UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

AJUSTE DA CONCENTRAÇÃO DE AMINOÁCIDOS DA DIETA DE ACORDO COM O RITMO CIRCADIANO DE SUÍNOS EM CRESCIMENTO E TERMINAÇÃO

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Mestre em zootecnia
Zootecnista

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Coorientador: Prof. Dr. Luan Sousa dos Santos

Tese Apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Zootecnia.

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: AJUSTE DA CONCENTRAÇÃO DE AMINOÁCIDOS DA DIETA DE ACORDO COM O RITMO CIRCADIANO DE SUÍNOS EM CRESCIMENTO E TERMINAÇÃO

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Jaboticabal, 07 de maio de 2021

DADOS CURRICULARES DO AUTOR

ALINI MARI VEIRA – nascida em Bauru – SP, no dia 8 de outubro de 1990. Ingressou no curso de Zootecnia na Universidade Federal do Ceará (UFC) no ano de 2010, graduando-se em dezembro de 2014. Durante a graduação, fez estágio na área de produção e nutrição de suínos, participou de projetos de pesquisa, foi membro da Empresa Junior de Zootecnia da UFC, foi membro do Núcleo de Ensino e Estudos em Suinocultura da UFC de agosto de 2010 a junho de 2014. Durante o período de agosto de 2012 a julho de 2014 foi bolsista de iniciação científica (CNPq) sob orientação do Prof. Dr. Pedro Henrique Watanabe. Em março de 2015 iniciou o curso de Mestrado no Programa de Pós-graduação em Zootecnia, da Faculdade de Ciências Agrárias e Veterinárias/UNESP – Campus de Jaboticabal, como bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Obtendo o título de Mestre em Zootecnia em fevereiro de 2017 sob orientação do Prof. Dr. Luciano Hauschild. Mesmo ano que iniciou o doutoramento no Programa de Pós-graduação em Zootecnia, da Faculdade de Ciências Agrárias e Veterinárias/UNESP – Campus de Jaboticabal, como bolsista do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, número do processo: 141289/2017-1) no período de março de 2017 a junho de 2018. Durante o doutorado foi professora bolsista do Departamento de Zootecnia da Faculdade de Ciências Agrárias e Veterinárias/UNESP – Campus de Jaboticabal, sendo responsável e ministrando as disciplinas de Suinocultura e Zootecnia de Suínos para estudantes de graduação durante o período de fevereiro a junho de 2018. Ainda durante o doutorado foi bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, número do processo: 2017/18734-1) de julho de 2018 a abril de 2021. Defendendo a presente tese no dia 7 de maio de 2021 sob orientação do Prof. Dr. Luciano Hauschild.

“ Se você tem conhecimento, deixe os outros acenderem suas velas nele. ”

Margaret Fuller

Aos meus queridos animais (pigs ❤), todo o meu respeito e gratidão!

À minha mãe Emilia, por todo apoio, incentivo e amor incondicional.

Dedico!

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À Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de Jaboticabal, por toda infraestrutura fornecida para elaboração dessa tese.

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Ao meu orientador Prof. Dr. Luciano Hauschild, por toda essa trajetória de orientação na pós-graduação, por todas as oportunidades de crescimento profissionais e pessoais, por todo o tempo dedicado à minha formação e às nossas pesquisas, por todo respeito e, principalmente, por sua humildade. São ensinamentos que levarei sempre comigo. Muito obrigada! *“Pouco conhecimento faz com que as pessoas se sintam orgulhosas. Muito conhecimento, que se sintam humildes. É assim que as espigas sem grãos erguem desdenhosamente a cabeça para o céu, enquanto que as cheias as baixam para a terra. ” Leonardo da Vinci.*

Ao meu coorientador, colega de pós-graduação e amigo querido Prof. Dr. Luan Sousa dos Santos, por participar de todas as etapas desse trabalho e sempre me amparar em todos os momentos de necessidade (profissionais e pessoais). Obrigada pela amizade, por toda a contribuição para minha formação acadêmica e por sempre acreditar no meu trabalho!

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“ Quem acolhe um benefício com gratidão, paga a primeira prestação da sua dívida. ”

Sêneca

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Plano de alimentação sequencial com variação no nível de aminoácidos a cada 12 horas para suínos em crescimento"**, protocolo nº 012570/18, sob a responsabilidade do Prof. Dr. Luciano Hauschild, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 06 de setembro de 2018.

Vigência do Projeto	15/10/2018 a 07/01/2019
Espécie / Linhagem	Suínos / Comercial
Nº de animais	70
Peso / Idade	25 – 100kg / 60 a 150 dias
Sexo	Machos castrados
Origem	Granjas comercial / Minas Gerais

Jaboticabal, 06 de setembro de 2018.

Fabiana Pilarski

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Plano de alimentação sequencial com variação no nível de aminoácidos a cada 12 horas para suínos em crescimento"**, protocolo nº 08335/19, sob a responsabilidade do Prof. Dr. Luciano Hauschild, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 04 de julho de 2019.

Vigência do Projeto	01/08/2019 a 30/08/2019
Espécie / Linhagem	Suínos / Comercial
Nº de animais	24
Peso / Idade	25 – 40 kg / 60 a 90 dias
Sexo	Machos castrados
Origem	Granja comercial / Minas Gerais

Jaboticabal, 04 de julho de 2019.

Fabiana Pilarski

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AJUSTE DA CONCENTRAÇÃO DE AMINOÁCIDOS DA DIETA DE ACORDO COM O RITMO CIRCADIANO DE SUÍNOS EM CRESCIMENTO E TERMINAÇÃO

RESUMO – Na nutrição de suínos, as rações são formuladas de acordo com o estágio de crescimento dos animais e, predominantemente, uma única dieta é fornecida por um longo período dentro de um sistema de alimentação por fases. Nesse sistema, quando o objetivo é otimizar o desempenho de um lote de animais os nutrientes são, normalmente, fornecidos em excesso principalmente ao final de cada fase. Dessa forma, devido ao custo elevado dos alimentos, aliado a excreção de elementos poluidores, novas estratégias nutricionais devem ser propostas e avaliadas. Dentro desse contexto, programas que permitem fornecer uma dieta que atenda a exigência diária dos animais têm se demonstrados promissores uma vez que permitem reduzir custos e excreção de elementos poluidores. Nesse contexto de nutrição de precisão, torna-se relevante destacar que a eficiência de utilização dos nutrientes pode variar ao longo do dia, de acordo com as variações do estado metabólico (catabolismo e anabolismo) dos animais determinado pelo ciclo circadiano. Portanto, o ajuste da concentração nutricional da dieta às flutuações do estado metabólico dos suínos ao longo do dia pode ser uma estratégia promissora para melhorar o uso racional de nutrientes e consequentemente reduzir custos e impacto ambiental. Dessa forma, com essa tese objetivou-se avaliar os efeitos do ajuste da concentração de aminoácidos da dieta de acordo com o ritmo circadiano do estado metabólico de suínos sobre o metabolismo e desempenho zootécnico. Para isso foram realizados dois ensaios experimentais: (i) metabolismo e (ii) desempenho e composição corporal. No ensaio de metabolismo o objetivo foi avaliar balanço de nitrogênio e metabolismo de nutrientes de suínos alimentados com dietas com diferentes concentrações de aminoácidos nas refeições da manhã e da tarde, respectivamente. Um total de 24 suínos machos (peso corporal inicial de 35 ± 3,32 kg) foram alojados em gaiolas metabólicas e submetidos a três programas de alimentação (8 animais por tratamento). Um programa de alimentação diário (DP), onde uma dieta atendendo 100% das exigências em aminoácidos (AA) foi fornecida nas refeições da manhã e da tarde; e dois programas de alimentação sequencial (SEQ): programa de

alimentação sequencial com redução e aumento em 40% na concentração de AA (SEQ_{LH}) no período da manhã e da tarde, respectivamente; e programa de alimentação sequencial com aumento e redução em 40% na concentração de AA no período da manhã e da tarde (SEQ_{HL}), respectivamente. O experimento metabólico teve duração de 21 dias, consistindo em 7 dias de adaptação e 14 dias de experimento. O balanço de nitrogênio (N) foi realizado utilizando o método de coleta total. Coletas de sangue foram realizadas nos dias 7 e 14 com duas coletas em cada dia: a primeira em jejum (pré-prandial) e a segunda 2,5 horas após a primeira refeição do dia (pós-prandial). O metabolismo de nutrientes foi mensurado por meio de análise das concentrações séricas ou plasmáticas de lactato, glicose, triglicerídeos, proteína total, albumina e ureia presentes no sangue. Os animais do SEQ_{HL} apresentaram uma tendência de menor ganho de peso diário (11%, $P = 0,06$), e tiveram uma pior eficiência alimentar (10%, $P = 0,02$) comparado aos DP e SEQ_{LH}. Contudo, os animais do SEQ_{LH} e DP apresentaram desempenho similares ($P < 0,05$). A excreção urinária de N foi mais alta (17%, $P = 0,03$) e a retenção de N (%) tendeu a ser menor para os animais do SEQ_{HL} comparado aos do SEQ_{LH} (7%, $P = 0,08$). Contudo, os animais do DP e SEQ_{LH} apresentaram balanço de N similares ($P < 0,05$). As concentrações plasmáticas de glicose pré-prandial foram maiores para os animais do SEQ_{HL} comparado aos do DP e SEQ_{LH} (5%, $P = 0,04$). No dia 7, as concentrações séricas de proteína total e albumina pré-prandial foram maiores para os animais do SEQ_{LH} comparado aos o DP e SEQ_{HL} (43 e 38%; $P < 0,01$). Os animais do SEQ_{HL} tiveram maior concentração sérica de ureia pós-prandial do que os do DP e SEQ_{LH} (28%; $P < 0,01$). Nesse primeiro estudo conclui-se, portanto, que o fornecimento de dietas com baixo e alto nível de AA nas refeições da manhã e da tarde, respectivamente, pode melhorar o metabolismo da proteína de suínos em crescimento. No segundo estudo objetivou-se avaliar o desempenho, a composição corporal e o balanço de nutrientes de suínos alimentados com dietas com diferentes níveis de decréscimo e aumento na concentração de AA nas primeiras e últimas 12 horas do dia, respectivamente. Um total de 68 suínos machos castrados (peso corporal inicial de 25 ± 2,67 kg) foram distribuídos em um delineamento de blocos ao acaso (peso corporal) em quatro tratamentos (17 animais por tratamento): programa de alimentação diário (DP), onde uma dieta contendo 100% da concentração de AA foi fornecida 24 horas por dia; e

três programas de alimentação sequencial (SEQ), com diferentes níveis de variação na concentração de AA, em que os suínos receberam uma dieta com redução (-20, -30, -40%) da concentração de AA de 0000 a 1159 h (período 1; P1) e aumento (+20, +30, +40%) de 1200 a 2359 h (período 2; P2), respectivamente. O experimento teve duração de 82 dias (25 a 100 kg), dividido em três fases experimentais. As análises estatísticas consistiram em avaliar a comparação entre os programas de alimentação (DP vs SEQ) e as respostas linear e quadrática de acordo com o aumento da concentração dos níveis de AA na dieta. Durante a fase 1, o consumo médio diário de ração, consumo médio diário de lisina, peso corporal e proteína corporal tenderam (P <0,09) a aumentar de forma quadrática com o aumento da variação nos níveis de AA da dieta, enquanto o lipídio corporal aumentou (P <0,03) quadraticamente com o aumento da variação nos níveis de aminoácidos da dieta. Durante a fase 2, a eficiência alimentar aumentou (P = 0,01) linearmente com o aumento da variação nos níveis de AA da dieta, enquanto que o ganho de peso e ganho de proteína tenderam (P = 0,09) a aumentar de maneira linear com a variação na dieta os níveis de AA aumentaram. Durante a fase 3, os animais do SEQ, quando comparados ao grupo DP, apresentaram desempenho e composição corporal semelhantes (P > 0,05), contudo uma tendência para maior excreção de N foi observada (12%; P = 0,05). Portanto, os resultados do presente estudo indicam que o ajuste das concentrações de AA na dieta de acordo com o ritmo circadiano (estado de catabolismo e anabolismo) melhora a deposição de proteína para suínos de 25-50 kg. Além disso, este ajuste tende a aumentar ganho de peso diário, peso e proteína corporal para suínos de 50-70 kg. Essas melhores respostas foram observadas em redução e aumento de 40% nas primeiras e nas últimas doze horas do dia, respectivamente, na recomendação dos níveis de aminoácidos da dieta.

Palavras-chave: anabolismo, catabolismo, exigência nutricional, programas nutricionais, suinocultura.

ADJUSTMENT IN THE DIET AMINO ACIDS CONCENTRATION ACCORDING TO THE CIRCADIAN RHYTHM OF GROWING-FINISHING PIGS

ABSTRACT – In swine nutrition, feeds are formulated according to the growth stage of the animals and, predominantly, a single diet is provided for a long period within a phase feeding system. In this system, when the objective is to optimize the performance of a group of animals, nutrients are normally supplied in excess mainly at the end of each phase. Thus, due to the high cost of feeds, combined with the excretion of polluting elements, nutritional strategies must be proposed and evaluated. Within this context, programs that allow to provide a diet that meets the daily requirement of animals have shown to be promising since they allow to reduce costs and excretion of polluting elements. In this context of precision nutrition, it is important to highlight that the efficiency of nutrient use may vary throughout the day, according to the variations in the metabolic state (catabolism and anabolism) of the animals determined by the circadian cycle. Therefore, adjusting the nutritional concentration of the diet to fluctuations in the metabolic status of pigs throughout the day may be a promising strategy to improve to improve the rational use of nutrients and consequently reduce costs and environmental impact. Thus, with this thesis aimed to evaluate the effects of adjusting the concentration of nutrients in the diet according to the circadian rhythm of the metabolic state of pigs on metabolism and growth performance. For this, two experimental tests were performed: (i) metabolism and (ii) performance and body composition. In the metabolism assay, the objective was to evaluate nitrogen balance and nutrient metabolism of pigs fed diets with different amino acid concentrations in the morning and afternoon meals, respectively. A total of 24 male pigs (initial body weight 35 ± 3.32 kg) were housed in metabolic cages and submitted to three feeding programs (8 animals per treatment). A daily feeding program (DP), which a diet with 100% of amino acid (AA) requirements was provided in morning and afternoon meals; and two sequential feeding programs (SEQ): sequential feeding program with 40% reduction and increase in AA concentration (SEQ_{LH}) in the morning and afternoon, respectively; and sequential feeding program with 40% increase and decrease in AA

concentration in the morning and afternoon (SEQ_{HL}), respectively. The metabolic experiment lasted 21 days, consisting of 7 days of adaptation and 14 days of experiment. The nitrogen (N) balance was performed using the total collection method. Blood collections were performed on days 7 and 14 with two collections each day: the first after fasting (preprandial) and the second 2.5 hours after the first meal of the day (postprandial). Nutrient metabolism was measured by analyzing the serum or plasma concentrations of lactate, glucose, triglycerides, total protein, albumin and urea present in the blood. The animals from SEQ_{HL} showed a tendency for lower daily weight gain (11%, $P = 0.06$), and had a worse feed efficiency (10%, $P = 0.02$) compared to the DP and SEQ_{LH}. However, SEQ_{LH} and DP animals showed similar performance ($P < 0.05$). Urinary N excretion was higher (17%, $P = 0.03$) and N retention (%) tended to be lower for SEQ_{HL} animals compared to SEQ_{LH} animals (7%, $P = 0.08$). However, animals from DP and SEQ_{LH} showed similar N balance ($P < 0.05$). Plasma concentrations of preprandial glucose were higher for SEQ_{HL} animals compared to DP and SEQ_{LH} animals (5%, $P = 0.04$). On day 7, serum concentrations of total protein and preprandial albumin were higher for SEQ_{LH} animals compared to DP and SEQ_{HL} animals (43 and 38%; $P < 0.01$). The animals from SEQ_{HL} had higher serum concentration of postprandial urea than those from DP and SEQ_{LH} (28%; $P < 0.01$). Therefore, in this first study it was concluded that the provision of diets with low and high levels of AA in the morning and afternoon meals, respectively, can improve the protein metabolism of growing pigs. In the second study the objective was to evaluate the performance, body composition and nutrient balance of pigs fed diets with different levels of decrease and increase in AA concentration in the first and last 12 hours of the day, respectively. A total of 68 barrows (initial body weight 25 ± 2.67 kg) were distributed in a randomized block design (body weight) in four treatments (17 animals per treatment): daily feeding program (SD), where a diet containing 100% of the AA concentration was provided 24 hours a day; and three sequential feeding programs (SEQ), with different levels of variation in the concentration of AA, in which the pigs received a diet with a reduction (-20, -30, -40%) in the concentration of AA from 0000 to 1159 h (period 1; P1) and increase (+20, +30, +40%) from 1200 to 2359 h (period 2; P2), respectively. The experiment lasted 82 days (25 to 100 kg), divided into three experimental phases. Statistical analyzes consisted of evaluating the comparison between feeding programs

(DP vs SEQ), and linear and quadratic responses according to the increase in the concentration of AA levels in the diet. During phase 1, average daily feed intake, daily lysine intake, body weight and body protein tended ($P < 0.09$) to increase quadratically with increasing variation in dietary AA levels, while body lipid increased ($P < 0.03$) quadratically with increasing variation in dietary AA levels. During phase 2, feed efficiency increased ($P = 0.01$) linearly with increasing variation in dietary AA levels, while weight gain and protein gain tended ($P = 0.09$) to increase linearly with increasing variation in dietary AA levels. During phase 3, the animals in the SEQ, when compared to the DP group, showed similar performance and body composition ($P > 0.05$), however a tendency for a higher N excretion was observed (12%; $P = 0.05$). Therefore, the results of the present study indicate that adjusting dietary AA concentrations according to the circadian rhythm (catabolism and anabolic state) improves protein deposition for pigis from 25-50 kg. In addition, this adjustment tends to increase daily weight gain, body weight and body protein for pigs from 50-70 kg. These better responses were observed in a 40% decrease and increase in the first and last twelve hours of the day, respectively, in the diet amino acid levels recommendation.

