UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

SISTEMA DE ALIMENTAÇÃO SEQUENCIAL COM AJUSTES NUTRICIONAIS PARA SUÍNOS EM CRESCIMENTO E TERMINAÇÃO DE DUAS LINHAGENS GENÉTICAS SUBMETIDOS A CONDIÇÃO DE ESTRESSE CÍCLICO POR CALOR

Alícia Zem Fraga

Zootecnista

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UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: SISTEMA DE ALIMENTAÇÃO SEQUENCIAL COM AJUSTES NUTRICIONAIS PARA SUÍNOS EM CRESCIMENTO E TERMINAÇÃO DE DUAS LINHAGENS GENÉTICAS SUBMETIDOS A CONDIÇÃO DE ESTRESSE CÍCLICO POR CALOR

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Jaboticabal, 23 de fevereiro de 2018

DADOS CURRICULARES DO AUTOR

ALÍCIA ZEM FRAGA – Nascida em Muriaé, Minas Gerais, no dia 20 de Março de 1993. Em 2011 ingressou no curso de graduação em Zootecnia da Universidade Federal de Viçosa, onde obteve o título de destaque na apresentação e defesa da Monografia “Piglets' behavior in maze with glass walls in function of weaning age” orientada pelo Dr. Aloísio Soares Ferreira e defendida no ano de 2016. Nesse mesmo ano iniciou o Mestrado na Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista “Júlio de Mesquita Filho” (FCAV–UNESP), Campus de Jaboticabal, orientada pelos professores Dr. Luciano Hauschild e Dr. Paulo Henrique Reis Furtado Campos (coorientador). No Mestrado foi bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), com período sanduíche no Instut National de la Recherche Agronomique França (INRA UMR PEGASE), sob supervisão da phD. Nathalie Le Floc'h.

À minha mãe, por me dar asas e sólidas raízes.

Dedico

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SUMÁRIO

CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

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CERTIFICADO

Certificamos que o Projeto intitulado **"Sistema de alimentação sequencial com ajustes nutricionais para suínos em crescimento e terminação de duas linhagens genéticas submetidos a condição de estresse cíclico por calor"**, protocolo nº 18.077/16, 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 junho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da 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 14 de dezembro de 2016.

Vigência do Projeto	15/02/2017 a 15/05/2017
Espécie / Linhagem	Suínos
Nº de animais	78
Peso / Idade	15 aos 120 kg
Sexo	Macho e fêmea
Origem	Granja Comercial Parceira

Jaboticabal, 14 de dezembro de 2016.

Profª Drª Lizandra Amoroso
Coordenadora – CEUA

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RESUMO

SISTEMA DE ALIMENTAÇÃO SEQUENCIAL COM AJUSTES NUTRICIONAIS PARA SUÍNOS EM CRESCIMENTO E TERMINAÇÃO DE DUAS LINHAGENS GENÉTICAS SUBMETIDOS A CONDIÇÃO DE ESTRESSE CÍCLICO POR CALOR

RESUMO: Objetivou-se com esse estudo avaliar o sistema de alimentação sequencial como uma ferramenta para atenuar os efeitos do estresse cíclico por calor em suínos de duas linhagens genéticas nas fases de crescimento e terminação. Oitenta suínos machos castrados de 22kg (± 2,5) PV foram distribuídos em um delineamento experimental inteiramente casualizado, em esquema fatorial 3x2 (3 programas de alimentação e 2 linhagens genéticas; 3 PA e 2 LG) sendo o animal a unidade experimental. Os PA foram: Controle (**CON**), alta-gordura/baixa-proteína bruta (**AG/BP**) e Alimentação Sequencial [**SEQ**, de 1800 às 1000 h dieta controle (Tempo-Período 1, TP1) e de 1000 às 1800 h dieta AG/BP (TP2)]. A temperatura foi 22.3 ± 0.4ºC para TP1 e 30.2 ± 0.5ºC para TP2. A duração do período experimental foi de 84 dias subdividido em três fases: crescimento 1 (0 a 20 dias), crescimento 2 (21 a 48 dias) e terminação (49 a 83 dias). O consumo de ração foi registrado em tempo real pelo sistema automático de alimentação (AIPF). Os animais foram pesados no início e no término de cada fase experimental e nos dias 0, 35 e 75 foi mensurada a composição corporal. Houve interação entre LG e Tempo-Período (TP) para consumo médio de ração (CMR) por hora ($P < 0,05$). A 22ºC o CMR não se diferiu entre linhagens ($P > 0,05$) contudo, a 30ºC foi maior para linha B ($P < 0,05$). Houve interação ($P < 0,05$) entre PA e LG para custo energético de ganho de massa magra. De 35 a 75 dias, SEQ apresentou similar custo para ganho de massa magra para linha A quando comparado com CON ($P > 0,05$). No entanto para ambas as linhas, SEQ apresentou maior custo em relação ao CON ($P < 0.05$). Nas fases de crescimento 1 e 2 não houveram diferenças (P> 0,05) entre AG/BP e CON para CDR e GDP. Porém na fase de terminação maiores resultados para essas variáveis, além de maior custo de energia para ganho de peso, foram observados para AG/BP ($P < 0,05$). Na fase de crescimento 2, SEQ apresentou maior GDP ($P = 0,04$) com similar custo de energia para ganho ($P = 0,24$) quando comparado com programa CON. Na terminação, a alimentação SEQ permitiu reduzir o consumo de EL ($P < 0,01$), mas com GDP semelhante ($P > 0,05$) ao AG/BP. Além disso, SEQ apresentou custo de ganho de energia similar ao CON ($P > 0,05$) nessa fase. Massa gorda e ganho de massa gorda foram semelhantes entre SEQ e AG/BP ($P > 0,05$) e ambos maiores que CON ($P < 0,05$). O fornecimento de diferentes dietas ajustadas à variação da temperatura ao longo do dia é uma abordagem eficiente para atenuar o efeito do estresse térmico, mas com subsequente carcaças mais gordas. Além disso, em relação à eficiência energética para a deposição de massa magra, a alimentação sequencial é um programa eficiente a ser aplicado para a linha genética menos tolerante ao estresse térmico.

Palavras-chave: genótipo, nutrição, temperatura ambiente

ABSTRACT

SEQUENTIAL FEEDING SYSTEM WITH NUTRITIONAL ADJUSTMENTS FOR PIGS GROWING AND FINISHING PHASES OF TWO GENETIC LINES SUBMITTED TO CYCLICAL HEAT STRESS

ABSTRACT: This study aimed at evaluating the sequential feeding system as a tool to attenuate the effects of cyclic heat stress in two genetics of growing-finishing pigs. Eighty 22kg (± 2.5) BW barrows were assigned in a completely randomized experimental design, in factorial scheme 3x2 (3 feeding programs and 2 genetic lines; 3FP and 2GL) being the animal the experimental unit. The FP were: Control (**CON**), high-fat/low-crude protein (**HF/LP**) and Sequential Feeding **[SEQ**, from 1800 to 1000 h control diet (Time-Period 1, TP1) and from 1000 to 1800 h HF/LP diet (TP2)]. The temperature was 22.3 ± 0.4ºC for TP1 and 30.2 ± 0.5ºC for TP2. The experimental period lasted 84 days subdivided into three phases, growing 1 (0 to 20d), growing 2 (21 to 48d), and finishing phase (49 to 83d). Feed intake was recorded using Automated Feeder System. Pigs were weighed at beginning and end of each experimental phase and on days 0, 35 and 75 were measured the body composition. There was interaction between GL and time-period (TP) for average feed intake (AFI) per hour ($P < 0.05$). At 22ºC the AFI did not differ between lines ($P > 0.05$), but at 30ºC the line B pigs had higher values than line A ($P < 0.05$). There was interaction ($P < 0.05$) between FP and GL for energy cost of lean gain. From 35 to 75d, SEQ had similar energy cost of lean gain for line A compared to CON (P > 0.05). However, for both lines, SEQ had higher the energy cost of lean than CON (P < 0.05). In growing 1 e 2 there was not difference ($P > 0.05$) between HF/LP and CON for ADFI and ADG. But in finishing phase, higher results for these variables and energy cost for gain were observed for HF/LP ($P < 0.05$). In growing 2, SEQ had higher ADG ($P = 0.04$) and similar energy cost of gain ($P = 0.24$) compared to CON program. In finishing phase, the SEQ allowed reducing NE intake ($P < 0.01$) and had a similar ADG ($P > 0.05$) than HF/LP. In addition, SEQ had similar energy cost of gain compared to CON ($P > 0.05$) in this phase. Fat mass and fat mass gain were similar between SEQ and HF/LP ($P > 0.05$), but both higher than CON ($P < 0.05$). In conclusion, the supply of different diets adjusted to the variation of the temperature over the day is an efficient approach to attenuate the heat stress effect, but with subsequent fatter carcasses. In addition, regarding energy efficiency for lean deposition the sequential feeding is an efficient program to be applied for the genetic line less tolerant to heat stress.

Keywords: ambient temperature, genotype, nutrition

1. CAPÍTULO 1 - INTRODUÇÃO

1.1. Contextualização

Durante a última década, a produção de suínos em países tropicais e subtropicais tem aumentado consideravelmente para suprir a crescente demanda por produtos de origem animal impulsionada pelo crescimento demográfico e melhoria da renda per capita da população. Apesar dos desafios enfrentados pela indústria de suínos nos países em desenvolvimento (preço de matérias-primas, crises econômicas e problemas socioambientais), acredita-se que a produção de suínos nessas regiões continuará a sustentar o crescimento mundial futuro da cadeia suinícola (ABCS, 2015).

A produção de carne suína no Brasil cresceu 4,95% em 2015 (em relação ao ano anterior), totalizando 3,5 milhões de toneladas, posicionando o país como quarto maior produtor e exportador mundial de carne suína (ABIEC, 2015; ABPA, 2015). Embora esse cenário seja promissor, as condições ambientais são consideradas fatores limitantes para maior eficiência da produção de suínos em regiões de clima quente. Adicionalmente, dependendo das limitações de investimentos em tecnologias (ambiência e alimentação) os animais podem não conseguir expressar seu máximo potencial genético e, consequentemente, apresentarem índices produtivos aquém do esperado.

