YGOR HENRIQUE DE PAULA

ESTRATÉGIAS NUTRICIONAIS PARA IMUNOMODULAÇÃO E APROVEITAMENTO DE NUTRIENTES EM SUÍNOS: NOVAS ABORDAGENS PARA O USO DA NUTRIÇÃO DE PRECISÃO E β-MANANASE

LAVRAS - MG
2025

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

Orientador
Prof. Dr. Vinícius de Souza Cantarelli

Coorientadora
Profa. Dra. Ines Andretta

LAVRAS - MG
2025

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

de Paula, Ygor Henrique.
Estratégias nutricionais para imunomodulação e aproveitamento de nutrientes em suínos : novas abordagens para o uso da nutrição de precisão e beta-mananase / Ygor Henrique de Paula. - 2025.
123 p. : il.

Orientador: Vinícius Cantarelli
Coorientadora: Ines Andretta

Tese (Doutorado) - Universidade Federal de Lavras, 2025.
Bibliografia.

1. Nutrição. 2. Saúde intestinal. 3. Enzimas exógenas. 4. Resposta imune. 5. Microbioma. I. Cantarelli, Vinícius. II. Andretta, Ines. III. Universidade Federal de Lavras. IV. Título.

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NUTRITIONAL STRATEGIES FOR IMMUNOMODULATION AND NUTRIENT UTILIZATION IN PIGS: NEW APPROACHES FOR THE USE OF PRECISION NUTRITION AND B-MANNANASE

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

Aprovado em 13 de junho de 2025.

Dr. Vinícius de Souza Cantarelli – Universidade Federal de Lavras - UFLA
Dra. Ines Andretta – Universidade Federal do Rio Grande do Sul – UFRGS
Dr. Márvio Lobão Teixeira de Abreu – Universidade Federal de Lavras – UFLA
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Prof. Dr. Vinícius de Souza Cantarelli
Orientador

LAVRAS - MG

2025

Aos meus amados pais e irmão, e todos aqueles
que me ajudaram ao longo desta jornada!
Dedico!

AGRADECIMENTOS

A vida é feita de ciclos, e, para que novos se iniciem, outros devem se encerrar. Aqui encerro um importante ciclo de minha vida, ao qual tenho muito orgulho e boas lembranças da trajetória que tive. Houve um grande amadurecimento pessoal e profissional. Acredito que sejam os frutos de todo o esforço plantado.

Ao todo, foram mais de 23 anos em processo de formação. Agradeço a todos os profissionais da educação que passaram pelo meu caminho, desde a Prof. Sebastiana Chaves, a quem me alfabetizou, até o Prof. Dr. Vinícius Cantarelli, que me orientou durante a pós-graduação. A educação transformou a minha realidade, e sou imensamente grato a todos vocês por terem me mostrado esse caminho.

Muito mais do que dados sobre nutrição e saúde intestinal, esta tese é a forma física (ou digital) da conquista de um sonho vivido intensamente por toda a minha família. Agradeço aos meus pais, Otogair e Regina, que, na sua simplicidade, me deram os princípios mais valiosos que eu poderia ter. Sou extremamente grato a Deus por ter me dado vocês de presente. Agradeço ao meu irmão, César, que sempre foi meu exemplo. Se estou onde estou, devo muito a você. À minha cunhada Caroline, obrigado pela amizade e por ser um ponto de apoio. À minha sobrinha Clara, sou grato pelo seu sorriso, que recarrega minhas energias quando a vejo. No mais, tanto a vocês quanto aos demais familiares, agradeço por todo o suporte e compreensão nos momentos de ausência. Mesmo não estando presente, nunca deixei de pensar em vocês por um só momento.

Agradeço ao meu orientador, Prof. Dr. Vinícius Cantarelli, por ter sido, além de um direcionador acadêmico, um grande amigo. Junto a ele, tive o privilégio de conviver, nestes anos, com a Profa. Ines Andretta, o Prof. Matheus Costa e o Prof. Luciano Hauschild. Me sinto honrado em dizer que estive ao lado deste time durante esses anos. Obrigado pela confiança e por todas as oportunidades concedidas.

Aos demais membros da banca de defesa, professores Dr. Márvio Abreu e Dr. Cesar Garbossa, agradeço pela disponibilidade e considerações.

À linha de pesquisa *Animal Science and Intestinal Health* (ASIH), da qual me orgulho em fazer parte, pela promoção do meu desenvolvimento pessoal e profissional. Ao Núcleo de Estudos em Suinocultura (NESUI), pelas amizades e experiências vividas ao longo destes cinco anos.

Sou grato pelas alegrias e desafios enfrentados. Neste contexto, a presença dos meus amigos foi determinante. Obrigado a todos os amigos da UFLA, LabSui, Lezo e Canadá que

estiveram comigo nesta trajetória. Em especial, à Jéssica Barbosa, Iva Carla e Ismael França. Sempre serei grato por todos os conselhos e ajuda!

À Universidade Federal de Lavras e à University of Saskatchewan, pelas portas abertas.

