence decrease the incidence of post-weaning diarrhea (Heo et al., 2008; Wu et al., 2015). In the current study, the substitution for the animal and fish proteins sources with MVP reduced crude protein levels of phase 1 diet in approximately two percentage points (Tables 1 and 2), this could explain the differences in fecal score, fecal consistency, and diarrhea indices observed during the second-week post-weaning. In this regard, it has been shown that post-weaning pigs performance were not affected by reducing the protein level from 23% to 21% (Nyachoti et al., 2006). Thus, this was not a confounding factor in the current study as all essential amino acids were nearly identical among dietary treatments.

On the other hand, concerning sources of protein, Rist et al. (2014) showed that the use of FM as the main protein source in the swine diet might be in favor of increasing

pathogenic bacteria in the small intestine. In the same way, Cao et al. (2016) found that the proportion of observations assigned to the genus Escherichia/Shigella was much higher in pigs fed FM than those fed soybean meal and cottonseed meal. This is related with a greater or lesser protein fermentation too, being that in the study of Wen et al. (2018) diets supplemented with FM rather than soy protein concentrate were more fermentable in the large intestine. In this study, the FM was one of the main sources of protein used, which could be related to the greater diarrhea incidences observed in the CONT group.

Volatile fatty acid analysis

As shown in Table 7, the total concentration of volatile fatty acid was greater (P = 0.003) in feces from pigs fed CONT compared to MVP100. This reflected in greater (P < 0.020) concentrations of acetate, isobutyrate, and isovalerate acids for CONT. Pigs fed the low budget had a greater (P = 0.025) concentration of valerate acid compared to the high budget.

In the present study, the changes in microbial fermentation were evaluated through the VFA concentrations in fecal samples. The VFAs are the fermented products of undigested nutrients such as protein and carbohydrates in the gastrointestinal tract (Le et al., 2005; Nyachoti et al., 2006). Thus, the quantity and quality of the nutritional components of the diet are related to microbial activity and hence VFA concentrations. It has been shown that increasing the dietary protein level, usually in nursery diets, raised the concentration of VFAs in the intestinal tract of piglets and that the source of protein may be a related factor (Nyachoti et al., 2006; Wen et al., 2018). In this regard, Wen et al. (2018) showed that the highest concentrations of VFAs (acetic, butyric, and valeric) occurred in piglets fed a diet containing a high level of FM. In the current study, the total concentration of VFAs was greater in the CONT group, with greater concentrations of acetate, isobutyrate, and isovalerate acids. These results may be related to the contents of FM in the CONT diet that increased the microbial

fermentation in the intestine and produced more bacterial metabolites. Furthermore, the presence of NSP enzymes in MVP blend and a possible reduction of crude protein content by substituting the animal and fish proteins sources with MVP maybe part of our explanation of the results obtained as well.

Immune responses

Plasma concentration of IgG, IgA, TNF-α, IL-4, and IL-6 did not differ among dietary treatments (Table 8). Plasma concentrations of IgG were lower (P = 0.013) in pigs fed the high budget compared with low budget. Plasma concentrations of IgA tended to be lower (P = 0.087; 0.070) in CONT and low budget compared to MVP100 and high budget, respectively.

In relation to the immune system, early weaning stress is associated with poor immunocompetence (McLamb et al., 2013; Pohl et al., 2017). An immature immune system and the interruption of the supply of immunoglobulins and other components from sow milk contributes to an inappropriate immunological response to pathogens, which can result in intestinal disorders and diseases (Stokes et al., 2001). Furthermore, intestinal inflammation during the immediate post-weaning period increases proinflammatory cytokine expression (Pié et al., 2004; Hu et al., 2013). Different dietary protein sources seem to have some effect on the immune response of weanling pigs (Che et al., 2012; Wu et al., 2015). The inclusion of SDP at 2.5%, 4% and 5% of the diet for the first weeks post-weaning, reduced intestinal inflammatory cytokines and unspecific immune molecules (IgG, IgA, C3) in weaned pigs (Touchette et al., 2002; Peace et al., 2011; Che et al., 2012). According to Pérez-Bosque et al. (2016), the efficacy of SDP in animal feed appears to be related mainly to an improved barrier function of the gut mucosa and the modulation of the mucosal immune response. In the present study, even using SDP in the CONT diet, there were statistical differences, only numerical reductions in IgG, IgA, and TNF-α among dietary treatments, however sampling as d 13 may have been to late to see any residual effects of SDP feed in the phase 1 diet. This

indicates that the use of MVP as a protein source for weanling pigs and a low budget, result in little or no adverse effects on the immune system.

Collectively, data from this study demonstrates that the substitution with MVP protein blend can be used as a cost effective alternative to animal and fish proteins in nursery pig diets without compromising overall growth performance, immune status, and post-weaning diarrhea. Furthermore, a low feeding budget can be used without compromising overall nursery pigs' growth performance and results in significant feed cost savings.

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Table 1. Exp 1: Experimental diets (as-fed basis).

Ingredient, %	Phase 1			Phase 2			Phase 3			Phase 4
	CONT	MVP	MVP100	CONT	MVP	MVP100	CONT	MVP	MVP100	COMMON
Corn	40.189	39.977	39.898	45.230	45.054	43.244	52.460	53.441	53.218	53.977
SBM, 47% CP	16.000	16.000	16.000	19.500	19.500	19.500	28.000	28.000	28.000	28.950
Plasma Protein, Spray-dried	5.000	5.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SD Blood Meal	1.000	1.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Fish Meal	4.500	0.000	0.000	4.500	0.000	0.000	4.000	0.000	0.000	0.000
Proplex DY - ADM Yeast Prod.	4.000	4.000	4.000	2.500	2.500	2.500	2.000	2.000	0.000	0.000
ADM PROPLEX MVP	0.000	4.500	10.000	0.000	4.500	8.000	0.000	2.500	5.000	0.000
DDGS - 7% Fat	2.500	2.500	2.500	7.500	7.500	7.500	7.500	7.500	7.500	10.000
Soybean Oil	3.190	2.715	2.660	2.968	2.498	2.163	2.539	2.249	2.164	0.000
Swine Grease	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.000
Dried Whey	20.000	20.000	20.000	12.500	12.500	12.500	0.000	0.000	0.000	0.000
Lysine-HCL	0.218	0.221	0.458	0.539	0.533	0.466	0.419	0.480	0.415	0.435
DL-Methionine	0.200	0.191	0.255	0.280	0.267	0.217	0.186	0.199	0.176	0.150
L-Threonine	0.013	0.005	0.135	0.180	0.167	0.127	0.118	0.138	0.108	0.140
L-Tryptophan	0.034	0.027	0.077	0.081	0.072	0.066	0.051	0.051	0.043	0.015
L-Valine	0.000	0.000	0.092	0.115	0.087	0.064	0.034	0.048	0.015	0.000
L-Isoleucine	0.026	0.000	0.000	0.099	0.070	0.000	0.000	0.000	0.000	0.000
Limestone	0.872	1.326	1.245	0.978	1.431	1.413	0.844	1.254	1.231	1.415
MonoCalcium Phosphate	0.490	0.770	0.910	0.430	0.720	0.640	0.200	0.490	0.480	0.560
Salt (Sodium Chloride)	0.350	0.350	0.350	0.400	0.400	0.400	0.450	0.450	0.450	0.350
Zinc Oxide	0.322	0.322	0.322	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Copper sulfate	0.000	0.000	0.000	0.095	0.095	0.095	0.095	0.095	0.095	0.080
Choline Chloride, Dry 70%	0.040	0.040	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Trace Mineral Premix[1]	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Vitamin Premix[2]	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250

Se 600 premix[3]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Phytase[4]	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084
ADM Bacillus LS[5]	0.000	0.000	0.000	0.050	0.050	0.050	0.050	0.050	0.050	0.000
CitriStim[5]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.000
Kemgest[6]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.000
Carbadox – 10g/lb	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Banmith dewormer – 48g/lb[7]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
Clarify larvicide[8]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070
Calculated Nutrients										
ME, Kcal/kg	3417	3417	3417	3395	3395	3395	3395	3395	3395	3391
CP, %	23.53	23.23	21.86	21.82	21.51	22.27	23.08	22.16	22.47	21.52
Total Lys, %	1.64	1.65	1.64	1.60	1.60	1.61	1.55	1.53	1.53	1.43
SID[9] Lys	1.46	1.47	1.46	1.42	1.42	1.42	1.34	1.34	1.34	1.25
SID Met:Lys	35.62	34.69	41.78	41.55	40.85	40.14	38.81	38.81	38.06	35.14
SID M+C:Lys	59.59	59.86	60.27	59.86	59.86	59.86	59.70	59.70	59.70	58.12
SID Thr:Lys	59.59	59.86	60.27	59.86	59.86	59.86	59.70	59.70	59.70	62.24
SID Tryp:Lys	19.86	19.73	19.86	19.72	19.72	19.72	20.15	20.15	20.15	18.14
SID Iso:Lys	54.79	55.10	55.48	54.93	54.93	55.63	56.72	56.72	58.21	59.84
SID Val:Lys	69.86	71.43	67.12	66.90	66.90	66.90	67.16	67.16	67.16	66.36
Ca,%	0.80	0.80	0.80	0.80	0.80	0.80	0.65	0.65	0.65	0.75
P, %	0.69	0.67	0.68	0.63	0.62	0.62	0.54	0.53	0.54	0.55
Avail. Phos., %	0.60	0.60	0.60	0.50	0.50	0.50	0.37	0.37	0.37	0.35
Lactose, %	14.00	14.00	14.00	8.75	8.75	8.75	0.00	0.00	0.00	0.00

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.
[3]Provided 0.3 ppm Se
[4]Provided 600 FTU per kg of the diet
[5]ADM, 77 West Wacker Drive, Suite 4600, Chicago, Illinois 60601, USA.