Key-words: anabolism, catabolism, nutritional requirement, nutritional programs, swine production.

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1.1 Introdução

As previsões indicam que em 2050 a população mundial passará a ter aproximadamente 10 bilhões de pessoas, o que representará um aumento de 22% comparado a população atual (ONU, 2019). Nesse sentido, a produção de alimentos impreterivelmente terá que aumentar de forma a acompanhar esse aumento da população mundial. Contudo, essa produção adicional de alimentos, tanto de origem vegetal quanto animal, deverá continuar adotando e buscando técnicas de produção que reduzam e minimizem os impactos ambientais causados pelos sistemas de produção. Nesse contexto, o conhecimento do potencial nutritivo dos alimentos associado ao ajuste da composição nutricional das dietas à exigência nutricional dos animais é fundamental para avançar para uma nutrição mais precisa que reduza custo e impacto ambiental na produção de suínos.

Na nutrição atual dos suínos, as dietas fornecidas aos animais apresentem boa digestibilidade e alto valor nutricional. No entanto, ainda existem oportunidades para reduzir custos e impacto ambiental no que se refere a forma como a composição nutricional das dietas é ajustada às exigências nutricionais dos animais. Atualmente, o sistema de alimentação por fases é o que predomina na produção de suínos. Nesse sistema os animais são alimentados com uma única dieta por um longo período, onde parte da fase os animais recebem nutrientes acima das suas respectivas exigências. Nesse sentido, vários estudos têm demonstrados que um sistema de alimentação que permite ajustes diários da composição nutricional das dietas às exigências nutricionais dos animais permite reduzir custo e impacto ambiental na produção de suínos comparado ao convencional por fases (Andretta et al., 2014; Pomar et al., 2014). Contudo, sistemas que considerem possíveis variações nas exigências nutricionais dos animais ao longo do dia ainda são pouco avaliados.

Aspectos da fisiologia animal, incluindo a motilidade do trato gastrointestinal, funções renais, hepáticas e secreção de hormônios são influenciados pelo ciclo circadiano, podendo acontecer com menores ou maiores frequências e intensidades de acordo com o período do dia (Reppert e Weaver, 2002). Dentro desse contexto, especula-se que o ritmo circadiano da alimentação e do estado metabólico têm impacto direto na digestão, absorção e metabolismo dos nutrientes. Isso sugere que essas variações circadianas podem afetar a demanda por nutrientes dos suínos ao longo do dia. Portanto, o presente estudo aportará informações importantes relacionadas ao impacto do ajuste da concentração de nutrientes da dieta de acordo com o ritmo circadiano, no metabolismo e desempenho de suínos em crescimento e terminação.

1.2 Revisão de Literatura

1.2.1 Programas nutricionais para suínos

O Brasil é um dos maiores produtores de grãos do mundo, com uma produção que atingiu cerca de 113 milhões de toneladas de soja e 100 milhões de toneladas de milho no ano de 2019 (IBGE, 2020). Desse total de soja e milho produzidos, 16% e 88% são destinados a alimentação animal, respectivamente (CONAB, 2020). Devido ao alto valor nutricional associado a qualidade desses grãos produzidos no Brasil, as rações utilizadas para alimentação dos suínos apresentam alto valor nutricional e boa digestibilidade. Contudo, embora a produção de suínos no Brasil possa contar com ingredientes nobres (milho e soja), a alimentação não muito diferente de outros países representa a maior parte dos custos de produção (aproximadamente 70%). Além da necessidade da redução desses custos, outro aspecto relevante é necessidade de reduzir também a excreção de elementos poluidores, tais como nitrogênio (N) e fósforo (P).

Atualmente, o programa nutricional mais utilizado na alimentação de suínos é a alimentação por fases, que consiste em fornecer um número sucessivo de dietas de acordo com as fases de crescimento dos animais. Nesse sistema os animais são alimentados com uma única dieta por um longo período. Nesse sentido, ao adotar esse sistema assumimos que a exigência é constante durante uma determinada fase. No entanto, a exigência nutricional dos animais em termos de concentração de nutrientes é dinâmica uma vez que apresenta redução diária em função da idade do animal (Figura 1; Hauschild et al., 2010). Nesse contexto, vários estudos têm demonstrado que o aumento no número de fases por permitir um melhor ajuste da composição nutricional das dietas às exigências nutricionais dos animais permite reduzir custo e impacto ambiental (Pomar e Barnett, 1994; Van der Peet-Schwering et al., 1999; Montminy et al., 2005). Os benefícios podem ser ainda maiores quando se trabalha com programas nutricionais de ajuste da dieta à exigência individual nutricional diária dos animais, reduzindo custos e excreção de N e P (Andretta et al., 2014; Pomar et al., 2014; Santos et al., 2018).

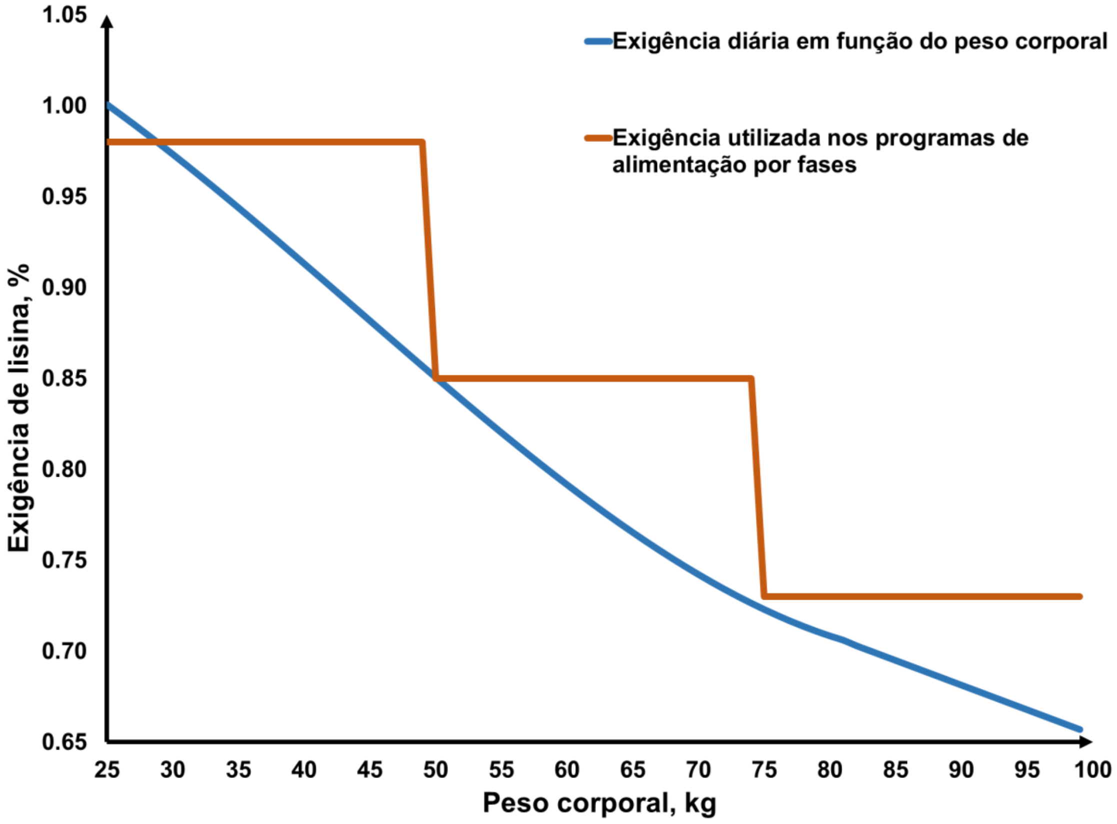

Figura 1. Exigência de lisina digestível ileal estandardizada (%) de suínos dos 25 aos 100 kg de peso corporal e concentração ideal diária estimada com o modelo do NRC (2012) e por fase de crescimento (NRC, 2012).

Além dos programas nutricionais de ajuste à exigência diária, estratégias que alteram a concentração de nutrientes da dieta ao longo do dia têm sido avaliadas (Silva et al., 2021). Esse tipo de estratégia pode ser aplicada utilizando o método de alimentação sequencial, que consiste em fornecer dietas com diferentes composições nutricionais por um determinado período dentro de 24 horas (Bouvarel et al., 2008). O uso de alimentação sequencial ao longo do dia baseia-se no princípio de que a digestão, absorção e metabolismo dos nutrientes apresentam flutuações ao longo do dia. O ajuste da composição nutricional da dieta ao ciclo circadiano dos animais, pode ser uma alternativa para otimizar o uso dos nutrientes, uma vez que todas as funções fisiológicas sofrem flutuações ao longo do dia.

1.2.2 Ciclo circadiano

As atividades fisiológicas e comportamentais dos animais se ajustam ao movimento de rotação da terra, que tem duração de aproximadamente 24 horas. Essa periodicidade é denominada ciclo circadiano e é controlada por um relógio central interno localizado no núcleo supraquiasmático (suprachiasmatic nuclei, SCN) do hipotálamo (Reppert e Weaver, 2002). Esse relógio central interno responde a estímulos externos e internos que podem ser chamados de *zeitgebers* (Aschoff, 1966). Os *zeitgebers* são fundamentais no ciclo circadiano, sendo o fotoperíodo um dos mais importantes *zeitgebers* externos, uma vez que a luz influencia todas as funções fisiológicas do organismo.

O ritmo circadiano nos mamíferos funciona de forma que o estímulo externo (luz) controla o relógio circadiano central, que por sua vez comanda os relógios localizados nos órgãos periféricos (Figura 2). O SCN recebe o estímulo fótico da retina e transmite essa informação para células em outras regiões do cérebro e órgãos periféricos por meio de uma variedade de mecanismos (Welsh et al., 2010). Dentre esses mecanismos, destaca-se as conexões neuronais, sinais endócrinos, ritmos de temperatura corporal e ciclo atividade-repouso (Dibner et al., 2010).

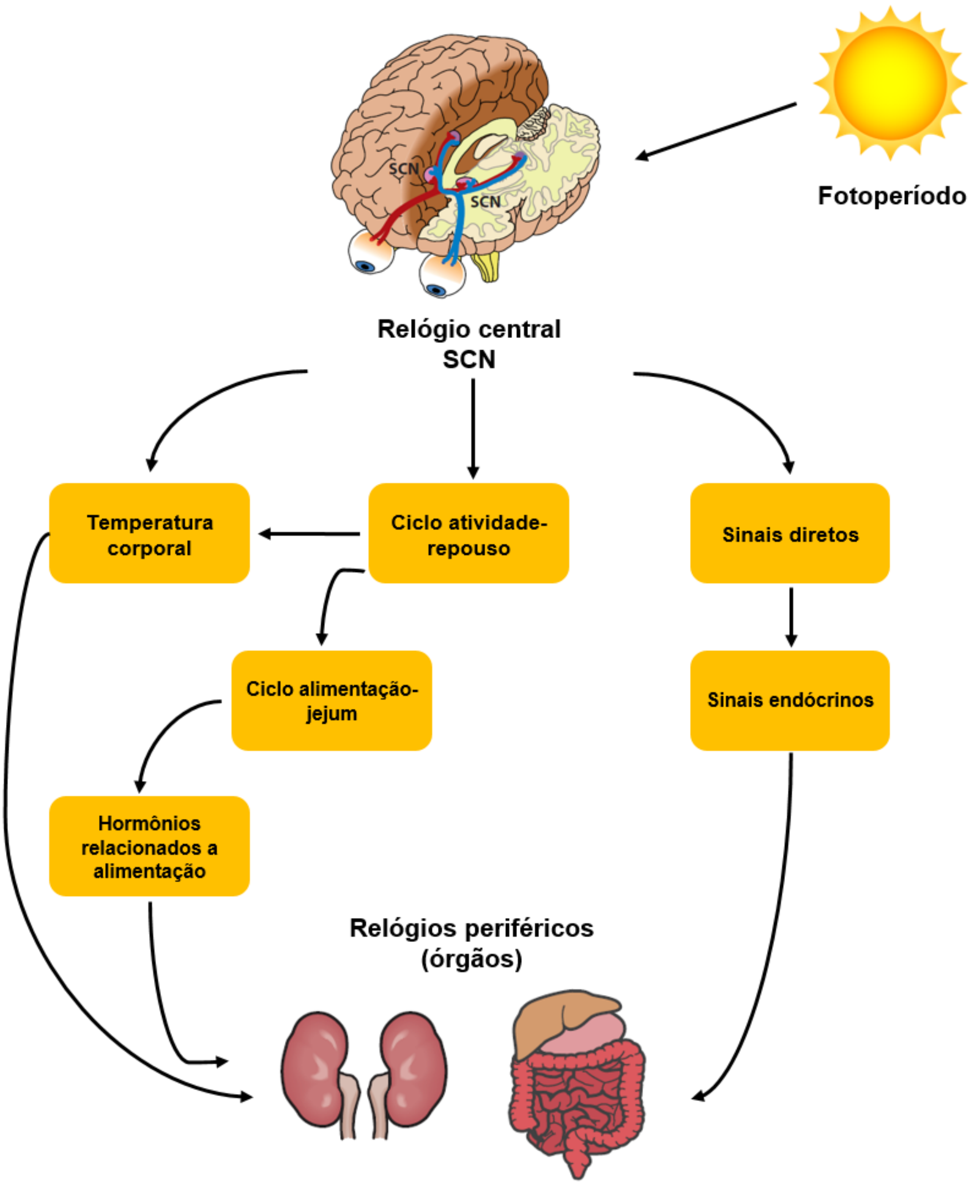

Figura 2. Mecanismos de comunicação entre relógio circadiano central e periféricos. Adaptado de Dibner et al. (2010).

Dentre os mecanismos de comunicação entre relógio circadiano central e periféricos, o ciclo atividade-repouso e os sinais endócrinos são os que apresentam grandes implicações na área da nutrição. O ciclo atividade-repouso controla o ciclo alimentação-jejum, sendo esse um importante *zeitgeber* interno para os relógios periféricos de órgãos, como fígado, pâncreas e rins (Tahara e Shibata, 2013).