É possível afirmar que a melhora ou até mesmo a manutenção da resposta animal em condições climáticas adversas, dependem da busca por novas tecnologias que possam minimizar esse impacto. Com a publicação do 4º relatório do *Intergovernmental Panel on Climate Change* (IPCC) em 2007, está evidente que a mudança climática global está efetivamente ocorrendo e provavelmente, é uma das maiores ameaças do século XXI. As previsões indicam aumento da temperatura global média de 0,8-2,6 °C até 2050, bem como uma maior amplitude e frequência de ondas de calor do verão (IPCC, 2013).

Dados obtidos na Estação Agroclimatológica UNESP-Jaboticabal (SP) indicam que a temperatura média no município de Jaboticabal é de 27ºC durante o dia (atingindo 30ºC entre 13 e 14 h), enquanto à noite a temperatura média é de 22ºC (Figura 1). Este cenário é similar na maior parte das regiões brasileiras, com amplitude térmica diária de cerca de 10ºC (INMET, 2009).

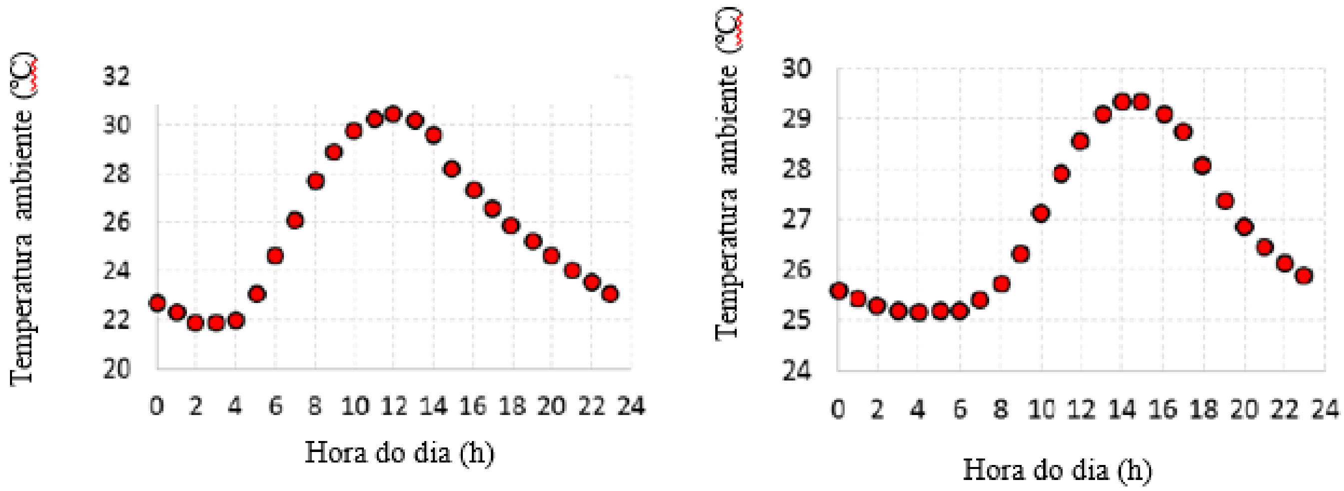

Figura 1. Distribuição da temperatura ao longo do dia no município de Jaboticabal (SP). Dados obtidos na Estação Agroclimática UNESP (2016).

Alterações dessa natureza podem impactar negativamente a sustentabilidade e produtividade dos sistemas de produção de suínos em regiões de clima quente, visto que o estresse por calor pode resultar em efeitos deletérios no desempenho dos animais. Nesse sentido, um estudo de meta análise demonstrou que a cada grau Celsius de aumento na temperatura ambiente (entre 25 e 30°C) o consumo de alimento e ganho de peso apresentaram redução de 45 e 25 g/dia, respectivamente, em suínos de 50 kg de peso vivo (RENAUDEAU et al., 2011).

Estudos têm demostrado que, além de mudanças no desempenho, os estados metabólicos e fisiológicos dos animais também são alterados em situações de estresse por calor. Quando expostos a altas temperaturas, suínos apresentam alterações na sensibilidade à insulina (PEARCE et al., 2013), menores níveis circulantes de hormônios tiroidianos e, consequentemente, menor metabolismo basal (BERNABUCCI et al., 2010). Além disso, são observadas mudanças no potencial de deposição de proteína e gordura na carcaça (LE BELLEGO et al., 2002; RHOADS et al., 2013) principalmente para as novas linhagens de suínos disponíveis no mercado, visto que, por apresentarem maior deposição de tecido muscular e produzirem elevada quantidade de calor metabólico, são mais susceptíveis ao estresse por calor (BROWN-BRANDL et al., 2003).

Dado o fato da produção de suínos apresentar grande importância socioeconômica e ambiental no Brasil, estudos com objetivo de avaliar estratégias alimentares para atenuar os efeitos negativos do estresse por calor, podem auxiliar no desenvolvimento de produtos e tecnologias voltados para o avanço de uma cadeia produtiva sustentável em regiões de clima quente.

1.2. Efeitos do estresse por calor no desempenho e respostas fisiológicas de suínos em fase de crescimento e terminação

A temperatura ambiental elevada está entre os fatores climáticos mais importantes que influenciam o desempenho de suínos (RENAUDEAU et al., 2010; CAMPOS et al., 2014a). Ao serem expostos a condições de alta temperatura, suínos mantêm a homeotermia por meio de ajustes comportamentais, fisiológicos e metabólicos para diminuir a produção e/ou aumentar a dissipação de calor.

Com o aumento na intensidade do estresse térmico, a concentração dos hormônios tiroidianos T3 e T4 diminuem, uma vez que estão relacionados com aumento do metabolismo e, consequentemente, agem estimulando a termogênese. Com baixos níveis de T3 e T4, há decréscimo da atividade da bomba ATPase Na^+/K^+ dependente o que acarreta em redução da hidrólise de ATP pelas células animais, resultando em declínio da taxa metabólica. Adicionalmente, observa-se que outros hormônios tais como as catecolaminas, hormônio de crescimento e glicocorticoides também têm seus níveis reduzidos em condições prolongadas de calor (BERNABUCCI et al., 2010).

A temperatura ambiental elevada também pode desencadear efeitos negativos sobre o desempenho de suínos em fase de crescimento e terminação em condições de alimentação a vontade. Campos et al. (2014b) observaram que suínos em crescimento, quando expostos à alta temperatura (30ºC) em relação a condição termoneutra (24ºC), apresentaram, respectivamente, menor consumo de ração (1500 e 2003 g/dia) e ganho de peso (449 e 684 g/dia). Dessa maneira, houve piora em 14% na conversão alimentar (CA) dos animais mantidos à 30ºC. Em outro estudo, foi observado impacto negativo de 20% na CA dos animais alojados em locais com temperaturas ambientais superiores a 30ºC, quando comparados com suínos criados em temperatura entre 20 e 30ºC (RENAUDEAU et al., 2011).

1.3. Programas Nutricionais e Alimentares para Atenuação dos Efeitos do Estresse por Calor em Suínos: Estado da Arte e Perspectivas de Desenvolvimento Científico

Em termos de nutrição animal, os efeitos do ambiente térmico no metabolismo dos animais e nas exigências nutricionais são raramente considerados. Majoritariamente, as dietas são formuladas em função da fase de crescimento dos animais (i.e., programa de alimentação por fases), independentemente das interações animal-ambiente. Para suínos em crescimento, por exemplo, uma única dieta é fornecida para animais entre 30 e 50 kg de peso vivo (dieta crescimento I) e outra para animais entre 50 e 70 kg de peso vivo (dieta crescimento II; ROSTAGNO et al., 2011). Além disso, nesses programas de alimentação, os nutrientes são geralmente fornecidos em excesso, a fim de suprir as exigências dos animais com maior demanda nutricional em um grupo de indivíduos. Essa prática, porém, conduz à formulação de dietas com alto valor termogênico e, portanto, inapropriadas para regiões de clima quente.

Em ensaios calorimétricos, no qual estima-se a partição e utilização energética dos animais, foi possível observar que suínos em crescimento raramente retêm mais do que 50% da ingestão de energia metabolizável (EM), sendo o restante perdido na forma de calor. Parte dessa perda de calor é devido a mantença, atividade física e incremento calórico, que inclui a transformação dos nutrientes dietéticos para a deposição de proteínas e lipídeos, além do custo de energia (ATP) desses processos. Devido ao gasto energético associado ao *turnover* protéico, a deposição de proteína é energeticamente menos eficiente em relação a deposição dos lipídeos (VAN MILGEN et al., 2003). Dessa maneira, em virtude das diferenças energéticas no metabolismo dos macronutrientes, expressar o conteúdo energético das dietas em termos de energia líquida representa, de forma mais precisa, a energia disponível nos ingredientes da ração (MOEHN et al., 2005).

Estratégias nutricionais podem ser utilizadas com o objetivo de melhor adequação das dietas frente às condições ambientais. Como forma de garantir o atendimento das exigências nutricionais em função da redução do consumo de alimento pelos animais, uma estratégia é aumentar os níveis dos nutrientes (ex:

aumento da densidade energética) nas rações em condições de estresse por calor (TAVARES et al., 2000). Essa estratégia pode ser realizada pela suplementação de energia por meio do uso de óleos e gorduras que, além de aumentar o valor energético da dieta, permite uma redução da produção de calor metabólico devido ao baixo incremento calórico desses ingredientes.