[6]Kemin Industries, Inc. Des Moines, Iowa USA 50317
[7]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet
[8]Clarifly (Central Life Sciences, Schaumburg, IL) provided 4.7 ppm (Phase 4) diflubenzuron in the diet
[9]SID = standardized ileal digestible.

Table 2. Exp 2: Experimental diets (as-fed basis).

Ingredient, %	Phase 1		Phase 2		Phase 3				Phase 4
					High Budget		Low Budget		
	CONT	**MVP100**	**CONT**	**MVP100**	**CONT**	**MVP100**	**CONT**	**MVP100**	**COMMON**
Corn	38.381	37.405	41.677	38.847	54.462	54.472	42.937	42.967	53.812
SBM, 47% CP	16.000	16.000	19.500	19.500	26.000	26.000	26.000	26.000	29.000
Plasma Protein, Spray-dried	5.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SD Blood Meal	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
Fish Meal	4.500	0.000	4.500	0.000	4.000	0.000	4.000	0.000	0.000
Proplex DY - ADM Yeast Prod.	4.000	4.000	2.500	2.500	2.000	0.000	2.000	0.000	0.000
ADM PROPLEX MVP	0.000	10.000	0.000	8.000	0.000	5.000	0.000	5.000	0.000
DDGS - 7% Fat	2.500	2.500	7.500	7.500	7.500	7.500	7.500	7.500	10.000
Soybean Oil	3.000	2.900	3.000	2.650	2.340	2.445	2.080	2.180	0.000
Swine Grease	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.100
Dried Whey	22.000	22.000	16.500	16.500	0.000	0.000	12.400	12.400	0.000
Lysine-HCL	0.210	0.450	0.460	0.430	0.395	0.435	0.285	0.325	0.435
DL-Methionine	0.210	0.275	0.250	0.230	0.170	0.185	0.155	0.165	0.150
L-Threonine	0.050	0.165	0.160	0.140	0.120	0.140	0.065	0.085	0.140
L-Tryptophan	0.034	0.080	0.060	0.066	0.045	0.050	0.025	0.030	0.030
L-Valine	0.000	0.105	0.060	0.064	0.020	0.040	0.000	0.015	0.000
L-Isoleucine	0.025	0.035	0.050	0.000	0.000	0.000	0.000	0.000	0.000
Limestone	0.830	0.985	0.800	1.120	0.980	1.240	0.940	1.200	1.415
MonoCalcium Phosphate	0.490	1.330	0.430	0.900	0.365	0.890	0.010	0.530	0.560
Salt (Sodium Chloride)	0.350	0.350	0.400	0.400	0.450	0.450	0.450	0.450	0.350
Zinc Oxide	0.322	0.322	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Copper sulfate	0.000	0.000	0.095	0.095	0.095	0.095	0.095	0.095	0.080
Choline Chloride, Dry 70%	0.040	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Trace Mineral Premix[1]	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125

Vitamin Premix[2]	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Se 600 premix[3]	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Phytase[4]	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084	0.084
CitriStim[5]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.000
Kemgest[6]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.000
Carbadox – 10g/lb	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Banmith dewormer 48g/lb[7]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
Clarify larvicide[8]	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070
Calculated Nutrients									
ME, Kcal/kg	3466	3466	3446	3445	3395	3395	3395	3395	3396
CP, %	23.95	21.74	22.39	22.35	22.97	22.08	23.26	22.36	21.54
Total Lys, %	1.69	1.64	1.61	1.61	1.53	1.52	1.52	1.51	1.43
SID[9] Lys	1.50	1.50	1.45	1.45	1.35	1.35	1.35	1.35	1.25
SID Met:Lys	35.23	41.95	39.49	40.18	37.49	38.14	36.72	37.01	35.12
SID M+C:Lys	58.08	58.25	58.38	58.32	58.23	58.32	58.41	58.15	58.08
SID Thr:Lys	60.49	60.26	60.36	60.46	60.13	60.35	60.22	60.43	62.23
SID Tryp:Lys	19.14	19.03	19.17	19.16	19.25	19.23	19.21	19.19	19.30
SID Iso:Lys	56.55	55.05	56.43	55.39	58.90	56.75	62.63	60.49	59.84
SID Val:Lys	69.02	65.11	66.38	65.82	67.22	66.41	68.57	67.41	66.35
Ca,%	0.85	0.85	0.80	0.80	0.75	0.75	0.75	0.75	0.75
P, %	0.74	0.76	0.67	0.68	0.60	0.61	0.58	0.59	0.55
Avail. Phos., %	0.54	0.53	0.45	0.44	0.34	0.34	0.35	0.35	0.35
Lactose, %	16.03	16.03	12.03	12.03	0.00	0.00	9.04	9.04	0.00

[1]Provided per kg of diet available minerals: iron, 121.3 mg; zinc, 121.2 mg; manganese, 15.0 mg; copper, 11.33 mg; iodine, and 0.46 mg.
[2]Provided per kg of diet: vitamin A, 6,614 IU; vitamin D3, 661 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 9 mg; pantothenic acid, 22 mg; niacin, 33 mg and B12 38.6 mg.
[3]Provided 0.3 ppm Se
[4]Provided 600 FTU per kg of the diet
[5]ADM, 77 West Wacker Drive, Suite 4600, Chicago, Illinois 60601, USA.

[6]Kemin Industries, Inc. Des Moines, Iowa USA 50317
[7]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet
[8]Clarifly (Central Life Sciences, Schaumburg, IL) provided 4.7 ppm (Phase 4) diflubenzuron in the diet
[9]SID = standardized ileal digestible.

Table 3. Exp 1: Main effects of dietary treatments on weaned piglets growth performance.[1]

Item	CONT	MVP	MVP100	SEM	P-value[2]
Initial BW, kg	6.446	6.464	6.455	0.0102	0.421
Period, d0 - 7					
BW d7, kg	7.226^{y}	7.112^{yz}	7.004^{z}	0.0647	0.065
ADG, kg/d	0.112^{a}	0.093^{ab}	0.078^{b}	0.0090	0.043
ADFI, kg/d	0.123^{y}	0.106^{z}	0.103^{z}	0.0062	0.062
G:F, kg/kg	0.913 ± 0.0442	0.858 ± 0.0425	0.793 ± 0.0461		0.188
Period, d7 - 14					
BW d14, kg	8.918	9.104	9.004	0.1055	0.477
ADG, kg/d	0.242^{b}	0.284^{a}	0.286^{a}	0.0109	0.010
ADFI, kg/d	0.342	0.362	0.344	0.0106	0.343
G:F, kg/kg	0.727^{b} ± 0.0227	0.793^{a} ± 0.0218	0.837^{a} ± 0.0218		0.005
Period, d14 - 21					
BW d21, kg	11.376	11.363	11.576	0.1715	0.619
ADG, kg/d	0.363^{a} ± 0.0137	0.323^{b} ± 0.0132	0.367^{a} ± 0.0132		0.043
ADFI, kg/d	0.485 ± 0.0134	0.479 ± 0.0128	0.502 ± 0.0139		0.443
G:F, kg/kg	0.747^{a} ± 0.0183	0.682^{b} ± 0.0183	0.740^{ab} ± 0.0191		0.035
Period, d21 - 28					
BW d28, kg	14.393	14.642	14.814	0.2295	0.435
ADG, kg/d	0.431 ± 0.0167	0.479 ± 0.0174	0.463 ± 0.0167		0.141
ADFI, kg/d	0.637	0.694	0.655	0.0205	0.148
G:F, kg/kg	0.682 ± 0.0287	0.690 ± 0.0287	0.707 ± 0.0276		0.822
Period, d28 - 35					
BW d35, kg	17.513	17.922	17.863	0.3021	0.590
ADG, kg/d	0.446	0.469	0.436	0.0172	0.392
ADFI, kg/d	0.788 ± 0.0329	0.800 ± 0.0316	0.846 ± 0.0316		0.402
G:F, kg/kg	0.580^{a} ± 0.0208	0.590^{a} ± 0.0199	0.518^{b} ± 0.0199		0.032
Period, d35 - 42					
BW d42, kg	22.322	22.839	22.689	0.3794	0.614
ADG, kg/d	0.687	0.703	0.689	0.0202	0.836
ADFI, kg/d	1.022	1.054	1.046	0.0277	0.705
G:F, kg/kg	0.672	0.668	0.663	0.0114	0.855
Period, d0 - 42					
ADG, kg/d	0.378	0.390	0.386	0.009	0.632
ADFI, kg/d	0.567	0.583	0.583	0.583	0.621
G:F, kg/kg	0.666	0.670	0.665	0.0051	0.706

[1] **CONT**= Diet with animal and fish protein sources; **MVP**= Diet with the replacement of fish proteins with a fortified protein blend; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2]**P-values =** Overall diet effect among treatments.

ab – Letters indicate significant differences at $P \leq 0.05$ using Duncan's means separation test.

yz – Letters indicate tendencies ($0.05 < P \leq 0.10$) using Duncan's means separation test.

Table 4. Exp. 2: Main effects of dietary treatments and feeding programs on weaned piglets growth performance.