Enquanto que os sinais endócrinos agem de forma direta entre relógio circadiano central e periféricos (Dibner et al., 2010). Embora as vias para os sinais endócrinos sejam diretas e conhecidas (hipotálamo-hipófise-órgãos periféricos), as vias moleculares pelas quais o ciclo alimentação-jejum se conecta com os relógios periféricos ainda são pouco conhecidas. Contudo, algumas suposições tem sido levantadas destacando que os hormônios e metabólitos relacionados ao metabolismo de nutrientes podem apresentar uma influência importante nesses processos (Bailey et al., 2014).

1.2.3 Ritmo circadiano de alimentação

O ciclo atividade-repouso determina o comportamento do ciclo alimentação-jejum nas diferentes espécies de animais. Os animais que estão em atividade durante o período de luz, podem apresentar dois tipos de padrões de alimentação que foram previamente descritos por Aschoff (1957). O padrão *bigeminus*, onde o primeiro pico de alimentação ocorre no início da atividade e é superior ao segundo pico de alimentação, que ocorre no final do período ativo, e o padrão *alternans*, onde o primeiro pico de alimentação é menor que o segundo. Os suínos são animais que estão em atividade durante o período de luz, sendo assim classificados como animais de hábitos diurnos. Dentro do período ativo, esses animais apresentam um pico maior de alimentação ao final da atividade (Wangsness et al., 1980; Feddes et al., 1989; Andretta et al., 2016), sendo classificados como animais de padrão de alimentação *alternans*. Esse tipo de comportamento com dois picos sendo o maior ao final da tarde implica em um maior consumo de ração no período da tarde (Figura 3).

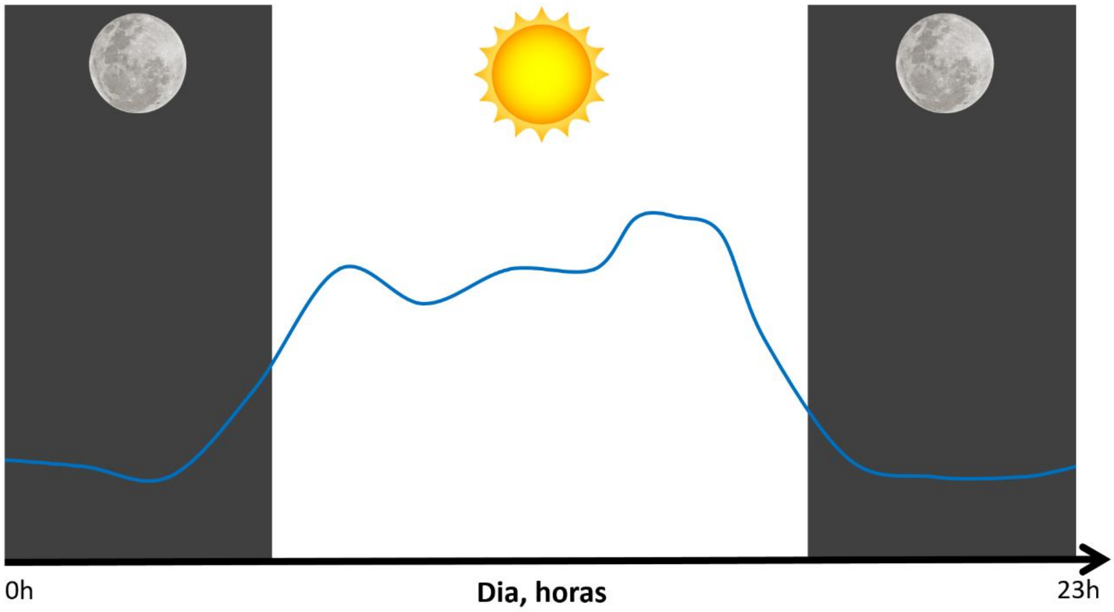

Figura 3. Variação circadiana de ocupação do comedouro de suínos em crescimento e terminação. Fonte: Adaptado de Andretta et al., (2016).

O padrão de alimentação é fortemente regulado pelo ciclo atividade-repouso (Dibner et al., 2010), uma vez que este influencia no balanço de energia (Albrecht, 2012), resultando nas flutuações do ciclo alimentação-jejum. Este, por sua vez, influencia os relógios periféricos e o funcionamento dos órgãos, devido a secreção dos hormônios relacionados a alimentação. Dentre esses, destaca-se como os principais hormônios reguladores a leptina e grelina (Jha et al., 2015), os quais são fortemente influenciados pela alimentação (Jha et al., 2015; Boumans et al., 2017). Além do ciclo alimentação-jejum, o relógio circadiano central também tem influência direta no funcionamento dos órgãos periféricos, por meio da secreção de hormônios que irão atuar na digestão, absorção e metabolismo dos nutrientes (Bailey et al., 2014). Dentre esses, a melatonina e o cortisol são hormônios com grande importância nas flutuações do metabolismo dos nutrientes ao longo do dia, uma vez que influenciam no estado metabólico dos animais (Boumans et al., 2017).

A melatonina tem uma variação circadiana similar em mamíferos de hábitos noturnos e diurnos, sendo secretada em maior quantidade no período de escuro, induzindo animais diurnos ao repouso, enquanto o mesmo não ocorre em animais noturnos (Jha et al., 2015). Diante disso, os suínos apresentam um ritmo circadiano com picos noturnos de melatonina (Tast et al., 2001). A melatonina está envolvida na regulação do metabolismo energético, na secreção da insulina e na sincronização de processos metabólicos com fases sensíveis e resistentes à insulina (Seraphim et al., 2000; Cipolla-Neto et al., 2014). Esse processo metabólico desencadeado pela melatonina contribui para a biossíntese das células e consequentemente tecidos.

O cortisol, por outro lado, é secretado antes do início da atividade, onde uma maior secreção ocorre pouco antes do amanhecer em mamíferos diurnos e do anoitecer em mamíferos noturnos (Dallman et al., 2004). Os suínos, assim como os demais mamíferos diurnos, apresentam um ritmo circadiano de cortisol com pico antes do amanhecer e níveis mais elevados pela manhã (Koopmans et al., 2005). O cortisol está envolvido em diversas funções fisiológicas, porém se tratando de nutrição e crescimento animal podemos destacar os mecanismos metabólicos de degradação de moléculas complexas para manter os níveis de glicose e aminoácidos no sangue (Roldan et al., 2014).

Portanto, essas variações circadianas nos sinais endócrinos no caso da melatonina e cortisol influenciam no estado metabólico dos animais (Figura 4). Dessa forma, os ritmos circadianos da melatonina e do cortisol tem significativa participação nas flutuações do estado anabólico e catabólico dos animais durante as 24 horas do dia (Boumans et al., 2017).

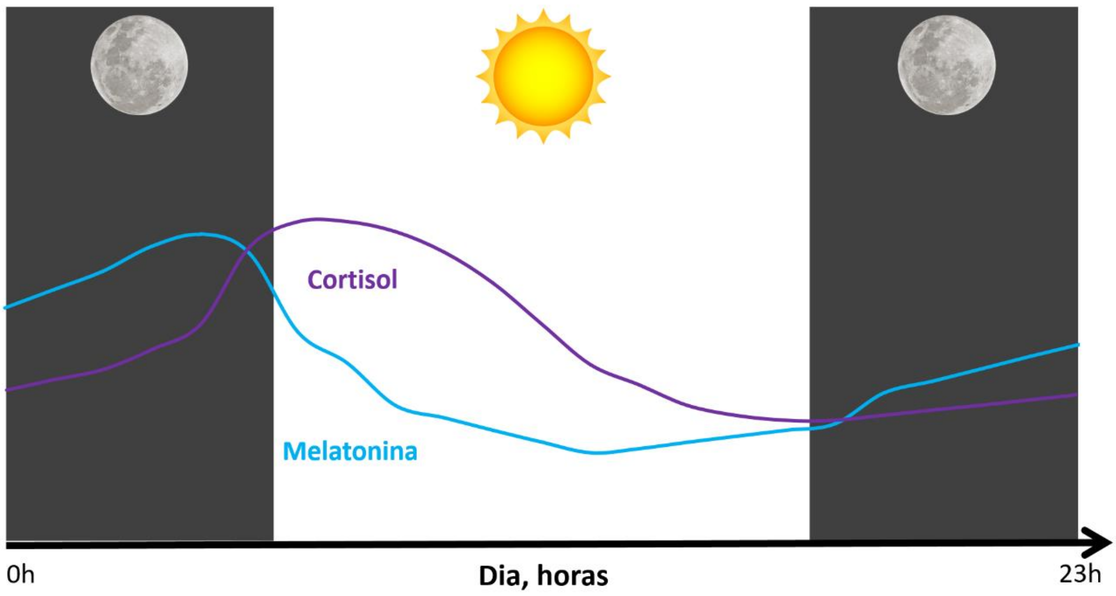

Figura 4. Variações circadianas da melatonina e cortisol em suínos. Fontes: Koopmans et al. (2005); Zhang et al. (2018).

1.2.4 Ritmo circadiano do estado metabólico

As flutuações no estado metabólico promovem a renovação celular e são absolutamente necessárias para a sobrevivência dos animais (Sanders e Wride, 1995; Jacobson et al., 1997). Essa complexa renovação das células ocorre de forma dinâmica, onde o processo de degradação de componentes celulares é equilibrado por processos de biossíntese conhecidos como catabolismo e anabolismo (Theorell, 2009). O catabolismo é a fase de degradação, em que moléculas complexas, como carboidratos, gorduras e proteínas são convertidas em produtos finais mais simples, como glicose, aminoácidos e ureia (Nelson e Cox, 2014). Enquanto o anabolismo é a fase de biossíntese, em que precursores simples formam moléculas complexas (Nelson e Cox, 2014).

O catabolismo e o anabolismo são controlados por dois sistemas distintos (Theorell, 2009), em que o primeiro é coordenado pelo eixo hipotálamo – hipófise - adrenal (hypothalamic – pituitary - adrenocortical; HPA) e o segundo pelo eixo hipotálamo – hipófise - gonadal (hypothalamic – pituitary - gonadal; HPG). O eixo HPA se estende do hipotálamo, passando pela hipófise, até o córtex adrenal (Jacobson e Sapolsky, 1991). Nesse eixo o hipotálamo estimula a hipófise a liberar o hormônio adrenocorticotrófico (adrenocorticotropic hormone; ACTH) através do fator de liberação de corticotrofina (corticotropin - releasing factor; CRF). Por fim, o ACTH estimula o córtex adrenal a secretar cortisol, resultando em catabolismo.

Por outro lado, o eixo HPG se estende do hipotálamo, passando pela hipófise, até as gônadas. O hipotálamo, através do hormônio liberador de gonadotrofina (gonadotropin - releasing hormone; GnRH), estimula a hipófise a secretar os hormônios que atuam nas gônadas. Esse estimulo, além do sistema reprodutivo, influencia também no anabolismo (Dhillo et al., 2005). Além disso, o anabolismo também é influenciado pelo hormônio do crescimento (growth hormone, GH; Theorell, 2009). A secreção do GH ocorre, principalmente, durante o repouso (Strobl e Thomas, 1994), sendo que este é fortemente influenciado pela melatonina em mamíferos diurnos (Dawson e Encel, 1993; Bubenik et al., 2000).

Sendo assim, o ritmo circadiano dos sinais endócrinos afeta o estado metabólico dos animais, por meio de flutuações na absorção e metabolismo dos nutrientes ao longo dia. Dentro desse contexto, estudos tem relatado variações circadianas no metabolismo da glicose (Qian and Scheer, 2016; Mason et al., 2020), peptídeos (Qandeel et al., 2009; Hussain e Pan, 2015), aminoácidos (Zhang et al., 2017) e lipídios (Pan e Hussain, 2009; Douris et al., 2011). No entanto, pouco se sabe sobre como essas variações circadianas podem afetar a demanda por nutrientes dos suínos ao longo do dia.

1.2.5 Problemática do tema pesquisado

O ajuste das dietas às exigências nutricionais diárias dos animais permite reduzir custos e excreção de elementos poluidores ao ambiente (Andretta et al., 2014; Pomar et al., 2014). Contudo, esse sistema negligencia o fato que a exigência nutricional dos animais pode variar ao longo do dia, de acordo com as variações do estado metabólico (catabolismo e anabolismo) determinado pelo ciclo circadiano. Dessa forma, ao fornecer uma dieta com concentração de nutrientes constante ao longo do dia os animais podem estar recebendo excesso ou deficiência de nutrientes em parte do dia (Figura 5).

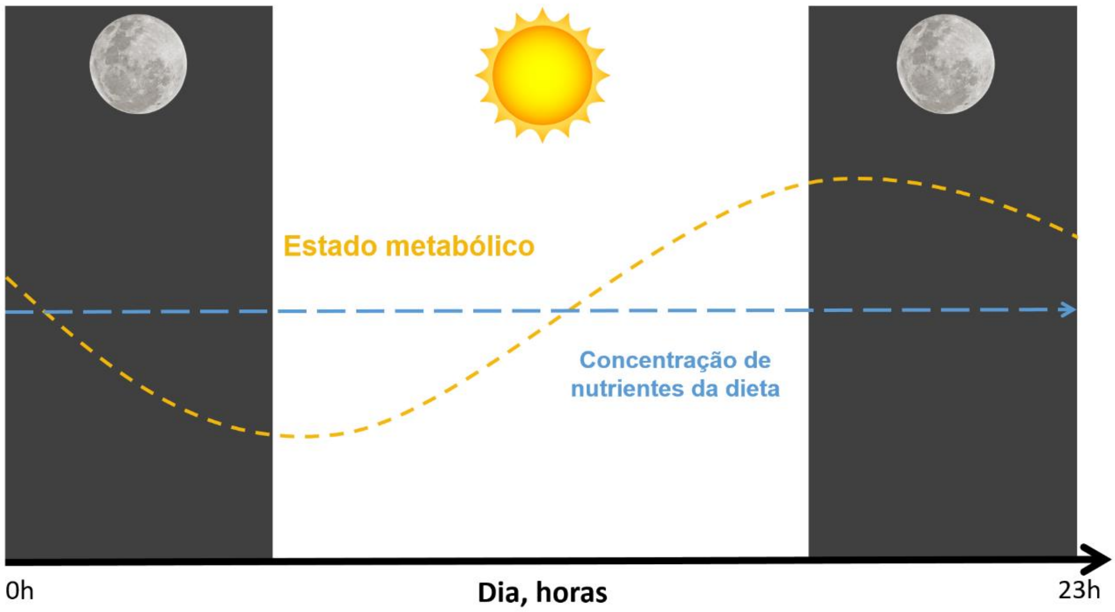

Figura 5. Variações circadianas do estado metabólico de suínos. Fonte: Adaptado de Boumans et al. (2017).

1.2.6 Hipótese

Diante do exposto, nossa hipótese foi que o ajuste da concentração de aminoácidos da dieta às flutuações do estado metabólico dos suínos ao longo do dia melhora o uso racional desses nutrientes, os índices zootécnicos e reduz a excreção de elementos poluidores no meio ambiente.

1.2.7 Objetivos

Geral – Avaliar as respostas de suínos submetidos ao ajuste da composição de aminoácidos da dieta de acordo com o ritmo circadiano.

Específicos

- Avaliar o balanço de nitrogênio e metabólitos sanguíneos de suínos alimentados com dietas com diferentes concentrações de aminoácidos nas refeições da manhã e da tarde, respectivamente, em comparação ao sistema de alimentação diária.

- Avaliar desempenho, composição corporal e balanço de nutrientes de suínos em crescimento e terminação alimentados com dietas com diferentes níveis de decréscimo e aumento na concentração de aminoácidos nas primeiras e últimas 12 horas do dia, respectivamente.