A suplementação de óleo e gordura também pode reduzir a velocidade do trânsito do alimento (possibilitando maior tempo para a digestão e absorção dos nutrientes) o que, possivelmente, resulta em melhor conversão alimentar (ADEOLA & ORBAN, 1995). Entretanto, é importante mencionar que existem limites para a adição de óleos e gorduras, uma vez que níveis acima do recomendado podem diminuir a digestibilidade dos nutrientes em virtude da menor ação das enzimas digestivas (JANSEN et al., 2007) além de poder acarretar comprometimento na elaboração das rações devido a menor durabilidade dos pellets. (BRIGGS et al.,1999).

Outra estratégia consiste na formulação de dietas suplementadas com aminoácidos industriais com base no conceito de proteína ideal que, quando comparadas às dietas com base em proteína bruta, possuem baixo incremento calórico (RODRIGUES et al., 2012). Com o excesso de nitrogênio no organismo do animal em razão da alta concentração aminoacídica, intensificam-se as reações de transaminação, desaminação, síntese e excreção de uréia. Devido ao alto gasto energético dessas reações (ex: são necessários 4 ATP para cada molécula de NH_3 incorporada ao ciclo da uréia), o metabolismo proteico é considerado altamente calorigênico (VAN MILGEN, 2001).

É importante considerarmos que a modulação nutricional com a finalidade de diminuir a produção de calor aumenta a disponibilidade de energia líquida para deposição de gorgura (ZHANG et al., 2011). Cada grama de redução na ingestão de proteína bruta por exemplo, resulta em 1,7 kcal a menos de energia na forma de calor porém de 1,12 kcal a mais de energia líquida disponível para deposição de gordura (LE BELLEGO et al., 2001). Além disso, por demandarem de maior controle técnico e gerencial, essas estratégias nutricionais ainda são pouco adotadas nos sistemas de produção de suínos convencionais. Somado ao fato que o constante melhoramento genético dos suínos resulta em alterações nas exigências proteicas e energéticas dos animais, torna-se necessário reavalia-las periodicamente (GONÇALVES et al., 2015).

Além do ajuste nutricional, outro aspecto importante para permitir uma alimentação com baixo incremento calórico, consiste no método de fornecimento das dietas ao longo do dia. Noirot et al. (1998) relataram que, além do fornecimento de uma única dieta, existem três outros métodos de distribuição de alimentos: divisão física do comedouro (Figura 1a; onde em cada espaço são fornecidas duas dietas com concentrações nutricionais diferentes), alimentação pela mistura proporcional de duas ou mais dietas (Figura 1b; também com concentrações nutricionais diferentes) e a alimentação sequencial (Figura 1c).

O primeiro método, divisão física do comedouro, tem relação estrita com a capacidade do animal consumir o alimento de forma a atender sua necessidade nutricional. No caso da energia, por exemplo, tem sido verificado em estudos com suínos que a ingestão voluntária está associada ao nível energético da dieta (QUINIOU & NOBLET, 1999). Porém, nas fases de crescimento e terminação os suínos podem ingerir mais que suas reais necessidades, o que pode resultar em desperdício de nutrientes com impacto ambiental ocasionado pela excreção do excesso.

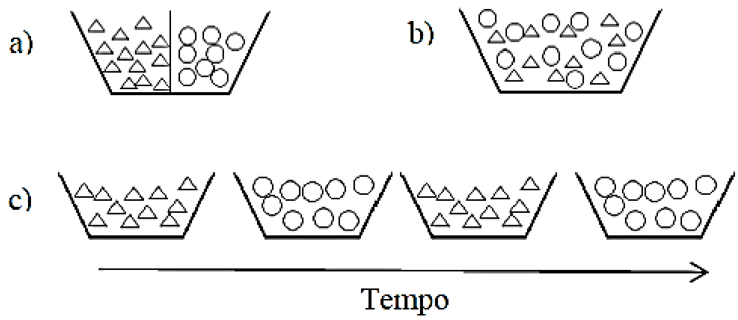

Figura 1. Esquema representando os três métodos de distribuição de alimentos, sendo: a) divisão física do comedouro; b) mistura proporcional; e c) alimentação sequencial.

O segundo método refere-se à mistura de duas dietas com densidades nutricionais diferentes. Em suínos, com a utilização de sistemas inteligentes de alimentação de precisão – *Automated Intelligent Precision Feeder* (AIPF), essa mistura pode ser feita de forma fixa ou variável, em sistemas de alimentação em grupos por fases ou individual diária (ANDRETTA et al., 2014).

O terceiro método aplica-se à alimentação sequencial, que consiste na prática de alternar diferentes dietas durante o dia (BOUVAREL et al., 2008). Partindo do pressuposto que em condições de clima tropical e subtropical os animais são submetidos a variações cíclicas da temperatura ambiente, caracterizadas por temperaturas acima da faixa de termoneutralidade durante o período diurno e temperaturas mais amenas durante o período noturno, acredita-se que o fornecimento de dietas ajustadas à variação da temperatura ambiental pode resultar em benefícios no desempenho dos animais.

Sob essa perspectiva, com o objetivo de atenuar o efeito do estresse por calor em suínos em fase de crescimento e terminação, estudos com alimentação sequencial baseados na modulação nutricional da dieta utilizando sistemas como o AIPF, poderão fornecer informações importantes para o estabelecimento de estratégias nutricionais e alimentares para produção de suínos em clima quente.

1.4. Interação nutrição, genética e ambiente: pilares da produção animal

Além dos ajustes nutricionais e tecnológicos para atenuação dos efeitos do estresse por calor, o genótipo dos suínos também é um fator de grande importância uma vez que exerce influência sob capacidade de adaptação dos animais ao ambiente térmico. Van Millgen et al. (1998) ao estimar a produção de calor em suínos de diferentes genótipos, constataram que a mesma aumentou linearmente com o aumento da percentagem de músculo na carcaça. Dessa maneira, entende-se que genótipos modernos selecionados para alta deposição de tecido magro possuem elevada produção de calor metabólico sendo portanto, menos adaptados as condições de calor quando comparados aos genótipos nativos não selecionados (RENAUDEAU et al., 2007).

Com o desafio de aliar importantes pilares na produção animal (nutrição, genética e condições de ambiente) com avanços tecnológicos sustentáveis para a produção, o presente projeto também consistiu no estudo de diferentes genótipos de suínos em fase de crescimento e terminação para avaliação dos efeitos do estresse por calor.

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CAPÍTULO 2 - Sequential feeding with a high-fat/low-crude protein diet as a tool to attenuate the effects of cyclic heat stress in two lines of growing-finishing pigs

Esse capítulo é apresentado de acordo com as normas da revista Journal of Animal Science

Running head: Sequential feeding to heat stressed pigs

Sequential feeding with a high-fat/low-crude protein diet as a tool to attenuate the effects of cyclic heat stress in two lines of growing-finishing pigs[1]

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ABSTRACT: This study aimed at evaluating the sequential feeding system as a tool to attenuate the effects of cyclic heat stress in two genetics of growing-finishing pigs. Eighty 22kg (± 2.5) BW barrows were assigned in a completely randomized experimental design, in factorial scheme 3x2 (3 feeding programs and 2 genetic lines; 3FP and 2GL) being the animal the experimental unit. The FP were: Control (**CON**), high-fat/low-crude protein (**HF/LP**) and Sequential Feeding **[SEQ**, from 1800 to 1000 h control diet (Time-Period 1, TP1) and from 1000 to 1800 h HF/LP diet (TP2)]. The temperature was 22.3 ± 0.4ºC for TP1 and 30.2 ± 0.5ºC for TP2. The experimental period lasted 84 days subdivided into three phases, growing 1 (0 to 20d), growing 2 (21 to 48d), and finishing phase (49 to 83d). Feed intake was recorded using Automated Feeder System. Pigs were weighed at beginning and end of each experimental phase and on days 0, 35 and 75 were measured the body composition. There was interaction between GL and time-period (TP) for average feed intake (AFI) per hour ($P < 0.05$). At 22ºC the AFI did not differ between lines ($P > 0.05$), but at 30ºC the line B pigs had higher values than line A ($P < 0.05$). There was interaction ($P < 0.05$) between FP and GL for energy cost of lean gain. From 35 to 75d, SEQ had similar energy cost of lean gain for line A compared to CON. However for both lines, SEQ had higher the energy cost of lean than CON. In growing 1 e 2 there was not difference ($P > 0.05$) between HF/LP and CON for ADFI and ADG. But in finishing phase, higher results for these variables and energy cost for gain were observed for HF/LP ($P < 0.05$). In growing 2, SEQ had higher ADG ($P = 0.04$) and similar energy cost of gain ($P = 0.24$) compared to CON program. In finishing phase, the SEQ allowed reducing NE intake ($P < 0.01$) and had a similar ADG ($P > 0.05$) than HF/LP. In addition, SEQ had similar energy cost of gain compared to CON ($P > 0.05$) in this phase. Fat mass and fat mass gain were similar between SEQ and HF/LP ($P > 0.05$), but both higher than CON ($P < 0.05$). In conclusion, the supply of different diets adjusted to the variation of the temperature over the day is an efficient approach to attenuate the heat stress effect, but with subsequent fatter carcasses. In addition, regarding energy efficiency for lean deposition the sequential feeding is an efficient program to be applied for the genetic line less tolerant to heat stress.

Keywords: ambient temperature, genotype, nutrition

INTRODUCTION

Pig production has considerably increased in tropical and subtropical areas to support the greater global demand for animal products (FAO, 2010). However, high ambient temperature significantly limits pig production productivity and efficiency in these areas due to its negative effects on pig feed intake, metabolism and growth rate (Campos et al., 2017). Therefore, a myriad of genetic (Renaudeau et al., 2007), environmental (Shaffer et al., 2017) and nutritional strategies have been evaluated to attenuate the effects of heat stress in pigs. Regarding nutritional strategies, increasing dietary energy and reducing crude protein content (Renaudeau et al., 2012) in the diets have been shown to attenuate feed intake and growth depression in growing-finishing pigs reared under high ambient temperature conditions.

