

**UNIVERSIDADE ESTADUAL PAULISTA JÚLIO DE MESQUITA FILHO
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL**

**IMPACTO DO DESAFIO SANITÁRIO NA VARIABILIDADE
DO DESEMPENHO, DA COMPOSIÇÃO CORPORAL E DAS
EXIGÊNCIAS DE LISINA DE SUÍNOS EM CRESCIMENTO**

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Jaboticabal, 10 de julho de 2023

DADOS CURRICULARES DO AUTOR

Manoela Trevisan Ortiz, filha de Derlis Adelfo Ortiz Bareiro Jr e Eliana Cristina Trevisan Ortiz, nasceu no dia 12 de novembro de 1997, na cidade de Porto Ferreira, São Paulo, Brasil. Em abril de 2016, ingressou no curso de Zootecnia na Universidade Federal de Lavras – UFLA, graduando-se em junho de 2021. Em agosto de 2021, iniciou o Mestrado em Ciência Animal na Universidade Estadual Paulista – UNESP, câmpus de Jaboticabal, sob orientação do Prof. Dr. Luciano Hauschild e coorientação dos pós-doutorandos Dr. Antonio Diego Brandão Melo e Dr. Danilo Alves Marçal, dedicando-se à área de Produção e Nutrição de Suínos. No dia 11 de agosto de 2022, submeteu-se à banca para qualificação da Dissertação e no dia 10 de julho de 2023 realizou sua defesa.

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“Deixo o futuro criar raiz”.

5 a seco

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É possível reencontrar o propósito.

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IMPACTO DO DESAFIO SANITÁRIO NA VARIABILIDADE DO DESEMPENHO, DA COMPOSIÇÃO CORPORAL E DAS EXIGÊNCIAS DE LISINA DE SUÍNOS EM CRESCIMENTO

RESUMO – Com a intensificação da produção animal, é cada vez mais comum que suínos estejam expostos a patógenos ou alojados em condições inadequadas – situações que podem levar à ativação do sistema imune. Objetivou-se com esse estudo avaliar o impacto de um desafio sanitário na variabilidade do desempenho, da composição corporal e das exigências de lisina (Lys) de suínos em crescimento. Foram utilizados dados de uma população de 40 fêmeas suínas em crescimento ($25,4 \pm 3,7$ kg) alojadas em condições sanitárias (CS) adequadas ($n = 20$), ou em CS precárias e inoculadas com 5 mL de 2×10^9 UFC de *Salmonella* Typhimurium ($n = 20$). Dados de consumo de ração diário (CRD) e de peso corporal (PC) dessa população foram ajustados utilizando uma função polinomial de primeira ordem. A deposição proteica (DP) foi estimada em função do ganho de peso diário (GPD) estimado e da relação entre DP e GPD observados. Essas variáveis foram integradas em uma equação fatorial para estimar as exigências diárias de Lys de cada indivíduo. Durante a primeira semana experimental, animais em CS precárias apresentaram maior temperatura retal e maior e menor concentrações séricas de haptoglobina e albumina, respectivamente, que os animais em CS adequadas. O desafio sanitário também reduziu o PC final em 9,5%, o GPD em 20,3%, o CRD em 8,5% e a DP em 20,3%. Os suínos alojados em CS precárias tiveram exigências de Lys 19,4% menores no 28º dia experimental e 9,9% menores durante toda fase experimental em relação aos alojados em CS adequadas. Em relação à variabilidade individual, suínos em CS precárias apresentaram CV maiores para PC (2,6%), GPD (9,7%), CRD (6,0%), DP (9,7%) e exigências de Lys (7,9%) durante o experimento. Além disso, as diferenças entre as exigências de Lys dos animais mais e menos exigentes foram de 17% nas CS precárias e de 14% nas CS adequadas. Esses resultados demonstram como um desafio sanitário pode reduzir PC, GPD, CRD, DP e as exigências de Lys, além de aumentar a variabilidade do desempenho, da composição corporal e das exigências nutricionais de suínos em crescimento.

Palavras-chave: condições sanitárias, deposição proteica, método fatorial, *Salmonella* Typhimurium

IMPACT OF A SANITARY CHALLENGE ON THE VARIABILITY OF PERFORMANCE, BODY COMPOSITION AND LYSINE REQUIREMENTS OF GROWING PIGS

ABSTRACT – Due to the intensification of livestock production, it became common that pigs are exposed to pathogens or housed in poor sanitary conditions (SC) – situations that can lead to the activation of the immune system. This study aimed to evaluate the impact of a sanitary challenge in the variability of growth performance, body composition and lysine (Lys) requirements of growing pigs. Data from 40 growing female pigs (25.4 ± 3.7 kg) that were either raised in Good SC ($n = 20$) or raised under poor SC and inoculated with 5 mL of 2×10^9 UFC of *Salmonella* Typhimurium ($n = 20$) were used. Individual daily feed intake (DFI) and BW data from this population were smoothed by linear regression. Protein deposition (PD) was estimated considering predicted average daily gain (ADG) and the relation between observed PD and ADG. These variables were integrated in a factorial equation to estimate daily individual Lys requirements. During the first experimental week, animals in the poor SC had higher rectal temperature, higher haptoglobin and lower albumin concentrations than the animals in the Good SC. The sanitary challenge also caused reductions of 9.5% for final BW, 20.3% for ADG, 8.5% for DFI, and 20.3% for PD. For poor SC pigs, Lys requirements were 19.4% lower on day 28 and 9.9% lower throughout the experimental phase if compared to Good SC pigs. When analyzing individual pig variability, it was observed that animals in the poor SC had higher CV for final BW (2.6%), ADG (9.7%), DFI (6.0%), PD (9.7%), and for Lys requirements (7.9%) throughout the phase. Moreover, the differences in Lys requirements between the least and the most demanding pigs were 17% in the poor SC, and 14% in the Good SC. These results demonstrate how a sanitary challenge may reduce BW, ADG, DFI, PD, and Lys requirements; it also enhances the variability of growth performance, body composition and Lys requirements of growing pigs.

Keywords: factorial method, protein deposition, *Salmonella* Typhimurium, sanitary conditions

CAPÍTULO 1 – Considerações gerais

1 INTRODUÇÃO

A intensificação da produção animal, aliada à menor capacidade das raças modernas de se adaptar a perturbações ambientais, pode comprometer ainda mais os resultados de suínos expostos a condições de desafio sanitário (Campos *et al.*, 2017). Esses desafios podem estimular a ativação do sistema imune (SI) e, consequentemente, a produção de citocinas pró-inflamatórias, situações que podem impactar negativamente o desempenho e a eficiência desses animais (Johnson, 2012).

Dentre os desafios sanitários que podem acometer suínos em crescimento, os mais comuns de serem encontrados a campo são condições precárias de alojamento, desafios entéricos e respiratórios (Rodrigues *et al.*, 2022), ou uma combinação destes. As infecções respiratórias geralmente são subclínicas, já que é comum que o SI atue apenas nos tecidos afetados (Blanchard *et al.*, 1992). Apesar disso, as perdas econômicas podem ser expressivas, principalmente aquelas relacionadas a penalidades no abate (Rodrigues *et al.*, 2022). As infecções entéricas são mais observadas na fase pós-desmame, porém, as salmoneloses podem persistir durante toda a fase produtiva (Rodrigues *et al.*, 2022). As salmoneloses afetam as células do epitélio intestinal, o que geralmente resulta em diarreia (Luppi, 2017). Já as condições precárias de alojamento podem ser resultado da falta de protocolos de limpeza e desinfecção, de falhas no cronograma vacinal, ou uma combinação de fatores (van der Meer *et al.*, 2020), e podem expor os animais a agentes biológicos, como vírus e bactérias, e não-biológicos, como gases nocivos e poeira (Rodrigues *et al.*, 2022).

A magnitude do impacto do desafio sanitário no desempenho animal depende da fase produtiva e do tipo de desafio (Rodrigues *et al.*, 2022). Dados de estudos recentes, conduzidos com suínos em diversos estágios de crescimento e com diferentes tipos de desafio sanitário, mostram que o estímulo do SI pode resultar em reduções entre 6 e 26% no peso corporal (PC) final, 22 e 37% no ganho de peso diário (GPD), 9 e 22% no consumo de ração diário (CRD) e 4 e 10% na eficiência alimentar (van der Meer *et al.*, 2020; Rodrigues *et al.*, 2021; da Cunha Valini *et al.*, 2023).