Item	DIET[1]		BUDGET[2]			P-value[3]		
	CONT	MVP100	H	L	SEM	DIET	BUDGET	DIET x BUDGET
Initial BW, kg	5.881	5.887	5.880	5.888	0.0062	0.458	0.328	0.527
Period, days 0 to 7								
BW day 7, kg	6.686	6.151	6.376	6.460	0.0475	<.0001	0.227	0.788
ADG, kg/d	0.115	0.038	0.071	0.082	0.0068	<.0001	0.281	0.727
ADFI, kg/d	0.135	0.078	0.100	0.112	0.0041	<.0001	0.060	0.431
G:F, kg/kg	0.851	0.468	0.629	0.691	0.0483	<.0001	0.376	0.137
Period, days 7 to 14								
BW day 14, kg	8.712	8.399	8.635	8.475	0.0645	0.003	0.096	0.622
ADG, kg/d	0.289	0.321	0.323	0.288	0.0098	0.033	0.021	0.780
ADFI, kg/d	0.316	0.318	0.320	0.313	0.0073	0.897	0.509	0.068
G:F, kg/kg	0.913	1.011	1.010	0.914	0.0250	0.013	0.014	0.162
Period, days 14 to 21								
BW day 21, kg	11.171	10.965	11.212	10.925	0.1529	0.353	0.202	0.236
ADG, kg/d	0.351	0.367	0.368	0.350	0.0168	0.530	0.456	0.204
ADFI, kg/d	0.486	0.484	0.498	0.472	0.0152	0.912	0.249	0.720
G:F, kg/kg	0.723	0.763	0.746	0.740	0.0195	0.171	0.839	0.098
Period, days 21 to 28								
BW day 28, kg	14.207	13.809	14.213	13.802	0.1733	0.121	0.111	0.229
ADG, kg/d	0.434	0.406	0.429	0.411	0.0083	0.032	0.148	0.634
ADFI, kg/d	0.629	0.605	0.620	0.614	0.0106	0.119	0.709	0.222
G:F, kg/kg	0.688	0.672	0.691	0.669	0.0114	0.335	0.177	0.495
Period, days 28 to 35								
BW day 35, kg	17.717	17.185	17.482	17.419	0.1967	0.072	0.822	0.182
ADG, kg/d	0.501	0.482	0.467	0.517	0.0153	0.390	0.034	0.600
ADFI, kg/d	0.739	0.699	0.697	0.741	0.0129	0.041	0.025	0.423

G:F, kg/kg	0.682	0.695	0.677	0.700	0.0133	0.513	0.229	0.905
Period, days 0 to 35								
ADG, kg/d	0.338	0.323	0.332	0.329	0.0055	0.065	0.795	0.170
ADFI, kg/d	0.461	0.437	0.447	0.451	0.0070	0.022	0.722	0.259
G:F, kg/kg	0.735	0.742	0.745	0.732	0.0053	0.334	0.102	0.510

[1]**CONT**= Diet with animal and fish protein sources; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2]**H**= High budget (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig); **L**: Low budget: (Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig).

[3]**P-values =** main effect and interaction probabilities.

Table 5. Exp. 2: Cost-benefit analyses of protein sources in nursery diet and feeding programs for weaned piglets (all values in American dollars).[1]

Item (Exp. 1)	CONT	MVP	MVP100	SEM	P-value[3]
Feed cost for 42d, US$/pig	10.391[a]	9.959[a]	9.360[b]	0.1546	0.0002
Feed cost/kg gain, US$/kg	0.659[a]	0.611[b]	0.579[c]	0.0014	<.0001

Item (Exp. 2)								
Budget[2]	HIGH		LOW		SEM	P-value		
Diet	CONT	MVP100	CONT	MVP100		DIET	BUDGET	DIET x BUDGET
Feed cost for 35d, US$/pig	7.692	6.331	6.460	5.813	0.0737	<0.0001	<0.0001	0.003
Feed cost/kg gain, US$/kg	0.638	0.570	0.558	0.509	0.0008	<0.0001	<0.0001	0.120

[1]**CONT**= Diet with animal and fish protein sources; **MVP**= Diet with the replacement of fish proteins with a fortified protein blend; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2]**H**= High budget (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig); L: Low budget: (Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig).

[3]**P-values =** main effect and interaction probabilities.

abc – Letters indicate significant differences at $P \leq 0.05$ using Duncan's means separation test.

Table 6. Exp. 2: Main effects of dietary treatments and feeding programs on fecal scores, diarrhea incidenc, and treatment rate of weaned piglets.

Item	DIET[1]		BUDGET[2]			P-value[3]		
	CONT	MVP100	H	L	SEM	DIET	BUDGET	DIET x BUDGET
Period, days 0 to 7								
Fecal Score[4]	1.296	1.163	1.194	1.265	0.0547	0.104	0.368	0.897
Fecal Score 1, %	58.973	58.939	60.073	57.839	3.0377	0.994	0.609	0.982
Fecal Score 2, %	19.832	25.092	22.494	22.430	2.6741	0.181	0.987	0.229
Fecal Score 3, %	12.047	8.160	9.156	11.051	1.5807	0.099	0.408	0.278
Fecal Score 4, %	9.148	7.809	8.277	8.680	1.6765	0.579	0.867	0.343
Diarrhea incidence, %[5]	4.521	4.408	3.926	5.003	1.0260	0.939	0.468	0.923
Treatment rate, %[6]	5.75	5.95	8.43	3.27	1.893	0.942	0.07	0.999
Period, days 7 to 14								
Fecal Score	1.612	1.235	1.347	1.500	0.0870	0.007	0.229	0.683
Fecal Score 1, %	42.485	63.686	55.266	50.905	3.2500	<.0001	0.355	0.949
Fecal Score 2, %	31.181	26.561	31.222	26.520	2.2174	0.158	0.151	0.590
Fecal Score 3, %	14.383	6.389	7.986	12.785	1.6551	0.003	0.055	0.721
Fecal Score 4, %	11.951	3.364	5.525	9.790	1.7521	0.003	0.102	0.372
Diarrhea incidence, %	8.362	2.891	3.118	8.135	0.9312	0.001	0.001	0.020
Treatment rate, %	33.43	22.02	21.83	33.63	5.136	0.134	0.121	0.372
Period, days 14 to 21								
Fecal Score	1.929	1.286	1.500	1.714	0.1480	0.007	0.319	0.105
Fecal Score 1, %	40.289	69.099	59.294	50.094	4.7346	<.0001	0.186	0.104
Fecal Score 2, %	40.944	25.357	29.923	36.378	3.6537	0.007	0.228	0.057
Fecal Score 3, %	16.437	4.830	10.272	10.995	3.5115	0.031	0.886	0.939
Fecal Score 4, %	2.330	0.714	0.510	2.534	1.1030	0.314	0.211	0.707
Diarrhea incidence, %	9.573	2.431	2.877	9.127	2.3739	0.047	0.079	0.387
Treatment rate, %	17.36	9.62	10.52	16.47	3.461	0.131	0.24	0.905

Period, days 21 to 28								
Fecal Score	1.452	1.167	1.357	1.262	0.0652	0.006	0.315	0.612
Fecal Score 1, %	52.698	73.583	63.226	63.056	3.3614	0.001	0.972	0.554
Fecal Score 2, %	39.524	25.941	31.020	34.444	2.7783	0.003	0.395	0.887
Fecal Score 3, %	6.230	0.000	4.683	1.548	1.8517	0.029	0.247	0.247
Fecal Score 4, %	1.548	0.476	1.071	0.952	0.6646	0.269	0.901	0.387
Diarrhea incidence, %	4.299	1.620	2.910	3.009	0.8871	0.047	0.938	0.396
Treatment rate, %	14.29	12.3	12.6	13.99	3.086	0.655	0.754	0.450
Period, days 28 to 35								
Fecal Score	1.238	1.095	1.167	1.177	0.0652	0.139	0.998	0.315
Fecal Score 1, %	65.941	80.992	74.393	72.540	4.1382	0.019	0.755	0.983
Fecal Score 2, %	33.662	18.532	25.130	27.063	4.0317	0.016	0.739	0.862
Fecal Score 3, %	0.397	0.476	0.476	0.397	0.4501	0.902	0.902	0.187
Fecal Score 4, %	-	-	-	-	-	-	-	-
Diarrhea incidence, %	0.619	0.562	0.298	0.884	0.3867	0.918	0.298	0.338
Treatment rate, %	9.62	10.12	10.71	9.03	3.57	0.923	0.742	0.772
Period, days 0 to 35								
Fecal Score	1.505	1.189	1.313	1.382	0.0585	0.001	0.417	0.545
Fecal Score 1, %	52.077	69.260	62.451	58.887	2.4213	<.0001	0.312	0.642
Fecal Score 2, %	33.029	24.297	27.958	29.367	1.9570	0.006	0.617	0.400
Fecal Score 3, %	9.899	3.971	6.515	7.355	1.1721	0.002	0.618	0.536
Fecal Score 4, %	4.995	0.210	3.077	4.391	0.0701	0.031	0.239	0.800
Diarrhea incidence, %	5.475	2.382	2.626	5.232	0.8165	0.015	0.037	0.238
Treatment rate, %	16.090	12.002	12.818	15.278	3.429	0.557	0.365	0.689

[1]**CONT**= Diet with animal and fish protein sources; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2] **H**= High budget (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig); L: Low budget: (Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig).

[3] **P-values =** main effect and interaction probabilities.

[4]Fecal consistency categories: score one = firm and shaped, score two = soft and shaped, score three = loose, and score four = watery, where scores of 1 and 2 represented normal feces and scores of 3 and 4 represented diarrhea (Pedersen and Toft, 2011).

[5]Total number of daily diarrheal piglet observations for the period divided by the number of piglet days in the period, and the quotient multiplied by 100.

[6]Total number of piglets treated for the period divided by the number of piglet days in the period, and the quotient multiplied by 100.

Table 7. Exp. 2: Main effects of dietary treatments and feeding programs on the content of volatile fatty acid in d 13 fecal samples of weaned piglets.

Item	DIET[1]		BUDGET[2]		SEM	P-value[3]		
	CONT	MVP100	H	L		DIET	BUDGET	DIET x BUDGET
Acetate, mmol/L	74.406	65.885	69.368	70.924	2.4800	0.020	0.660	0.568
Propionate, mmol/L	29.800	26.621	27.225	29.196	1.4391	0.126	0.339	0.609
Isobutyrate, mmol/L	2.130	1.681	1.950	1.861	0.1203	0.012	0.605	0.331
Butyrate, mmol/L	14.786	14.786	14.095	14.051	0.9558	0.298	0.974	0.398
Isovalerate, mmol/L	2.607	1.972	2.328	2.251	0.1760	0.015	0.761	0.226
Valerate, mmol/L	2.651	2.565	2.280	2.936	0.1988	0.761	0.025	0.492
Total VFAs, mmol/L	126.380	112.083	117.245	121.218	4.5926	0.033	0.544	0.547
Acetate, % of total	59.114	59.237	59.393	58.958	0.7633	0.910	0.690	0.854
Propionate, % of total	23.612	23.524	23.136	24.000	0.6903	0.929	0.381	0.878
Isobutyrate, % of total	1.670	1.530	1.693	1.506	0.0913	0.286	0.156	0.090
Butyrate, % of total	11.484	11.709	11.830	11.363	0.4463	0.723	0.464	0.413
Isovalerate, % of total	2.040	1.796	2.024	1.812	0.1394	0.222	0.289	0.075
Valerate, % of total	2.080	2.205	1.925	2.361	0.1147	0.447	0.010	0.501

[1]**CONT**= Diet with animal and fish protein sources; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2]**H**= High budget (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig); L: Low budget: (Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig).