However, on the one hand these studies were usually performed in pigs exposed to constant high ambient temperature and individually housed in experimental pens or metabolic crates (Renaudeau et al., 2011) that do not represent the environmental conditions under which pigs are commercially raised. In tropical and subtropical areas, pigs are usually group-housed in semi-open buildings and then often exposed to daily fluctuation in ambient temperature (i.e. high ambient temperature during the day and moderate temperature during the night period). On the other hand, these studies did not consider adjusting the nutritional content of diets according to ambient temperature variation throughout the 24-h day period. The technique of alternating different diets over the day is known as sequential feeding (Bouvarel et al., 2008). In this regard, this study aimed to evaluate if adjusting the diet composition according to daily variation in ambient temperature could be a strategy to attenuate the negative effects of cyclic heat stress in pig production.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the Ethical Committee for the Care and Use of Experimental Animals of the School of Agricultural and Veterinarian Sciences of São Paulo State University (protocol No. 18.077/16).

Animals and Housing

The study was carried out in the experimental facilities of the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, Brazil. A total of 78 barrows (22 ± 2.5 kg) from two different commercial genetic lines (Line A and Line B, Agroceres PIC Inc.) were used. Both lines consisted of a Large White × Landrace × Duroc × Pietrain multiple cross with Line A pigs having a greater proportion of Pietrain genes than those from line B. Pigs were housed in a single 95 m^2 pen (density 1.19 m^2/animal), with a full concrete floor in a temperature-controlled room. The pen was equipped with five automatic feeders (Automatic and Intelligent Precision Feeder [AIPF]; University of Lleida, Lleida, Spain) and ten beat ball drinkers distributed all over the pen. The photoperiod was fixed to 12 h of artificial light (0600 to 1800 h).

The temperature-controlled room was equipped with an evaporative pad cooling system (Big Dutchman) and electric heaters, automated controlled to maintain the ambient temperature constant at 22ºC from 1800 to 1000 h and at 30ºC from 1000 to 1800 h. These temperatures and time periods aimed at simulating a cyclic heat stress that pigs are usually exposed in hot climate areas. During the experiment, ambient temperature and relative humidity in the room were recorded every 10 min using two data loggers (HOBO, Onset Computer Corporation, Bourne, MA).

Experimental Design

Pigs remained in the experiment during 102 days that consisted in 18-d adaptation period and a subsequent 84-d experimental period. The experimental period was divided into three phases according to the growing stage of the animals: growing phase 1, from 0 to 20 d; growing phase 2, from 21 to 48 d; and finishing phase, from 49 to 83 d.

At the beginning of the experiment, 39 pigs of each genetic line were randomly assigned to receive one of the feeding programs: control (**CON**), in which pigs received a control diet from 0000 to 2400 h; high-fat/low-crude protein (**HF/LP**) in which pigs received a high-fat/low-crude diet from 0000 to 2400 h; and sequential feeding (**SEQ**) in which pigs received the control diet from 1800 to 1000 h (22ºC) and the high-fat/low-crude diet from 1000 to 1800 h (30ºC). Irrespective of the feeding program, feed and water were provided *ad libitum* throughout the adaptation and experimental periods. Each AIPF consisted of a single-space feeder in which precision Archimedes screw conveyors delivered and simultaneously blended volumetric amounts of up to four feeds stored in independent containers located in the upper part of the AIPF (Pomar et al., 2011). The AIPF identifies each pig when its head is introduced into the feeder and then delivers feeds in response to each animal request according to the assigned experimental feeds (see Experimental diets section topic). In this way, any pig in the pen could access any of the feeders and receive the feed prescribed for that animal.

One serving consisted of the amount of feed delivered on each effective serving request (serving size was 25 g). A time lag (18 seconds) was imposed to ensure that pigs consumed each serving before requesting a new one. One meal included all servings delivered during each feeder visit. Pigs tend to leave the feeder hopper empty or to only leave very small amounts of feed after at each visit. The feeders were calibrated weekly to convert feed volumes into feed weights. All feeders were designed to provide meals to all animals regardless of the feeding program. The use of exclusive identification codes allowed recording individual feed intake over the trial. This feature allowed all animals to be housed in the same pen in a single group.

Experimental Diets

For each growth phase (growing 1, growing 2 and finishing), two corn and soybean meal-based experimental diets were formulated (control and high-fat/low-crude protein; Table 1) to meet the nutritional requirements of the animals according to NRC (2012) recommendations. The control diets were formulated without the use of oil and crystalline AA; whereas the high-fat/low-crude protein diet were formulated with 4% of soybean oil and with the supplementation of crystalline AA to obtain lower CP content than those of the control diets.). Within growth phase, diets were formulated with similar SID Lys:NE and Lys/AA (Met, Met + Cys, Thr and Trp).

Crude protein (CP), ether extract (EE), starch (ST), crude fiber (CF) and total amino acids (AA) of ingredients were analyzed before diets formulation by Near-Infrared Reflectance Spectroscopy (NIRS). The coefficient of digestibility of AA were calculated according to Sauvant et al. (2002). The nutritional composition of the raw materials used in the formulation, except for the values obtained by analysis (corn, soybean meal and wheat bran), was obtained from the Brazilian Poultry and Swine Tables (Rostagno et al., 2011). The feed was steam-pelleted at 2.5 mm.

Performance and Body Composition

Pigs were weighed at arrival, at the beginning and at the end of each experimental phase. Total body lean and fat mass were measured on day 0, 35 (0 to 35; period 1) and 70 d (36 to 70; period 2) in 24 pigs of each genetic line (16 animals per feeding program), through the use of a DXA densitometry device (Hologic Discovery Instrument, Hologic Inc., Bedford, MA). In this procedure, pigs were overnight fasted and then anesthetized with acepromazine (tranquilizer), xylazine hydrochloride (sedative) and ketamine hydrochloride (anesthetic) during the scans. Pigs were scanned in the ventral decubitus position. Ultrasound images were collected for backfat thickness and loin depth evaluation by the use of ALOKA ultrasound equipment (500v series with a linear probe of 3.5 - MHzZ, 13.5 -cm; Aloka Ultrasound Company, Wallingford, CT). The measurements were taken at the boundary of the thoracic and lumbar vertebrae (P2 point,) at six centimeters from of the midline (ABCS, 1973).

Analytical Procedures

Samples of offered feed were taken every week and pooled per phase for DM, EE and CF content determination according to AOAC (1990) methods. Crude protein content was calculated by multiplying total N (analyzed by Leco Corporation, St. Joseph, MI, USA) by 6.25. Gross energy was measured using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL, USA). The energy contents of diets were calculated according to Sauvant et al. (2002).

Calculations and Statistical Analysis

Feed intake was calculated using the feeding information of each pig (AIPF Software). The days on which pigs were scanned (body composition) were not considered in the analysis. Because the 22 vs. 30°C time-periods within the day had different duration (16 hours at 22°C and 8 hours at 30°C), average feed intake (AFI) was expressed per hour for comparisons.

Lean mass and fat mass deposition were calculated by the difference between the respective body constituents estimated from DXA readings at the beginning and end of each period. Within growth phase and body composition period, ADG, CP intake, NE intake, and average daily lean and fat deposition were calculated by dividing the corresponding variables by the duration of experimental phase or period.

The presence of outliers was evaluated through the residual analysis of data and by daily records of anomalies. The BoxCox e Cramer-von Mises tests were used to verify homogeneity of the variances and normality of the studentized residuals, respectively. For the analysis of variance, was used the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). For the analysis for AFI/hour regarding the time-period effect the follow model was used:

$$Y_{ijmk} = \mu + X + FP_i + GL_j + TP_m + (FP\times GL)_{ij} + (FP\times TP)_{im} + (GL\times TP)_{jm} + (FP\times GL\times TP)_{ijm} + P(FP\times GL)_{ijk} + e_{ijmk};$$

and for the other variables the model was as follow,

$$Y_{ijk} = \mu + X + FP_i + GL_j + (FP\times GL)_{ij} + P(FP\times GL)_{ijk} + e_{ijk};$$

where μ is the global media, X is the weight of the animals at the arrival (as covariate) and the fixed effects of feeding programs (FP; C, HF/LP or SEQ), genetic lines (GL; Line A or Line B), time-period of day (TP) and their interactions. The individual pig [P $(FP\times GL)_{ijk}$] was considered as random effect. The MIXED model included the effect of experimental phase (performance) or period (body composition) as a repeated effect, and a compound symmetry covariance structure was used to account for the experimental unit effect over time. The results were considered statistically significant if $P < 0.05$. When there were differences between feeding programs ($P < 0.05$), adjusted means were compared using the Tukey-Kramer test.

RESULTS

Animals and Climatic Conditions

Because of health problems, data from one HF/LP pig of genetic line B was not considered in the performance analysis. Due to adaptation problems to the AIPFs, growing phase 1 data from two HF/LP pigs (one of each genetic line), and overall data from two CON pigs (one of each genetic line) were not included in the performance analysis. In the third body composition evaluation, one HF/LP pig of genetic line B was not scanned due to resistance to anesthesia.

Room ambient temperature averaged 22.3 ± 0.4ºC from 1800 to 1000 h; and 30.2 ± 0.5ºC from 1000 to 1800 h. Relative humidity averaged 70.35 ± 3.20%. These values are in accordance with the objectives of the experiment.

Performance

The effect of initial BW as covariate was significant *(P < 0.01)* for all performance and body composition parameters. On days 20, 48 and 83 of the experiment the average BWs of the pigs were 45.73 ± 1.54, 74.11 ± 4.40 and 106.88 ± 5.80 kg, respectively. Irrespective of growth phase, interaction between genetic line and time-period within the day was observed for AFI (P < 0.05; Table 2). While at the 22^{o}C-period both lines had similar feed intake, at 30^{o}C-period line B pigs had greater feed intake than line A. Except for CP intake during the finishing-phase, no interaction between feeding program and genetic line was found for the performance (P > 0.05). In growing-phase 1 (0 to 20 d), ADFI, ADG and feed efficiency were not affected by feeding program (P > 0.05; Figure 1). Crude protein intake was similar between SEQ and HF/LP pigs (P > 0.05) and both were lower than CON (0.295 vs. 0.349 kg/d on average; P < 0.05). Net energy intake and energy cost of gain were also similar between SEQ and HF/LP (P > 0.05) and both were greater than CON (15.8 vs 13.7 MJ/d and 20.8 vs 18.4 MJ NE/kg of gain, respectively; P < 0.05).