Entretanto, as informações sobre como um desafio sanitário pode aumentar a

variabilidade do desempenho e das exigências nutricionais de uma população são escassas na literatura. Sabendo que esses cenários são comuns de serem encontrados a campo e que podem representar consequências econômicas na produção suinícola, é importante estudar e mensurar a magnitude do impacto do desafio sanitário na variabilidade do desempenho e das exigências nutricionais dos suínos.

2 REVISÃO DE LITERATURA

2.1 Impactos do desafio sanitário

Saúde animal pode ser definida como a ausência de doença a partir de exames clínicos e testes diagnósticos (Petersen *et al.*, 2004). Entretanto, os suínos podem sofrer inflamações subclínicas difíceis de serem detectadas, mas que também podem ativar o SI, impactando no consumo e crescimento (Le Floc'h *et al.*, 2004). Por isso, classificar o “status” de saúde desses animais é importante para estabelecer quais estratégias nutricionais serão utilizadas durante a fase de crescimento.

A exposição a patógenos e antígenos estimula o SI do animal e gera respostas como aumento na temperatura e o catabolismo de proteínas corporais (Black *et al.* 1999). Essas respostas resultam de alterações metabólicas induzidas pela cascata de citocinas, podendo incluir aumento da degradação de proteínas ou diminuição da síntese proteica, aumento da síntese hepática de proteínas de fase aguda (PFA) e aumento da desaminação de aminoácidos (AA) glicogênicos - dentre eles os AA essenciais metionina, cisteína, triptofano (Trp), isoleucina, valina, histidina e fenilalanina (Klasing & Johnstone, 1991; Lochmiller & Deerenberg, 2000; Le Floc'h *et al.*, 2004; van der Peet-Schwering *et al.*, 2019).

Condições de desafio podem resultar na redução do consumo de ração, da absorção intestinal de AA e da síntese de proteínas destinadas ao crescimento animal, além de estimular perdas de proteínas endógenas no trato gastrointestinal (TGI), degradação de proteínas para utilização no SI e oxidação de AA em órgãos e tecidos, o que impacta negativamente o crescimento animal, além de alterar as exigências de AA (van der Peet-Schwering *et al.*, 2019).

Parte dessas mudanças pode ser explicada pela produção das citocinas pró-inflamatórias. Elas estimulam a secreção de hormônios relacionados à saciedade, como a colecistocinina (que age periféricamente em seus receptores e diminui o

consumo e o esvaziamento gástrico), glucagon e insulina (Wong e Pinkney, 2004). As citocinas também podem alterar a função de barreira das “tight junctions” (Turner, 2009) e, na tentativa de manter a integridade da barreira intestinal, a síntese de mucinas aumenta, diminuindo, dessa forma, a disponibilidade de AA para outras funções corporais (Faure *et al.*, 2007; Dharmani *et al.*, 2009).

Pastorelli *et al.* (2012) conduziram uma meta-análise com 122 artigos para avaliar o impacto de seis diferentes modelos de desafio sanitário no consumo de ração e no crescimento de suínos. Os tipos de desafio sanitário consistiam em desafio por lipopolissacarídeos (LPS), condições precárias de alojamento, infecções bacterianas no TGI, doenças respiratórias, micotoxicoses e infecções por parasitas. Nos cenários de condições precárias de alojamento ou situação de infecções bacterianas no TGI, o maior impacto sobre o desempenho dos animais foi devido a mudanças na eficiência alimentar (74,4% e 74,8%, respectivamente) relacionadas a um impacto na exigência de manutenção e na digestibilidade de nutrientes. Contudo, nos outros tipos de desafio a queda do desempenho pôde ser explicada, em grande parte, pela redução no consumo de ração.

2.1.1 Digestibilidade de aminoácidos

O TGI passa por alterações fisiológicas durante uma inflamação sistêmica, que podem incluir mudanças na motilidade, na permeabilidade, na produção de mucinas e na expressão de algumas enzimas digestivas (Turner, 2009; Rakhshandeh & de Lange, 2011). As citocinas pró-inflamatórias envolvidas nas respostas imunes podem aumentar a síntese de mucinas – e, consequentemente, reduzir a disponibilidade de AA para outras funções corporais (Faure *et al.*, 2007) – e diminuir a motilidade do TGI e a absorção de nutrientes (Rakhshandeh & de Lange, 2011). A inflamação sistêmica também altera as funções pancreáticas e diminui a produção de enzimas digestivas (Fitzal *et al.*, 2003); entretanto, estudos mais recentes não confirmaram esse tipo de resposta (Rakhshandeh & de Lange, 2011).

A ativação imune sistêmica pode ter impacto negativo na digestibilidade de energia, proteína e AA, com reduções na ordem de 1 a 7% (Williams *et al.*, 1997a; Le Floc'h *et al.*, 2014; Kampman-van de Hoek *et al.*, 2016; van der Meer *et al.*, 2016; van der Meer, 2017). Essa redução pode ser explicada pelo impacto da ativação imune na secreção e atividade de enzimas digestivas e na reciclagem de proteínas endógenas

(van der Klis & Jansman, 2002).

Por outro lado, existem estudos que confirmam que desafios sanitários sistêmicos não afetam a habilidade do TGI de hidrolisar e absorver nutrientes (Sandberg *et al.*, 2007), consequentemente não afetando sua digestibilidade. Rakhshandeh *et al.* (2014) observaram que o desafio por injeção intramuscular de LPS de *Escherichia coli* (a cada 48 h, por 7 dias) em suínos em crescimento não impactou a digestibilidade aparente ileal ou a digestibilidade estandardizada ileal de proteína bruta, lisina (Lys), metionina, metionina + cisteína, treonina, arginina, isoleucina ou leucina.

2.1.2 Eficiência de utilização dos aminoácidos

A ineficiência na utilização de AA é um processo que pode ser explicado pelo catabolismo inevitável de AA (Moughan, 1999) ou devido à variação do potencial de crescimento entre os animais (Pomar *et al.*, 2003). Para a Lys, essa ineficiência é estimada em 25% e está associada a perdas endógenas basais e de renovação do TGI (NRC, 2012). Para esse AA, a eficiência de utilização de 75% foi estimada a partir de estudos com suínos em crescimento em condições controladas (Bikker *et al.*, 1994; Mohen *et al.*, 2000), é independente do PC (Doumad *et al.*, 1996; Mohen *et al.*, 2000) e pode ser maior em animais com maior potencial de crescimento (Mohen *et al.*, 2000).

A resposta imune afeta de forma distinta a utilização de cada AA. Williams *et al.* (1997b) conduziram um estudo com leitões de 10 a 25 kg expostos ou não a vetores de transmissão de antígenos e recebendo 4 níveis diferentes de Lys na dieta. Os autores observaram que a eficiência de utilização da Lys para retenção de nitrogênio (N) corporal nos suínos com baixa ou alta ativação do SI foi similar entre os grupos.

Rakhshandeh *et al.* (2014) investigaram a eficiência de utilização dos AA sulfurados (SAA) metionina e cisteína para deposição proteica de suínos em crescimento, desafiados ou não com LPS de *E. coli* e recebendo 5 níveis diferentes de metionina e cisteína na dieta. Os resultados indicam que o desafio diminuiu a deposição proteica dos animais, mas não influenciou a eficiência de utilização dos SAA.

De Ridder *et al.* (2012) conduziram um estudo para estimar a eficiência de utilização de Trp para deposição de proteína de suínos em crescimento, desafiados ou não com LPS de *E. Coli*. Nesse estudo foi observado que, nos três primeiros dias

após o desafio, tanto a ativação do SI como a diminuição dos níveis de Trp dietético diminuíram a deposição proteica dos animais. Isso pode indicar uma menor eficiência na utilização de Trp durante a resposta imune e, conseqüentemente, aumento das exigências de Trp durante o período de desafio. Para atingir uma deposição proteica de 65 g/dia, foram calculadas exigências de Trp de 1,09 g/dia para suínos não desafiados e de 1,17 g/dia para os desafiados (de Ridder *et al.*, 2012).