1.2.8 Estruturação da tese

Para avaliar a hipótese e os objetivos da presente tese, dois ensaios experimentais foram realizados: i) ensaio de metabolismo, onde foram avaliados desempenho, balanço de nitrogênio e metabólitos sanguíneos; ii) ensaio de desempenho e composição corporal, no qual foi avaliado desempenho, composição corporal e balanço de nutrientes.

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CAPÍTULO 2 - The sequential feeding system with adjust in the diet amino acid concentration according to the circadian rhythm can improve protein metabolism in pigs: I. Metabolism assay

Este capítulo é apresentado de acordo com as normas da **Revista Animal Feed Science and Technology.**

The sequential feeding system with adjust in the diet amino acid concentration according to the circadian rhythm can improve protein metabolism in pigs: I. Metabolism assay

Abstract

Sequential feeding system varying amino acids concentration may allow better adjustments of pig's diet nutritional composition according to the physiological state determined by a circadian rhythm. Therefore, we aimed with this study to evaluate the growth performance, nitrogen (N) balance, and blood metabolites of pigs fed diets with low and high or vice versa in the morning and afternoon meals, respectively, compared to the daily feeding system. Twenty-four male pigs (35 ± 3.32 kg) were distributed in a randomized block design on the basis of body weight in three treatments: Daily feeding program (DP), with a diet with 100% of the amino acids (AA) requirements provided in the morning and afternoon meals; sequential feeding program with 40% decrease and increase (SEQ_{LH}) of the AA requirements in the diet in morning and afternoon meals, respectively; and sequential feeding program with 40% increase and decrease (SEQ_{HL}) of the AA requirements in the diet in morning and afternoon meals, respectively. The metabolism trial lasted 21 days, consisting of 7 days adaptation and 14 days of experiment. During experiment, blood samples were collected twice on days 7 and 14. The first collection performed under fast (pre-prandial), and the second was carried out two hours and thirty minutes after the meal (postprandial). Growth performance, N balance and blood metabolites were evaluated. Pigs in teh SEQ_{HL} presented a tendency for a lower average daily gain (ADG; 11%, P=0.06), and had a worse gain:feed ratio (G:F; 10%, P = 0.02) than pigs in the DP and SEQ_{LH}, whereas SEQ_{LH} and DP pigs had similar performance (P < 0.05). The urinary N excretion was higher (17%, P = 0.03) and N retention (% of absorbed) tended to be lower (7%, P = 0.08) for SEQ_{HL} pigs than for the DP and SEQ_{LH} groups. The

SEQ_{LH} and DP pigs had similar N balance ($P < 0.05$). The pre-prandial glucose plasma concentrations were higher (5%, $P = 0.04$) for pigs in the SEQ_{HL} than DP and SEQ_{LH} pigs. On day 7, pre-prandial total protein and albumin serum concentrations were higher (43 and 38%; $P < 0.01$) for pigs in the SEQ_{LH} than DP and SEQ_{HL} pigs. SEQ_{HL} group had higher postprandial urea serum concentrations (28%, $P < 0.01$) than pigs in the DP and SEQ_{LH}. These findings suggest that the varying the diet amino acid content over the day affects nutrient metabolism and performance in pigs. It is proposed that sequential feeding program with pigs receiving low and high AA diet level in the morning and afternoon meals, respectively, improve protein metabolism and may help to improve growth performance of pigs.

Key words: albumin, blood variables, glucose, nitrogen balance, urea, swine nutrition, total protein.

Abbreviations

AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; D, day; DP, daily feeding program; FP, feeding program; G:F, gain:feed ratio; HPLC, high performance liquid chromatography; Lys, lysine; Met, methionine; Met + Cys, methionine + cysteine; N, nitrogen; NIRS, near infrared spectroscopy; P, phosphorus; SEQ, sequential feeding program; SEQ_{HL}, sequential feeding program with 40% increase and 40% decrease of the estimated AA requirements in the diet; SEQ_{LH}, sequential feeding program with 40% decrease and 40% increase of the estimated AA requirements in the diet; SID, standardized ileal digestible; Thr, threonine; Trp, Tryptophan; Val, valine.

Introduction

Several physiological and behavioral activities of the animals are controlled by an internal clock that responds to external stimuli, named *zeitgebers*, with a periodicity of 24 hours (Aschoff, 1957). The *zeitgebers* are fundamental in the circadian rhythm and the light-dark cycle is one of the most important *zeitgebers*. The light influences the hormonal secretion (Dibner et al., 2010), and consequently animal's physiological functions. The pattern of the catabolic and anabolic state is one of the metabolic events which may vary over the day according to the circadian rhythm. The catabolic state is highest in the morning with gradual reduction over the day while a gradual increase is observed in the anabolism (Boumans et al., 2017).

This metabolic behavior over the day may suggest that more and less nutrient should be supplied during the catabolic (morning) anabolic (afternoon) state, respectively, in order to have a rational use of nutrients. Therefore, feeding pigs diets adjusted according to the circadian rhythm with low and high amino acid concentration in the period of catabolic and anabolic state, respectively, could be a strategy to improve protein deposition and reduce nitrogen (N) excretion. The sequential feeding method, which consists of providing diets with different nutritional compositions over a given period, can be use in order to provide different diets over the day (Bouvarel et al., 2008). Therefore, we aimed with this study to evaluate the performance, N balance, and nutrients metabolism of pigs fed diets with low and high or vice versa in the morning and afternoon meals, respectively, compared to the daily feeding system.

Material and methods

The experimental protocol was evaluated and approved by Animal Use Ethics Committee at São Paulo State University, SP, Brazil (protocol number: 08335/19).

Animals, housing and experimental design

This metabolism trial was conducted at the Swine Studies Laboratory of São Paulo State University, Brazil. Twenty-four male pigs (Agroceres PIC, Rio Claro, Brazil) with an average initial body weight (BW) of 35 ± 3.32 kg were assigned on the basis of BW to a randomized block design in three blocks, being the block one with two pigs and blocks two and three with three pigs each, distributed in three treatments totalizing eight animals for each treatment. Pigs were housed individually in metabolic cages (0.60 × 1.60 m), similar to those designed by Pekas (1968), in a temperature controlled room. Each cage was equipped with a drinker (beat ball model) and a feeder (stainless steel self-feeder). The metabolic cages had separate compartments for collection of feces and urine.

The trial lasted 21 days, consisting of seven days of adaptation to the metabolism cages, feed regimen and experimental diets, followed by 14 days of experiment. During the first five days of adaptation, pigs were fed with diet that met 100% of nutritional requirements, while, during the last two days of adaptation and during the experiment, the feed corresponded to experimental treatments.

Feeding programs, nutritional requirements, and diets

The daily nutritional requirement curve for pigs from 25 to 100 kg of body weight was estimated using the NRC model (NRC, 2012). From this, were formulated two experimental feeds (A and B) with different nutrients concentrations (Table 1), however to contain similar net energy concentrations and similar ratios for all amino acids (AA).

The standardized ileal digestible (SID) AA contents were used to formulate the feeds, which were obtained by analyzing the total AA content of the ingredients and the ileal digestibility coefficients recommended by Rostagno et al. (2017) to estimate the content of SID AA. The nutritional composition of raw materials (corn and soybean meal) used in formulation, except for AA and protein values, was obtained from Rostagno et al. (2017). Feed A was formulated to satisfy 100% of the requirements for minerals, vitamins and energy, and 140% of the requirements for AA at 25 kg of body weight. Feed B was formulated to satisfy 100% of the requirements for minerals, vitamins and energy, and 60% of the requirements for AA at 100 kg of body weight. Feeds were pelleted at 5 mm.

The required daily concentration of nutrients and energy were estimated with a mathematical model (NRC, 2012) for pigs from 35 to 50 kg of BW. Dietary treatments were obtained by blending the two experimental feeds (A and B) in the required proportions to provide 100% of the daily requirements for minerals, vitamins, and energy, and to provide the AA level evaluate in each treatment. The calculate blend was adjusted daily (at 0600 h) for the daily feeding program (DP) group, and adjusted according to circadian rhythm for the sequential feeding program (SEQ). In the SEQ the blend was adjusted in the morning (at 0600 h) and afternoon (at 1400 h). In the DP a diet with 100% of estimated AA requirements was provided in morning and afternoon. In the SEQ, two strategies were evaluated: sequential feeding program with 40% decrease and 40% increase (SEQ_{LH}) of the estimated AA requirements in the diet in morning and afternoon, respectively; and sequential feeding program with 40% increase and 40% decrease (SEQ_{HL}) of the estimated AA requirements in the diet in morning and afternoon, respectively.

Daily feed allowance during the adaptation and experimental period was set 4% (as fed-basis) of individual BW, corresponding to approximately 3 times their daily energy requirement for maintenance (i.e., 0.44 MJ ME/kg of $BW^{0.75}$/day; (NRC, 1998). The BW was updating each day during the adaptation considering an average daily weight gain of 700 g/day, which was the average daily gain of pigs from arrival in the laboratory until they were housed in metabolic cages. During the experiment, individual BW was updated each day considering the individual daily weight gain of each pig during the adaptation. The daily feed allotment was divided into six equal portions and offered at 0600, 0800 and 1000 h corresponding to morning meals, and at 1400, 1600 and 1800 h, corresponding to afternoon meals. Pigs had unlimited access to water at all times.

Experimental measurements

Performance

Pigs were weighed at the beginning and end of the adaptation, as well as at the end of the experiment. The performance was evaluated through average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F) considering the 14 days of experiment.

Nitrogen balance

The N balance was evaluated through total collection method, in which samples of feces and urine were collected in the last days of the experiment. For the feces collection, on the afternoon of day 8 of experiment, pigs received feed mixed with 1% of ferric oxide as an indigestible marker in order to identify the beginning of collection. On the afternoon of day 13 of experiment, pigs received feed mixed with 1% ferric oxide again to identify the end of collections, totaling 5 days of collection. The feces collection commenced once the marked feces appeared after the feed with ferric oxide on day 8 and terminated immediately when the marked feces appeared after the feed with ferric oxide on day 13 of experiment. The feces were collected twice a day, packed in identified plastic bags, weighed and stored in a freezer at a temperature of −20º C. At the end of the experiment, the feces of each individual were thawed, homogenized and dried at 55° C for 72 hours for further analysis.

The urine collection was initiated and terminated on days 10 and 14 of experiment, respectively. The urine was collected in plastic buckets containing 20 mL of 6M hydrochloric acid (HCl) solution, in order to prevent the proliferation of microorganisms that could cause N volatilization and alter the urine content. Urine was collected once a day, the total urine was weighed and a sample of 20% of the total content was sub-sampled, packed in an identified plastic bottle and stored in a freezer at −20º C. At the end of the experiment, the samples urine samples of each individual were thawed, homogenized and frozen again for further analysis.

Blood sampling

On the morning of days 7 and 14 of experiment, blood samples were collected via the jugular vein. On each day, blood samples were collected twice, with the first collection performed before the 0600 h meal under 9-hour fast (pre-prandial). The second collection was carried out two hours and thirty minutes after the 0600 h meal (postprandial), following the methodology proposed by (Morales et al., 2016). Thus, specifically on the days that blood samples were collected, the pigs were fed at 0600, 0830 and 1000 h on morning. A total of 10 mL of blood were collected, 5 mL for serum extraction and 5 mL for plasma extraction, which were separated from whole blood by centrifugation at 2.800 × *g* for 15 minutes. The plasma and serum samples were identified and stored at −20 °C until analysis.

Analytical Procedures

Representative samples of corn and soybean meal were collected before formulation of the feeds. These samples were submitted to near infrared spectroscopy (NIRS) analysis to determine total AA contents that was used in feeds formulation. Representative samples of feeds A and B were collected and analyzed for dry matter (method 930.15) and crude protein as N × 6.25 (method 968.06) according to AOAC (1990). The SID AA composition (excluding tryptophan) of the feeds was determined by high performance liquid chromatography (HPLC) based on the chromatography method described by White et al. (1986).

The fecal samples were analyzed for dry matter (method 930.15) and crude protein as N × 6.25 (method 968.06). While urine samples were analyzed for crude protein as N × 6.25 (method 968.06). Both were used the methodology according to AOAC (1990). Blood variables analyzes were performed using an automatic biochemical evaluator (Labmax Plenno, Labtest Diagnóstica, Lagoa Santa, Brazil). Quantitative determination of lactate and glucose were performed by enzymatic analysis (lactate oxidase; glucose oxidase) in plasma using commercial reagents (Enzymatic lactate, Ref. 138; Glucose Liquiform, Ref. 133; Labtest Diagnóstica, Lagoa Santa, Brazil). Quantitative determination of triglycerides and urea were performed by enzymatic analysis (glycerol-3-phosphate oxidase; urease) in serum using commercial reagents (Triglycerides Liquiform, Ref. 87; Urea UV Liquiform, Ref. 104; Labtest Diagnóstica, Lagoa Santa, Brazil). Albumin and total protein were analyzed by colorimetry in serum using commercial reagents (Albumin, Ref. 19; Total protein, Ref. 99; Labtest Diagnóstica, Lagoa Santa, Brazil).

Calculations and statistical analysis

All data were examined for normality, homogeneity of variance, and presence of outliers. Data were analyzed using a mixed linear model (MIXED procedure, SAS 9.3 Software, SAS Institute Inc), considering as fixed effect the feeding program, and the block as a random effect. Blood variables, performed on the same animal throughout the experiment (days 7 and 14), were specified as a repeated effect, using the best variance / covariance matrix based on the lowest Akaike Information Criteria (AIC; MIXED procedure, SAS 9.3 Software, SAS Institute Inc). The adjusted means were compared by the Tukey test and the effects was considered significant at $P \leq 0.05$. A trend toward significance was considered at $0.05 < P \leq 0.10$ for performance and N balance variables.

Results

All pigs presented good health and consumed their daily amount of feed during the adaptation and experiment. During all the metabolism trial, the temperature of the room remained constant (22º ± 1º C).

Performance

Feeding programs did not affect ADFI (P = 0.88, Table 2). However, SEQ_{HL} pigs showed a tendency for a lower ADG (11%, P=0.06) and worse G:F (10%, P = 0.02) than pigs in the DP and SEQ_{LH}, whereas SEQ_{LH} and DP pigs had similar results (P < 0.05).

Nitrogen balance

The feeding program did not affect (P > 0.05; Table 3) the fecal N excretion, N absorbed, N retained and N retention (% of intake). However, when compared to DP and SEQ_{HL}, SEQ_{LH} pigs had a lower urinary N excretion (17%, P = 0.03) and showed a tendency for a higher N retention (% of absorbed; 7%, P = 0.08).

Plasm and serum variables

Pre-prandial

The glucose plasma concentrations were affected by the feeding programs (P = 0.04; Table 4), wherein pigs in the SEQ_{HL} had greater (5%) concentrations than DP and SEQ_{LH} pigs. The triglycerides and urea serum concentrations were affected by the collection day (P < 0.01) with greater concentrations on day 14 than day 7 (60% and 37%, respectively). There was a feeding program and collection day interaction for total protein serum concentrations (P = 0.04), wherein on day 14 there was not effect (P = 0.52), while on day 7, the value of this variable was higher (43%; P < 0.01) for pigs in the SEQ_{LH} than DP and SEQ_{HL} pigs. A feeding program and collection day interaction was detected for albumin serum concentrations (P = 0.03), wherein on day 14 were not influenced (P = 0.42), while on day 7, the value of this variable was higher (38%; P < 0.01) for SEQ_{LH} pigs than DP and SEQ_{HL} groups.

Postprandial

The triglycerides serum concentrations were affected by the collection day (P < 0.01; Table 5) with greater (45%) concentration on day 14 compared to day 7. The total protein and albumin serum concentrations were affected by the collection day (P < 0.01) with greater (28% for total protein and 30% for albumin) concentration on day 7 compared to day 14. The urea serum concentrations were affected by the feeding programs (P < 0.01), whereas pigs in the SEQ_{HL} group had higher (28%) concentrations than pigs in the DP and SEQ_{LH} groups.