Ao Programa de Pós-Graduação em Zootecnia (PPGZ), pela oportunidade concedida.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Centre de Recherche en Infectiologie Porcine et Avicole (CRIPA), pela concessão das bolsas de estudo.

Enfim, agradeço a Deus por me permitir finalizar mais uma etapa, sempre iluminando meu caminho e me dando força, saúde e discernimento diante das dificuldades.

AUTOBIOGRAFIA

Ygor Henrique de Paula – nascido em 02 de setembro de 1997, na cidade de Bambuí, Minas Gerais, filho de Otogair José de Paula e Regina Perpétua Leite Paula.

Ingressou em fevereiro de 2012, nos cursos técnicos em Agricultura e em Zootecnia no Instituto Federal de Minas Gerais (IFMG) *Campus* Bambuí, concluindo sua formação em janeiro de 2015.

Em fevereiro de 2015, ingressou no curso de Medicina Veterinária no Centro Universitário de Patos de Minas (UNIPAM), graduando-se em janeiro de 2020.

Em março de 2020, iniciou o curso de Mestrado em Zootecnia na área de Produção e Nutrição de Não Ruminantes, na Universidade Federal de Lavras (UFLA), obtendo o título de mestre em janeiro de 2022.

Em março de 2022, iniciou o curso de Doutorado em Zootecnia na área de Nutrição e Produção de Não Ruminantes, na Universidade Federal de Lavras (UFLA), com foco de pesquisa na saúde intestinal de suínos. Em agosto de 2024, iniciou o Doutorado Sanduíche na University of Saskatchewan, no Canadá.

Em 13 de junho de 2025, submeteu-se à defesa de tese para a obtenção do título de Doutor.

“O importante é não parar de questionar. A curiosidade tem sua própria razão de existir."
— Albert Einstein

RESUMO

Esta tese teve como objetivos avaliar os efeitos da alimentação de precisão sobre parâmetros bioquímicos, indicadores oxidativos, respostas imunes e microbiota intestinal, além de investigar o impacto da β-mananase (BM) sobre o desempenho, o balanço de nutrientes, biomarcadores metabólicos, estado oxidativo e respostas inflamatórias em suínos nas fases de crescimento e terminação. No primeiro experimento, com alimentação de precisão, 50 suínos machos inteiros (23,2 ± 3,12 kg) foram distribuídos entre dois tratamentos: um grupo controle (n=25), alimentado segundo um programa de fases (PF), e um grupo submetido à alimentação de precisão individual diária (DFI, n=25), formulada para atender 100% das exigências nutricionais estimadas de cada animal. Amostras de sangue e fezes (n = 7 por tratamento) foram coletadas para análise de biomarcadores metabólicos, estresse oxidativo, citocinas e biomarcadores inflamatórios, e composição e abundância da microbiota intestinal. Os níveis séricos de fator de necrose tumoral-α (TNF-α) e interleucina-10 (IL-10) foram mais elevados no grupo PF no dia 104 ($P < 0,050$). Além disso, a diversidade alfa foi reduzida para suínos PF entre os dias 20 e 103 ($P = 0,012$), enquanto a idade se mostrou um preditor consistente das variações na diversidade beta ($P < 0,0001$). A alimentação de precisão também modulou a abundância relativa de determinados gêneros bacterianos. Em síntese, a alimentação de precisão individual resultou em menor ativação inflamatória e influenciou de forma dinâmica a diversidade microbiana intestinal ao longo do tempo. No segundo experimento, com adição de β-mananase, 50 suínos machos inteiros (23,02 ± 0,42 kg) foram alimentados com uma dieta controle (sem adição enzimática, n=25) ou com dieta suplementada com BM (300 g/tonelada, n=25), durante 104 dias divididos em cinco fases. O desempenho e a composição corporal foram avaliados em cada uma das fases de alimentação. Amostras de sangue (n = 7 por tratamento) foram coletadas para análise de parâmetros bioquímicos, oxidativos e imunes, enquanto amostras fecais (n = 7 por tratamento) para avaliação de biomarcadores inflamatórios. A adição de BM na dieta promoveu melhor eficiência alimentar ($P = 0,048$). No dia 104, suínos suplementados com BM apresentaram maior espessura de toucinho ($P = 0,003$), maior massa lipídica e mineral ($P < 0,05$), além de tendência de aumento na massa proteica ($P = 0,076$) e na massa de proteína + água ($P = 0,072$). Considerando todo o período experimental, BM resultou em maior ganho de proteína ($P = 0,049$), e maior retenção de nitrogênio ($P = 0,035$). BM promoveu aumento na atividade de glutationa redutase no dia 98 ($P = 0,058$), e de glutationa peroxidase no dia 83 ($P = 0,016$). Houve redução de TNF-α no dia 104 ($P = 0,003$) e de IL-10 nos dias 21 ($P = 0,005$), 26 ($P = 0,011$) e 104 de experimento ($P = 0,044$) para BM, bem como menor concentração fecal de calprotectina aos 103 dias ($P = 0,053$). Portanto, a BM contribui para a melhoria do desempenho e da composição corporal de suínos, promovendo o incremento da massa muscular, utilização dos nutrientes e redução de respostas inflamatórias.