Outros AA envolvidos na resposta imune podem ter sua demanda e eficiência de utilização alteradas durante um desafio. A glutamina atua na expressão de genes relacionados à redução do estresse oxidativo (Wang *et al.*, 2008), serve como substrato para o ciclo de Krebs e como fonte de energia para os enterócitos (Watford, 2015). Já a treonina tem papel crítico na manutenção da função da barreira intestinal, por ser o AA mais abundante na mucina (Bortoluzzi *et al.*, 2018). Além disso, o metabolismo de ambos AA produz ácidos graxos (AG) de cadeia curta como metabólitos – o acetato, que atua como precursor da síntese de outros AGs, e o butirato, fonte de energia para os colonócitos (Davila *et al.*, 2013; Pieper *et al.*, 2016; Oliphant & Allen-Vercoe, 2019; Chalvan-Demersay *et al.*, 2021). A arginina participa da modulação da microbiota de matrizes (Luise *et al.*, 2020) e de suínos em terminação (Hu *et al.*, 2019), além de atuar na síntese de poliaminas (Chalvan-Demersay *et al.*, 2021) envolvidas na síntese proteica e de DNA (Portune *et al.*, 2016; Beaumont & Blachier, 2020).

2.1.3 Manutenção

A exigência de manutenção de um animal pode ser definida como a quantidade de nutrientes necessária para manutenção de funções vitais do corpo, com o objetivo de que o balanço entre os ganhos e as perdas basais seja zero (ARC, 1981). Algumas funções de manutenção incluem metabolismo basal, renovação celular e manutenção do potencial de membrana e do equilíbrio ácido-base (Velayudhanet *et al.*, 2015). Durante um desafio sanitário, os animais podem produzir quantidades significativas de compostos nitrogenados e proteínas (citocinas, anticorpos, PFA), que podem alterar as exigências de AA para manutenção (van der Peet-Schwering *et al.*, 2019).

Nos experimentos conduzidos por Rakhshandeh *et al.* (2014) para verificar o impacto do estímulo do SI por LPS de *E. coli* na digestibilidade e utilização de SAA, os autores encontraram um aumento de 23% da exigência de SAA para manutenção de

animais desafiados em relação aos animais controle. Uma possível explicação para esse aumento é a utilização da cisteína como precursora da síntese de glutatona, um composto não-proteico envolvido na resposta imune (Rakhshandeh *et al.*, 2014; van der Peet-Schwering *et al.*, 2019). Contudo, no caso do Trp, de Ridder *et al.* (2012) observaram que, embora os animais desafiados tiveram uma redução na eficiência de utilização desse AA para deposição proteica, as exigências de manutenção não diferiram entre os grupos.

As PFA têm mais fenilalanina, tirosina, Trp e treonina em sua composição quando comparadas às proteínas corporais e musculares. As imunoglobulinas também têm mais Trp, leucina, valina, cisteína, treonina e serina em sua composição em relação à proteína corporal ou muscular (van der Peet-Schwering *et al.*, 2019). Por isso, durante o desafio imune, o balanço entre a menor necessidade de AA destinados ao crescimento (menor deposição proteica) e a maior necessidade de AA destinados à ativação do SI é o que determina o impacto da resposta imune nas exigências de AA.

2.1.4 Desempenho animal

A queda do desempenho animal durante um desafio sanitário pode ser explicada, em grande parte, pela redução no consumo de ração (Pastorelli *et al.*, 2012). Entretanto, diferentes tipos de desafio sanitário envolvem mecanismos distintos (Pastorelli *et al.*, 2012). Nos desafios associados a patógenos e antígenos, a redução no consumo provavelmente acontece devido à resposta de fase aguda mediada por citocinas (Klasing, 1998). Já condições precárias de alojamento resultam em constante ativação do SI, devido ao aumento da pressão microbiana, o que também pode levar à redução do apetite dos animais (Klasing *et al.*, 1991).

Em mamíferos, as citocinas pró-inflamatórias interleucina 1 (IL-1), interleucina 6 (IL-6) e fator de necrose tumoral (TNF) têm efeito anorético (Klasing & Johnstone, 1991). Apesar dos mecanismo de ação não serem completamente elucidados, a IL-1 provavelmente reduz o consumo por meio de prostaglandinas (Hellernstein *et al.*, 1989), mas o mesmo não acontece com a IL-6 (McCarthy, 2000). Já o TNF- α age no hipotálamo reduzindo o consumo e favorecendo o catabolismo de substratos energéticos à partir da ativação de vias de sinalização inflamatórias (Romanatto *et al.*, 2007).

Em um experimento com diferentes condições de alojamento (boas vs precárias),

leitões alojados em condições precárias apresentaram redução de 26,3% no PC ao final do experimento, de 9% no CRD, de 22% no GPD e de 10% na eficiência alimentar quando comparados a leitões alojados em condições sanitárias (CS) boas (van der Meer *et al.*, 2020). Leitões inoculados com $3,3 \times 10^9$ UFC de *Salmonella* Typhimurium (ST) tiveram reduções de 6,6% no PC final, 34,6% no GPD e 21,9% no CRD quando comparados aos animais controle (Rodrigues *et al.*, 2021). Fêmeas suínas em crescimento inoculadas com 2×10^9 UFC de ST apresentaram reduções de 22% no CRD, 37% no GPD e 4% na eficiência alimentar na primeira semana após o desafio (da Cunha Valini *et al.*, 2023).

2.1.5 Síntese e deposição proteica

Das citocinas pró-inflamatórias, a IL-1, a IL-6 e o TNF- α são as principais envolvidas na regulação do metabolismo proteico (Le Floc'h *et al.*, 2004), podendo agir localmente, para ampliar a resposta imune celular, ou sistemicamente, alterando o comportamento, metabolismo e secreções neuroendócrinas (Johnson, 1997). O TNF- α é responsável pelo catabolismo do músculo esquelético (Klasing, 1988), pelo aumento da absorção hepática de AA e pela síntese de PFA (Andus *et al.*, 1991). A IL-1 é responsável pela síntese de PFA e inibição da síntese de proteína no músculo (Le Floc'h *et al.*, 2004), e a IL-6 age em sinergismo com a IL-1 na síntese de PFA no fígado, sendo considerada o mediador primário da resposta de fase aguda, além de estimular a absorção de AA pelos hepatócitos (Johnson, 1997; Le Floc'h *et al.*, 2004).

Em estudo conduzido com suínos de 6 a 112 kg, Williams *et al.* (1997c) investigaram a influência de diferentes níveis de ativação do SI (baixo e alto) e de diferentes níveis de Lys dietética na eficiência da utilização de Lys para máximo ganho de PC, deposição proteica e eficiência alimentar. Os níveis de Lys necessários para maximizar o ganho de PC e a eficiência alimentar foram de 2 a 6 g maiores para os animais não desafiados. Os autores observaram que as alterações metabólicas induzidas pela resposta imune perduraram até as fases mais tardias do desenvolvimento (crescimento e terminação), quando se espera que os animais sejam menos susceptíveis a antígenos patogênicos. A maior exigência de Lys dietética para suínos não desafiados está associada à maior deposição proteica e não a uma menor eficiência de utilização desse AA, o que já havia sido observado pelos autores em estudos anteriores (Williams *et al.*, 1997a; Williams *et al.*, 1997b).

Rakhshandeh *et al.* (2014) verificaram que animais com SI ativado necessitaram de 8% a mais de SAA diariamente para máxima síntese proteica em relação aos animais que não tiveram ativação do SI. Essa diferença se deu pela menor capacidade de deposição proteica dos animais desafiados (Williams *et al.* 1997b).

Stuart *et al.* (2015; 2016) e Rakhshandeh (2015) conduziram estudos para mensurar o impacto do desafio com o vírus da Síndrome Reprodutiva e Respiratória de Suínos (PRRSV) e com LPS de *E. coli* na deposição proteica e no metabolismo de fêmeas suínas. O desafio por PRRSV reduziu a deposição proteica em 35%, enquanto o desafio por LPS não teve efeito significativo na deposição.

Van der Meer (2017) também observou redução de 10% da deposição proteica entre suínos alojados em diferentes CS. Animais alojados em CS boas depositaram 124 g/dia, enquanto animais alojados em CS ruins depositaram 111 g/dia.

De forma geral, pode-se afirmar que a resposta imune reduz a deposição proteica em suínos e que essa redução varia de acordo com o tipo de desafio aplicado (van der Peet-Schwering *et al.*, 2019).

2.2 Utilização do Método Fatorial na avaliação do impacto do desafio na exigência nutricional

Em uma população de suínos, a exigência de um nutriente pode ser definida como a quantidade necessária deste nutriente para atender propósitos específicos de produção, como taxa de crescimento e conversão alimentar ideais, ou máximo lucro (Hauschild *et al.*, 2010). Atualmente, dois métodos têm sido propostos para estimar a exigência de nutrientes em suínos em crescimento (Remus *et al.*, 2019): método dose resposta e método fatorial.