In growing-phase 2 (21 to 48 d), the feeding programs did not affect ADFI neither feed efficiency (P > 0.05). Pigs in the SEQ had ADG similar than HF/LP pigs (1.035 kg/d on average; P = 0.69) and greater than CON (1.049 vs 0.964 kg/d; P = 0.04), whereas ADG did not differ between HF/LP and CON pigs (P = 0.24). Crude protein intake was similar between SEQ and CON pigs and both were greater than in HF/LP pigs (0.417 vs 0.367 kg/d on average; P < 0.05). Net energy intake was similar between SEQ and HF/LP pigs (P > 0.05) and both were higher than in CON pigs (20.28 vs 17.41 MJ/d; P < 0.05). The energy cost of gain was lower in SEQ and CON pigs compared to HF/LP pigs (18.38 vs 20.12 MJ NE/kg gain on average; P < 0.01).

In the finishing-phase (49 to 83 d), SEQ pigs had similar ADFI, ADG and energy cost of gain than HF/LP and CON pigs (P > 0.05); whereas HF/LP pigs had greater ADFI (2.41 vs. 2.16 kg/d; P < 0.01), ADG (0.984 vs. 0.897 kg/d; P = 0.03) and energy cost of gain (24.56 vs

21.51 MJ NE/gain, *P* = 0.02) than CON pigs. There was an interaction between feeding program and genetic line for CP intake (*P* = 0.03) that was not affected by feeding program in pigs from the genetic line B; while in pigs from genetic line A it was similar between SEQ and HF/LP pigs (0.299 kg/d; *P* = 0.07) and lower in SEQ than in CON pigs (0.250 vs 0.386 kg/d; *P* < 0.01). Net energy intake was greater in HF/LP than in SEQ than CON pigs (24.11 vs. 21.56 vs. 19.23 MJ/d; *P* < 0.05).

When analyzing the entire experimental period (0 to 83 d), ADFI, ADG and feed efficiency were not affected by feeding programs (*P* > 0.05). Compared to CON pigs, SEQ and HF/LP pigs had lower CP intake (0.352 vs 0.394 kg/d; *P* < 0.05), greater NE intake (19.67 vs 16.79 MJ/d on average; *P* < 0.05) and higher energy cost of gain (21.41 vs 19.34 MJ/kg; *P* < 0.05).

In regard, to genetic line effect on growth performance, irrespective of growth phase, pigs from genetic line B had greater ADFI, ADG and NE intake than pigs from genetic line A (*P* < 0.05). In growing-phase 1, genetic line B pigs were more efficient (0.49 vs 0.47 kg/kg; *P* = 0.04) and had lower energy cost of gain (19.51 vs 20.58 MJ NE/kg gain; *P* = 0.04) than those from genetic line A. In contrast, animals from genetic line A were more efficient (0.54 vs 0.48 kg/kg; *P* < 0.01) and had lower energy cost of gain (17.96 vs 19.94 MJ NE/kg gain; *P* < 0.01) than genetic line B pigs in growing phase 2. No difference between genetic lines in feed efficiency (*P* = 0.85) and energy cost of gain (*P* = 0.06) were observed in the finishing phase. When analyzing the entire experimental period, pigs from genetic line B had greater ADFI, ADG, NE and CP intake, and higher energy cost of gain than pigs from genetic line A (*P* < 0.05).

Body composition

The results of body composition are presented in Table 4. Except for feed conversion (MJ NE/kg lean mass) in period 2 and total period, there was no interaction between feeding program and genetic line ($P > 0.05$),

In period 1 (0 to 35 d) feeding programs did not affect lean mass, backfat thickness, loin depth and lean mass gain of pigs ($P > 0.05$). Fat mass and fat mass gain were similar between SEQ and HF/LP pigs ($P > 0.05$); whereas SEQ had greater fat mass (9.56 vs 8.61kg; $P = 0.04$) and fat mass gain (0.16 vs 0.13kg/d; $P = 0.04$) than CON pigs (Figure 2). Energy cost of lean mass gain was greater in SEQ and HF/LP than in CON pigs (26.50 vs 23.22 MJ NE/kg; $P < 0.01$).

In period 2 (36 to 70 d), feeding programs did not affect lean mass, loin depth and lean mass gain ($P > 0.05$). Pig in SEQ and HF/LP feeding programs had greater fat mass and fat mass gain compared to CON pigs (16.56 vs. 14.14 kg and 0.20 vs. 0.14 kg/d on average, respectively; $P < 0.05$). Feeding programs did not influence energy cost of lean mass gain in pigs from genetic line A whereas genetic line B, SEQ and HF/LP pigs had greater energy cost of lean mass gain than CON pigs (30.67 vs 23.42 MJ NE/kg lean mass gain; $P < 0.01$).

In regard to total period of evaluation (0 to 70 d), feeding programs did not affect lean mass gain ($P = 0.22$). Pigs in SEQ and HF/LP feeding programs had greater fat mass gain than CON pigs (0.18 vs 0.14 kg/d; $P < 0.01$). There was an interaction between feeding program and genetic line for energy cost (NE) of lean mass gain ($P = 0.04$). Pigs from genetic line A had greater energy cost of lean mass gain in the SEQ than in CON (20.49 vs. 19.50 MJ NE/kg lean mass gain; $P = 0.04$), while no differences were observed between SEQ and HF/LP neither between HF/LP and CON. Pigs from genetic line B had greater energy cost of lean mass gain in the SEQ and HF/LP compared to the CON feeding program (21.85 vs. 19.19 MJ NE/kg lean mass gain; $P < 0.01$).

When analyzing genetic line effects on body composition, irrespective of growth phase, fat mass and fat mass gain were greater in genetic line B than genetic line A pigs ($P < 0.05$). Backfat thickness were not influenced by genetic lines in period 1 ($P > 0.05$), but in period 2 greater values of these variable were observed in line B pigs ($P < 0.05$). Despite of similar lean mass gain between genetic lines in periods 1 and 2 ($P > 0.05$), greater loin depth was observed in genetic line A pigs in period 2 (67.88 vs 64.09 mm; $P = 0.03$). However, in the total period evaluation, line B pigs had greater lean mass gain than line A (0.73 vs 0.68 kg/d; $P = 0.03$).

DISCUSSION

Overview of the cyclic stress condition by heat and sequential feeding system

The study aimed to evaluate if adjusting the nutritional content of diets according to daily ambient temperature variation could be a strategy to attenuate the effects of cyclic heat stress in pigs. For that pigs, room ambient temperature was maintained at 22ºC from 1800 to 1000 h and at 30ºC-period from 1000 to 1800 h, and pigs were assigned to one of the three feeding programs: CON, HF/LP or SEQ. The technique of altering diets in the SEQ feeding program was possible due to the use of the Automatic and Intelligent Precision Feeders. Our first hypothesis was that the increase of dietary energy content would compensate the reduced feed intake in pigs exposed to diurnal high ambient temperatures; and the reduction of dietary crude protein content would decrease the thermic effect of feeding contributing then to attenuate the diurnal effects of high ambient temperature. Secondly, we hypothesized that feeding pigs a HF/LP diet exclusively on the periods of high ambient temperature (instead of during the entire day) would reduce the costs of this nutritional strategy contributing to its effective implementation on commercial production systems.

Effect of a high-fat/low-crude protein sequential feeding on pig performance and body composition

According to our results, feeding programs had negligible effects on pig performance during growing-phase 1 (Figure 1), suggesting that the cyclic heat stress was not critical in this phase. In fact, the magnitude of high ambient temperature effects increases as body weight increases in association with a greater metabolic rate and feed consumption (Renaudeau et al., 2011). In the growing-phase 2, SEQ fed pigs had similar ADG and better energy cost of gain compared to HF/LP fed pigs (Figures 1B and 1E). As di and tripeptides are more efficiently absorbed by basolateral membrane than the free amino acids (Frenhani & Burini, 1999), the absence of the best performance of HF/LP fed pigs may be due a reduction dietary protein that may limit peptide availability to gut cells for synthesis of intestinal constitutive proteins (Otto et al., 2003). Therefore, although the mechanism is still unclear, it seems that the supplementation of the diets with industrial amino acids may compromise the performance of pigs mainly in growing phases by do not provide all requirements of crude protein.

In this experimental phase, when compared to those fed control diet, SEQ fed pigs had higher ADG (10%) and similar energy cost of gain. As mentioned anteriorly, these results cannot be explained by the effect of dietary protein reduction (HF/LP) on animal response in a caloric stress condition. It could be hypothesized that the SEQ program affected ADG by having influence on the hormonal secretion cyclic variation over the day. Greater circulating levels of insulin in the first hour of the day was observed (Koopmans et al., 2005). In this sense, there is a nonnegligenciable variation in insulin-stimulated AA and glucose utilization during the day-cycle which is characterized by greater efficiency of nutrient metabolism in the first hours of the day (Koopmans et al., 2006). In addition, protein diet variation over the day (higher protein diet level in the morning and lower in the evening) increase insulin levels in the morning with consequent better body weight gain (Xie et al., 2015). Due to different CP diet levels for the SEQ programs (17% less for HF/LP than CON diet), we understand that the supply of different concentrations of CP together with hormonal variation throughout the day, might have improve the efficiency of nutrient utilization with consequence effect on ADG.