Palavras-chave: Nutrição; Saúde intestinal; Enzimas exógenas; Resposta imune; Microbioma.

ABSTRACT

This thesis aimed to evaluate the effects of precision feeding on biochemical parameters, oxidative indicators, immune responses, and intestinal microbiota, as well as to investigate the impact of β-mannanase (BM) on performance, nutrient balance, metabolic biomarkers, oxidative status, and inflammatory responses in pigs during the growing and finishing phases. In the first experiment, involving precision feeding, 50 entire male pigs (23,2 ± 3,12 kg) were assigned to two treatments: a control group (n=25), fed according to a phase-feeding program (PF), and a group subjected to daily individual precision feeding (DFI, n=25), formulated to meet 100% of the estimated nutritional requirements of each animal. Blood and fecal samples (n = 7 per treatment) were collected for the analysis of metabolic biomarkers, oxidative stress, cytokines, inflammatory biomarkers, and intestinal microbiota composition and abundance. Serum levels of tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) were higher in the PF group on day 104 ($P < 0.050$). Moreover, alpha diversity was reduced in PF pigs between days 20 and 103 ($P = 0.012$), while age proved to be a consistent predictor of variations in beta diversity ($P < 0.0001$). Precision feeding also modulated the relative abundance of certain bacterial genera. In summary, individual precision feeding resulted in lower inflammatory activation and dynamically influenced intestinal microbial diversity over time. In the second experiment, involving β-mannanase supplementation, 50 entire male pigs (23.02 ± 0.42 kg) were fed either a control diet (without enzyme addition, n=25) or a BM-supplemented diet (300 g/ton, n=25) for 104 days divided into five feeding phases. Performance and body composition were assessed at each feeding phase. Blood samples (n = 7 per treatment) were collected for the analysis of biochemical, oxidative, and immune parameters, while fecal samples (n = 7 per treatment) were used to assess inflammatory biomarkers. BM supplementation improved feed efficiency ($P = 0.048$). On day 104, pigs supplemented with BM showed greater backfat thickness ($P = 0.003$), higher lipid and mineral mass ($P < 0.05$), and a tendency toward increased protein mass ($P = 0.076$) and protein + water mass ($P = 0.072$). Over the entire experimental period, BM resulted in greater protein gain ($P = 0.049$) and higher nitrogen retention ($P = 0.035$). BM also promoted an increase in glutathione reductase activity on day 98 ($P = 0.058$) and glutathione peroxidase activity on day 83 ($P = 0.016$). There was a reduction in TNF-α on day 104 ($P = 0,003$) and in IL-10 on days 21 ($P = 0,005$), 26 ($P = 0,011$), and 104 ($P = 0,044$) in the BM group, as well as lower fecal calprotectin concentration on day 103 ($P = 0,053$). Therefore, BM contributes to improved performance and body composition in pigs by promoting increased muscle mass, better nutrient utilization, and reduced inflammatory responses.

Keywords: Nutrition; Gut health; Exogenous enzymes; Immune response; Microbiome.

IMPACTOS SOCIAIS, TECNOLÓGICOS, ECONÔMICOS E CULTURAIS

Os custos com alimentação nas fases de crescimento e terminação representam a maior parcela dos investimentos na produção suinícola. Diante disso, a avaliação precisa das exigências nutricionais dos animais, aliada à formulação de dietas adequadas, torna-se uma estratégia essencial não apenas para otimizar o uso da ração, mas também para promover avanços tecnológico, econômico, social e cultural. Do ponto de vista econômico, essa abordagem contribui diretamente para a redução dos custos de produção, tornando a atividade mais rentável. No aspecto tecnológico, a adoção de práticas nutricionais de precisão promove a aplicabilidade da associação entre *softwares* e *hardwares* ao cotidiano de um sistema de produção de suínos. Além disso, assim como o uso de enzimas exógenas – com destaque para a β-mananase – demonstram inovação ao promover o uso mais eficiente dos nutrientes da dieta, sem comprometer o desempenho animal. Essas estratégias também apresentam relevante impacto social e ambiental, ao reduzirem a excreção de nutrientes no ambiente, colaborando para sistemas de produção mais sustentáveis e alinhados com as demandas da sociedade por alimentos produzidos de forma ética e responsável. Além disso, melhoram o bem-estar dos animais ao favorecerem a saúde intestinal, reduzirem processos inflamatórios e otimizarem a fisiologia geral, o que também repercute positivamente na qualidade do produto final consumido pela população. Culturalmente, tais inovações têm potencial para transformar práticas tradicionais da suinocultura, influenciando a forma como produtores lidam com a alimentação animal e promovendo uma maior valorização do conhecimento científico aplicado no campo.

SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

Feed costs during the growing and finishing phases represent the largest portion of investment in swine production. In this context, accurately assessing the animals' nutritional requirements, combined with the formulation of appropriate diets, becomes an essential strategy not only to optimize feed utilization but also to promote technological, economic, social, and cultural advancements. From an economic perspective, this approach directly contributes to reducing production costs, making the activity more profitable. From a technological standpoint, the adoption of precision nutrition practices enables the integration of software and hardware into the daily routines of swine production systems. In addition, the use of exogenous enzymes — particularly β-mannanase — represents a significant innovation by promoting more efficient use of dietary nutrients without compromising animal performance. These strategies also have significant social and environmental impacts, as they reduce nutrient excretion into the environment, contributing to more sustainable production systems that are aligned with society's demand for ethically and responsibly produced food. Furthermore, they improve animal welfare by supporting gut health, reducing inflammatory processes, and optimizing overall physiology — all of which positively influence the quality of the final product consumed by the population. From a cultural standpoint, such innovations have the potential to transform traditional swine farming practices, influencing how independent producers and cooperatives approach animal nutrition and fostering a greater appreciation for scientific knowledge applied in the field.

RESUMO INTERPRETATIVO

Estratégias nutricionais para imunomodulação e aproveitamento de nutrientes em suínos: novas abordagens para o uso da nutrição de precisão e β-mananase

Elaborado por **Ygor H. de Paula** e orientado por **Vinícius de Souza Cantarelli**

ESTRATÉGIAS NUTRICIONAIS PARA IMUNOMODULAÇÃO E APROVEITAMENTO DE NUTRIENTES EM SUÍNOS: NOVAS ABORDAGENS PARA O USO DA NUTRIÇÃO DE PRECISÃO E β-MANANASE

Determinadas **estratégias nutricionais** podem minimizar a **excreção de componentes da dieta nas fezes** que, previamente, não eram absorvidos pelo trato gastrointestinal. Esses nutrientes, quando não absorvidos, são capazes de **modular o microbioma intestinal** – servindo de substrato para as bactérias – e, consequentemente, promover **respostas imunes.** Além dos danos teciduais que podem ser promovidos, a **ativação exacerbada** do sistema imunológico gera um gasto energético desnecessário, mobilizando **energia do ganho de massa** muscular para **processos inflamatórios.**

ARTIGO 1

Avaliação dos efeitos da alimentação de precisão individual sobre a resposta imune, microbioma, biomarcadores metabólicos e estresse oxidativo em suínos nas fases de crescimento e terminação.

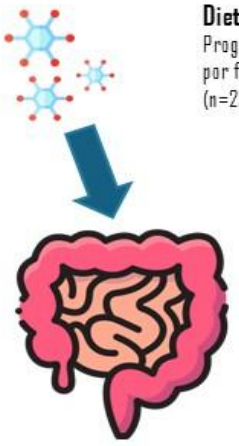
Programa de alimentação por fases (PF)
Níveis fixos de lisina digestível por período (n=25);

Alimentação Individual Diária (DFI)
Dieta ajustada diariamente para atender à exigência de lisina digestível de cada indivíduo (n=25)

DFI apresentou menores respostas pró e anti-inflamatórias. A microbiota intestinal foi modulada de acordo com a idade, com uma redução progressiva na diversidade alfa e uma diversidade beta significativamente distinta no dia 103.

ARTIGO 2

Avalição do impacto da β-mananase (BM) sobre o desempenho, balanço de nutrientes, biomarcadores metabólicos, estresse oxidativo e as respostas inflamatórias de suínos nas fases de crescimento e terminação.



Dieta controle (CON)
Programa convencional de alimentação por fases com níveis normais de energia (n=25);

Dieta suplementada com β-mananase (BM)
Dietas formuladas com composição idêntica, exceto por uma redução de 35 kcal de energia líquida, compensada pela adição de BM (300 g/ton) (n=25).

BM promoveu melhor eficiência alimentar, maior aproveitamento dos nutrientes e ganho de massa corporal. Também favoreceu a retenção de nitrogênio e reduziu processos inflamatórios.