No método fatorial, as exigências diárias são obtidas para um indivíduo, em um período específico, ao combinar as exigências calculadas para manutenção e produção (Pomar *et al.*, 2003). Quando se objetiva extrapolar as exigências nutricionais estimadas pelo método fatorial para uma população, é comum utilizar o indivíduo médio como referência (Hauschild *et al.*, 2010). Entretanto, embora os níveis recomendados de Lys para minimizar a conversão alimentar sejam próximos aos níveis de exigência do animal médio, o ganho de peso é maximizado quando a exigência do indivíduo no percentil 82 é considerada (Hauschild *et al.*, 2010). Porém, os impactos de um desafio sanitário não são iguais em todos os suínos de uma

população, o que pode resultar em aumento na variabilidade do desempenho de um grupo desafiado. Nesse caso, é possível avaliar o impacto do desafio sobre a variabilidade das exigências nutricionais a partir das exigências individuais estimadas. Entretanto, as equações disponíveis na literatura não levam em consideração como o desafio sanitário pode afetar as exigências de manutenção e de crescimento dos suínos, o que dificulta ainda mais a escolha do animal que melhor representa a população nesse cenário.

3 OBJETIVO GERAL

Nossa hipótese é de que o desafio sanitário altera a variabilidade do desempenho, da composição corporal e das exigências nutricionais de suínos. Levando em consideração que a *Salmonella* spp. é o patógeno entérico mais presente na cadeia suinícola e que condições precárias de alojamento são comuns a campo, objetiva-se com esse trabalho avaliar o impacto de um desafio sanitário com ST e baias sujas na variabilidade do desempenho, da composição corporal e das exigências de lisina de suínos em crescimento.

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CAPÍTULO 2 – A salmonella-challenge impacts the variability of performance, body composition and lysine requirements variability of growing pigs under poor housing conditions

ABSTRACT – Reduced performance for pigs under challenging sanitary conditions (SC) is a well-documented response, but little is known about its effect on the variability in performance and nutrient requirements. This study aimed to evaluate the impact of a sanitary challenge in the variability of growth performance, body composition and lysine (Lys) requirements of growing pigs. Data of 40 animals from a population of 120 growing female pigs (25.4 ± 3.7 kg) that were either raised in good sanitary condition (SC) ($n = 20$) or inoculated with 5 mL of 2×10^9 UFC of *Salmonella* Typhimurium (ST) and raised under poor SC (ST + Poor; $n = 20$) in a 28-day trial. For this study, only the pigs fed the control diet and which had their body composition measured were used. Observed individual daily feed intake (DFI) and BW data from this population were smoothed by linear regression. Protein deposition (PD) was estimated considering predicted average daily gain (ADG) and the relation between observed PD and ADG. These variables were integrated in a factorial equation to estimate daily individual Lys requirements. During the first experimental week, animals in the ST + Poor SC had higher rectal temperature, higher haptoglobin and lower albumin concentrations than the animals in the good SC. The sanitary challenge also caused reductions of 9.5% for final BW, 20.3% for ADG, 8.5% for DFI, and 20.3% for PD. For ST + Poor SC pigs, Lys requirements were 19.4% lower on day 28 and 9.9% lower throughout the experimental phase if compared to Good SC pigs. When analyzing individual pig variability, it was observed that animals in the ST + Poor SC had higher CV for final BW (+19.2%), ADG (+41.5%), DFI (+22.0%), PD (+41.5%), and for Lys requirements (+29.0%) throughout the phase. Moreover, the differences in Lys requirements between the least and the most demanding pigs were 17% in the ST + Poor SC, and 14% in the Good SC. These results demonstrate that despite the sanitary challenge reducing BW, ADG, DFI, PD, and Lys requirements, it enhances the variability of growth performance, body composition and Lys requirements of growing pigs.

Key words: factorial method, protein deposition, *Salmonella* Typhimurium, sanitary conditions

5 INTRODUCTION

The intensification of livestock production has increased animal exposure to pathogens and, consequently, immune system (IS) activation. Poor housing conditions results in reduced feed intake and growth when compared to healthy animals (Le Floc'h *et al.*, 2004), and may lead to expressive productive and economic losses. During immune response activation, amino acids are redistributed away from muscle protein synthesis towards tissues involved in inflammatory and immune responses (Le Floc'h *et al.*, 2004). In swine production, the most common enteric challenge is caused by *Salmonella* spp. (Rodrigues *et al.*, 2022), which stimulates changes in metabolic pathways that result in increased syntheses of acute-phase protein (APP) and immunoglobulin (Rodrigues *et al.*, 2021).

According to Williams *et al.* (1997c), growing pigs with lower IS activation may need 6 g more of daily lysine (Lys) than animals with higher IS activation. This greater Lys requirement is not due to a less efficient Lys utilization, but because of their greater anabolic endocrine status and capacity to deposit protein (Williams *et al.*, 1997b; 1997c), that can be up to 40% higher than in animals with a more intense activation of the IS (Williams *et al.*, 1997a). When thinking from an individual point of view, the animal's capacity of resistance and resilience to a sanitary challenge are also factors that could explain the variation in Lys requirements. Nevertheless, previous studies have investigated how these factors affect feed intake (Sandberg *et al.*, 2006; Nguyen-Ba *et al.*, 2020a; Nguyen-Ba *et al.*, 2020b), leaving a lack of information to relate them with the variability in Lys requirements.

For pig populations, nutrient requirements can be defined as the amount of nutrients needed for specified production purposes (Hauschild *et al.*, 2010). The factorial method is usually composed of the sum of maintenance and production requirements (Remus *et al.*, 2019), and can be used to obtain daily requirements for an individual pig, that best represents the population, at a specific point in time (Hauschild *et al.*, 2010). In healthy animals, the variability in the Lys requirements can be associated with individual differences in the efficiency of Lys utilization (Remus *et al.* 2021) or with differences in the lipid to protein deposition ratio (Bertolo *et al.*, 2005). However, for sanitary challenged pigs there are no information about the impact of the IS activation on the variability of the demand for Lys in a pig population.

We hypothesized that a sanitary challenge may affect the variability in Lys requirements of growing pigs, as well as animal performance, immune response, body composition and protein deposition (PD). Therefore, the aim of this study was to evaluate the impact of a sanitary challenge on the variability of growth performance, body composition and Lys requirements for growing pigs.

6 MATERIAL AND METHODS

All experimental procedures applied in the trial followed the Brazilian National Council of the Control of Animal Experimentation (CONCEA) and were reviewed and approved (protocol no. 4784/20) by the Ethical Committee on Animal Use (CEUA) of Faculdade de Ciências Agrárias e Veterinárias (FCAV/UNESP – Jaboticabal, SP, Brazil).

6.1 Animals, housing and experimental design

The simulation was conducted using a subset of 40 growing female pigs with an initial body weight (BW) of 25.4 ± 3.7 kg from a large study ($n = 120$ pigs) previously described by da Cunha Valini *et al.* (2023).

The criteria adopted to choose these 40 animals was the ones fed the control diet and which had their body composition measured in the original study. It consisted in a project evaluating the effects of increased dietary tryptophan (Trp), threonine (Thr), and methionine (Met) supplementation on performance, body composition, metabolism, and immune responses of group-housed growing pigs under challenging sanitary condition (SC).

Briefly, the pigs were blocked by initial BW and housed into two solid concrete floor rooms equipped with exhaust fans and evaporative pad cooling system set to keep a constant ambient temperature of 22 °C during the whole experimental period of 28 days. There were ten nipple drinkers and four automated and intelligent precision feeders (AIPF, Exafan, Spain) in each room. After arriving at the experimental site, a transponder with a unique identification was put at each animal's right ear using a tag plier. When the animal entered the AIPF, the machine recognized the transponder, which was a plastic button tag containing passive transponders of radio frequency identification (Andretta *et al.*, 2016).

For the sanitary challenge, each room represented a different SC [Good and

Salmonella Typhimurium (ST) + Poor]. At the ST + Poor SC, a sanitary challenge was imposed to the animals – the pigs were inoculated with a single oral dose of 5 mL containing 2×10^9 CFU of ST, manure from a commercial pig farm with low health status was spread on the floor at the beginning of the experiment, and the room was not washed throughout the experimental period. At the Good SC, the room was washed daily and the team members were only allowed to wear clean clothes and footwear before entering the room. Animals were not inoculated with ST, but received orally 5 mL of the growth medium used to culture the ST, the Brain Heart Infusion, in order to simulate the same management conditions applied at the ST + Poor SC.