In the finishing phase, the results of the present study confirm that using HF/LP diets compared to CON improve feed intake (+12%) and weight gain (+10%) for pigs raised under high ambient temperatures (Figures 1A and 1B). These results are consistent with previous literature (Spencer et al., 2001) who also reported performance improvement in heat-stressed finishing pigs fed high-fat diets. Between this diets, the difference of NE intake was 20% (19.23 vs. 24.11 MJ/d) which also resulted in higher values of fat mass and fat mass gain for HF/LP program (Figures 1A and 1B; period 2 and the entire period). These results are in agreement with those obtained by Campbell et al. (1988) and Le Bellego et al. (2001), who observed higher deposition of fat in the carcass with increase of NE levels in the diets. However, the literature shows great variability of results related to NE intake and pig carcass composition. Possibly factors such as genetic, age at slaughter, environmental temperature and condition of housing (group or individual) may interfere in the composition of the carcass. In addition, the HF/LP diet was formulated for higher NE content (see Table 1) which possibly accentuated these results. Although fed pigs HF/LP had better ADG and ADFI, different NE content of the diet should be better evaluated in other studies to try to optimize energy cost of gain compared to control diet. However, understanding how pigs housed group respond for energy in heat stress may not be simple due many factors that can affect it (animal competition for feed, space, sanitary conditions, etc.).

In this finishing phase, in contrast to our results where the feed efficiency was not affected by feeding programs, Le Bellego et al. (2002) observed better feed conversion (6.5%) for pigs of 64 to 100 kg BW fed with low CP and high NE diets compared to control diet under temperature of 29°C. In another study, Kerr et al. (2003) did not observe difference in feed efficiency between diets varying CP and EM content (16% CP; 12% CP + AA and 12% CP) for pigs kept at a constant temperature of 33°C. In fact, many factors affected feed efficiency and diet energy concentration is only one of them (Patience, 2012). Thus, this seems to be more pertinent to define feed efficiency in terms of net energy cost of gain (Patience, 2012).

In our experimental conditions, a worst energy cost of gain was observed for fed pigs HF/LP diet (+14%) compared to CON (Figure 1E; finishing phase). This is consequence of the lower increased of ADG compared to ADFI (10 vs 12%) associated with the greater NE intake (+25%) for HF/LP fed pigs, which also resulted in greater fat mass e gain for this program (Figures 2A and 2B; last and entire periods). This worst energy cost for fed pigs HF/LP diet disagrees with previous results of Le Bellego et al. (2002), who reported identical values for energy cost compared control diet (protein and energy) for pigs housed individually in an constant temperature over the day (29°C). The present results of this study were obtained with pigs housed in group and, unlike of Le Bellego et al. (2002), the ADFI for pigs fed HF/LP improved compared to those fed CON diet (Figure 1A). It should be noted that pigs housed individually compared to group showed different performances (Bornett et al., 2000) and this difference may become more evident in a limiting conditions (temperature, nutrition, health challenge, etc). As the animals at high temperature have their consumption regulated by the heat dissipation capacity, the higher ADFI of the pigs when fed by the HF/LP diet can be explained by the lower caloric increment of the lipid besides the increase of the palatability of the feed with the inclusion of soybean oil (Wolp et al., 2012; Kerr et al. 2015). In addition, sequential feeding allowed reducing NE intake (-12%) and had a similar ADFI, ADG and energy cost of gain in relation to HF/LP program for finishing phase (Figure 1). This better result on NE intake can be explained by the simple fact of fed pigs with different diets over the 24h period (control diet at 22ºC and HF/LP at 30ºC period; SEQ) which allowed reducing it for whole period.

In relation to lean mass and gain, results of this study showed that were not affect by feeding programs. This results agree with previous result of Silva et al. (2017), who reported similar lean mass gain for pigs fed higher AA diet content in the morning (from 1200h pm to 1200h am) and lower in the evening (from 1200h am to 1200h pm) compared to control diet over the day. Consequently similar results for lean mass and gain obtained in our study for HF/LP and CON programs, indicating that reduction of up to 4% CP with adequate amino acid supplementation does not affect the lean composition of the pig carcass under cyclic heat stress.

Effect of a genetic line on performance and body composition of pig exposed to a cyclic heat stress

The results of this study showed that genetic lines responded differently to cyclic thermal stress. The higher AFI per hour at 30ºC and higher final BW (11%) could suggest that line B is more tolerant to heat stress. Indeed the difference between lines was accentuated over the experimental period, with higher ADFI, ADG, NE intake and consequently, higher values of fat mass and gain for line B. Therefore, the lower capacity of thermoregulation of line A associated to the different CP level of feeding programs, may have determined the interaction for CP intake in finishing phase. The different results of CP intake between programs only for line A in this experimental phase, may be due to their lower heat tolerance and consequently, their greater sensitivity to composition of diets.

In relation to body composition, although lean gain was similar between lines in periods 1 and 2, higher loin depth was observed in the period 2 for the animals of the line A indicating that the genetic potential of this line (higher % Pietrain) was expressed although of ambient temperature oscillations. In the entire period was observed higher lean mass gain for line B (+ 7%), but this may have occurred due to the higher performance and adaptation to the experimental condition of this line.

Regardless of the genetic line, fed pigs SEQ presented higher final body weight (+ 4.0 kg per animal) compared to CON, but with a significant effect on fat mass and gain. Despite this, the SEQ could be an interesting economic advantage in the pork market which benefits the total BW instead of leaner meat. However, studies are needed to evaluate the dynamics of the feeding behavior of pigs fed SEQ program, in order to better understand how the variation of NE and CP during day could contribute with the composition of the pig carcass under heat stress. In relation to HF/LP, due the similarity of the performance results, the present experiment confirms that the supply of different diets adjusted to the variation of the temperature and hormonal secretion along the day (SEQ feeding system), can attenuate the negative effects of cyclic heat in pig production. As feed represents great part of the cost in pig production, sequential program could also be economically advantageous compared to HF/LP because it allow reduce the NE diet concentration (less oil).

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Table 1. Ingredients and composition of experimental diets[1]

	Growing 1		Growing 2		Finishing	
Itens	Control	HF/LP	Control	HF/LP	Control	HF/LP
Ingredient composition, as-fed basis, %						
Corn	62.09	67.69	64.20	70.61	69.74	76.38
Soybean meal	32.96	22.01	29.89	19.13	24.30	13.70
Wheat bran	2.00	2.00	2.96	2.00	3.29	2.00
Soy oil		4.00		4.00		4.00
Dicalcium phosphate	0.90	1.25	0.91	1.28	0.80	1.15
Calcium carbonate	0.90	0.74	0.91	0.73	0.82	0.66
Salt	0.40	0.41	0.38	0.38	0.35	0.36
Dextrin	0.50	0.50	0.50	0.50	0.50	0.50
Biolys, 56.6%		0.72		0.70		0.66
DL-Methionine		0.09		0.09		0.08
L-Threonine		0.25		0.24		0.23
L-Tryptophan		0.09		0.09		0.08
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05
Mineral and Vitamin Premix[2]	0.20	0.20	0.20	0.20	0.15	0.15
Chemical composition[3]						
ME, MJ/kg	13.30	14.23	13.29	14.29	13.36	14.33
NE, MJ/kg	8.87	9.97	9.10	10.05	8.93	10.04
Dry matter, %	88.02	88.51	88.00	88.48	87.93	84.40
Crude protein, %	22.49	18.86	21.44	17.81	19.48	15.85
Ether extract, %	3.95	7.95	4.01	8.02	4.15	8.16
Ash, %	5.39	4.92	5.27	4.78	4.78	4.29
Crude fiber, %	3.89	3.34	3.89	3.35	3.77	3.21
SID[4] Lys calculated, %	0.98	1.10	0.85	0.94	0.73	0.82
SID Met calculated, %	0.28	0.32	0.25	0.28	0.21	0.24
SID Met + Cys calculated, %	0.55	0.62	0.48	0.53	0.42	0.48
SID Thr calculated, %	0.59	0.66	0.52	0.57	0.47	0.52
SID Trp calculated, %	0.18	0.20	0.15	0.17	0.13	0.15
Lysine content (g/Mcal of NE)	4.62	4.62	3.91	3.91	3.42	3.42
Ca, %	0.66	0.66	0.59	0.59	0.52	0.52
Total P, %	0.43	0.44	0.38	0.38	0.33	0.34
Digestible P, calculated, %	0.23	0.23	0.20	0.20	0.17	0.17

[1]The diets were: control and high-fat/low-crude (HF/LP).
[2] Mineral vitamin supplement (per kg of diet): Vit. A (5.250 UI); Vit. D3 (750 UI); Vit. E (11 UI); Vit. K3 (1.5 mg); Vit. B1 (1 mg); Vit. B2 (2,4 mg); Vit. B6 (1 mg); Niacin (30 mg); Pantothenic acid (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).
[3]All calculated values were by the EvaPig software program (version 1.3.1.4; INRA, Saint-Gilles, France).
[4]SID = standardized ileal digestible.

Table 2. Effect of feeding programs, genetic lines and period of day on the average feed intake (AFI) of growing and finishing pigs[1]

Feeding Programs[2]:	Control		HF/LP		SEQ C/HF/LP		Line A		Line B		RSD[3]	Statistical Analysis[4]						
Period of day:	22ºC	30ºC	22ºC	30ºC	22ºC	30ºC	22ºC	30ºC	22ºC	30ºC		FP	GL	TP	FP×GL	FP×TP	GL×TP	FP×GL×TP
Growing phase 1																		
AFI, kg/hour	68.20	64.37	66.01	72.80	67.65	78.03	66.65	65.68	67.92	77.79	6.44	0.13	0.01	0.07	0.76	0.08	0.03	0.48
Growing phase 2																		
AFI, kg/hour	79.63	77.65	81.89	92.18	89.05	82.78	80.52	65.46	85.21	104.26	4.22	0.29	<0.01	0.69	0.84	0.33	<0.01	0.12
Finishing phase																		
AFI, kg/hour	102.07	87.32	95.73	106.48	89.08	108.36	96.24	76.82	92.34	127.29	5.93	0.67	<0.01	0.16	0.87	0.07	<0.01	0.51
Total experiment																		
AFI, kg/hour	82.36	77.83	84.80	86.92	81.94	89.74	81.20	69.38	81.85	103.30	6.36	0.18	<0.01	0.09	0.73	0.71	<0.01	0.23

[1]Initial weight as covariate was significant for all variables; $P<0.01$.
[2]Control: control diet from 0000 to 2400 h; HF/LP: high-fat/low-crude diet from 0000 to 2400 h; Sequential Feeding (C/HF/LP): 1800 to 1000 h control diet and 1000 to 1800 h high-fat/low-crude diet.
[3]Residual Standard Deviation.
[4]Data were analyzed using a linear MIXED model including the fixed effects of covariate, feeding programs (FP; n=3), genetic line (GL; n=2), time-period of day (TP; n=2), the interaction between FP and GL (FP×GL), FP and PD (FP×TP), GL and PD (GL×TP) and triple interaction FP×GL×TP.