[3]**P-values =** main effect and interaction probabilities.

Table 8. Exp. 2: Main effects of dietary treatment and feeding programs on the immune response of weaned piglets.

Item	DIET[1]		BUDGET[2]			P-value[3]		
	CONT	MVP100	H	L	SEM	DIET	BUDGET	DIET x BUDGET
IgG, g/L	6.812	8.158	6.213	8.757	0.6624	0.159	0.013	0.710
IgA, g/L	0.201	0.261	0.263	0.199	0.0243	0.087	0.070	0.116
TNF-alpha, pg/mL	71.501	78.199	75.623	74.078	5.7120	0.418	0.850	0.468
IL-6, pg/mL	211.379	205.688	214.572	202.494	11.4382	0.723	0.454	0.919
IL-4, pg/mL	57.984	54.188	55.657	56.515	2.6695	0.317	0.818	0.921

[1]**CONT**= Diet with animal and fish protein sources; **MVP100=** Diet with the replacement of all the animal and fish proteins with a fortified protein blend.

[2]**H=** High budget (Phase 1: 2.3 kg/pig; Phase 2: 4.5 kg/pig; Phase 3: 6.8 kg/pig); L: Low budget: (Phase 1: 1.1 kg/pig; Phase 2: 2.3 kg/pig; Phase 3: 3.4 kg/pig).

[3]**P-values =** main effect and interaction probabilities.

ARTIGO 2 - RELATIVE BIOAVAILABILITY OF L-lysine SULFATE IS EQUIVALENT TO THAT OF L-lysine HCl FOR NURSERY PIGLETS

JORGE YAIR PÉREZ PALENCIA

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Relative bioavailability of L-lysine sulfate is equivalent to that of L-lysine HCl for nursery piglets[1]

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ABSTRACT: Supplementary L-lysine sources include L-lysine HCl and L-lysine sulfate. L-lysine sulfate contains at least 50% L-Lys and other components as residues from the fermentation process, other amino acids, and other organic and inorganic substances, being an alternative to L-Lys HCl. The aim of this study was to evaluate the relative bioavailability (RBV) of L-Lys sulfate in comparison with L-Lys HCl and its effects on performance, blood parameters, intestinal functionality, and the apparent total tract digestibility in nursery piglets. A total of 168 female piglets (DB90×PIC337), weaned at 22 d (BW= 6.29 ± 0.41 kg), were distributed in seven dietary treatments and eight replicates, with three pigs per pen. The experimental period of 42 d was divided into two phases (phase 1, days 0 to 21; phase 2, days 21 to 42). The basal diet (CON) was lysine-deficient formulated to meet 73% of standardized ileal digestible Lys requirements. For the other diets, the CON was supplemented with three levels (80%, 90%, and 100% of standardized ileal digestible Lys requirements) of L-Lys sulfate (70% L-Lys) or L-Lys HCl (79% L-Lys). There were no significant differences ($P > 0.05$) in the performance and concentrations of plasma urea and creatinine between the L-Lys sources. The RBV of L-Lys sulfate relative to L-Lys HCl was not different and was 106%, 119% and 117% for effects on ADG, G:F and plasma urea, respectively. Lys deficiency resulted in a greater ($P < 0.05$) incidence of diarrhea, while pigs supplemented with Lys sulfate or Lys HCl showed greater ($P < 0.05$) villus height in the jejunum when compared to those receiving the CON. Diets supplemented with L-Lys sulfate had greater ($P < 0.05$) apparent total tract digestibility of dry matter, gross energy and crude protein. In conclusion, the RBV of L-Lys sulfate for effects on ADG, G:F, and plasma urea is equivalent to that of L-Lys HCl for nursery piglets.

Keywords: diarrhea, intestinal morphometry, limiting amino acid, nutrition, piglet

INTRODUCTION

The main objective of pork production is to provide lean tissue for human consumption. Lean tissue growth is the factor that most greatly influences the pigs' amino acid requirements, especially for Lys (NRC, 2012). Lys is the first limiting amino acid in diets for weaned piglets, and is essential for carcass muscle growth (Wu, 2013). Its adequate balance in the diet becomes important to meet the amino-acid requirements in pigs' different stages of growth.

Supplementary synthetic Lys is used to supply formulations deficits of this amino acid in diets (Baker et al., 1993; Eklunda at al., 2010), to reduce CP content of the diet (Hansen et al., 1993; Nyachoti et al., 2006), and reduce nitrogen (**N**) excretion (Jin et al., 1998; Shriver et al., 2003). In many cases, supplemental Lys increases feed efficiency and consequently is cost effective (Junghans et al., 2007; Yan et al., 2014). Supplementary Lys sources include L-Lys HCl (78% L-Lys) and L-Lys sulfate (> 50% L-Lys). L-Lys sulfate is obtained in a fermentation process similar to L-Lys HCl, but the processing forms differ mainly in the recovery and purification steps. At the end of processing, L-Lys sulfate contains at least 50% L-Lys in the base and some components such as other amino acids, residues from the fermentation process (dried microbial cells), macromolecules, and other organic and inorganic substances that could have some beneficial effect on the digestive and absorptive processes of piglets in the post-weaning period and improve their performance (Whittemore and Moffat 1976; Smiricky-Tjardes et al., 2004; Liu et al., 2007).

For the best utilization and strategic use of these products, knowledge about their relative bioavailability (**RBV**) is necessary, especially when formulating diets that provide the greatest economic return. Bioavailability studies necessitate feeding graded levels of the nutrient in question and the linear response area is defined by a minimum of three levels of the independent variable (Baker, 1986). However, studies on RBV of Lys from L-Lys sulfate

and L-Lys HCl in the post-weaning phase are limited for nursery piglets. The aim of the present study was to evaluate the RBV of L-Lys sulfate in comparison with L-Lys HCl and its effects on performance, blood parameters, intestinal functionality, and the apparent total tract digestibility in nursery piglets.

MATERIALS AND METHODS

All experimental procedures for the present study were approved by the Ethics Committee on Animal use of the Federal University of Lavras under the Protocol 076-16.

The experiment was performed in the weaning facilities of the Experimental Center on pig farming (*Centro Experimental de Suínos*) at the Department of Animal Science of the Federal University of Lavras, in Lavras, MG, Brazil. A total of 168 female piglets from a commercial lineage of high genetic value (DB90×PIC337), were weaned at 22 d of age (mean BW= 6.29 ± 0.41 kg) and placed on trial. The pigs were housed in raised decks pens (120 cm × 114 cm) with fully plastic slatted floor. Each pen had an adjustable nipple drinker and a galvanized steel trough. The rooms underwent a cleaning and disinfection program prior to the arrival of pigs.

To characterize the environment, a thermohygrometer associated with a black globe was installed in each room. Data were recorded twice a day (0700; 1600 hours) for the subsequent wet-bulb globe temperature (**WBGT**). Temperature and ventilation of rooms were controlled using heaters and adjustment of windows. The average minimum and maximum temperatures in the first 21 d of the experiment were, respectively, 22.4 and 28.3 °C, while in the second period (22 to 42 d) were 21.7 and 25.3 °C. The calculated WBGT was 71.88 and 69.72 for the first and second phase of the experiment.

The experimental period of 42 d was divided into two phases (phase 1, days 0 to 21; phase 2, days 22 to 42). The experimental design was a randomized complete block, with seven dietary treatments and eight replicates per treatment, with three pigs per experimental pen. The basal diet (**CON**) was lysine-deficient formulated to meet 73% of standardized ileal digestible Lys requirements, but adequate in other AA and energy (NRC, 2012). For the other dietary treatments, the CON was supplemented with three levels (80, 90, and 100% of standardized ileal digestible Lys requirements) of L-Lys sulfate (70% L-Lys) or L-Lys HCl (79% L-Lys). For the initial phase, the CON diet was formulated containing 0.987% SID-Lys, the diets supplemented with either L-Lys sulfate or L-Lys HCl had 1.080%, 1.215% and 1.350% SID-Lys, respectively, and replaced kaolin in the CON diet. For the second phase, the CON diet was formulated containing 0.90% SID-Lys, the diets supplemented with L-Lys sulfate or L-Lys HCl had 0.984%, 1.107% and 1.230% SID-Lys, respectively. The L-Lys sulfate and L-Lys HCl were obtained from a commercial company and had 70% and 79% of L-Lys in the base (CJ do Brasil Ind. e Com. de Produtos Alimentícios Ltda. São Paulo, Brazil). Ingredients and nutritional composition of diets are presented in Table 1 and 2. The analyzed free Lys content of the diets was used in all calculations. The pigs had *ad libitum* access to feed and water throughout the experimental period.

Pigs were weighed on the first day of the experiment, and at 7, 14, 21, 28, and 42 d. The same scale was used for all weighing. The provided diet and the leftovers were quantified daily in order to calculate ADG, ADFI, and G:F. At days 14, 28, and 42, blood samples from one pig per pen were collected for analysis of plasma urea and creatinine. Approximately 8 mL blood was collected by jugular vein puncture in heparinized tubes. In the laboratory, plasma samples were then collected by centrifuging at 3,000 × g, 15 min, 4°C, allocated into 1.5 mL microcentrifuge tubes. Plasma urea and creatinine analyses were performed through an automated analyzer (Labmax 240, Hirose Electric System Co.,Ltd. 1-9-6, Ebisuminami,

Nbf Ebisuminami Building. Gf. Shibuya-ku, 150-0022, Japan), using the enzymatic and kinetic methods, respectively.