The control diet consisted in a corn-soybean meal-based diet supplemented with crystalline amino acids, minerals and vitamins, formulated according to the National Research Council (2012) for pigs from 25 to 50 kg and was offered *ad libitum* throughout the trial.

6.2 Data collection

All animals were individually weighted weekly (days 0, 7, 14, 21 and 28) and had their individual daily feed intake (DFI) recorded by the AIPFs. Pigs from each treatment (n = 10) whose BW was closest to the group's average BW in the beginning of the trial (D0) had their body composition measured at the first and the last day of the experiment by dual-energy X-ray absorptiometry (DXA, GE Lunar Prodigy Advance, Madison, USA). Body protein content was estimated by converting the lean mass obtained with DXA into its chemical equivalent, using the equation described by Pomar and Rivest (1996).

In order to validate the sanitary challenge, during the first experimental week the rectal temperature was measured daily (6:00 AM) and on day 7, after 6 hours of fasting, blood was collected from the jugular vein for haptoglobin and albumin dosage. Blood samples were collected in 8-mL serum tubes (BD Vacutainer; NJ, USA) and allowed to clot for 1 h. After centrifugation for 10 min at $3000 \times g$ at 4 °C (Novatecnica, NT 835, Piracicaba, SP, Brazil), serum samples were collected and stored at -80 °C for further analysis. Serum haptoglobin and albumin concentrations were determined in sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by da Cunha Valini *et al.* (2023).

6.3 Individual animal characterization

Individual predicted daily body weight (BW) was estimated by a linear function of time fitted to data from the five weightings of each pig. Daily gain (DG) was estimated by the first derivative of this function. Individual daily feed intake (DFI) was estimated by a linear function of time fitted to data from daily feed consumption, excluded the data from days in which the animals were fasted (e.g.: inoculation or DXA days).

PD was predicted as a function of predicted DG, as follows:

$$PDp = [DGp \times PD/DG] \times 1000,$$

where PDp is the predicted protein deposition, DGp is the predicted DG and PD/DG is the relation between observed PD and observed DG.

6.4 Lysine requirements prediction

Daily Lys requirements were obtained based on the equations described by Remus *et al.* (2019), adapted from van Milgen *et al.* (2008), as follows:

$$Lys_{req} \text{ (g/day)} = [(0.313 \times DFI \times 0.88)_{(1)} + (0.0045 \times BW^{0.75})_{(2)} + (0.0239 \times BW^{0.75})_{(3)}] + [(0.07 \times PD)_{(4)}/0.72_{(5)}],$$

where: ₍₁₎Basal endogenous losses; ₍₂₎Integument Lys losses; ₍₃₎Basal renewal of body protein losses; ₍₄₎Lys concentration on the protein deposited; ₍₅₎Efficiency of Lys utilization. Predicted PD values were used.

Lys requirements in function of Lys concentration in the diet were obtained as follows:

$$Lys \text{ (\%)} = (Lys_{req}/DFIp)/10,$$

where DFIp is the predicted DFI.

6.5 Statistical analysis

Statistical analysis were performed in Minitab® 18 Statistical Software (2017). Mean values of rectal temperature, APP concentration (haptoglobin and albumin), and predicted performance variables (BW, ADG, DFI, and PD) were analyzed using Student's t-test for 2 samples. Differences between means were considered significant

if $P \leq 0.05$. Using Pearson's correlation test, we evaluated the correlation coefficient between the observed and the predicted PD of each SC. We also evaluated the correlation coefficient between haptoglobin concentration, PD and Lys (%), using data from both SC. The correlations were considered significant if $P \leq 0.05$. To evaluate the impact of a sanitary challenge on the variability of Lys requirements, each animal's average Lys requirements (%) went through CV and normal distribution analysis.

7 RESULTS

One animal in the Good SC was excluded from the database due to health problems not related to the study.

7.1 *Impact of the sanitary challenge on immune response and growth performance*

Animals in the ST + Poor SC had higher ($P = 0.05$) rectal temperatures when compared to the animals in the Good SC (Figure 1).

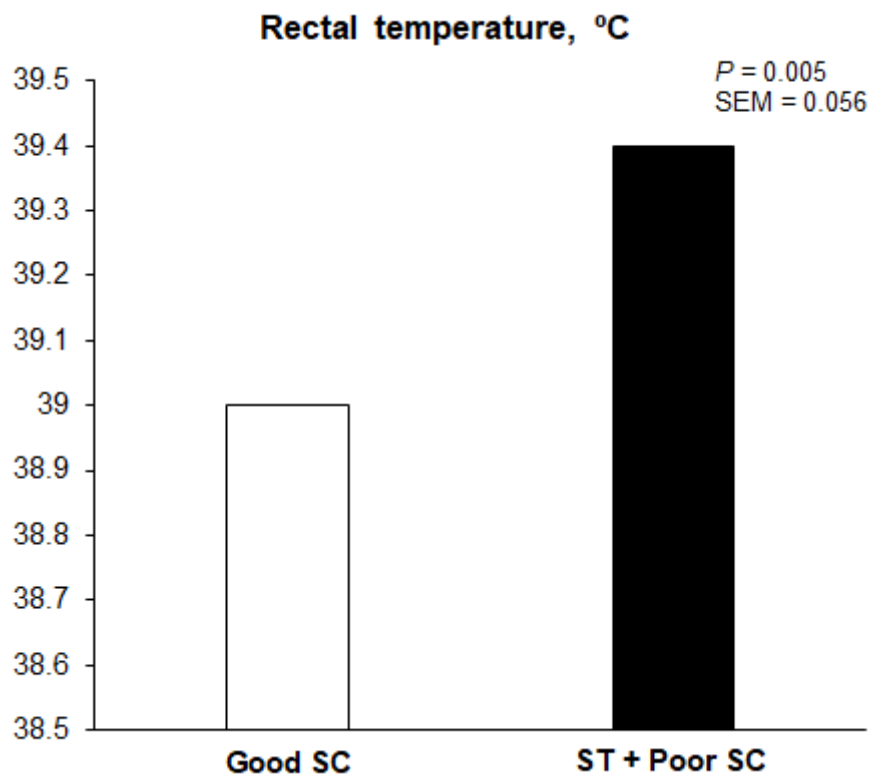


Figure 1. Mean rectal temperature (°C) of growing pigs in different sanitary conditions throughout the first experimental week (days 1 to 7 after the sanitary challenge).

The concentrations of the serum APP showed that the animals in the ST + Poor SC presented a higher ($P < 0.001$) concentration of haptoglobin (Figure 2a) and a lower ($P = 0.001$) concentration of albumin than the animals in the Good SC (Figure 2b).

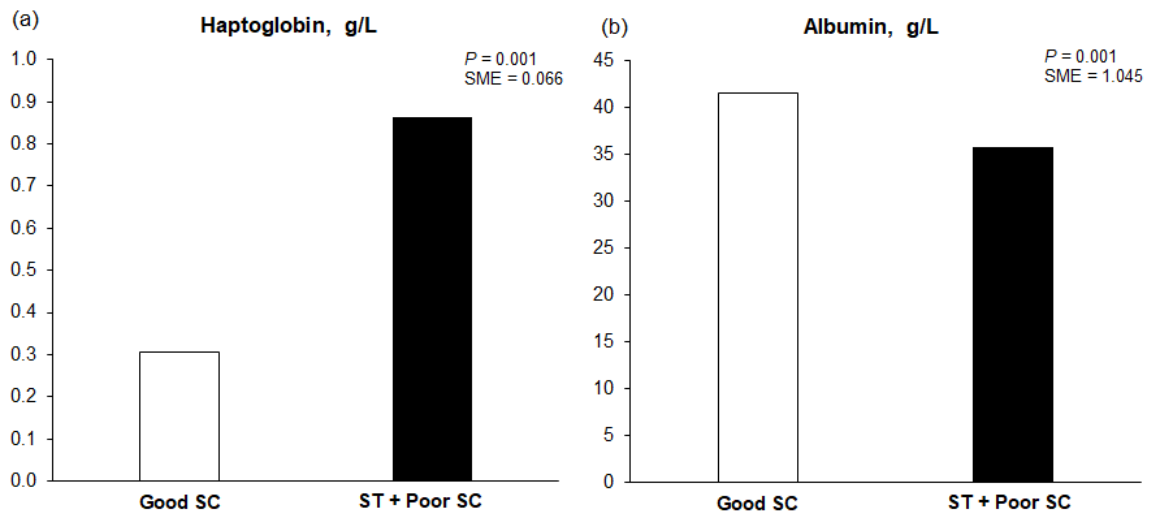


Figure 2. Mean acute phase proteins serum concentration (g/L) of growing pigs in different sanitary conditions on day 7 of experiment. (a) Concentration of haptoglobin; (b) Concentration of albumin.