Table 3. Effect of feeding program and genetic lines on performance of growing and finishing pigs[1]

Item	Feeding programs[2]: Genetic Line:	Control A	Control B	HF/LP A	HF/LP B	SEQ C/HF/LP A	SEQ C/HF/LP B	RSD[3]	Statistical Analysis (P-value)[4] FP	GL	FP×GL
Number of animals		13	13	13	13	13	13	-			
Growing phase 1 (30 to 45 kg BW; 21 days)									FP	GL	FP×GL
Initial BW, kg		29.42	30.07	29.89	30.51	29.44	30.22	1.51	0.52	0.07	0.98
Final BW, kg		43.48	47.27	44.51	47.18	44.15	47.79	3.37	0.82	<0.01	0.83
ADFI, kg/d		1.47	1.63	1.46	1.67	1.53	1.72	0.22	0.41	<0.01	0.94
ADG, kg/d		0.675	0.823	0.702	0.793	0.702	0.839	0.10	0.75	<0.01	0.70
Feed efficiency, kg/kg		0.46	0.51	0.48	0.48	0.46	0.49	0.71	0.71	0.04	0.33
CP intake, kg/d		0.331	0.366	0.281	0.310	0.259	0.326	0.05	<0.01	<0.01	0.46
NE intake, MJ/d		13.05	14.40	14.54	16.65	15.22	16.79	2.14	<0.01	<0.01	0.82
Energy cost of gain, MJ NE/kg gain		19.35	17.53	20.70	21.00	21.70	20.00	2.22	<0.01	0.04	0.44
Growing phase 2 (45 to 75 kg BW; 28 days)											
Initial BW, kg		43.48	47.27	44.51	47.18	44.15	47.79	3.37	0.82	<0.01	0.83
Final BW, kg		68.56	76.15	72.34	76.94	72.14	78.50	5.73	0.18	<0.01	0.67
ADFI, kg/d		1.71	2.12	1.88	2.24	1.92	2.29	0.30	0.09	<0.01	0.94
ADG, kg/d		0.896	1.032	0.992	1.051	1.000	1.098	0.12	0.04	<0.01	0.55
Feed efficiency, kg/kg		0.54	0.49	0.54	0.47	0.53	0.48	0.20	0.80	<0.01	0.72
CP intake, kg/d		0.366	0.457	0.340	0.394	0.389	0.456	0.06	<0.01	<0.01	0.58
NE intake, MJ/d		15.59	19.23	18.91	22.49	17.83	21.88	3.04	<0.01	<0.01	0.96
Energy cost of gain, MJ NE/kg gain		17.20	18.61	18.92	21.31	17.77	19.90	2.04	<0.01	<0.01	0.69
Finishing phase (45 to 105 kg BW; 35 days)											
Initial BW, kg		68.56	76.15	72.34	76.94	72.14	78.50	5.73	0.18	<0.01	0.67
Final BW, kg		97.02	110.20	104.46	113.66	103.25	112.68	8.57	0.07	<0.01	0.66
ADFI, kg/d		1.99	2.32	2.21	2.60	2.09	2.44	0.28	0.01	<0.01	0.94
ADG, kg/d		0.816	0.977	0.920	1.047	0.892	0.980	0.10	0.04	<0.01	0.54
Feed efficiency, kg/kg		0.41	0.42	0.42	0.41	0.43	0.40	0.20	0.26	0.85	0.22
CP intake, kg/d		0.386	0.456	0.347	0.414	0.250	0.445	0.09	0.02	<0.01	0.03

NE intake, MJ/d	17.75	20.71	22.08	26.14	19.62	23.50	2.73	<0.01	<0.01	0.75
Energy cost of gain, MJ NE/kg gain	21.78	21.24	24.16	24.95	22.01	24.08	2.07	<0.01	0.06	0.09
Total experiment (84 days)										
Initial BW, kg	29.42	30.07	29.89	30.51	29.44	30.22	1.51	0.52	0.07	0.98
Final BW, kg	97.02	110.20	104.46	113.66	103.25	112.68	8.57	0.07	<0.01	0.66
ADFI, kg/d	1.72	2.02	1.86	2.17	1.84	2.15	0.40	0.07	<0.01	1.00
ADG, kg/d	0.796	0.944	0.875	0.967	0.864	0.972	0.17	0.11	<0.01	0.56
Feed efficiency, kg/kg	0.48	0.48	0.49	0.46	0.47	0.46	0.28	0.23	0.85	0.22
CP intake, kg/d	0.361	0.426	0.324	0.374	0.299	0.409	0.08	<0.01	<0.01	0.08
NE intake, MJ/d	15.46	18.11	18.60	21.80	17.55	20.72	3.86	<0.01	<0.01	0.89
Energy cost of gain, MJ NE/kg gain	19.50	19.19	21.42	22.35	20.49	21.35	2.79	<0.01	<0.01	0.32

[1]Initial weight as covariate was significant for all variables; *P*<0.01.

[2]Control: control diet from 0000 to 2400 h; HF/LP: high-fat/low-crude diet from 0000 to 2400 h; Sequential Feeding (C/HF/LP): 1800 to 1000 h control diet and 1000 to 1800 h high-fat/low-crude diet.

[3]Residual Standard Deviation.

[4]Data were analyzed using a linear MIXED model including the fixed effects of covariate, feeding programs (FP; n=3), genetic line (GL; n=2), the interaction between FP and GL (FP×GL) and phases were specified as a repeated effect.

Table 4. Effect of feeding program and genetic lines on body composition of growing and finishing pigs[1]

Item	Feeding programs [2]: / Genetic Line:	Control A	Control B	HF/LP A	HF/LP B	SEQ C/HF/LP A	SEQ C/HF/LP B	RSD[3]	Statistical Analysis (P-value)[4] FP	GL	FP×GL
Number of animals		8	8	8	8	8	8	-			
Initial Condition											
Average BW, kg		29.57	30.00	29.95	30.47	29.60	30.12	1.39	0.63	0.13	0.55
Lean Mass, kg		25.32	25.83	25.45	25.65	25.35	25.50	1.30	0.81	0.33	0.72
Fat Mass, kg		4.10	4.01	4.14	4.36	4.11	4.23	1.26	0.14	0.29	0.25
Backfat thickness, mm		8.96	8.28	8.76	8.57	8.85	8.51	0.89	0.98	0.22	0.73
Loin depth, mm		35.60	33.93	36.61	36.28	36.52	32.68	3.54	0.28	0.17	0.38
Period 1 (0 to 35 d)											
Average BW,kg		56.55	58.10	57.93	59.40	57.58	62.49	4.23	0.21	0.08	0.43
Lean Mass, kg		47.38	48.57	48.13	48.51	47.98	51.36	3.29	0.31	0.17	0.42
Fat Mass, kg		8.40	8.82	8.97	10.04	8.85	10.28	3.10	0.03	0.01	0.43
Backfat thickness, mm		11.18	11.08	11.92	11.95	12.18	12.53	1.46	0.07	0.79	0.91
Loin depth,mm		47.37	47.75	50.50	50.59	51.33	51.33	5.88	0.13	0.94	0.87
Lean mass gain, kg/d		0.62	0.68	0.64	0.67	0.64	0.74	0.07	0.21	0.06	0.30
Fat mass gain, kg/d		0.12	0.14	0.14	0.16	0.14	0.17	0.03	0.03	<0.01	0.53
Feed conversion, MJ NE/kg lean mass		23.31	23.12	25.56	27.76	26.10	26.57	2.33	<0.01	0.18	0.40
Period 2 (36 to 70 d)											
Average BW,kg		85.32	93.04	91.44	92.69	90.32	98.83	7.12	0.11	0.02	0.31
Lean Mass, Kg		71.00	76.47	74.66	73.40	74.16	79.28	5.55	0.27	0.12	0.18
Fat Mass, Kg		13.07	15.21	15.40	17.87	14.86	18.10	2.06	<0.01	<0.01	0.74
Backfat thickness, mm		13.30	15.18	14.88	17.01	15.72	17.33	2.23	0.02	0.02	0.95
Loin depth,mm		70.00	63.06	69.06	63.88	67.57	65.33	4.36	0.57	0.03	0.64
Lean mass gain, kg/d		0.68	0.80	0.76	0.71	0.75	0.80	0.03	0.36	0.17	0.20
Fat mass gain, kg/d		0.13	0.18	0.18	0.22	0.17	0.22	0.03	<0.01	<0.01	0.81
Feed conversion, MJ NE/kg lean mass		23.71	23.42	25.49	30.85	26.11	30.48	3.38	<0.01	<0.01	0.04
Total period (0-70 d)											
Initial BW, kg		29.57	30.00	29.95	30.47	29.60	30.12	1.39	0.63	0.13	0.55
Final BW, kg		85.32	93.04	91.44	92.69	90.32	98.83	7.12	0.11	0.02	0.31
Lean mass gain, kg/d		0.65	0.74	0.70	0.69	0.69	0.77	0.06	0.22	0.03	0.10

Fat mass gain, kg/d	0.13	0.16	0.16	0.19	0.15	0.20	0.03	<0.01	<0.01	0.76
Feed conversion, MJ NE/kg lean mass	23.45	23.38	25.63	29.21	26.26	28.53	2.97	<0.01	<0.01	0.04

[1]Initial weight as covariate was significant for all variables; *P*<0.01.
[2]Control: control diet from 0000 to 2400 h; HF/LP: high-fat/low-crude diet from 0000 to 2400 h; Sequential Feeding (C/HF/LP): 1800 to 1000 h control diet and 1000 to 1800 h high-fat/low-crude diet.
[3]Residual Standard Deviation.
[4]Data were analyzed using a linear MIXED model including the fixed effects of covariate, feeding programs (FP; n=3), genetic line (GL; n=2), the interaction between FP and GL (FP×GL) and phases were specified as a repeated effect

Figure 1. a) ADFI (kg/d), b) ADG (kg/d), c) CP intake (kg/d), d) NE intake (MJ/d) and e) Energy cost of gain (MJ NE/kg gain) in each experimental phases (growing 1, growing 2 and finishing phase) and entire experiment.