Daily, throughout the 42d experiment, fecal consistency was visually examined at the same time, by the same person, and was scored on a scale of 1 to 5 (Halas et al., 2010). Diarrhoea was recorded when a piglet developed pasty or watery fecal consistency (score 4 to 5). The incidence of diarrhea (%) was calculated as the sum of the total number of daily diarrheal piglet observations over the period divided by the number of piglet days in the period, and the quotient multiplied by 100. The incidence of diarrhea was calculated in the first week and throughout the experimental period.

On day 42 of the experiment, one pig with BW closest to the pen average weight was chosen from each experimental pen and euthanized, totaling 56 pigs. The slaughter was performed through stunning by electronarcosis (> 300 V, 1.25 A, for 6 s) followed by exsanguination. The slaughter was performed in a commercial slaughterhouse in the municipality of Lavras (Minas Gerais, Brazil) accompanied by a veterinarian. After evisceration, samples of about 3cm long were collected from the jejunum (location relative to the of íleo-cecal junction) to perform histological analyses (villus height, crypt depth and villus:crypt ratio). After careful removal of the luminal content and washing with saline solution, the jejunum samples were fixed in 10% formaldehyde for 24 h and transferred to 70% alcohol solution until the slides were prepared. The histological analysis was performed in paraffin-embedded segments, sectioned at 4 μm and stained with hematoxylin and eosin stain, based on Luna (1968). The slides were photographed through the trinocular microscope (CX31, Olympus Optical do Brasil Ltda., São Paulo, SP, Brazil) and digital image capture camera (SC30, Olympus Optical do Brasil Ltda., São Paulo, SP, Brazil). The villus height and crypt depth were measured through the AxionVision SE64 4.9.1 software, using 15 well-

oriented villi and crypts per tissue. The villus:crypt ratio was calculated and all analyses were performed by a single person.

The apparent total tract digestibility (**ATTD**) of dry matter, crude protein, and gross energy of experimental diets were calculated according to the indirect evaluation method with the chromium oxide (**Cr_2O_3**) indicator. In the last week of the experiment, chromium oxide at 0.3% was added to the feed at the expense of corn. After the presence of the indicator was detected in the feces through the color change in all the pens, samples were collected once a day for three consecutive days in each pen (days 37, 38, and 39). The fecal samples were processed (homogenized, dried, and ground) and together with samples of experimental diets, were subjected to the analysis of dry matter, crude protein, gross energy, and chromium quantification. The following formula was used to calculate ATTD of CP for example: ATTD = 100 - [100 × (DC/CF × PF/PD)], where ATTD is the apparent total tract digestibility; DC - % Cr_2O_3 in the diet; CF - % Cr_2O_3 in feces; PF -% crude protein in feces and PD - % crude protein in the diet (Zhang and Adeola, 2017).

The diets and fecal samples were analyzed for dry matter and N content following the methods of AOAC (2000). Crude protein content was calculated by multiplying the N content by 6.25. Gross energy was determined using bomb calorimetry (IKA C5000). The total AA content of the diets was quantified by high performance liquid chromatography, as outlined by Liu et al. (2007). Calcium and phosphorus in diets were determined according to the methods of AOAC (2000). The chromium was measured by atomic absorption spectrometry, using a spectrophotometer (Varian SpectrAA 100, Varian Australia Pty Ltd, Mulgrave, Victoria, Australia) according to the method reported by Williams et al. (1962).

Statistical analyses of the data were performed by ANOVA, using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Differences were considered significant if $P \leq 0.05$ and were described as tendencies if $0.05 < P \leq 0.10$. Orthogonal-polynomial contrasts were used to

determine the linear and quadratic effects of L-Lys levels and sources on the studied variables. Data were fitted in the multivariate linear regression model with the following equation: $y = a + b_1x_1 + b_2x_2$, where y = performance criterion (ADG, G:F, plasma urea), a = common intercept, b_1 = slope of L-Lys HCl, x_1 = value for L-Lys HCl, b_2 = slope of L-Lys sulfate, and x_2 = value for L-Lysine sulfate. The RBV of L-Lys sulfate as compared with L-Lys HCl was calculated for the 42-d experimental period and using the analyzed free Lys content of the diets. The RBV was calculated as the ratio of their linear slopes (i.e., $b_2/b_1 \times 100$) as described by Littell et al. (1997). The results were considered significant if $P < 0.05$. For the variable incidence of diarrhea, the influence of each treatment on the occurrence of diarrhea was analyzed by applying the generalized linear model in the GENMOD procedure of SAS.

RESULTS AND DISCUSSION

Maximum and minimum temperatures in the first 21 d of the experiment were, respectively, 22.4 and 28.3 °C, while in the second period (21 to 42 d) were 21.7 and 25.3 °C. The calculated WBGT was 71.9 and 69.7 for the first and second phase of the experiment. These environmental parameters indicate that the pigs were kept in a comfortable thermoneutral environment, so that the environment did not represent an influencing factor in the test results.

The animal performance results are presented in Table 3. In the first week, the pigs supplemented with L-Lys sulfate had higher ($P < 0.05$) feed intake than pigs supplemented with L-Lys HCl, with a tendency for greater daily weight gain ($P = 0.91$) and d7 BW ($P = 0.08$). There were no significant differences in piglet performance when comparing supplementation of L-Lys sources and CON. When considering the two nursery periods (days 0 to 21 and days 22 to 42), pigs supplemented with L-Lys sulfate or L-Lys HCl had higher (*P*

< 0.05) feed efficiency in relation to animals fed with the CON diet, but no significant differences in ADG or ADFI. Moreover, there were no significant differences in piglet performance between the tested L-Lys sources and levels.

In swine production, weaning is a stressful event in which pigs are subjected to nutritional changes and immunological and psychological challenges that lead to low feed intake, diarrhea and decreased performance (Smith et al., 2010). In the present study, the consequences of these stressors were similar for all pigs. Pig growth performance was not significantly different among the treatments. However, supplementation of L-Lys sulfate produced a greater DFI, which is extremely important in this first week after weaning and could promote a positive residual effect on subsequent performance. For the two nursery periods, Lisine supplemented pigs had improved feed efficiency. This was expected as Lys is the first limiting amino acid for pigs, and deficiencies in the intake of this amino acid reduce pig performance.

Plasma urea concentrations were significantly lower ($P < 0.01$) at all three time points (d 14, 28, and 42) for pigs supplemented with L-Lys sulfate or L-Lys HCl in relation to those fed with the CON diet (Table 4). Creatinine levels were not influenced ($P > 0.05$) by Lys supplementation in the experimental diets. There were no significant effects of Lys source on plasma urea and creatinine concentrations.

Due to its close relationship with urinary nitrogen excretion, plasma urea can be used as an important metabolic indicator in swine (Zervas and Zijlstra, 2002). According to Cai et al. (1994), the concentration of plasma urea is inversely correlated with the use of proteins, which in turn is influenced by the quality and quantity of the ingested protein. Thus, the lower the plasma urea concentrations, the greater the use of nitrogenous components of the diet. In the present study, pigs fed with CON had greater plasma urea concentrations. Lys is the first

limiting amino acid, and deficiency in Lys would affect the amino acid balance and increase excretion of the remaining amino acids of the diet in the form of urea.

Creatinine is a nonprotein nitrogen, produced from creatine metabolism in the muscle cells and excreted in urine (Deminice et al., 2009). Creatinine has been related to muscular mass (Deguchi 1997) and its levels associated with the quality of dietary protein (Awosanya et al., 1999). Moreover, increases in serum levels of creatinine was observed with dietary L-glutamine supplementation in weaned piglets (Xiao et al., 2012). The results of this study indicate that creatinine is not a suitable indicator of the amino acid utilization of the diet.

The RBV curves in response to supplementary levels of L-Lys sulfate and L-Lys HCl are shown in Figures 1, 2 and 3. The variables ADG, G:F, and plasma urea for overall period were fitted to the multivariate linear regression model described by Littel et al. (1997). Based on the ADG response, the RBV of Lys from L-Lys sulfate was 106% (76% to 136%) in relative to L-Lys HCl, with a coefficient of determination of 0.93 (Figure 1). Based on the G:F response to L-Lys supplementation level, RBV was estimated in 119% (92% to 146%) for L-Lys sulfate in relative to L-Lys HCl, with a coefficient of determination equal to 0.96 (Figure 2). The RBV based on plasma urea concentrations was estimated in 117% (83% to 150%) for L-Lys sulfate in relation to L-Lys HCl, with a coefficient of determination equal to 0.96 (Figure 3). However, the values of RBV based on ADG, G:F, and plasma urea were not different ($P > 0.05$) from 100%, indicating that the RBV of L-Lys sulfate and L-Lys HCl are equivalents. That supporting the fact that they do not show differences when comparing their supplementation effects on pig performance and blood metabolites. These results agree with Smiricky-Tjardes et al. (2004), which evaluated the RBV of Lys from L-Lys sulfate (47.3% L-Lys) in relation to L-Lys HCl (78.5% L-Lys) in diets for piglets weighing 9.5 kg for 21 d. These authors found that RBV of Lys from L-Lys sulfate was not significantly different from RBV of Lys from L-Lys HCl based on the responses for ADG (99%) and G:F (97%).

In a similar study, Liu et al. (2007) evaluated the RBV of two supplementary sources of Lys in diets for pigs weighing from 10 to 20 kg, being L-Lys sulfate (50% L-Lys) and L-Lys HCl (78% L-Lys). In this research, the RBV of Lys from L-Lys sulfate in relation to L-Lys HCl was 101%, 105%, 104% and 95% for the variables ADG, G:F, plasma urea nitrogen and retained nitrogen, respectively. In a more recent study, RBV of Lys from L-Lys sulfate (54.5% L-Lys) in relation to L-Lys HCl (78% L-Lys) was estimated in 104% and 112% for the variables ADG and feed efficiency of growing pigs from 57 to 87 kg (Htoo et al., 2016). Together, these results corroborate with those of the present study, supporting the conclusion that there are no marked differences in RBV of Lys from L-Lys sulfate and L-Lys HCl when supplemented for growing pigs. Therefore, L-Lys sulfate can be used as a substitute for L-Lys HCl in the diet of growing pigs.