The sanitary challenge also worsened predicted animal performance. Predicted final BW was lower ($P = 0.015$) in the ST + Poor SC when compared to the Good SC (41.44 vs 45.77 kg, respectively). At the Good SC, predicted ADG was 779 g, with the lowest and highest values being 632 and 991 g, respectively. At the ST + Poor SC, predicted ADG was lower ($P < 0.001$), of 621 g, and the lowest and highest values were 379 and 885 g, respectively. At the Good SC, predicted average daily PD was 129 g, varying from 104 to 164 g, and at the ST + Poor SC, predicted average daily PD was lower ($P < 0.001$), of 103 g, with the lowest and highest values being 71 and 146 g, respectively. Nevertheless, predicted DFI was similar ($P = 0.176$) in both SC through the trial (1.536 kg/d in the Good SC and 1.406 kg/d in the ST + Poor SC).

7.2 Impact of sanitary challenge on individual pig variability

7.2.1 Performance

The CV for final BW were 10.93% in the Good and 13.52% in the ST + Poor SC (Figures 3a and 3b).

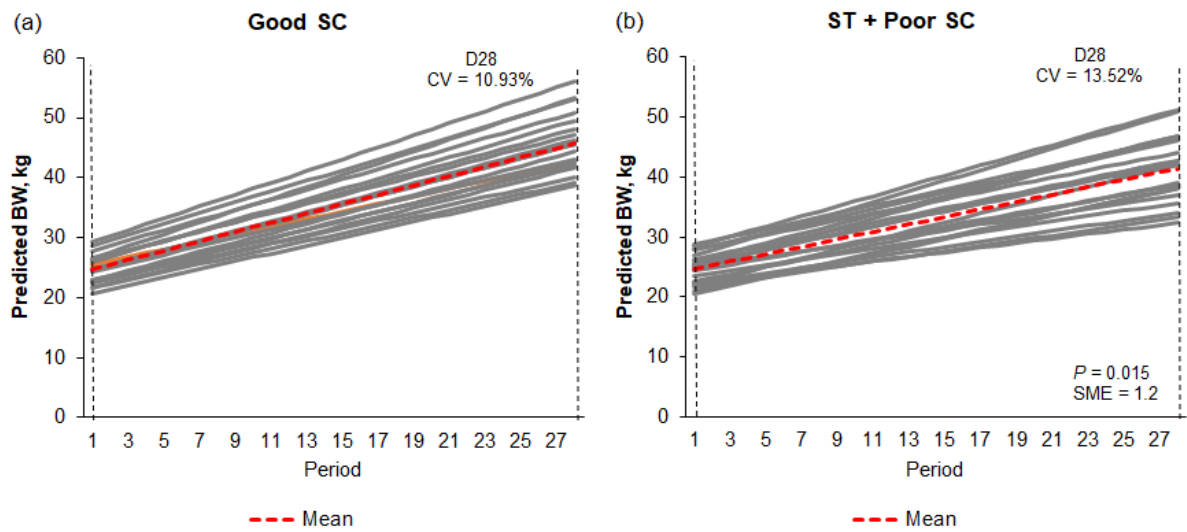


Figure 3. Predicted individual BW (kg) of growing pigs throughout the 28-day experimental period. a) Good sanitary condition; b) ST + Poor sanitary condition.

For DFI, the CV in the experimental period was 20.71% in the Good SC and 26.74% in the ST + Poor SC (Figures 4a and 4b).

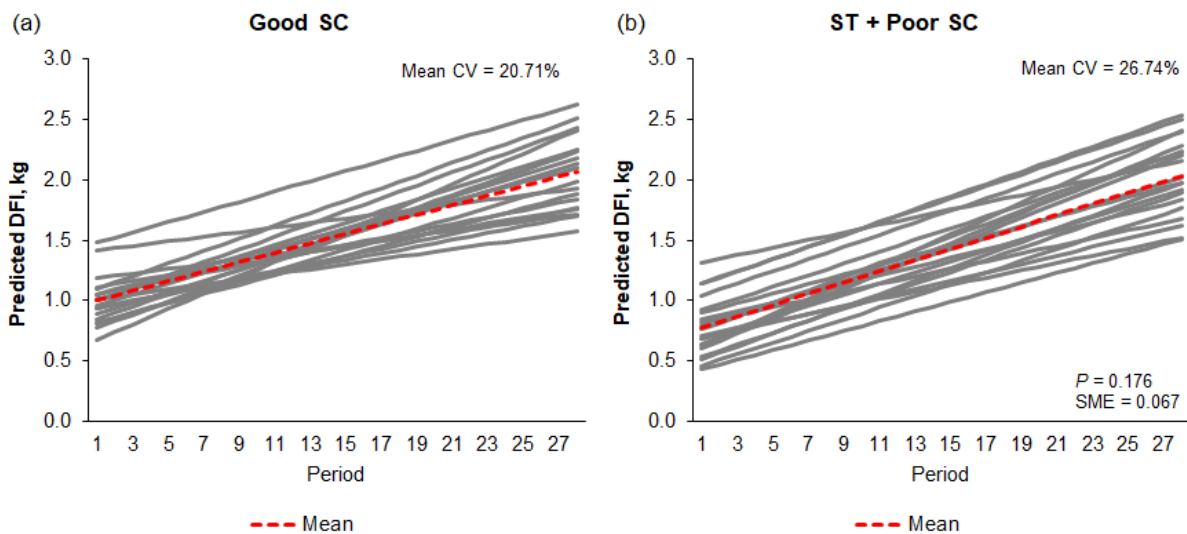


Figure 4. Predicted individual DFI (kg) of growing pigs throughout the 28-day experimental period. a) Good sanitary condition; b) ST + Poor sanitary condition.

The CV were similar for both ADG and PD, being of 13.7% in the Good SC and 23.4% in the ST + Poor SC (Figure 5).

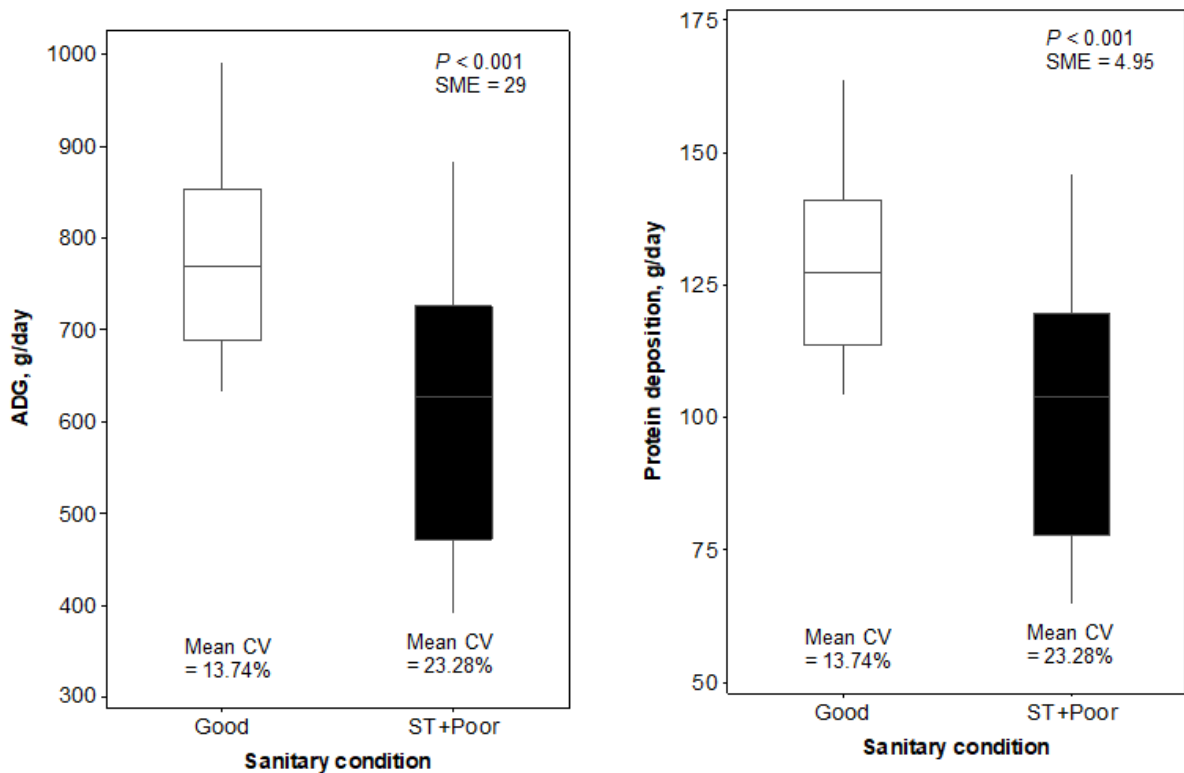


Figure 5. Predicted ADG (g/d) and protein deposition (g/d) of growing pigs in different sanitary conditions throughout the 28-day experimental period.