Figure 2. a) Fat mass (kg), b) Fat mass gain (kg/d) and c) Feed conversion (MJ NE/kg lean mass gain) in each period and entire experiment.

Figure 1.

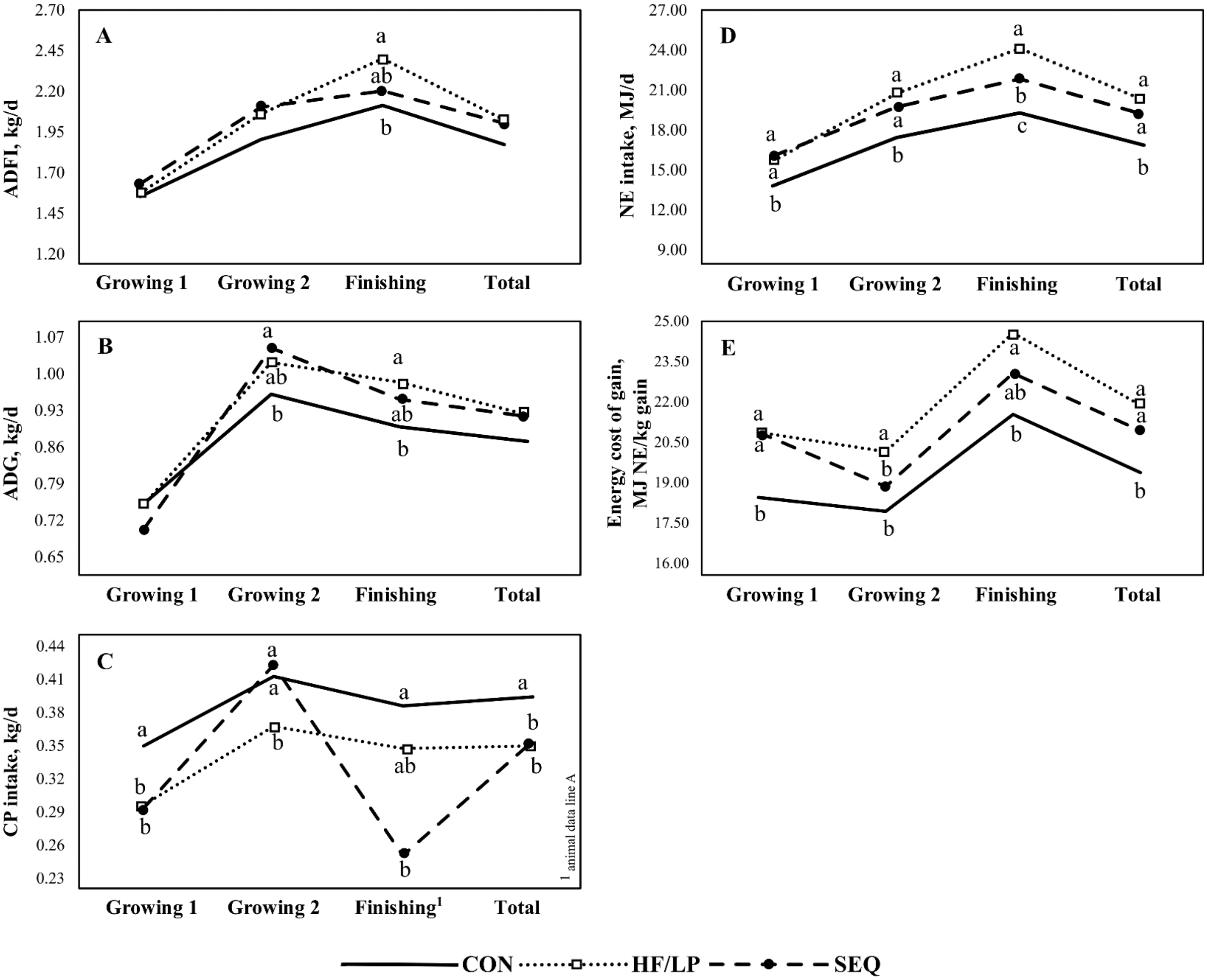
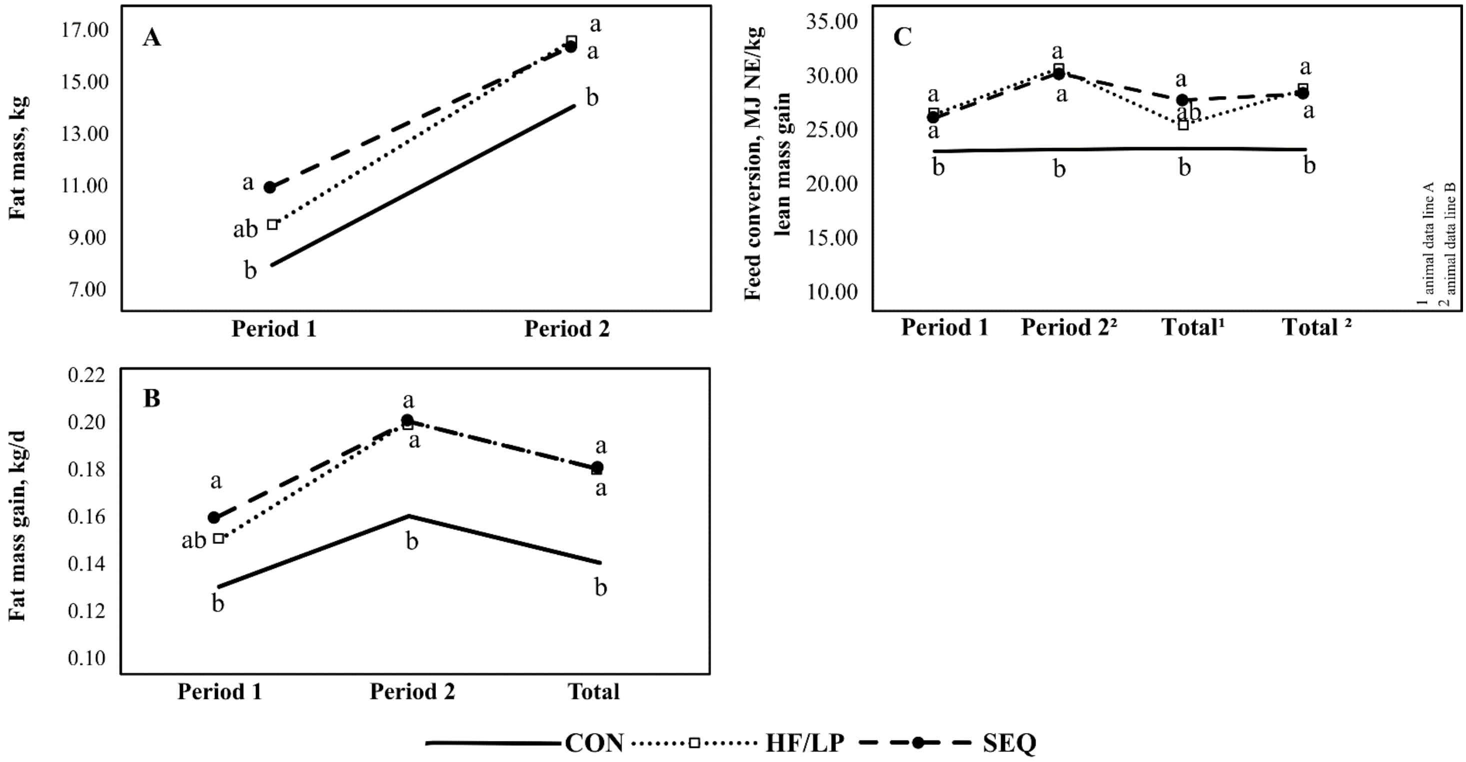

A
ADFI, kg/d
2.70
2.45
2.20
1.95
1.70
1.45
1.20
Growing 1
Growing 2
Finishing
Total
a
ab
b
B
ADG, kg/d
1.07
1.00
0.93
0.86
0.79
0.72
0.65
Growing 1
Growing 2
Finishing
Total
a
ab
b
a
ab
b
C
CP intake, kg/d
0.44
0.41
0.38
0.35
0.32
0.29
0.26
0.23
Growing 1
Growing 2
Finishing1
Total
1 animal data line A
D
NE intake, MJ/d
27.00
24.00
21.00
18.00
15.00
12.00
9.00
Growing 1
Growing 2
Finishing
Total
E
Energy cost of gain, MJ NE/kg gain
25.00
23.50
22.00
20.50
19.00
17.50
16.00
Growing 1
Growing 2
Finishing
Total
CON
HF/LP
SEQ

Figure 2.


A
Fat mass, kg
17.00
15.00
13.00
11.00
9.00
7.00
Period 1
Period 2
B
Fat mass gain, kg/d
0.22
0.20
0.18
0.16
0.14
0.12
0.10
Period 1
Period 2
Total
C
Feed conversion, MJ NE/kg lean mass gain
35.00
30.00
25.00
20.00
15.00
10.00
Period 1
Period 2²
Total¹
Total ²
1 animal data line A
2 animal data line B
CON
HF/LP
SEQ