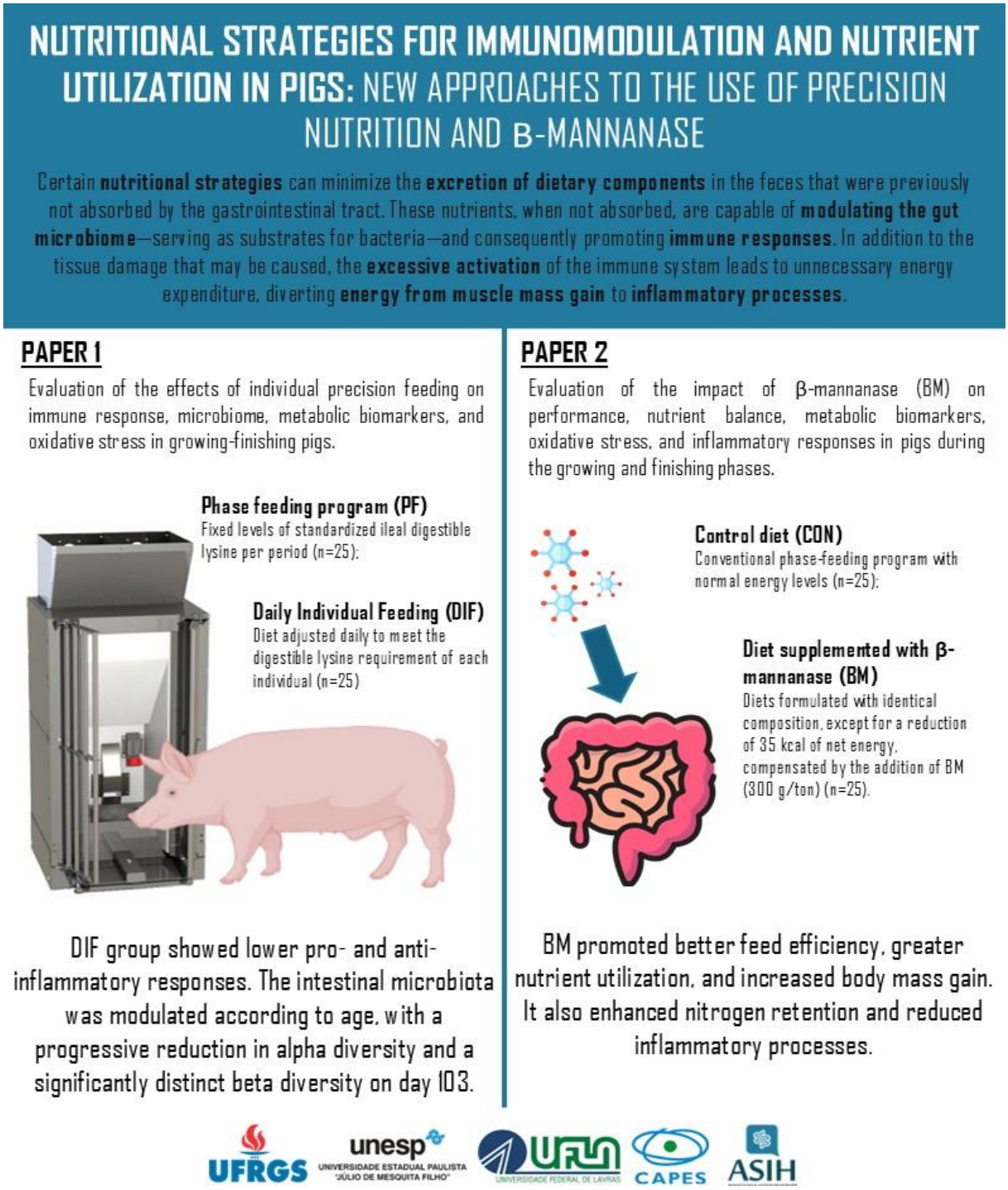
Dois experimentos avaliaram o efeito de estratégias nutricionais com potencial modulação sobre respostas imunes, microbiota e aproveitamento dos nutrientes da dieta de suínos em crescimento e terminação. Esta tese possui resultados que fornecem um novo olhar as estas tecnologias, e contribui para orientar tomadores de decisão na produção de suínos.

Tese de doutorado em Zootecnia na UFLA, defendida em 13 de junho de 2025

INTERPRETIVE SUMMARY

Nutritional strategies for immunomodulation and nutrient utilization in pigs: new approaches for the use of precision nutrition and β-mannanase

Designed by **Ygor H. de Paula** and supervised by **Vinícius de Souza Cantarelli**



Two experiments evaluated the effect of nutritional strategies with potential modulation of immune responses, microbiota, and nutrient utilization in the diet of growing and finishing pigs. This thesis provides new insights into these technologies and helps guide decision-makers in swine production.

PhD thesis in Animal Science at UFLA, defended on June 13, 2025

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CAPÍTULO 1

1 INTRODUÇÃO GERAL

Os custos com alimentação na fase de crescimento e terminação são os mais elevados entre todas as etapas da produção suína. Desta forma, a avaliação precisa das exigências nutricionais dos animais associada a formulação de dietas adequadas são estratégias fundamentais para aprimorar a eficiência na utilização da ração. Além disso, possibilita reduzir os custos de produção e minimizar a excreção de nutrientes, possibilitando um sistema de produção mais sustentável. Assim, os níveis nutricionais das dietas impactam não apenas o desempenho dos suínos e a digestibilidade dos nutrientes, mas também afetam diretamente o metabolismo dos animais nessa fase. Neste contexto, a dieta é ponto-chave como promotor de saúde.

A saúde intestinal é representada por uma interação química e biológica complexa multidirecional entre a dieta, a microbiota e o sistema imunológico. Estes elementos trabalham em conjunto, influenciando-se mutuamente a nível celular e molecular para orquestrar uma estabilidade imunometabólica intestinal. Por esta razão, a saúde intestinal regula a fisiologia geral e sistêmica do animal.