7.2.2 Lysine requirements

Predicted individual Lys requirements are shown in Figures 6a and 6b. For the Good SC, the average Lys requirements were 1.21%, 1.04% and 0.67% of the diets on D3 (beginning of acute response phase), D8 (beginning of chronic response phase) and D28, respectively, while the requirements for the animal in the 80-percentile of the population were 1.38% on D3, 1.13% on D8 and 0.72% on D28. Also, in this SC, the most demanding animal required 1.03% of the diet and the least demanding one required 0.80% throughout the experimental phase. At the ST + Poor SC, mean Lys requirements were 1.21% on D3, 0.96% on D8 and 0.54% on D28, and the 80-percentile animal in the population required 1.39%, 1.03% and 0.60% of Lys on D3, D8 and D28, respectively. Furthermore, in this SC the most and least demanding animals required 0.98% and 0.71% of the diet, respectively, throughout the experiment. On the first experimental week (days 1 to 7), the CV was 7.5% at the Good SC, and 11.5% at the ST + Poor SC, while from days 8 to 28, the CV were 13.5% and 17.9% at the Good and ST + Poor SC, respectively.

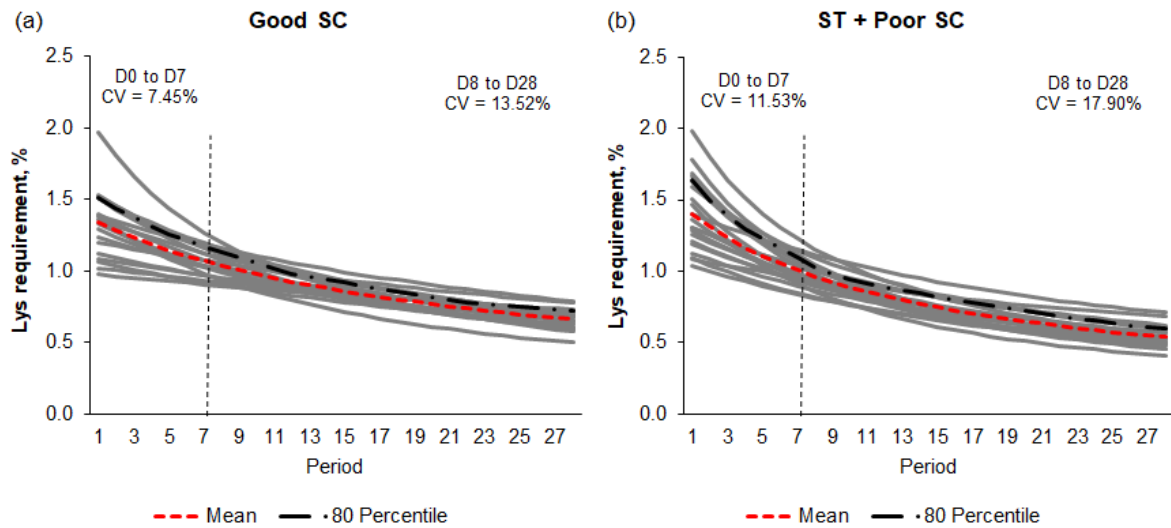


Figure 6. Predicted individual Lys requirements of growing pigs throughout the 28-day experimental period. a) Good sanitary condition; b) St + Poor sanitary condition.

Lys requirement data from both SC were normally distributed (Figures 7a and 7b). In the ST + Poor SC, the mean Lys requirement was lower (0.827% vs 0.913%, respectively) while the SD was greater than in the Good SC ($\pm 0.08\%$ vs $\pm 0.07\%$, respectively). For Good SC pigs, the +1 SD was 14% greater compared to -1 SD, while for ST + Poor SC this difference was 17%.

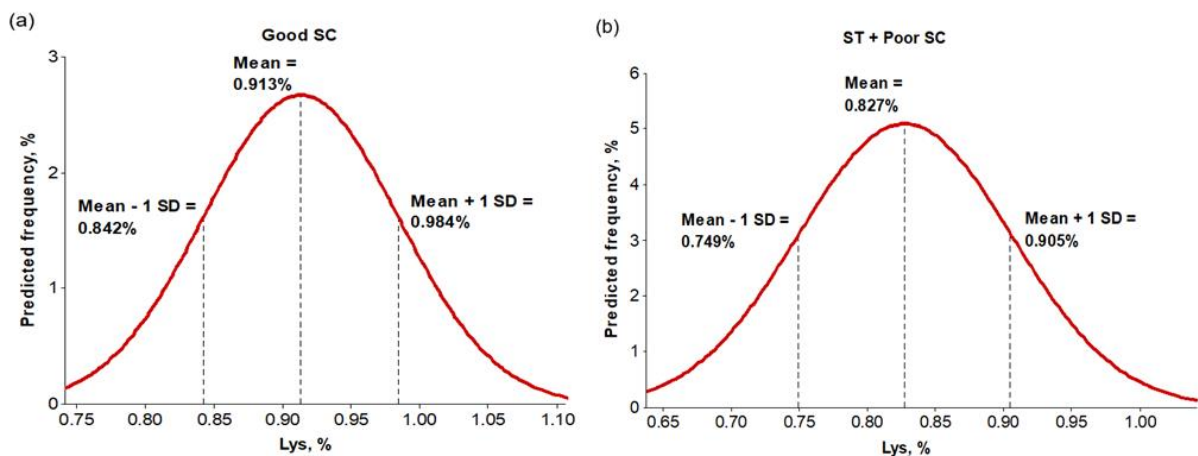


Figure 7. Frequency distribution of the predicted average individual Lys requirements of growing pigs. a) Good sanitary condition; b) ST + Poor sanitary condition.

7.3 Haptoglobin, PD and Lys (%) correlations

We found negative correlations between haptoglobin concentrations and PD (r

= -0.54; $P < 0.001$) and between haptoglobin concentration and Lys (%) ($r = -0.49$; $P = 0.002$) (Figure 8).

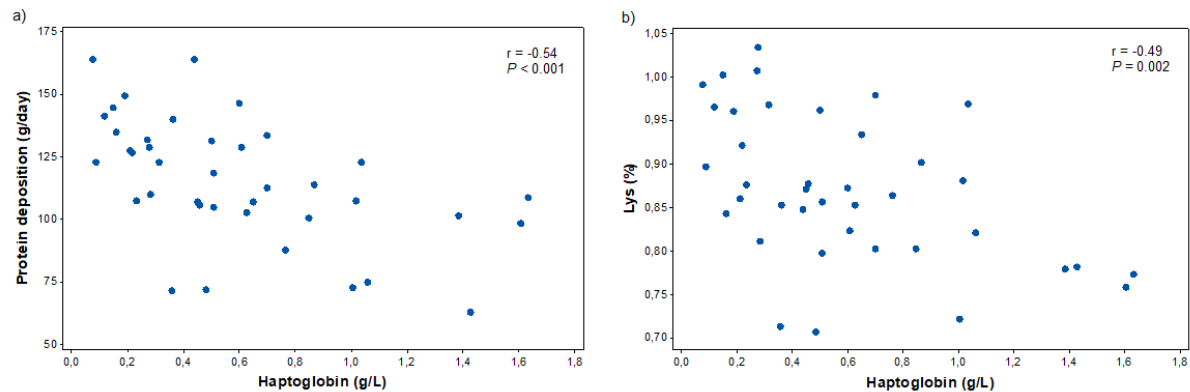


Figure 8. Correlation between serum concentration of haptoglobin (g/L), protein deposition (g/d) and Lys requirements (%) of growing pigs under sanitary challenge.