In previously cited articles, and in this study, L-Lys sulfate had different levels of Lys in the base product (47.3%, 50%, 54.5%, and 70%). Although the purity of the base product was variable, the RBV of the L-Lys sulfate relative to L-Lys HCl was not significantly different for these four trials. However, the relative contribution of nutrients from a given source may be related to purity level and therefore affect its bioavailability (Baker, 1986). Studies are needed to evaluate whether variation in the Lys percentage of L-Lys Sulfate may have some effect on RBV and how this would impact economic profitability by using a base produced with a higher or lower concentration of L-Lys.

The results for diarrhea incidence are shown in Figure 4. In the first week, there were differences in the diarrhea index among dietary treatments. Piglets fed with CON and those supplemented with L-Lys HCl at 80% had a greater ($P < 0.05$) rate of diarrhea in relation to pigs supplemented with L-Lys sulfate at 90% and 100% and with L-Lys HCl at 100%. For the total period, the pigs supplemented with L-Lys sulfate at 90% and 100% and with L-Lys HCl at 100% maintained a lower ($P < 0.05$) diarrhea rate.

There were significant treatment effects for villus height (Table 5). Pigs supplemented with L-Lys sulfate or L-Lys HCl had greater ($P < 0.05$) villus height in relation to those receiving the CON diet. The source and level of Lys did not affect villus height, crypt depth and villus:crypt ratio in the pigs evaluated.

Lys deficiencies during the weaning phase could compromise the intestinal health of piglets, causing a greater incidence of diarrhea and damaged jejunal morphometry. These harmful events could be triggered by an immune system less prepared to combat pathogenic agents causing imbalances in the intestinal microbiota, since pigs subjected to Lys deficiency can have decreased immune responses (Li et al., 2007; Liao et al., 2015). This is likely due to impairment of the synthesis of proteins related to the animal inflammatory responses, including serum antibody, inflammatory cytokines, TLRS system, and ERK1/2 and NF-κB signals (Han et al., 2018). It is also important to consider that the intestinal tissue has a high rate of cell turnover. Thus, for this process to occur efficiently, a constant supply of nutrients is necessary, including amino acids. Although Lys is more related to muscle protein synthesis, this amino acid is also metabolized by enterocytes, which may have direct or indirect effects on the dynamics of cell renewal and consequent state of intestinal villi. For instance, direct effects, being an important source of energy for the enterocyte (Stoll et al., 1998), or indirect, being a precursor for the amino acid synthesis of the glutamate family, main cellular fuels at the intestinal level.

He et al. (2013) found that dietary supplementation with Lys enhanced villus height and crypt depth in the jejunum and affected intestinal expression of cationic amino acid transporters, which could be related to the absorption of Lys and other basic amino acids. In a more recent study, Lys restriction (30% according to the NRC 2012) increased bacterial diversity (Yin et al., 2017). Thus, the greatest incidence of diarrhea presented in CON group, in the present study, might be associated with changes in the gut microbiome.

The ATTD of dry matter, gross energy and crude protein of diets supplemented with different levels and sources of L-Lys are presented in Table 6. When comparing the L-Lys sources with the CON diet, the ATTD of dry matter was greater ($P < 0.05$) in diets supplemented with L-Lys sulfate, while the ATTD of the gross energy and crude protein ratio tended ($P = 0.09$) to be greater in diets supplemented with L-Lys sulfate. There were no significant differences when comparing treatments supplemented with L-Lys HCl and the CON diet. The diets supplemented with L-Lys sulfate had greater ($P < 0.05$) ATTD of dry matter, gross energy and crude protein than diets supplemented with L-Lys HCl.

The fact that L-Lys sulfate favors ATTD could be related to the presence of other nutrients. During the production process of L-Lys sulfate, the fermented broth does not undergo the separation and purification processes, leading to a product containing other nutrients besides Lys, including mainly other essential amino acids (Leuchtenberger et al., 2005). There is also the presence of impurities from the fermentation process, such as dried microbial cells, macromolecules, and other organic and inorganic substances (Kumon et al., 1991). These additional components could influence the digestibility of nutrients. However, the concentrations of these components must be known to explore their effects on the digestive processes and other functions, including pig growth performance. Furthermore, it is important to highlight the association of these results with the incidence of diarrhea and intestinal morphometry. The pigs fed L-Lys sulfate had decreased incidence of diarrhea and greater villus height, which may have contributed to better utilization of feed nutrients, reflecting higher nutrient digestibility coefficients.

The overall results of this experiment indicate that the RBV of L-Lys sulfate for effects on ADG, G:F and plasma urea is equivalent to that of L-Lys HCl. Consequently, L-Lys sulfate can be used in substitution of L-Ly HCl as a supplementary source of Lys for

nursery piglets. Lys deficiency may compromise the intestinal functionality of nursery piglets with a greater incidence of diarrhea and damaged jejunal morphometry.

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Table 1. Experimental diets of phase 1, days 0 to 21 (as-fed basis)

Ingredients, %	CON	L-Lys sulfate			L-Lys HCl		
Corn, 7.88% CP	52.55	52.55	52.55	52.55	52.55	52.55	52.55
Micronized soy	11.66	11.66	11.66	11.66	11.66	11.66	11.66
Soybean meal	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Plasma, spray dried	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Whey dried, 70% lactose	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean oil	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral-vitamin premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate,18.5%	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Calcitic limestone	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Common salt	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Zinc oxide, 72%	0.34	0.34	0.34	0.34	0.34	0.34	0.34
L-Lys HCl, 79%	0.00	0.00	0.00	0.00	0.12	0.29	0.46
L-Lys sulfate, 70%	0.00	0.13	0.33	0.52	0.00	0.00	0.00
L-Threonine	0.13	0.13	0.13	0.13	0.13	0.13	0.13
L-Tryptophan	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L-Valine	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Methionine	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Kaolin	0.60	0.47	0.27	0.08	0.48	0.31	0.14
Calculated values[2]							
ME, kcal/kg	3400 (4093)	3400 (4093)	3400 (4093)	3400 (4093)	3400 (4093)	3400 (4093)	3400.00 (4093)
CP, %	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)
SID[3] Lys, %	0.99 (1.15)	1.08 (1.24)	1.22 (1.38)	1.35 (1.51)	1.08 (1.24)	1.22 (1.38)	1.35 (1.51)
SID Met, %	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)
SID Met + Cys, %	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)
SID Thr, %	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)
SID Trp, %	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)
SID Arg, %	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)
SID Val, %	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)
SID Ile, %	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)
SID Leu, %	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)
SID His, %	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)
SID Phe, %	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)

Lactose, %	10.50	10.50	10.50	10.50	10.50	10.50	10.50
Total calcium, %	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)
Total phosphorus, %	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)
Available phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium, %	0.35	0.35	0.35	0.35	0.35	0.35	0.35

[1]**Levels per kg of diet, mineral premix:** 0.08 g of iron, 0.04 g of manganese, 0.2 mg of cobalt, 0.11 g of zinc, 1.2 mg of iodine, and 0.35 mg of selenium.

Vitamin premix: 15,000 IU of vitamin A, 3,125 IU of vitamin D3, 87.5 IU of vitamin E, 3.13 mg of vitamin K3, 2.75 mg of vitamin B1, 0.01 g of vitamin B2, 0.025 g of vitamin pantothenic acid, 3.75 mg of vitamin B6, 37.5 mg of vitamin B12, 0.038 g of nicotinic acid, 3.75 mg of folic acid, and 0.5 mg of biotin.

[2] Values in parentheses are analyzed = gross energy (kcal/kg); total AA contents (%); total calcium and phosphorus (%).

[3] SID = standardized ileal digestible.

Table 2. Experimental diets for phase 2, days 21 to 42 (as-fed basis)

Ingredients, %	**CON**		**L-Lys sulfate**			**L-Lys HCl**	
Corn, 7.88% CP	61.94	61.94	61.94	61.94	61.94	61.94	61.94
Micronized soy	6.79	6.79	6.79	6.79	6.79	6.79	6.79
Soybean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Mineral-vitamin premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate,18.5%	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Calcitic limestone	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Common salt	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Zinc oxide, 72%	0.26	0.26	0.26	0.26	0.26	0.26	0.26
L-Lys HCl, 79%	0.00	0.00	0.00	0.00	0.11	0.26	0.42
L-Lys sulfate, 70%	0.00	0.12	0.30	0.47	0.00	0.00	0.00
L-Threonine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Kaolin	0.70	0.58	0.40	0.23	0.59	0.44	0.28
Calculated values[2]							
ME, kcal/kg	3350 (4729)	3350 (4619)	3350 (4776)	3350 (4751)	3350 (4896)	3350 (4762)	3350 (4727)
CP, %	19.00 (18.96)	19.00 (20.56)	19.00 (19.30)	19.00 (19.28)	19.00 (19.99)	19.00 (18.81)	19.00 (19.51)
SID[3] Lys, %	0.90 (0.97)	0.98 (1.05)	1.11 (1.18)	1.23 (3.30)	0.98 (1.05)	1.11 (1.18)	1.23 (1.30)
SID Met, %	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)
SID Met + Cys, %	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)
SID Thr, %	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)
SID Trp, %	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)
SID Arg, %	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)
SID Val, %	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)
SID Ile, %	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)
SID Leu, %	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)
SID His, %	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)
SID Phe, %	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)
Total calcium, %	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)
Total phosphorus, %	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)

Available phosphorus, %	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Sodium, %	0.28	0.28	0.28	0.28	0.28	0.28	0.28

[1]**Levels per kg of diet, mineral premix:** 0.08 g of iron, 0.04 g of manganese, 0.2 mg of cobalt, 0.11 g of zinc, 1.2 mg of iodine, and 0.35 mg of selenium.