Discussion

The circadian clock coordinates metabolic fluctuations over the day such as hormonal secretion (Holzberg and Albrecht, 2003; Dibner et al., 2010; Froy, 2010). Due to the hormonal secretion synchronized with the circadian clock, a higher catabolism and anabolism of nutrients can be observed at the beginning and end of the day in pigs, respectively (Boumans et al., 2017). Accordingly, our study was conducted to evaluate two sequential feeding strategies, with low and high, and with high and low diet AA concentration in catabolism and anabolism period, respectively, for growing pigs. Our hypothesis was that providing a lower AA concentration in the period of day where catabolic state is greater (morning meals), and higher where anabolic state is greater (afternoon meals), may improve protein metabolism, with consequent improvement in performance and N balance. According to our results, providing a low diet AA concentration in morning and a high in afternoon although improve protein metabolism do not affect performance and N balance.

Performance and nitrogen balance

In our study, an improvement in growth performance and N balance was observed in pigs receiving a sequence of low and high diet AA concentration (SEQ_{LH}) what was not observed in the high and low diet AA concentration (SEQ_{HL}). Within the circadian rhythm, the absorption of nutrients is also influenced by the light-dark cycle. Previous studies have shown that peptide absorption in rats is greater in the dark period (Pan et al., 2002; Qandeel et al., 2009; Hussain and Pan, 2015). In addition, the transport and absorption of AA in pigs behave similarly to the absorption of peptides in rats, being greater during the dark period (Zhang et al., 2017). For pigs, which are animals of daytime habits, the dark period coincides with the rest period. During rest, the levels of hormones involved in biosynthesis are increased which increase the anabolism (Theorell, 2009). This implies that increasing AA diet concentration during the afternoon meals may be essential for the protein synthesis that occur during the dark period. In the present study, we found that pigs receiving a diet with lower AA concentration at the end of the light period showed lower body weight gain and N retention. Although SEQ_{LH} was the feeding program with better results compared to SEQ_{HL}, the growth performance and N balance when compared to DP was similar. Our assumption is that the reduction in AA levels may have been extremely high in the morning meals. This may have impaired the animals from presenting a better performance compared to DP group. Further experiments with a smaller reduction in AA diet levels in the morning meals are needed to confirm this hypothesis.

Blood metabolites

The term pre-prandial is defined as a period when animal is in a fasting state (Schembre et al., 2020), while postprandial is defined as the period when the animal is in a feeding state (Hanefeld and Temelkova-Kurktschiev, 1997). In the present study, according to the metabolites evaluated in the pre-prandial period, we found that the carbohydrate and protein metabolism were affected by the feeding programs, while in the postprandial only protein metabolism was affected.

According to the results, the pre-prandial glucose plasma concentrations was great for SEQ_{HL} group compared to the other programs. In carbohydrate metabolism, blood glucose can be an indicator of nutritional status (Adeva-Andany et al., 2014). It is interesting to note that diets had different rations of carbohydrate and protein over the day. SEQ_{HL} due to the high proportions of feed B had a high carbohydrate and low protein concentration in afternoon meals.

Therefore, this may explain the great glucose concentration for pigs in the SEQ_{HL} in the pre-prandial evaluation. Indeed, consumption of a carbohydrates-rich meal in the evening led to significantly higher glucose and insulin response compared to its consumption in the morning (Kessler et al., 2017). However, this high plasma glucose concentration for SEQ_{HL} did not result in improve in growth performance. In fact, this could be more expected to have an alteration on body composition. This is assumption may be explain by the fact that animals are less active at night with consequent use of glucose excess to lipid synthesis. In addition, due to the fact that less AA was given in anabolic period, the energy not used for protein deposition is directed for fat deposition. Although in the present study was not observed modification in lipid metabolism, a previous study conducted by our group have shown that high-low AA diet sequency plan increase fat deposition (Silva et al., 2021). This suggest that pigs receiving low AA diet which has high amounts of carbohydrates at the end of the light period, in addition to reduce performance, may have an increase in the lipid deposition.

In protein metabolism, the amount of total protein and albumin in the blood are correlated with protein synthesis (Zhao et al., 2019), while urea is the final product of protein metabolism and may be indicative of catabolism (Coma et al., 1995). The pre-prandial total protein and albumin serum concentrations was higher for SEQ_{LH} group compared to the other feeding programs. This effect combined with greater body weight gain and a better N balance, suggests that protein synthesis was greater for the SEQ_{LH} group compared to SEQ_{HL} group. It may suggest that pigs receiving a high concentration of amino acids in the afternoon meals, can improve protein deposition.

In the present study, the postprandial results showed that the urea serum concentrations was great for SEQ_{HL} group compared to the other programs. The high blood urea concentration can be negatively correlated with the use of proteins and amino acids (Coma et al., 1995). This effect, combined with the worse performance and N balance for the SEQ_{HL} group, suggests that the excess of AA provided in the morning meals has not been efficiently utilized for protein deposition.

Conclusion

The findings reported in the present study suggest that the varying the diet amino acid content over the day affects nutrient metabolism and nitrogen balance in pigs. In this study, it was found that increasing and reducing AA diet in the morning and afternoon meals, respectively, affect nutrient metabolism and consequently reduce growth performance and N balance. In the other hand, reducing and increasing AA diet in the morning and afternoon meals, respectively, improve protein metabolism, but did not affect growth performance and N balance in pigs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Centesimal and nutritional composition of diets A and B.

Item	Diet A (High AA concentration)	Diet B (Low AA concentration)
Ingredient composition, as-fed basis, %		
Corn grain	64.50	91.00
Soybean meal	30.00	-
Soybean oil	0.20	0.80
Dicalcium phosphate	1.25	1.00
Limestone	0.60	0.55
Vitamin and mineral premix[1]	0.20	0.20
Salt	0.20	0.20
L-Lysine sulfate (54.6%)	1.02	0.37
L-Threonine	0.24	0.01
DL-Methionine	0.27	-
L-Tryptophan	0.05	0.02
L-Valine	0.12	-
Kaolin	1.29	5.79
Choline chloride (60%)	0.06	0.06
Chemical composition, %		
NE[2], kcal/kg	2472	2555
Crude Protein[3]	19.91	9.60
Available Phosphorus[2]	0.32	0.22
Calcium[2]	0.62	0.48
Chlorine[2]	0.17	0.17

Sodium[2]	0.10	0.10
SID Lys[4]	1.34	0.44
SID Thr[4]	0.80	0.28
SID Met[4]	0.50	0.17
SID Met + Cys[4]	0.77	0.32
SID Trp[5]	0.25	0.07
SID Val[4]	0.88	0.38

AA, amino acid; NE, net energy; SID, standardized ileal digestible; Lys, lysine; Thr, threonine; Met, methionine; Cys, cysteine; Trp, tryptophan; Val, valine.

[1] Mineral and vitamin supplement (per kg of diet): Vit. A (5,250 IU); Vit. D3 (750 IU); Vit. E (11 IU); Vit. K3 (1.5 mg); Vit. B1 (1 mg); Vit. B2 (2.4 mg); Vit. B6 (1 mg); Niacin (30 mg); B.C. Pantothenic (8.1 mg); Folic acid (0.53 mg); Biotin (0.05 mg); Vit. B12 (16.5 mcg); Copper (13.5 mg); Iodine (0.19 mg); Manganese (37.5 mg); Selenium (0.15 mg); Zinc (72 mg); Iron (72 mg); Cobalt (0.19 mg).

[2] Values estimated according to NRC model (2012).

[3] Crude protein as N × 6.25 according to AOAC (1990).

[4] Standardized ileal digestible (SID) amino acids concentration values were calculated using the total amino acids content of feeds A and B obtained by high performance liquid chromatography (HPLC) and the coefficients proposed by Rostagno et al. (2017).

[5] Standardized ileal digestible (SID) tryptophan concentration values were estimated from analyzed raw matters composition (corn and soybean meal) using the coefficients proposed by Rostagno et al., (2017).

Table 2. Performance of pigs submitted to adjustment of nutritional composition according to circadian rhythm using the sequential feeding method with different amino acid variations throughout the day.

Variables[1]	Feeding programs[2]			SEM	*P*-values[3]
	DP	SEQ_{LH}	SEQ_{HL}		
BW, kg					
At beginning of adaptation	34.60	34.55	34.61	2.25	0.99
At end of adaptation	42.19	42.20	41.99	2.67	0.97
At end of experiment	55.29	55.16	53.64	3.47	0.48
Performance[4]					
ADFI, kg/day	1.85	1.86	1.84	0.04	0.88
ADG, kg/day	0.93^{a}	0.92^{a}	0.83^{b}	0.06	0.06
G:F	0.50^{a}	0.49^{a}	0.45^{b}	0.01	0.02

[1] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

[2] Daily feeding program (DP) with a diet with 100% of estimated AA requirements was provided in the morning and afternoon; sequential feeding program with 40% decrease and 40% increase (SEQ_{LH}) of the estimated AA requirements in the diet in morning and afternoon, respectively; and sequential feeding program with 40% increase and 40% decrease (SEQ_{HL}) of the estimated AA requirements in the diet in morning and afternoon, respectively.

[3] Data were analyzed using a linear mixed model including the effect of feeding program (FP; n=3) as fixed effects and random effect of blocks. Least square means of 8 pigs per treatment. SEM, standard error of the mean

[4] Values referring to the experiment (14 days).

Table 3. Nitrogen balance of pigs submitted to adjustment of nutritional composition according to circadian rhythm using the sequential feeding method with different amino acid variations throughout the day.

Variables[1]	Feeding programs [2]			SEM	*P*-values[3]
	DP	SEQ_{LH}	SEQ_{HL}		
N intake, g/day	44.72	45.01	44.41	2.71	0.87
Fecal N, g/day	10.59	11.77	11.06	0.76	0.44
Urine N, g/day	9.26^{ab}	8.34^{a}	9.99^{b}	0.75	0.03
N absorbed, g/day	34.23	33.35	33.45	2.24	0.83
N retained, g/day	25.03	25.07	23.52	1.64	0.58
N efficiency, %	55.66	55.21	52.72	1.85	0.49
N efficiency, % of absorbed	72.81^{ab}	74.70^{a}	69.92^{b}	1.41	0.08

[1] N, nitrogen.

[2] Daily feeding program (DP) with a diet with 100% of estimated AA requirements was provided in the morning and afternoon; sequential feeding program with 40% decrease and 40% increase (SEQ_{LH}) of the estimated AA requirements in morning and afternoon, respectively; and sequential feeding program with 40% increase and 40% decrease (SEQ_{HL}) of the estimated AA requirements in morning and afternoon, respectively.

[3] Data were analyzed using a linear mixed model including the effect of feeding program (FP; n=3) as fixed effects and random effect of blocks. Least square means of 8 pigs per treatment.

Table 4. Pre-prandial[1] concentrations of metabolites in pigs submitted to adjustment of nutritional composition according to circadian rhythm using the sequential feeding method with different amino acid variations throughout the day.

Variables	Feeding programs [2]			SEM	*P*-values[3]		
	DP	SEQ_{LH}	SEQ_{HL}		FP	Time	FP × Time
Lactate[4], mg/dL							
Day 7	15.20	13.71	18.31	2.20	0.15	0.13	0.29
Day 14							
Glucose[4], mg/dL							
Day 7	71.09[a]	73.22[a]	76.03[b]	2.27	0.04	0.07	0.62
Day 14							
Triglycerides[5], mg/dL							
Day 7	26.88	26.00	26.62	2.46	0.93	<0.01	0.54
Day 14							
Total protein[5,6], g/dL							
Day 7	4.29[a]	4.81[b]	3.97[a]	0.29	<0.01	<0.01	0.04

Day 14							
Albumin[5,7], g/dL							
Day 7	1.81[a]	1.95[b]	1.75[a]	0.15	0.18	<0.01	0.03
Day 14							
Urea[5], mg/dL							
Day 7	7.41	8.30	9.30	1.03	0.21	<0.01	0.09
Day 14							

[1] Blood collection was performed after 9-hour fast.

[2] Daily feeding program (DP) with a diet with 100% of estimated AA requirements was provided in the morning and afternoon; sequential feeding program with 40% decrease and 40% increase (SEQ_{LH}) of the estimated AA requirements in morning and afternoon, respectively; and sequential feeding program with 40% increase and 40% decrease (SEQ_{HL}) of the estimated AA requirements in morning and afternoon, respectively.

[3] Data were analyzed using a linear mixed model including the effect of feeding program (FP; n=3), time (n=2), their interaction (FP × Time) as fixed effects, and random effect of blocks. The collection day was specified as a repeated effect. Least square means of 8 pigs per treatment. SEM, standard error of the mean.

[4] Analysis in plasma.

[5] Analysis in serum.

[a, b] Means with different small letters superscripts within a row are affected by feeding program ($P < 0.05$).

[6] *P-value* of slices Day 7 $P = 0.0006$ and Day 14 $P = 0.5229$.

[7] *P-value* of slices Day 7 $P = 0.0048$ and Day 14 $P = 0.4287$.

Table 5. Postprandial[1] concentrations of metabolites in pigs submitted to adjustment of nutritional composition according to circadian rhythm using the sequential feeding method with different amino acid variations throughout the day.

Variables	Feeding programs [2]			SEM	*P*-values[3]		
	DP	SEQ_{LH}	SEQ_{HL}		FP	Time	FP × Time
Lactate[4], mg/dL							
Day 7	18.48	18.75	17.89	3.03	0.95	0.06	0.19
Day 14							
Glucose[4], mg/dL							
Day 7	75.41	79.04	79.47	3.06	0.27	0.85	0.69
Day 14							
Triglycerides[5], mg/dL							
Day 7	30.57	30.48	30.69	2.72	0.99	<0.01	0.42
Day 14							
Total protein[5], g/dL							
Day 7	5.36	4.79	4.78	0.31	0.13	<0.01	0.21

Day 14							
Albumin[5], g/dL							
Day 7	2.25	2.00	2.12	0.15	0.30	<0.01	0.08
Day 14							
Urea[5], mg/dL							
Day 7	10.12[a]	9.30[a]	13.49[b]	1.17	<0.01	0.33	0.38
Day 14							

[1] Blood collection was performed 0230 h after the meal.

[2] Daily feeding program (DP) with a diet with 100% of estimated AA requirements was provided in the morning and afternoon; sequential feeding program with 40% decrease and 40% increase (SEQ_{LH}) of the estimated AA requirements in morning and afternoon, respectively; and sequential feeding program with 40% increase and 40% decrease (SEQ_{HL}) of the estimated AA requirements in morning and afternoon, respectively.

[3] Data were analyzed using a linear mixed model including the effect of feeding program (FP; n=3), time (n=2), their interaction (FP × Time) as fixed effects, and random effect of blocks. The collection day was specified as a repeated effect. Least square means of 8 pigs per treatment. SEM, standard error of the mean.

[4] Analysis in plasma.

[5] Analysis in serum.

[a, b] Means with different small letters superscripts within a row are affected by feeding program ($P < 0.05$).