8 DISCUSSION

8.1 Impact of sanitary challenge on population performance and lysine requirement

The data showed that pigs raised under a ST + Poor SC and inoculated with ST not only had higher rectal temperature, but also higher haptoglobin and lower albumin concentrations during the first experimental week. Similarly, elevated rectal temperature, higher haptoglobin and lower albumin concentrations were observed in nursery (Rodrigues *et al.*, 2021) and growing pigs (Wellington *et al.*, 2019) during the first week after being inoculated with ST. These results indicate that the ST inoculation and poor housing conditions successfully stimulated an immune response. During the acute-phase response, the levels of APP in blood plasma can either increase (positive APP; e. g.: haptoglobin) or decrease (negative APP; e. g.: albumin) (Rakhshandeh and de Lange, 2011). The production of APP in the liver is mediated by the proinflammatory cytokines interleukin 1 (IL-1) and 6 (IL-6) (Le Floc'h *et al.*, 2004). They act synergically, but IL-6 is the primary initiator of the acute-phase response (Le Floc'h *et al.*, 2004). IL-1 is also responsible for inducing fever and anorexia during inflammation (Le Floc'h *et al.*, 2004).

Our predicted data showed that the sanitary challenge worsened animal performance. Animals in the ST + Poor SC had a final predicted BW 9.5% lower than

the animals in the Good SC, as well as 20.3% lower predicted ADG, and, although numerically, 8.5% lower predicted DFI throughout the experimental phase. Similarly, barrows from 6 to 112 kg raised under poor housing conditions had 17.1% lower ADG and 11.2% lower ADFI than barrows raised under good housing conditions (Williams *et al.*, 1997c). Female growing pigs raised under poor housing conditions had reductions of 25.3% for BW at slaughter, 18.3% for ADG, and 8.2% for ADFI when compared to the animals raised under good housing conditions (Van der Meer *et al.*, 2020). For weanling pigs inoculated with ST, a reduction of 6.6% for BW, 34.6% for ADG, and 22.0% for ADFI was observed in relation to the control animals (Rodrigues *et al.*, 2021). These results indicate that sanitary challenges lead to anorectic behavior and reduced growth response, that can be related to a reduction in feed intake and reduced nutrient efficiency of utilization (Rodrigues *et al.*, 2021). Despite predicted DFI not being statistically lower, the numerical reduction in the ST + Poor SC group may be related to other observed responses, such as lower predicted ADG and PD, which showed a significant effect of the challenge. The reduction of growth performance observed in our study may be explained by the components of the sanitary challenge model. The pathogen-associated part results in the development of an acute-phase response mediated by cytokines, and the poor housing conditions can result in continuous activation of the IS due to increased microbial pressure (Pastorelli *et al.*, 2012). The reduced ADG, although partially explained by the reduced DFI, is also a result of changes in metabolism and nutrient fluxes for the synthesis of cytokines, antibodies and APP (Pastorelli *et al.*, 2012). The direct action of cytokines reduces protein synthesis in skeletal muscle, while increasing protein catabolism, which results in amino acid supply for the liver to synthesize APP, but also leads to reduced ADG (Pastorelli *et al.*, 2012).

As expected, a strong correlation between observed and predicted PD was found in both SC. We observed that the animals in the ST + Poor SC had 20.3% lower protein deposition (PD) than animals in the Good SC. A reduction in maximum PD was observed in 6 to 112 kg barrows raised under poor housing conditions in relation to animals raised under good housing conditions (Williams *et al.*, 1997c), and also in growing pigs inoculated with LPS of *Escherichia coli* in relation to control animals (Rakhshandeh *et al.*, 2014). These results can be explained by the formerly discussed redistribution of amino acid utilization from growth and protein accretion to IS activation

during a sanitary challenge. It is important to note that the magnitude of the reductions in animal performance and body composition may vary according to the challenge model used, the length of the experimental phase, and the age, sex and variability between animals.

It was observed that the animals in the Good SC had Lys requirements 19.4% higher on the last experimental day and 9.9% higher throughout the phase than the animals in the ST + Poor SC. Similarly, it was observed that during their growing-finishing phase, animals with low activation of the IS had Lys requirements for maximal PD 20% higher than animals with high activation of the IS (Williams *et al.*, 1997c). When modelling Lys requirements for growing male pigs challenged with *E. coli* LPS, Barcellos *et al.* (2021) found that control pigs required 18.12 g/d of this amino acid, while challenged pigs required only 8.62 g/d. The greater Lys requirements for the animals raised in Good SC is probably due to the greater conditions for the pigs to express their capacity of protein deposition and not to a lower efficiency of Lys utilization (Williams *et al.*, 1997b).

8.2 Impact of sanitary challenge on individual pig variability: lysine requirement and performance

When compared to the animals in the Good SC, pigs in the ST + Poor SC had higher CV for final BW (+19.2%), DFI (+22.5%), ADG (+41.5%) and for PD (+41.5%). Indeed, individual pigs may respond differently when raised under stressors (Wellock *et al.*, 2004; Hauschild *et al.*, 2020) which may explain the greater variability observed in our study for pigs in ST + Poor SC. To our knowledge, previous studies that evaluated how a sanitary challenge affected pig performance only reported the means of the performance in different conditions, but not the CV. Knowing the individual animal variability is an important piece of information to understanding the whole impact of a sanitary challenge in pig production and making more accurate decisions in these situations. Therefore, it would be interesting that future studies make this information available.

Pigs in the ST + Poor SC had a CV 35.4% higher for Lys requirements during the acute phase response, 24.5% higher during the chronic phase response and 29.0% higher throughout the experimental period, which indicates higher variability of Lys requirements in this population. From a nutritional point of view, this translates into

a higher percentage of animals receiving more or less Lys supply than they require. We also observed that throughout the experimental phase, the pig in the 80 percentile of the population had Lys requirements 8.1% and 9.9% higher in the Good and ST + Poor SC, respectively, than the average animal in both SC. When comparing different models using the factorial method, it was observed that, for healthy animals, the 82-percentile pig in the population had Lys requirements 16% higher than the average pig (Remus *et al.*, 2019). It was also observed that for maximum ADG, the 82-percentile pig in the population required 12% more Lys than the average animal (Hauschild *et al.*, 2010). The lower difference observed in the current study (8%) may be related to the better homogeneity of animals in the beginning of the trial compared to previous studies. However, an interesting finding is that there is a difference between SC when comparing the requirement for 82-percentil and average individual. Moreover, the difference between the -1 SD (least demanding pigs) and +1 SD (most demanding pigs) was 17% greater for ST + Poor pigs, while for Good pigs it was only 14%. Regarding the variability being greater for pigs in the ST + Poor SC, it might be more difficult to stablish the optimal recommendation of Lys levels for these animals in the conventional feed phases programs system. For instance, in this system only one diet is offered for all the pigs during a long period (Hauschild *et al.*, 2010). Indeed, it has been a challenge to choose what could be the best representative pigs to define the optimal level in the diet for feeding phase program. Regarding that the variability is increased for pigs under stressors, it makes this process still more difficult.

As mentioned before, challenged animals redistribute metabolism and nutrient fluxes for the synthesis of APP, antibodies and cytokines (Pastorelli *et al.*, 2012). Besides the phenotypic variability, pigs may also show metabolic variability, that can be explained in part by the difference in the potential of protein deposition between pigs of a same population (Remus *et al.*, 2021). This difference in protein deposition, as well as in amino acid requirements, may determine differences in immune responses. Regarding this aspect, it should be noted that the negative correlations observed between Lys requirement and haptoglobin may indicate better capacity of coping with a sanitary challenge for more demanding pigs.

Another factor to be considered is the reduced Lys requirement observed in the animals in the ST + Poor SC. Since Lys not only controls amino acid metabolism, but also regulates key hormones involved in pathways for muscle growth (Liao *et al.*,

2015), the reduction in Lys requirements can be associated to the reductions in ADG and PD observed in the ST + Poor SC group. The conditions of our study – poor housing conditions and presence of ST – are not uncommon in the field, and it might be interesting to reduce the diet Lys concentration in these scenarios. However, regarding the difficulty to define the best representative pig in the feeding phases system, using the precision feeding system may be a promising strategy for challenged pigs (Hauschild *et al.*, 2020). For instance, a previous study evaluating the precision feeding system compared to feeding phase system for heat stress challenged pigs showed the possibility to reduce SID Lys intake (19%) without compromising growth (Santos *et al.*, 2018), which could result in reduction of feed costs (Pomar *et al.*, 2021). However, we lack information of how this strategy would affect sanitary challenged animals, and thus further investigations should be conducted before applying it in the field.

9 CONCLUSIONS

Collectively, these results show how a sanitary challenge may reduce BW, ADG, DFI, PD, and Lys requirements of growing pigs, besides also enhancing the individual variability for growth performance, body composition and Lys requirements.

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