Vitamin premix: 15,000 IU of vitamin A, 3,125 IU of vitamin D3, 87.5 IU of vitamin E, 3.13 mg of vitamin K3, 2.75 mg of vitamin B1, 0.01 g of vitamin B2, 0.025 g of vitamin pantothenic acid, 3.75 mg of vitamin B6, 37.5 mg of vitamin B12, 0.038 g of nicotinic acid, 3.75 mg of folic acid, and 0.5 mg of biotin.

[2] Values in parentheses are analyzed = gross energy (kcal/kg); total AA contents (%); total calcium and phosphorus (%).

[3] SID = standardized ileal digestible.

Table 3. Weight, daily weight gain (ADG), daily feed intake (ADFI), and feed efficiency (G:F) of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to CON[1]

Item	CON[1]	L-Lys sulfate[2]			L-Lys HCl[3]			SEM	*P*-value[4]		
		80%	90%	100%	80%	90%	100%		C1	C2	C3
Initial BW, kg	6.288	6.288	6.291	6.291	6.288	6.288	6.29	0.0024	0.42	0.78	0.45
Period, days 0 to 7											
BW, kg day 7	6.935	7.033	7.025	6.958	6.778	6.810	6.999	0.0968	0.53	0.52	0.08
ADG, kg	0.109	0.124	0.121	0.113	0.081	0.090	0.119	0.0160	0.58	0.52	0.09
ADFI, kg	0.175	0.194	0.210	0.164	0.146	0.146	0.186	0.0152	0.42	0.39	0.02
G:F, kg/kg	0.604	0.643	0.583	0.601	0.541	0.498	0.586	0.0528	0.94	0.32	0.14
Period, days 0 to 21											
BW, kg day 21	11.696	11.833	12.215	12.116	11.559	11.635	12.153	0.1102	0.14	0.72	0.12
ADG, kg	0.258	0.264	0.280	0.279	0.253	0.255	0.278	0.0042	0.15	0.69	0.14
ADFI, kg	0.428	0.431	0.443	0.418	0.409	0.411	0.423	0.0062	0.88	0.54	0.28
G:F, kg/kg	0.603	0.615	0.640	0.670	0.615	0.620	0.650	0.0048	<0.01	0.04	0.14
Period, days 0 to 42											
BW, kg day 42	21.233	21.485	22.085	22.715	21.125	21.611	22.729	0.4699	0.12	0.28	0.48
ADG, kg	0.365	0.371	0.385	0.401	0.362	0.374	0.401	0.0115	0.12	0.29	0.48
ADFI, kg	0.661	0.655	0.664	0.636	0.619	0.630	0.662	0.0205	0.70	0.33	0.40
G:F, kg/kg	0.553	0.566	0.581	0.630	0.586	0.593	0.606	0.0097	<0.01	<0.01	0.73

[1]CON= basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

[2]Supplementation of L-Lys sulfate meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to NRC, 2012.

[3]Supplementation of L-Lys HCl meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[4]*P*-valuesof the orthogonal contrasts: C_1 = CON vs L-Lys sulfate; C_2 = CON vs L-Lys HCl; C_3 = L-Lys sulfate vs L-Lys HCl.

Table 4. Plasma urea and creatinine, in milligram per deciliter, of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to a basal diet

Item	CON[1]	L-Lys sulfate[2]			L-Lys HCl[3]			SEM	*P*-value[4]		
		80%	90%	100%	80%	90%	100%		C_1	C_2	C_3
Urea, day 14	24.38	21.75	14.75	11.63	18.38	12.25	9.25	1.8967	<0.01	<0.01	0.08
Creatinine, day 14	0.94	1.01	0.94	1.02	1.01	0.98	0.89	0.0488	0.39	0.71	0.49
Urea, day 28	27.88	20.38	18.75	15.50	21.50	18.13	15.00	1.7674	<0.01	<0.01	0.99
Creatinine, day 28	0.95	0.99	0.93	0.96	0.89	0.93	0.94	0.0352	0.88	0.47	0.25
Urea, day 42	22.75	18.88	17.00	14.63	19.88	17.25	15.50	1.4409	<0.01	<0.01	0.55
Creatinine, day 42	1.03	1.04	1.10	1.14	1.15	1.11	1.10	0.0473	0.26	0.10	0.41

[1]CON= basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

[2]Supplementation of L-Lys sulfate meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[3]Supplementation of L-Lys HCl meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[4]*P*-valuesof the orthogonal contrasts: C_1 = CON vs L-Lys sulfate; C_2 = CON vs L-Lys HCl; C_3 = L-Lys sulfate vs L-Lys HCl.

Table 5. Jejunal morphometry, in μm, of piglets on day 42 when supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to a basal diet

Item[1]	CON[2]	L-Lys sulfate[3]			L-Lys HCl[4]			SEM	*P*-value[5]		
		80%	90%	100%	80%	90%	100%		C1	C2	C3
A	344	439	389	419	412	418	419	22.9283	0.01	0.01	0.98
P	372	386	362	358	370	357	364	17.6582	0.87	0.69	0.74
R	0.99	1.14	1.13	1.25	1.18	1.25	1.21	0.0971	0.11	0.05	0.64

[1]A = villus height; P = crypt depth; R = villus height/crypt depth ratio.

[2]CON= basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

[3]Supplementation of L-Lys sulfate meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[4]Supplementation of L-Lys HCl meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[5]*P*-valuesof the orthogonal contrasts: C_1 = CON vs L-Lys sulfate; C_2 = CON vs L-Lys HCl; C_3 = L-Lys sulfate vs L-Lys HCl.

Table 6. Apparent total tract digestibility (ATTD) for dry matter (DM), gross energy (GE) and crude protein (CP) of diets supplemented with different levels and sources of L-lys for weaned pigs

Item	CON[1]	L-Lys sulfate[2]			L-Lys HCl[3]			SEM	*P*-value[4]		
		80%	90%	100%	80%	90%	100%		C1	C2	C3
DM	76.12	78.27	79.29	79.81	75.31	78.87	76.28	0.8785	0.01	0.50	<0.01
GE	76.19	77.22	78.22	79.19	75.71	77.29	74.41	1.0246	0.09	0.75	0.01
CP	65.65	69.21	68.09	68.57	65.12	67.71	63.09	1.4677	0.09	0.84	0.01

[1]CON= basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

[2]Supplementation of L-Lys sulfate meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[3]Supplementation of L-Lys HCl meeting 80%, 90% and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

[4]*P*-valuesof the orthogonal contrasts: C_1 = CON vs L-Lys sulfate; C_2 = CON vs L-Lys HCl; C_3 = L-Lys sulfate vs L-Lys HCl.

Figure 1. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on overall (d 0 - 42) daily weight gain (ADG) of weaned pigs.

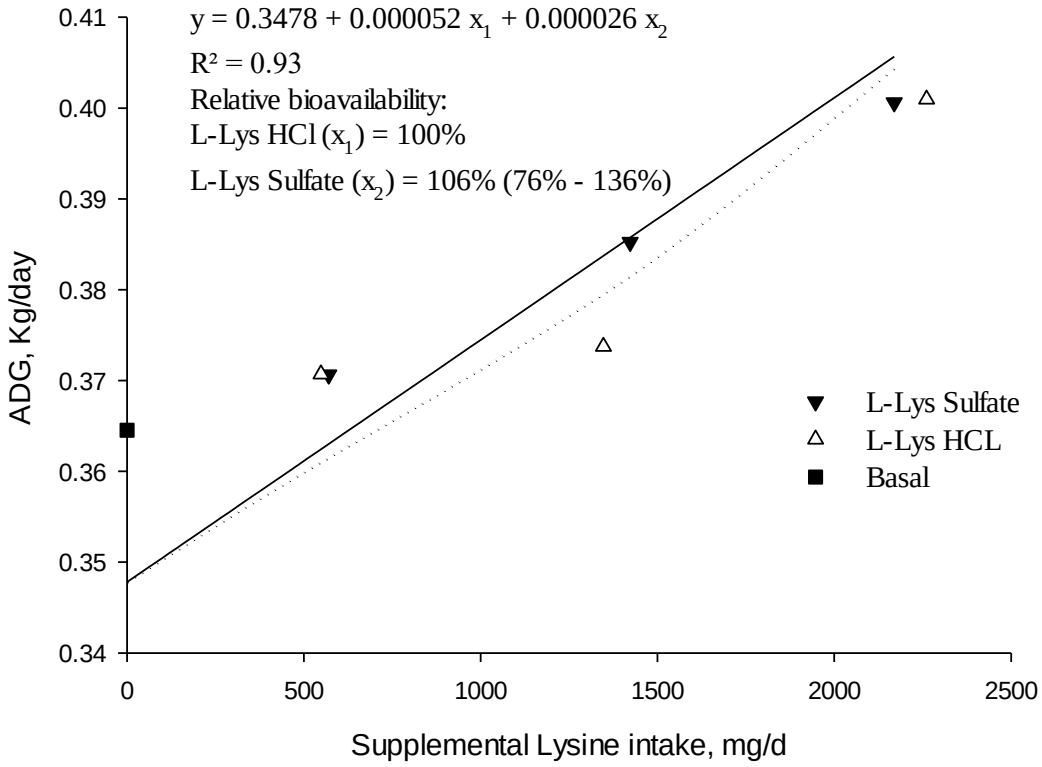
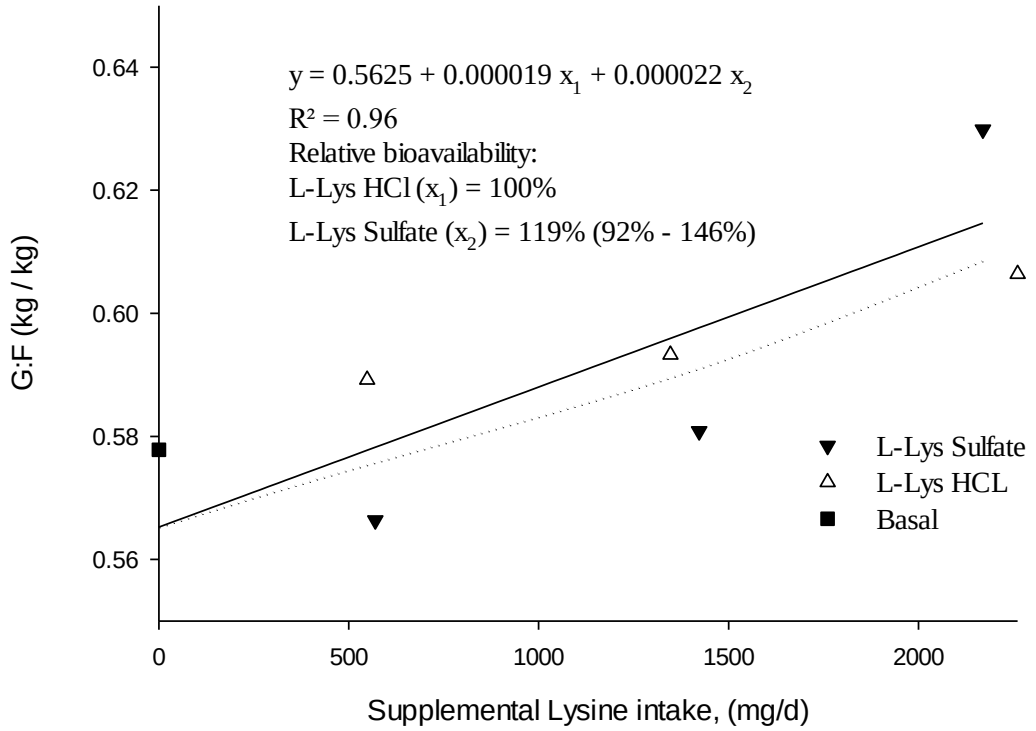