CAPÍTULO 3 - The sequential feeding system with adjust in the diet amino acid concentration according to the circadian rhythm can improve protein deposition in pigs: II. Growth assay

Este capítulo é apresentado de acordo com as normas da **Revista Animal Feed Science and Technology.**

The sequential feeding system with adjust in the diet amino acid concentration according to the circadian rhythm can improve protein deposition in pigs: II. Growth assay

Abstract

Sequential feeding system may allow adjustments of pigs' diet nutritional composition according to the differences in metabolic state over the day determined by the circadian rhythm. Therefore, our objective was to evaluate a sequential feeding program varying dietary amino acids (AA) concentration in the first and last 12 hours of the day, on performance, body composition, and nutrients balance of growing and finishing pigs. A total of 68 castrated male pigs (25 ± 2.67 kg) were distributed in a randomized block design in four treatments: daily feeding program (DP), in which pigs received a diet with 100% of the required daily concentration of standardized ileal digestible (SID) AA from 0000 to 2359 h; and three sequential feeding programs (SEQ) with different levels of variation in AA concentration, in which pigs received a diet with decrease (-20, -30, -40%) of the required daily concentration of SID AA from 0000 to 1159 h (period 1; P1), and increase (+20, +30, +40%) from 1200 to 2359 h (period 2; P2), respectively. Individual pigs were considered the experimental units and were fed *ad libitum* using automatic feeders. The experiment lasted 82 days, from 25 to 100 kg of body weight (BW), divided in three phases. Comparison between feeding programs (DP vs SEQ) and the linear and quadratic responses according to increased dietary AA levels concentration were evaluated. During phase 1, SEQ pigs had higher SID lysine (Lys) intake, bone mineral content, protein gain, crude protein (CP) intake, phosphorus (P) retention, and P efficiency than DP group ($P \leq 0.05$). On the same phase, pigs in SEQ showed a tendency for higher average daily feed intake (ADFI), average daily gain (ADG), BW, body protein, body lipid, lipid gain, nitrogen (N) retention, N excretion, and P intake compared to DP pigs (P =

0.10). During phase 1, ADFI, SID Lys intake, BW and body protein content tended (P < 0.09) to increase in quadratic manner as the variation in dietary amino acid levels increased, while body lipid increased (P < 0.03) quadratically as the variation in dietary amino acid levels increased. In the second phase, SEQ showed a tendency for higher average feed intake on P2 (AFI P2), ADG, BW, body protein, and P retention than pigs in DP (P = 0.10). During phase 2, gain:feed ratio (G:F) increased (P = 0.01) linearly as the variation in dietary amino acid levels increased, while ADG and BW tended (P = 0.09) to increase in linear manner as the variation in dietary amino acid levels increased. During phase 3, SEQ pigs compared to DP group had similar performance and body composition (P > 0.05), however a tendency for a higher N excretion (P = 0.06) was observed. Therefore, the results of this study indicate that SEQ with dietary AA variation, which decrease and increase in the first and the last twelve hours for the day, respectively, improve pig's performance, body composition and nutrient balance for pigs 25-70 kg.

Key words: anabolism, catabolism, lipid deposition, protein gain, swine nutrition

Abbreviations

AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; AFI P1, average feed intake on period 1; AFI P2, average feed intake on period 2; AIPF, automatic intelligent precision feeder; BW, body weight; CP, crude protein; DP, daily feeding program; DXA, dual energy x-ray absorptiometry; G:F, gain:feed ratio; HPLC, high performance liquid chromatography; Lin, linear effects; Lys, lysine; N, nitrogen; NIRS, near infrared spectroscopy; P, phosphorus; P1, period 1 (0000 to 1159 h); P2, period 2 (1200 to 2359 h); SEQ, sequential feeding programs; SEQ20, sequential feeding program with 20% of diet amino acid variation; SEQ30, sequential feeding program with 30% of diet amino acid variation; SEQ40, sequential feeding program with 40% of diet amino acid variation; SID, standardized ileal digestible; Quad, quadratic effects

Introduction

In swine nutrition programs, diets are predominantly formulated according to animal growth phase and a single diet is provided to the animals during the whole phase (feeding phase programs). In fact, the nutrient requirement may change every day due the dynamic of animal's growth (Hauschild et al., 2010). In this context, daily feeding programs may be and effective approach due to the better adjustment of the diet to the daily nutrient requirements of the animals allowing reduce costs and environmental impacts (Andretta et al., 2014; Pomar et al., 2014). However, this feed system does not consider the variations in voluntary feed intake and nutrient metabolism that occurs during the 24-hour-day cycle.

Several aspects of animal physiology, including motility of the gastrointestinal tract, nutrient metabolism, and hormone production, are influenced by the circadian system and may occur with lower or higher frequencies and/or intensities according to the time of day (Reppert and Weaver, 2002). The pattern of the metabolic state may vary throughout the day, being higher in the morning, inducing a catabolic state, and lower in the afternoon, inducing an anabolic state (Boumans et al., 2017). In addition, studies have shown that pigs are animals with diurnal feeding habits (Wangsness et al., 1980; Feddes et al., 1989; Andretta et al., 2016) where a higher feed intake has been observed before the lights turn off (Andretta et al., 2016).

Thus, feeding pigs with diets adjusted according to the circadian rhythm using the sequential feeding method, which allows provide a lower concentration of amino acids (AA) in the period of catabolic state, and a higher concentration of AA in the period of anabolic state, may improve performance, body composition, and nutrient utilization. Sequential feeding consists of providing diets with different nutritional compositions over a given period (Bouvarel et al., 2008). This strategy allows to provide diets with lower and higher concentration of AA in the first and last twelve hours of the day, respectively. Therefore, we aimed with this study to evaluate a sequential feeding program varying dietary AA concentration in the first and last 12 hours of the day, on performance, body composition, and nutrients balance of growing and finishing pigs.

Material and methods

The protocol for this experiment was evaluated and approved by Animal Use Ethics Committee at São Paulo State University, SP, Brazil (protocol number: 012570/18).

Animals, housing and experimental design

The study was conducted at the Swine Studies Laboratory of São Paulo State University, Brazil. Sixty-eight castrated male pigs (Agroceres PIC, Rio Claro, SP, Brazil) were housed in a single pen in a negative pressure shed with 95 m^2 with concrete floor. The temperature-controlled room was equipped with an evaporative pad cooling system (Big Dutchman, Araraquara, SP, Brazil). The photoperiod was fixed in 12 hours of artificial light (0600 to 1800 h), provided by three led lamps (240 cm length, 75W of power, 6400 lumens luminous flux), and 12 hours of darkness (1800 to 0600 h). Water was supplied by beat ball model drinkers and feed by five automatic intelligent precision feeders (AIPF; University of Lleida, Lleida, Spain), both were provided *ad libitum*.

All animals were equipped with an exclusive transponder (a plastic button tag containing passive transponders of radio frequency identification; Allflex, Joinville, SC, Brazil) that was inserted in the right ear of each pig using the specific tagger pliers. Each transponder had an exclusive code that allowed the feeders to identify and record data for each pig individually. Feeders were described by Pomar et al. (2011) and basically consists of a physical component and a logical component. The physical component consisted in a single-space feeder that simultaneously blended volumetric amounts of feeds stored in independent containers located in the upper part of the AIPF. The logical component consisted in a software allowed programming all commands executed by the feeders, as well as register them.

The AIPF identified each pig when its head was introduced into the feeder and then provided the diet according to the assigned experimental feeds (see next session) in response to each animal request. In this way, the animals could be housed in the same pen, and any pig could access any of the five feeders and receive the diet prescribed for that animal according to its treatment. Pigs were adapted to the automatic feeders five days before the trial.

The pigs had an average initial body weight (BW) of 25 ± 2.67 kg and were assigned on the basis of BW to a randomized block design in three blocks, being the block one with five pigs and blocks two and three with six pigs each, distributed in four treatments. A daily feeding program (DP), in which pigs received a diet with 100% of the required daily concentration of SID AA from 0000 to 2359 h; and three sequential feeding programs (SEQ) with different levels of variation in AA concentration, in which pigs received a diet with decrease (-20, -30, -40%) of the required daily concentration of SID AA from 0000 to 1159 h (period 1; P1), and increase (+20, +30, +40%) from 1200 to 2359 h (period 2; P2), respectively. The experimental unit was the individual pig, and each treatment had 17 replicates. The trial lasted 82 days and was divided in three phases, corresponding to phase 1 (25 to 50 kg), phase 2 (50 to 70 kg) and phase 3 (70 to 100 kg).

Nutritional requirements, diets, and feeding programs

The NRC model (NRC, 2012) were used to estimate the daily lysine (Lys) requirements curve for pigs from 25 to 100 kg of BW and to formulate the experimental feeds (A and B; Table 1). The formulation of these feeds was performed using each ingredient's standardized ileal digestible (SID) AA content, which was obtained by ingredient analysis (corn and soybean meal) and the SID AA value in Rostagno et al. (2017). The experimental feeds were formulated to contain similar net energy concentrations and similar ratios for all AA.

Feed A was formulated with high nutrients concentration to satisfy the requirements for minerals, vitamins, and energy for the average pig of population at the beginning of the growing phase (25 kg BW). Feed B was formulated with low nutrients concentration to satisfy the requirements for minerals, vitamins, and energy for the average pig of population at the end of the finishing phase (100 kg BW). Additionally, feed A was formulated to provide 140% of the ideal AA concentration, and the feed B was formulated to provide 60% of the ideal AA concentration. Feeds were pelleted at 5 mm.

Dietary treatments were obtained by blending feeds A and B in proportions necessary to obtain the four treatments. In the DP the required daily concentration of SID AA was adjusted daily, providing 100% of AA concentration during 24 hours per day. In the SEQ, the required daily concentration of SID AA was adjusted each 12 hours, providing a low AA concentration from 0000 to 1159 h (P1), and a high AA concentration from 1200 to 2359 h (P2). Three SEQ programs (SEQ20, SEQ30 and SEQ40) were evaluated with differents levels of variation in AA concentration (20, 30 and 40%) each 12 hours. Wherein, in P1 the AA concentration was decreased (-20, -30 and -40%) of the required daily concentration of SID AA, while in P2 the AA concentration was increased (+20, +30 and +40%) of the required daily concentration of SID AA.

In this regard, pigs from SEQ20, SEQ30 and SEQ40, recived diets whit 80, 70 and 60% of the required daily concentration of SID AA during P1, and 120, 130 and 140% of the required daily concentration of SID AA during P2. These feeding programs were defined to have a graded decrease (P1) and increase (P2) in diet AA levels between treatments (0, 20, 30, 40). However, the reason for not having equidistant levels between the first two treatments (0 and

20) was because there was no positive effect in a previous study with SEQ programs with levels of variation in AA concentration below 20% of the required daily concentration of SID AA (Silva et al., 2021).

Analytical procedures

Representative samples of corn and soybean meal were collected before formulation of the feeds. These samples were submitted to near infrared spectroscopy (NIRS) analysis to determine total AA values. From this, the SID AA coefficient of corn and soybean meal proposed by Rostagno et al. (2017) were used to calculate the SID AA values that was used in feeds formulation (see previous session). Representative samples of feeds A and B were collected throughout the experiment and at the end were homogenized and analyzed. The feeds were analyzed for dry matter (method 930.15) and crude protein (CP) as N × 6.25 (method 968.06; LECO model F528 N, Leco Corp., St. Joseph City, MI, US) according to AOAC (1990). Standardized ileal digestible tryptophan concentration values were estimated from analyzed raw matters composition (corn and soybean meal) using the coefficients proposed by Rostagno et al. (2017). The total AA composition (excluding tryptophan) of the feeds was determined by high performance liquid chromatography (HPLC) based on the chromatography method described by White et al. (1986). The SID AA coefficient proposed by Rostagno et al. (2017) were used to calculate the SID AA values.

Experimental measurements

The information of each visit on feeders, such as pig time of entry and exit and feed intake, was continuously registered by AIPF software. The pigs were weighted on the first day and at the end of each experimental phase (days 28, 54 and 82). Performance was evaluated through average daily feed intake (ADFI; kg/day), average feed intake on P1, that corresponds from 0000 to 1159h (AFI P1; kg/period), average feed intake on P2, that corresponds from 1200 to 2359 h (AFI P2; kg/period), SID Lys intake (g/day), average daily gain (ADG; kg/day), gain:feed ratio (G:F; kg/kg) and final BW (kg) in each phase. On days 0, 28, 54 and 82, total body fat, lean and mineral content were measured by dual energy X-ray absorptiometry (DXA; Hologic Discovery Instrument, Hologic, Inc., Bedford, MA, USA). Body composition was evaluated through body protein (kg), body lipid (kg), bone mineral content (kg), protein gain (g/day), protein in weight gain (%) and lipid gain (g/day).

Pigs were underwent fasting for eight hours to receive anesthesia for containment at the time of total body scanning. The anesthesia protocol used was intramuscular application and consisted of a tranquilizer (acepromazine), a sedative (xylazine hydrochloride) and a general anesthetic (ketamine hydrochloride). The acepromazine concentration administered in all measurements was 0.1 mg kg-1. The xylazine hydrochloride and ketamine hydrochloride changed with animal growth, being used: 1.5 mg kg-1 of xylazine hydrochloride and 15 mg kg-1 of ketamine hydrochloride with 25 kg; 1.0 mg kg-1 and 10 mg kg-1 with 50 and 70 kg; and 0.8 mg kg-1 and 7 mg kg-1 with 100 kg. After the scan, pigs had their temperature and respiratory rate monitored until they recovered completely of anesthesia.

Calculations and statistical analysis

The pig weight gain was calculated as the difference between the weights measured at the beginning and at the end of each phase divided by number of days. Feed intake data was calculated using the feeding information of each pig registered by AIPF software. Average feed intake (AFI) on P1 was obtained by consumption from 0000 h to 1159 h and AFI on P2 from 1200 h to 2359 h. From these values, SID Lys intake, CP intake and total P intake were calculated considering the amount of nutrients of each blended feeds provided.

The body lean and fat masses obtained by total body scan were converted to body protein and lipid mass following the methodology of Pomar and Rivest (1996). Lean and fat mass gain were calculated by the difference between the initial and final value in each experimental phase, divided by the number of days. Total body P value was estimated according to Letourneau-Montminy et al. (2015). Nitrogen (N) and phosphorus (P) excretion values were obtained by subtracting the respective nutrient retention and intake values. Nitrogen and P efficiency were calculated by relation between deposition and intake, respectively.

Data were analyzed as a randomized complete block design with fixed effect of feeding programs and random effect of blocks. All analysis were performed using SAS MIXED procedure (version 9.3; SAS Institute Inc., Cary, NC, USA). The GROUP statement was used to equalize variances between the means on the basis of the covariance parameter estimates and AIC values. Orthogonal-polynomial contrasts analysis were used to compare the daily feeding programs with sequential feedings programs (DP vs SEQ), and to determine the linear and quadratic responses according to increased dietary AA levels concentration. Regarding the levels of variation in dietary AA concentration, the decrease and increase in P1 and P2, respectively, were similar, therefore linear and quadratic analysis were evaluated in function only of the numbers, without positive or negative signals (0, 20, 30 and 40). Hence, three contrasts were constructed: DP vs SEQ programs, linear effects (Lin), and quadratic effects (Quad). Statistical differences were considered significant with $P \leq 0.05$, while $0.05 < P \leq 0.10$ was considered a tendency.

Results

The room ambient temperature averaged 22 ± 1.59 ºC from 25 to 50 kg, 21 ± 1.76 ºC from 50 to 70 kg, and 20 ± 1.84 ºC from 70 to 100 kg. These ambient temperature values were in accordance with the thermal comfort zone recommended for growing-finishing pigs. Five pigs, two from SEQ30 and three from SEQ40, were removed from the trial for presenting clinical signs not related to the experimental diets. Pigs had ADFI, ADG and body composition according to expected for the genetic line. The analyzed feed AA content was, on average, 8 and 4% below and above than expected for feeds A and B, respectively.

Performance

During the first experimental phase, when compared to DP, SEQ pigs had higher SID Lys intake (13.6 vs 12.0 g/day; P = 0.03; Table 2) and a tendency for higher ADFI (1.41 vs 1.29 kg/day; P = 0.10), ADG (0.70 vs 0.63 kg/day; P = 0.06) and BW (44.56 vs 42.37 kg; P = 0.09). On this same phase, the ADFI, AFI P2, SID Lys intake and BW tended (P = 0.09) to increase in quadratic manner as the variation in dietary amino acid levels increased. During the phase 2, when compared to DP, SEQ pigs presented a tendency for higher AFI P2 (1.10 vs 0.94 kg/period; P = 0.07), ADG (0.83 vs 0.74 kg/day; P = 0.10) and BW (65.78 vs 61.43 kg; P = 0.10). On this same phase, G:F increased (P = 0.01) linearly as the variation in dietary amino acid levels increased, while ADG and BW tended (P = 0.09) to increase in linear manner as the variation in dietary amino acid levels increased. During phase 3, SEQ pigs had higher AFI P2 when compared to DP group (1.42 vs 1.21 kg/period; P = 0.03). Considering the global experiment, when compared to DP pigs, SEQ pigs showed a tendency for higher AFI P2 (1.10 vs 0.96 kg/period; P = 0.07) and SID Lys intake (16.3 vs 14.8 g/day; P = 0.08).