Determinadas estratégias nutricionais podem minimizar a excreção de componentes da dieta que previamente não eram absorvidos pelo trato gastrointestinal. Estes compostos quanto não absorvidos são capazes de modular o microbioma intestinal – sendo substrato para as bactérias – e, consecutivamente promover respostas imunes. Além dos danos teciduais que pode promover, a ativação exacerbada do sistema imunológico gera um gasto energético desnecessário, mobilizando energia do ganho de massa muscular para processos inflamatórios.

A imunocastração representa uma alternativa à castração cirúrgica, promovendo bem-estar animal e influenciando o equilíbrio hormonal, o metabolismo e a resposta imune. Compreender esses efeitos, é fundamental para otimizar estratégias nutricionais e garantir saúde e desempenho dos suínos. Neste contexto, estratégias como a implementação da técnica de nutrição de precisão e o uso de enzimas exógenas - aqui sendo destacada a β-mananase - promovem uma melhor utilização dos nutrientes ofertados na dieta de suínos imunocastrados, com melhor saúde intestinal, sem que haja comprometimento do desempenho. Portanto, esta tese possui resultados inovadores que podem dar um novo olhar as referidas tecnologias, e contribuir ativamente para orientar tomadores de decisão na cadeia produtiva de suínos.

Sendo assim, os objetivos dos estudos apresentados nesta tese foram: avaliar os efeitos da alimentação de precisão individual sobre parâmetros bioquímicos, indicadores de estresse oxidativo, respostas imunes e composição da microbiota intestinal em suínos imunocastrados; e investigar o impacto da adição de β-mananase sobre o desempenho zootécnico, o balanço de nutrientes, biomarcadores metabólicos, estado de estresse oxidativo e respostas inflamatórias em suínos imunocastrados em fases de crescimento e terminação.

2 REVISÃO DE LITERATURA

2.1 Eficiência na utilização de nutrientes

Nas últimas décadas, a suinocultura passou por uma profunda transformação, abandonando os sistemas agrícolas tradicionais e adotando modelos de produção mais industrializados. Esse processo também impulsionou a industrialização da fabricação de rações. Com os avanços tecnológicos e o progresso nas pesquisas em nutrição de suínos, observou-se um notável aumento na eficiência produtiva e na viabilidade econômica da produção em larga escala (STRID ERIKSSON et al., 2005; GARCIA-LAUNAY et al., 2014; WU et al., 2020).

Atualmente, algumas das principais pautas de discussão no setor incluem a busca por maior eficiência alimentar, redução dos custos de produção e mitigação dos impactos ambientais (STRID ERIKSSON et al., 2005; GARCIA-LAUNAY et al., 2014; VAN DER HEIDEA; NØRGAARDA; MADSEN, 2025). Essa preocupação decorre dos desafios relacionados ao crescimento populacional global, à necessidade de preservar os recursos naturais e às restrições nas áreas disponíveis para cultivo (NIEMANN; KUHLA; FLACHOWSKY, 2011). Diante disso, produtores e profissionais da nutrição animal têm revisado seus programas de alimentação, visando diminuir a excreção de nutrientes no ambiente (POMAR et al., 2015; ANDRETTA et al., 2016).

Os avanços científicos na nutrição de suínos têm sido progressivamente aplicados à formulação de rações, permitindo que as exigências nutricionais dos animais sejam atendidas com maior precisão. Com isso, a abordagem nas pesquisas da área também evoluiu: o enfoque que antes era estático e voltado à conversão de nutrientes passou a incorporar modelos dinâmicos capazes de estimar as necessidades nutricionais dos suínos em diferentes fases de crescimento, níveis de produtividade e condições ambientais. Nesse contexto, estratégias nutricionais tornaram-se ferramentas essenciais para promover o desempenho, a saúde e a sustentabilidade na produção suinícola (WU et al., 2020).

2.2 Nutrição de precisão em suínos

Os programas de alimentação convencionais buscam potencializar o desempenho animal, fornecendo uma única ração a todos os suínos durante um longo período de tempo. Geralmente, estas dietas buscam atender as exigências dos animais mais exigentes (HAUSCHILD; POMAR; LOVATTO, 2010; MARIC et al., 2025). Todavia, as dietas deveriam ser ajustadas diariamente para atender as exigências nutricionais com maior precisão e, portanto, melhorar a eficiência da utilização dos nutrientes. Entretanto, o aumento do número de dietas complica o manejo alimentar e aumenta os custos de produção (POMAR; REMUS, 2019).

As exigências nutricionais dos suínos também sofrem grande variação entre indivíduos, mesmo em populações homogêneas em termos de idade e sexo (POMAR et al., 2003; BROSSARD et al., 2009). Assim, por mais que lidar com a variabilidade individual destas demandas nutricionais seja uma tarefa difícil, as técnicas de agricultura de precisão podem ser uma solução (WATHES et al., 2008).