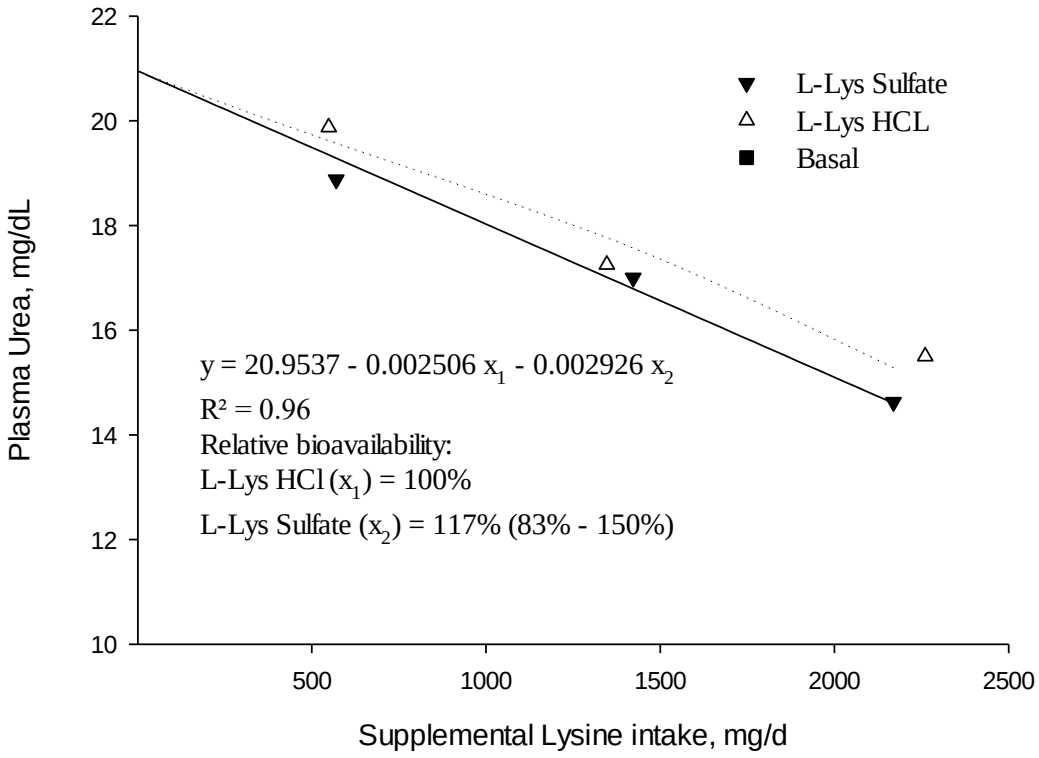
Figure 2. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on overall (d 0 - 42) feed efficiency (G:F) of weaned pigs.



..... L-Lys HCl

____ L-Lys sulfate

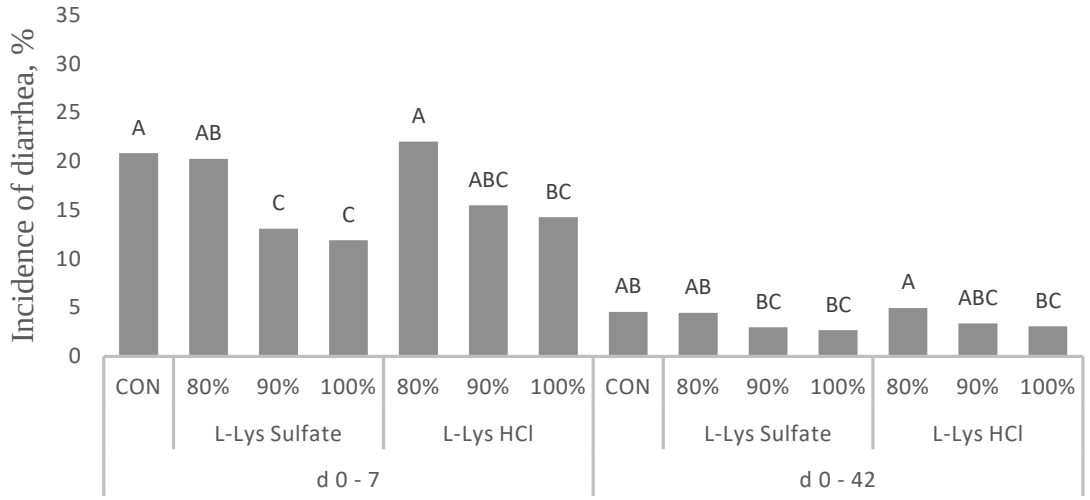
Figure 3. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on the plasma urea concentrations of weaned pigs.



..... L-Lys HCl

____ L-Lys sulfate

Figure 4. Incidence of diarrhea of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to CON pigs.



ANEXO A - CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

UNIVERSIDADE FEDERAL DE LAVRAS
PRÓ-REITORIA DE PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS
Cx.P.3037 - Lavras – MG – 37200-000 – (35) 3829-5182 cba@nintec.ufla.br

CERTIFICADO

Certificamos que a proposta intitulada " Biodisponibilidade da Lisina Sulfato e Lisina - HCl para otimizar o desempenho corporal, parâmetros sanguíneos, digestibilidade e morfometria intestinal de leitões ", protocolo nº 076/16, sob a responsabilidade de Vinícius de Souza Cantarelli, Jorge Yair Perez Palencia, Márvio Lobão Teixeira de Abreu e Maíra Reseade, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto homem), para fins de ensino e/ou pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas edificadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), do Ministério da Ciência, Tecnologia e Inovação (MCTI), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Pró-Reitoria de Pesquisa/UFLA, em reunião de 07/12/2016.

Vigência da autorização: de 01/12/2016 a 31/01/2018
Finalidade: ( ) Ensino (x) Pesquisa Científica
Espécie/linhagem/raça: Suíno / DB90 x PIC415
Número de animais aprovados: 176
Peso/Idade: 6 kg / 21 dias
Sexo: fêmea
Origem dos animais (documento apresentado pelo pesquisador responsável e arquivado pela CEUA): Fazenda São Paulo S.A. - Rod. Fernão Dias, km 634 - Oliveira/MG - Cx. Postal, 45 - CEP.: 35540-000. Responsável: Pollyana F. Alves de Sousa.

Prof. Juliano Vogas Peixoto
Presidente da Comissão de Ética no Uso de Animais CEUA

Universidade Federal de Lavras
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CERTIFICATE

We certify that the proposal entitled "Bioavailability of Lysine Sulfate and Lysine-HCl to optimize performance, body composition, blood parameters, digestibility and intestinal morphology of piglets" Protocol No. 076/16, under the responsibility of Vinicius de Souza Cantarelli, Jorge Yair Perez Palencia, Márvio Lobão Teixeira de Abreu and Maíra Resende, which involves the production, maintenance and / or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except man), for purposes of teaching and / or scientific research, is in accordance with the provisions of Law No. 11.794, of October 8, 2008, Decree No. 6899 of July 15, 2009, and with the standards required by the National Council Animal Experimentation Control (CONCEA), the Ministry of Science, Technology and Innovation (MCTI), and was approved by ETHICS COMMITTEE ON ANIMAL USE (CEUA) of the Dean of Research / UFLA in meeting 12/07/2016.

Authorization validity: 12/01/2016 to 01/31/2018
Finality: ( ) Teaching (x) Scientific research
Species / strain / breed: Swine / DB90 x PIC415
Number of approved animals: 176
Weight / Age: 6 kg / 21 days
Sex: female
Origin of animals (document presented by the responsible researcher and filed by CEUA): Fazenda São Paulo S.A. - Rod. Fernão Dias, km 634 - Oliveira/MG - Cx. Postal, 45 - CEP.: 35540-000. Responsável: Pollyana F. Alves de Sousa.

Prof. Juliano Vogas Peixoto
Presidente da Comissão de Ètica no Uso de Animais CEUA

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