Body composition

During phase 1, when compared to DP pigs, SEQ pigs had higher bone mineral content (0.76 vs 0.71 kg; P = 0.03; Table 3) and protein gain (120.0 vs 107.4 g/day; P = 0.03). When compared to DP pigs, SEQ pigs showed a tendency for higher body protein (6.44 vs 6.05 kg; P = 0.06), body lipid (10.03 vs 9.72 kg; P = 0.09) and lipid gain (145.5 vs 125.1 kg/day; P = 0.08) in the first experimental phase. On this same phase, the body lipid increased (P = 0.03) quadratically as the variation in dietary amino acid levels increased, while body protein tended (P = 0.06) to increase in quadratic manner as the variation in dietary amino acid levels increased.

During phase 2, SEQ pigs when compared to DP had higher bone mineral content (1.14 vs 1.05 kg; P = 0.04) and showed a tendency for higher body protein (9.84 vs 9.19 kg; P = 0.10). On this same phase, the body protein and protein gain tended (P = 0.10) to increase in linear manner as the variation in dietary amino acid levels increased. On phase 3, SEQ pigs when compared to DP pigs showed a tendency for a higher bone mineral content (1.64 vs 1.53 kg; P = 0.09).

Nutrient balance

During the first experimental phase, SEQ pigs compared to DP pigs had higher CP intake (220.7 vs 197.3 g/day; P = 0.05; Table 4), P retention (2.7 vs 2.4 g/day; P = 0.01), and P retention efficiency (42.19 vs 39.18 %; P = 0.05). On this same phase, SEQ pigs compared to DP pigs presented a tendency for higher N retention (19.0 vs 17.2 g/day; P = 0.06), N excretion (16.6 vs 14.6 g/day; P = 0.10) and P intake (6.7 vs 6.1 g/day; P = 0.07). On this same phase, the P excretion increased (P = 0.05) quadratically as the variation in dietary amino acid levels increased, while CP intake, N excretion and P intake tended (P = 0.08) to increase in quadratic manner as the variation in dietary amino acid levels increased. During second phase, P retention tended to be higher for SEQ than DP pigs (4.1 vs 3.5 g/day; P = 0.08). On this same phase, the N retention tended (P = 0.09) to increase in linear manner as the variation in dietary amino acid levels increased. During third phase, SEQ pigs compared to DP pigs had a worse N retention efficiency (43.29 vs 47.07 %; P = 0.04) and showed a tendency for a higher N excretion (30.9 vs 27.1 g/day; P = 0.06). During the global experiment, SEQ pigs compared to DP pigs showed a tendency for higher CP intake (279.6 vs 255.3 g/day; P = 0.10), N excretion (23.8 vs 21.2 g/day; P = 0.10) and P retention (3.8 vs 3.5 g/day; P = 0.09).

Discussion

Overview of the sequential feeding system

The physiology of the animals are controlled by an internal clock that responds to external stimuli (*zeitgebers*), with a 24-hour periodicity (Aschoff, 1966). The light-dark cycle is considered the most important signal due to its influence in the hormonal secretion and consequently in the catabolic and anabolic state (Boumans et al., 2017). Higher catabolism and anabolism have been observed at the beginning and at the end of the day in pigs fed *ad libitum*, respectively (Boumans et al., 2017). Accordingly, this study was conducted to evaluate the sequential feeding with decrease and increase AA diet concentration in the first (P1) and last twelve hours (P2) of the day. Our hypothesis was that providing a lower AA diet level in the period of day where catabolic state is greater (P1), and higher where anabolic state is greater (P2), may improve the nutrient efficiency, performance, and body composition of pigs. In the SEQ, although the pigs had better performance and body composition, N efficiency was not affected. However, SEQ pigs showed a better P efficiency in the beginning of growing-finishing phase (25 to 50 kg).

Performance and body composition

The higher ADFI (8%) by SEQ pigs during phase 1 of the experiment (25 to 50 kg) resulted in a higher SID Lys intake (12%) and, consequently, greater ADG (5%) and deposition of lipid (6%), protein (6%), and minerals (6%) compared to DP pigs. In adequate conditions of health and environment, pigs are able to regulate feed intake in order to meet their energy requirements (Kyriazakis and Whittemore, 2006). However, younger pigs may have feed intake limited by the capacity of the gastrointestinal tract (Whittemore et al., 2003). The nutrient requirements of SEQ pigs may have increased because of the improvement in performance, and consequently, a higher consumption of nutrients was necessary. However, as expected pigs also had a higher consumption of energy, resulting in an increase of lipids deposition. Lipid deposition in growing pigs shows sigmoid nonlinear behavior in function of age and tends to increase with increasing BW (Schinckel and de Lange, 1996; Suárez-Belloch et al., 2015; Menegat et al., 2020). However, the increase in lipid deposition can also be attributed to a higher feed intake, and consequently, energy (Quiniou et al., 2010; Lanferdini et al., 2013; Weiler et al., 2013).

As variation AA levels increased, the ADFI, BW, body protein, and body lipid increased quadratically in the first phase of the experiment. This could be related to the fact that extremely low levels of some AA (ex: tryptophan, leucine and valine) affect the feed intake of pigs (Le Floc'h and Seve, 2007; Sterndale et al., 2020; Yin et al., 2020). Therefore, in the present study the feed intake and consequently performance and body composition may have been affected when variation AA levels increased and a lower dietary AA concentration was used in P1 (e.g., SEQ40). However, this reduction in feed intake was not observed in the phases 2 and 3 of the experiment. This is likely that young animals suffer more in a severe AA restriction due to the limited capacity of increase consumption in a deficiency nutrient diet compared to older pigs (Henry, 1985; Yin et al., 2017).

During phase 2, the higher AFI P2 associated to higher dietary AA concentration in this period, can explain the linear increase in ADG, BW, G:F, and protein gain as variation AA levels increased, however the lipid gain was similar between the treatments. Protein deposition is mainly determined by the genetic potential of lean tissue growth and the proportion of AA and energy ingested (Ruusunen et al., 2007). Therefore, the reason that the SEQ pigs showed a better protein deposition (9% higher) compared to DP can be attributed to a higher intake of AA in the period of greatest anabolism, since there is a positive correlation between protein intake and protein synthesis rate (Ruiz-Ascacibar et al., 2017). However, decreased dietary AA concentration in P1 may have had also contributed for this better response in SEQ program.

Accordingly to the circadian rhythm, plasma glucose levels are lower in the morning compared with night (Stenvers et al., 2012), due to the active state in the morning and fasting period during the night. Additionally, insulin sensitivity in relation to glucose metabolism is lower at night than in the morning (Yoshino et al., 2014). Suggesting that the highest utilization of glucose within cells occurs during the morning. The diets in SEQ pigs were obtained by mixing two feeds (A and B) with similar energy levels. However, it should be noted that the diets had different proportion of carbohydrate and protein. Diets with higher proportions of feed B (P1) had a higher concentration of carbohydrate and lower protein, while the opposite is true for diets that had higher proportions of feed A (P2). This may suggest that providing a diet with a greater amount of carbohydrate in the period when insulin sensitivity is greater may be advantageous. In the case of protein, a better utilization can occur at the end of the day, during the anabolic state (Stenvers et al., 2012). Therefore, this indicate that providing a lower dietary AA concentration (more carbohydrate) in the first twelve hours (P1) and a higher dietary AA concentration (more protein) in the last twelve hours (P2) of the day allow to follow better the utilization of glucose and protein observed throughout the day.

In our study, the greater feed intake in P2 for SEQ pigs did not reflect in improvements in weight gain and protein gain during the phase 3 (70 to 100 kg) compared to DP. This may be related to the fact that the animals possibly reached the maximum protein gain in this phase or even in the previous phases. The pigs SEQ40, for example, showed similar protein gain in phase 2 and 3 (143.2 vs 143.9 g/day). Therefore, once the maximum protein gain was reached, the increment in AA intake obtained in SEQ programs likely resulted in an AA deamination due to the excess in the metabolism. Since protein deposition does not depend only on nutritional strategy and the genetic factor is indispensable, the adjust of the diet AA concentration according to the circadian rhythm of the catabolic and anabolic state can be an effective approach to be used when the animals have not yet reached the maximum protein deposition.

Nutrient balance

According to our results, the positive effect of SEQ on ADFI resulted in a tendency for greater N and P retention during phase 1. In addition, the positive effect of SEQ in feed intake in P2 combined with the AA and P levels increase in this period resulted in a higher N and P retention during phase 2. Thus, beside the feed intake effect, SEQ pig by ingesting less nutrients in P1 and more in P2 may had a greater availability of nutrients in the anabolic period. However, although feed intake in P2 was higher for SEQ pig during phase 3, N and P balance retention was worse compared to DP. Again, it could be explained by the maximum protein deposition that was reached in phase 2 of the experiment.

In our study the N efficiency utilization were similar between SEQ and DP, while the P utilization was improved for the SEQ programs during phase 1. Skeletal tissues express a strong circadian clock and numerous studies have shown that bone metabolic functions are regulated in a circadian manner (Zvonic et al., 2007; Aoyama and Shibata, 2017). Thus, P can be considered an essential nutrient that controls circadian function in both skeletal and non-skeletal peripheral tissues (Noguchi et al., 2018). Thus, an adequate intake of this nutrient during the day may improve the P retention and efficiency of utilization for pigs as observed in our study.

Overall, the adjustment of the diet AA and P concentrations according to the circadian rhythm (catabolism and anabolism state) can improve the nutrients intake allowing the animals to express their growth potential in the initial stages of growth. These findings suggest that SEQ program is a potential strategy to improve N and P deposition of pigs when compared with standard feeding programs. Additionally, as feed represents a large part of the cost of pig production, and the P component represent an important proportion of feed cost (Cordell et al., 2009; Zangarini et al., 2020). The SEQ feeding program may be economically advantageous than daily feeding and even standard programs since SEQ pigs had the best values for P efficiency in the beginning of growing phase (25 to 50 kg).

Conclusion

In summary, the results of the present study indicate the adjustment of the diet AA concentrations according to the circadian rhythm (catabolism and anabolism state) improve protein deposition and tended to increase ADFI and ADG for pig from 25-50 kg. Moreover, this adjustment tends to increase ADG, BW and body protein for pigs from 50-70 kg. These better responses were observed in 40% decrease and increase in the first and the last twelve hours for the day, respectively, in the diet amino acid levels recommendation. Therefore, the sequential feeding system with the adjust in the diet nutrient concentration according to the circadian rhythm is an effective approach for improving performance and body composition of growing pigs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Centesimal and nutritional composition of feeds A and B.

Item	Diet A (High AA concentration)	Diet B (Low AA concentration)
Ingredient composition, as-fed basis, %		
Corn grain	64.50	91.00
Soybean meal	30.00	-
Soybean oil	0.20	0.80
Dicalcium phosphate	1.25	1.00
Calcitic limestone	0.60	0.55
Vitamin and mineral premix[1]	0.20	0.20
Salt	0.20	0.20
L-Lysine sulfate (54.6%)	1.02	0.37
L-Threonine	0.24	0.01
DL-Methionine	0.27	-
L-Tryptophan	0.05	0.02
L-Valine	0.12	-
Kaolin	1.29	5.79
Choline chloride (60%)	0.06	0.06
Chemical composition, %		
Net energy[2], kcal/kg	2472	2555
Crude Protein[3]	19.91	9.60
Available Phosphorus[2]	0.32	0.22
Calcium[2]	0.62	0.48
Chlorine[2]	0.17	0.17

Sodium[2]	0.10	0.10
SID Lysine[4]	1.34	0.44
SID Threonine[4]	0.80	0.28
SID Methionine[4]	0.50	0.17
SID Methionine + cysteine[4]	0.77	0.32
SID Tryptophan[5]	0.25	0.07
SID Valine[4]	0.88	0.38

[1] Mineral and vitamin supplement (per kg of feed): Vit. A (5,250 IU); Vit. D3 (750 IU); Vit. E (11 IU); Vit. K3 (1.5 mg); Vit. B1 (1 mg); Vit. B2 (2.4 mg); Vit. B6 (1 mg); Niacin (30 mg); B.C. Pantothenic (8.1 mg); Folic acid (0.53 mg); Biotin (0.05 mg); Vit. B12 (16.5 mcg); Copper (13.5 mg); Iodine (0.19 mg); Manganese (37.5 mg); Selenium (0.15 mg); Zinc (72 mg); Iron (72 mg); Cobalt (0.19 mg).

[2] Values estimated according to NRC model (NRC, 2012).

[3] Crude protein as N × 6.25 according to AOAC (1990).

[4] Standardized ileal digestible (SID) amino acids concentration values were calculated using the total amino acids content of feeds A and B obtained by high performance liquid chromatography (HPLC) and the coefficients proposed by Rostagno et al. (2017).

[5] Standardized ileal digestible (SID) tryptophan concentration values were estimated from analyzed raw matters composition (corn and soybean meal) using the coefficients proposed by Rostagno et al. (2017).

Table 2. Performance of pigs in a daily feeding program (DP) or in a sequential feeding program (SEQ) with different levels of variation in amino acid (AA) concentration throughout the day[1].

	Variation in recommended amino acid levels in P1 and P2, %					*P-values*		
	DP	SEQ[3]						
		SEQ20	SEQ30	SEQ40			Levels[4]	
Variables[2]	0/0	-20/+20	-30/+30	-40/+40	SEM	DP vs SEQ	Lin	Quad
Initial Conditions								
BW, kg	25.29	25.20	25.90	25.26	0.55	0.79	0.93	0.32
Phase 1 (25 to 50 kg)								
ADFI, kg/day	1.29	1.32	1.50	1.40	0.06	0.10	0.41	0.08
AFI P1, kg/period	0.59	0.61	0.65	0.65	0.04	0.31	0.48	0.71
AFI P2, kg/period	0.71	0.77	0.85	0.76	0.04	0.12	0.86	0.09
SID Lys intake, g/day	12.0	12.7	14.5	13.6	0.66	0.03	0.32	0.08
ADG, kg/day	0.63	0.66	0.74	0.70	0.03	0.06	0.39	0.18
G:F, kg/kg	0.48	0.48	0.49	0.49	0.01	0.71	0.49	0.86

BW, kg	42.37	42.86	46.23	44.60	1.20	0.09	0.29	0.07
Phase 2 (50 to 70 kg)								
ADFI, kg/day	1.88	2.00	2.09	2.10	0.11	0.18	0.31	0.62
AFI P1, kg/period	0.95	0.91	1.00	1.01	0.06	0.72	0.25	0.58
AFI P2, kg/period	0.94	1.11	1.08	1.10	0.08	0.07	0.96	0.81
SID Lys intake, g/day	15.3	16.0	17.0	17.3	1.01	0.15	0.33	0.75
ADG, kg/day	0.74	0.76	0.83	0.90	0.05	0.10	0.08	0.84
G:F, kg/kg	0.39	0.39	0.40	0.42	0.01	0.20	0.01	0.93
BW, kg	61.43	62.27	66.88	68.19	2.65	0.10	0.09	0.58
Phase 3 (70 to 100 kg)								
ADFI, kg/day	2.50	2.63	2.63	2.59	0.11	0.34	0.78	0.92
AFI P1, kg/period	1.21	1.18	1.12	1.23	0.07	0.68	0.61	0.26
AFI P2, kg/period	1.21	1.43	1.49	1.34	0.11	0.03	0.49	0.33
SID Lys intake, g/day	17.9	19.2	19.1	18.4	0.84	0.27	0.48	0.77
ADG, kg/day	0.99	1.00	1.01	1.01	0.04	0.70	0.84	0.89

G:F, kg/kg	0.40	0.38	0.38	0.38	0.01	0.11	0.63	0.81
BW, kg	89.02	90.66	95.44	96.68	3.79	0.18	0.23	0.68
Global performance (25 to 100 kg)								
ADFI, kg/day	1.86	1.96	2.06	2.03	0.09	0.15	0.57	0.58
AFI P1, kg/period	0.91	0.90	0.92	0.96	0.05	0.78	0.35	0.85
AFI P2, kg/period	0.96	1.09	1.13	1.08	0.07	0.07	0.91	0.60
Daily SID Lys intake, g/day	14.8	15.8	16.7	16.5	0.82	0.08	0.51	0.55
ADG, kg/day	0.78	0.81	0.85	0.87	0.04	0.17	0.29	0.78
G:F, kg/kg	0.42	0.41	0.41	0.42	0.01	0.64	0.26	0.82

[1] Daily feeding program (DP), in which pigs received a diet with 100% of the required daily concentration of SID AA from 0000 to 2359 h; Sequential feeding programs (SEQ) with different levels of variation in AA concentration, in which pigs received a diet with decrease (-20, -30, -40%) of the required daily concentration of SID AA from 0000 to 1159 h (period 1), and increase (+20, +30, +40%) from 1200 to 2359 h (period 2), respectively. Least square means of 17 pigs per treatment.