A alimentação de precisão consiste no uso de técnicas de alimentação que possibilitam que a quantidade ideal de alimento, com uma composição adequada, seja disponibilizada a um grupo de animais ou a animais individualmente (PARSONS et al., 2007; CANGAR et al., 2008; ANDRETTA et al., 2014; POMAR et al., 2014; ANDRETTA et al., 2016). O uso desta técnica oferece benefícios imediatos, pois alimentar os suínos individualmente com dietas diárias adaptadas promove a redução da ingestão de lisina em mais de 25%, os custos de alimentação em mais de 8%, a excreção de nitrogênio e fósforo em quase 40% e emissão de gases de efeito estufa em 6%. Sendo ressaltado que todos estes benefícios são promovidos sem comprometer o desempenho (ANDRETTA et al., 2014; ANDRETTA et al., 2016; ANDRETTA et al., 2018).

O uso de técnicas de alimentação de precisão é uma estratégia para se reduzir o teor de proteína nas dietas (MONTEIRO et al., 2016). Portanto, é uma ferramenta que visa tornar os sistemas de produção mais sustentáveis, fornecendo rações com a composição de acordo com padrão de consumo e crescimento de cada indivíduo (POMAR et al., 2010).

2.2.1 Excesso de proteína e seus impactos no intestino

Os alimentos que entram no trato gastrointestinal possibilitam o fornecimento de nutrientes ao organismo. Desta maneira, as interações entre dieta, digestão, absorção, microbioma, barreira intestinal e sistema imune podem afetar a homeostase (FARRÉ et al.,

2020). A proteína é um nutriente limitante e oneroso na alimentação animal. O nível proteico da dieta se torna excedente quando o suíno não é capaz de digerir ou absorver tudo o que foi ofertado na ração. Seja por má qualidade da proteína ofertada e/ou pela limitação das enzimas digestivas e transportadores intestinais (HUMPHREY; ZHAO; FARIS, 2019).

A proteína que não é digerida se torna disponível para a fermentação no íleo e no intestino grosso, impactando negativamente a permeabilidade intestinal e a imunidade, devido ao aumento do pH (HEO et al., 2010a; HEO et al., 2010b) e por ser considerada um substrato para a fermentação bacteriana (KIM et al., 2012). Portanto, ocorre uma mudança nas populações de microrganismos em direção a populações mais proteolíticas e o aumento da produção de subprodutos da fermentação (WILLIAMS; VERSTEGEN; TAMMINGA, 2001). Desta forma, este excesso de proteína pode desempenhar um mecanismo precursor em patologias e um estado inflamatório no organismo (CAMILLERI, 2019). Em resumo, os efeitos provenientes do excesso de proteína nas dietas de suínos sobre o sistema imune está esquematizado na Figura 1.