[2] BW, body weight; ADFI, average daily feed intake; AFI P1, average feed intake in period 1 from 0000 to 1159 h; AFI P2, average feed intake in period 2 from 1200 to 2359 h; SID, standardized ileal digestible; Lys, lysine; ADG, average daily gain; G:F, gain:feed ratio.

[3] Levels of variation in AA concentration in relation to the required daily concentration of SID AA (NRC, 2012).

[4] Lin, linear effect; Quad, quadratic effect.

506 **Table 3**. Body composition of pigs in a daily feeding program (DP) or in a sequential feeding program (SEQ) with different levels of variation in
507 amino acid (AA) concentration throughout the day[1].

	Variation in recommended amino acid levels in P1 and P2, %							
	DP	SEQ[3]				*P-values*		
		SEQ20	SEQ30	SEQ40			Levels[4]	
Variables[2]	0/0	-20/+20	-30/+30	-40/+40	SEM	DP vs SEQ	Lin	Quad
Initial Conditions								
Body protein, kg	3.17	3.14	3.27	3.19	0.09	0.76	0.75	0.38
Body lipid, kg	6.38	6.37	6.50	6.35	0.11	0.83	0.91	0.31
Bone mineral content, kg	0.53	0.52	0.54	0.51	0.01	0.64	0.76	0.12
Phase 1 (25 to 50 kg)								
Body protein, kg	6.05	6.19	6.72	6.42	0.18	0.06	0.38	0.06
Body lipid, kg	9.72	10.00	10.84	10.06	0.30	0.09	0.90	0.03
Bone mineral content, kg	0.71	0.75	0.77	0.75	0.01	0.03	0.55	0.31
Protein gain, g/day	107.4	114.6	125.3	120.1	5.23	0.03	0.44	0.20

Protein in weight gain, %	16.9	16.6	16.7	17.0	0.16	0.51	0.12	0.48
Lipid gain, g/day	125.1	139.9	158.8	137.8	10.45	0.08	0.88	0.12
Phase 2 (50 to 70 kg)								
Body protein, kg	9.19	9.33	9.97	10.22	0.41	0.10	0.10	0.67
Body lipid, kg	14.27	14.47	15.95	15.98	0.84	0.16	0.17	0.44
Bone mineral content, kg	1.05	1.10	1.14	1.17	0.04	0.04	0.21	0.98
Protein gain, g/day	120.8	124.1	132.9	143.2	8.36	0.14	0.09	0.94
Protein in weight gain, %	16.2	16.1	15.9	15.8	0.27	0.37	0.49	0.87
Lipid gain, g/day	176.6	182.6	210.7	216.1	19.64	0.20	0.20	0.62
Phase 3 (70 to 100 kg)								
Body protein, kg	13.40	13.43	13.98	14.30	0.55	0.40	0.24	0.85
Body lipid, kg	22.79	23.71	25.64	25.13	1.38	0.17	0.44	0.45
Bone mineral content, kg	1.53	1.59	1.67	1.65	0.05	0.09	0.45	0.47
Protein gain, g/day	143.7	140.6	140.4	143.9	6.87	0.78	0.73	0.82
Protein in weight gain, %	14.6	14.3	13.8	14.3	0.29	0.13	0.93	0.14

Lipid gain, g/day	299.8	327.2	348.1	329.7	23.28	0.16	0.93	0.47
Global body composition (25 to 100 kg)								
Protein gain, g/day	126.5	127.5	132.1	136.1	6.04	0.41	0.29	0.96
Protein in weight gain, %	15.6	15.4	15.2	15.4	0.17	0.12	0.73	0.33
Lipid gain, g/day	202.2	215.2	235.6	229.5	16.29	0.16	0.51	0.48

[1] Daily feeding program (DP), in which pigs received a diet with 100% of the required daily concentration of SID AA from 0000 to 2359 h; Sequential feeding programs (SEQ) with different levels of variation in AA concentration, in which pigs received a diet with decrease (-20, -30, -40%) of the required daily concentration of SID AA from 0000 to 1159 h (period 1), and increase (+20, +30, +40%) from 1200 to 2359 h (period 2), respectively. Least square means of 17 pigs per treatment.

[2] Body protein and lipid estimated according to Pomar and Rivest (1996) from lean and fat mass measured by dual-energy X-ray absorptiometry measurements (DXA).

[3] Levels of variation in AA concentration in relation to the required daily concentration of SID AA (NRC, 2012).

[4] Lin, linear effect; Quad, quadratic effect.

Table 4. Nutrient balance of pigs in a daily feeding program (DP) or in a sequential feeding program (SEQ) with different levels of variation in amino acid (AA) concentration throughout the day[1].

	Variation in recommended amino acid levels in P1 and P2, %							
	DP	SEQ[3]				*P-values*		
		SEQ20	SEQ30	SEQ40			Levels[4]	
Variables[2]	0/0	-20/+20	-30/+30	-40/+40	SEM	DP vs SEQ	Lin	Quad
Phase 1 (25 to 50 kg)								
CP intake, g/day	197.3	206.2	235.7	220.3	10.65	0.05	0.34	0.07
N retention, g/day	17.2	17.9	19.8	19.2	0.86	0.06	0.29	0.22
N excretion, g/day	14.6	15.4	18.1	16.2	1.09	0.10	0.60	0.08
P intake, g/day	6.1	6.3	7.2	6.7	0.32	0.07	0.36	0.08
P retention, g/day	2.4	2.6	2.8	2.8	0.14	0.01	0.30	0.51
P excretion, g/day	3.7	3.7	4.3	3.8	0.23	0.38	0.63	0.05
N retention efficiency, %	53.91	52.73	52.52	54.17	1.39	0.62	0.46	0.57
P retention efficiency, %	39.18	42.43	40.85	43.29	1.34	0.05	0.64	0.21

Phase 2 (50 to 70 kg)								
CP intake, g/day	261.2	272.3	290.9	294.8	16.87	0.16	0.32	0.70
N retention, g/day	19.3	19.8	21.2	22.9	1.33	0.14	0.09	0.94
N excretion, g/day	22.8	24.1	25.5	25.0	1.67	0.24	0.68	0.63
P intake, g/day	8.4	8.7	9.4	9.5	0.53	0.17	0.31	0.66
P retention, g/day	3.5	3.8	4.0	4.4	0.27	0.08	0.11	0.78
P excretion, g/day	4.9	5.0	5.4	5.2	0.32	0.44	0.69	0.48
N retention efficiency, %	45.79	44.77	46.26	46.45	1.49	0.98	0.40	0.71
P retention efficiency, %	41.86	41.75	42.45	44.08	1.57	0.58	0.27	0.80
Phase 3 (70 to 100 kg)								
CP intake, g/day	320.3	341.1	339.7	330.1	15.01	0.30	0.58	0.81
N retention, g/day	22.9	22.5	22.4	23.0	1.10	0.78	0.73	0.82
N excretion, g/day	27.1	31.1	31.9	29.6	1.85	0.06	0.54	0.49
P intake, g/day	10.8	11.4	11.4	11.1	0.51	0.37	0.64	0.86
P retention, g/day	4.5	4.6	4.9	4.6	0.22	0.49	0.98	0.37

P excretion, g/day	6.0	6.5	6.5	6.4	0.39	0.25	0.82	0.97
N retention efficiency, %	47.07	43.54	41.96	44.36	1.66	0.04	0.71	0.31
P retention efficiency, %	44.10	42.33	43.14	41.76	1.66	0.35	0.80	0.58
Global nutrient balance (25 to 100 kg)								
CP intake, g/day	255.3	270.5	286.1	282.4	13.94	0.10	0.52	0.55
N retention, g/day	20.2	20.4	21.1	21.7	0.96	0.41	0.29	0.96
N excretion, g/day	21.2	23.2	24.9	23.4	1.45	0.10	0.90	0.35
P intake, g/day	8.4	8.8	9.2	9.1	0.45	0.16	0.58	0.56
P retention, g/day	3.5	3.7	3.9	3.9	0.17	0.09	0.31	0.78
P excretion, g/day	4.8	5.0	5.3	5.2	0.30	0.27	0.72	0.52
N retention efficiency, %	48.22	45.98	46.01	47.30	1.05	0.12	0.36	0.61
P retention efficiency, %	42.11	41.92	42.48	42.77	0.97	0.79	0.52	0.90

[1] Daily feeding program (DP), in which pigs received a diet with 100% of the required daily concentration of SID AA from 0000 to 2359 h; Sequential feeding programs (SEQ) with different levels of variation in AA concentration, in which pigs received a diet with decrease (-20, -30, -40%) of the required daily concentration of SID AA from 0000 to 1159 h (period 1), and increase (+20, +30, +40%) from 1200 to 2359 h (period 2), respectively. Least square means of 17 pigs per treatment.

[2] CP, crude protein; N, nitrogen; P, phosphorus. Body protein estimated according to Pomar and Rivest (1996) from lean mass measured by dual-energy X-ray absorptiometry measurements (DXA) and converted to nitrogen. Total body phosphorus value was estimated according to Létourneau-Montminy et al. (2015). Nitrogen and phosphorus efficiency were obtained by relation between deposition and intake phosphorus and protein, respectively.

[3] Levels of variation in AA concentration in relation to the required daily concentration of SID AA (NRC, 2012).

[4] Lin, linear effect; Quad, quadratic effect.

Supplementary material

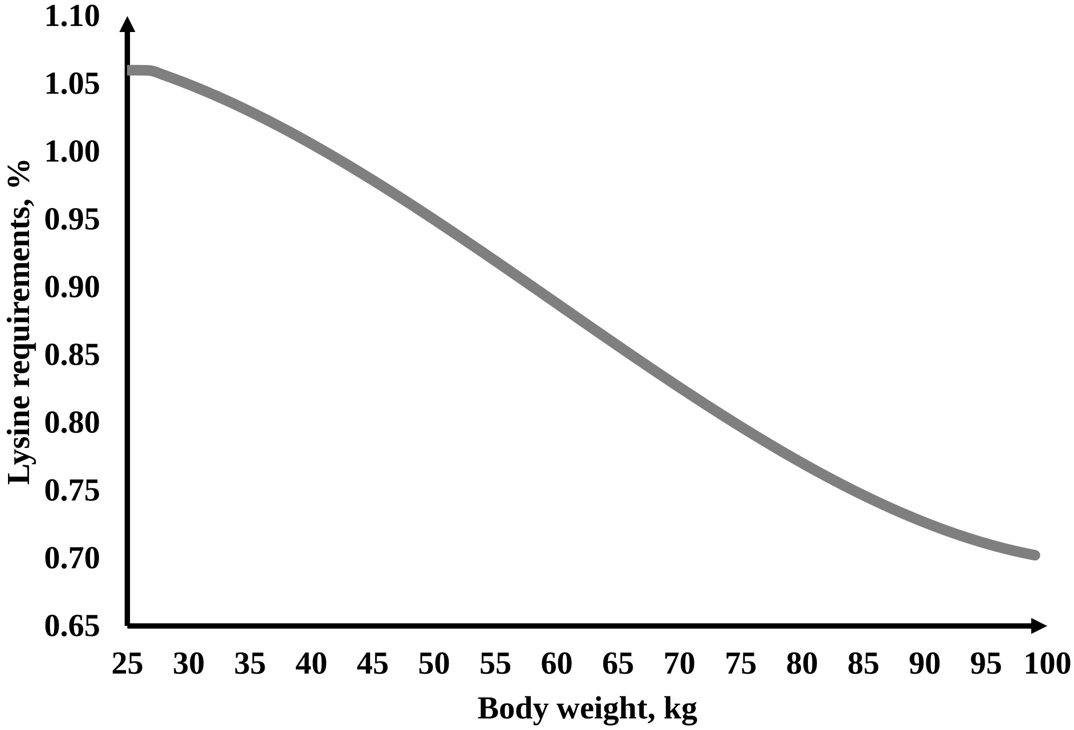

Figure 1. Daily lysine requirements for barrow pigs from 25 to 100 kg of body weight estimated according to NRC model (NRC, 2012).

CAPÍTULO 4 – CONSIDERAÇÕES FINAIS

No atual cenário de crescimento demográfico e perspectivas para aumento da produção de alimentos, onde o conceito de sustentabilidade deve ser levado em consideração, o ajuste da composição nutricional da dieta à exigência nutricional dos animais é uma importante ferramenta para obtenção de bons resultados. Dentro dos programas nutricionais para suínos, o ajuste da composição nutricional da dieta realizado diariamente traz benefícios ambientais e econômicos quando comparado ao ajuste de acordo com a fase crescimento do animal. No entanto, estratégias que reduzam ainda mais custos e impacto ambiental, com possibilidade de aumentar as respostas de crescimento dos suínos devem ser estudadas.

Dentro desse contexto, objetivou-se com a presente tese avaliar uma estratégia de ajuste da composição de aminoácidos da dieta ao longo do dia para suínos em crescimento e terminação. Essa estratégia pode ser justificada com base no ritmo circadiano, que orquestra todas as atividades fisiológicas dos animais dentro de um período de, aproximadamente, 24 horas. Dentro do ritmo circadiano, os nutrientes podem ser melhor absorvidos em determinado momento ao longo de um dia. De acordo com as variações circadianas, em animais de comportamento diurno, tem-se observado um catabolismo e anabolismo mais acentuado durante o dia e a noite, respectivamente.

No segundo capítulo dessa tese, o objetivo foi avaliar balanço de nitrogênio e metabolismo de nutrientes de suínos alimentados com dietas com diferentes concentrações de aminoácidos nas refeições da manhã e da tarde, respectivamente. Com esse capítulo concluiu-se que o fornecimento de dietas com baixo e alto nível de aminoácidos nas refeições da manhã e da tarde, respectivamente, melhora o metabolismo da proteína de suínos em crescimento.

No terceiro capítulo, objetivou-se avaliar o desempenho, a composição corporal e o balanço de nutrientes de suínos alimentados com dietas com diferentes níveis de decréscimo e aumento (20, 30 e 40%) na concentração de aminoácidos nas primeiras e últimas 12 horas do dia, respectivamente. Os resultados deste estudo indicaram que o ajuste das concentrações de aminoácidos na dieta de acordo com o ritmo circadiano (estado de catabolismo anabolismo) melhora a deposição de proteína para suínos de

25-50 kg. Além disso, este ajuste tende a aumentar ganho de peso diário, peso e proteína corporal para suínos de 50-70 kg. Essas melhores respostas foram observadas em redução e aumento de 40% nas primeiras e nas últimas doze horas do dia, respectivamente, na recomendação dos níveis de aminoácidos da dieta.

Com base nos estudos realizados na presente tese, o ajuste da composição de aminoácidos da dieta de acordo com o ritmo circadiano de suínos pode ser uma estratégia nutricional para auxiliar os programas de alimentação de suínos em crescimento. No entanto, por se tratar de uma alternativa ainda pouco avaliada, mais estudos são necessários para que de fato essa estratégia possa ser aplicada na produção de suínos.