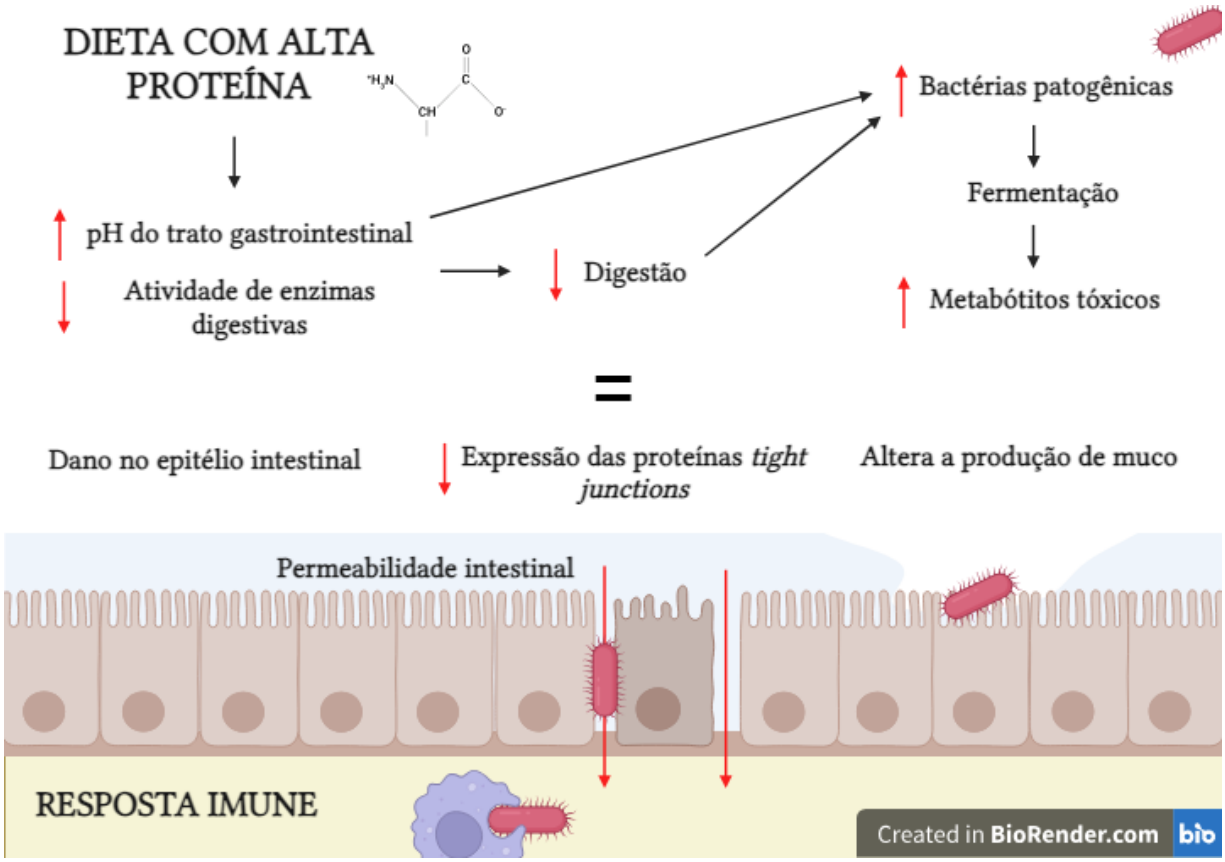

Figura 1. Representação dos efeitos provenientes de uma dieta com alto nível protéico sobre os principais mecanismos que se relacionam a ativação da resposta imune. Adaptado de Xia et al., 2022. Criado com BioRender.com.

2.2.1.1 Impactos na barreira intestinal

O intestino não realiza apenas os processos de digestão e absorção de nutrientes, mas também é uma importante defesa contra a entrada de substâncias nocivas, como bactérias patogênicas e toxinas (XIA et al., 2022). Dietas com alto nível proteico podem causar uma elevação do pH do trato gastrointestinal, promovendo uma diminuição da atividade de enzimas digestivas (YU; ZHU; HANG, 2019). Promove-se assim, uma redução da capacidade de digestão e absorção. Além disso, também demonstram efeitos sobre a mucosa intestinal, sendo capaz de alterar o número de células caliciformes (FRANCE; TURNER, 2017; XIN et al., 2017).

A alteração da composição da mucina aumenta a probabilidade do surgimento de patologias associadas a microrganismos patogênicos, tal como diminui a função imune. Estudos demonstraram que o consumo de níveis elevados de proteínas tem a capacidade de afetar a síntese de mucina (XIN et al., 2017).

Como um importante componente das *tight junctions*, as proteínas zônula ocludens-1 (ZO-1), ocludina e claudina e o citoesqueleto desempenham um papel importante na barreira intestinal. Dietas ricas em proteína tem a capacidade de regular negativamente a expressão destas proteínas das células epiteliais intestinais. Desta forma, ocorre uma perturbação na integridade da mucosa intestinal, a deixando mais permeável aos diferentes componentes no lúmen do intestino (BUCKLEY; TURNER, 2018).

Níveis excessivos de proteína também levam a alterações na morfologia intestinal. Atrofia das vilosidades, aumento da profundidade das criptas, assim como diminuição da relação entre altura das vilosidades e profundidade das criptas são observações relatadas em alta inclusão proteica. E desta forma, estes danos facilmente levam a uma disfunção na capacidade digestiva e absortiva do intestino (WU et al., 2015).

Wu et al. (2015) demonstraram que o aumento no teor de proteína bruta na dieta de leitões em fase de creche, promoveu a redução da expressão gênica de ZO-1 e ocludina no jejuno e íleo. Consequentemente, houve aumento da incidência de diarreia. Desta forma, as mudanças na síntese e funcionalidade das *tight junctions* podem estar relacionadas à quantidade de proteína antigênica presente em fontes proteicas vegetais (XIA et al., 2022).

O farelo de soja é a principal fonte de proteína utilizada em formulações para suínos, pois apresenta um ótimo perfil de aminoácidos e de digestibilidade. No entanto, a morfologia intestinal e a imunidade dos animais podem ser afetados negativamente com base na quantidade que é utilizada. Isto ocorre devido à presença de componentes antinutricionais neste ingrediente,

como fitatos, inibidores de tripsina e oligossacarídeos (ADEYEMO; ONILUDE, 2013; DENG et al., 2022; ROCHA; DUARTE; KIM, 2022).

Estudos demonstraram que a presença das proteínas antigênicas β-conglicinina e glicinina na dieta de leitões após o desmame reduziram a expressão de claudina e ZO-1 no duodeno, jejuno e íleo. Assim quanto maior a dose presente na dieta, maiores serão os efeitos posteriores (WANG et al., 2014). Estes componentes não apenas inibem diretamente a expressão das proteínas das *tight junctions*, mas também na fosforilação da cadeia leve da miosina (*myosin light chain* - MLC). Desta forma, as proteínas das *tight junctions* são degradadas pela endocitose celular e sua distribuição nas células é alterada, resultando assim em aumento da permeabilidade da barreira intestinal (DU et al., 2016).

2.2.1.2 Impactos na microbioma intestinal

O trato gastrointestinal dos suínos é formado por uma comunidade de microrganismo complexa, a qual pode ser alterada pela dieta consumida e pelos processos digestivos e absortivos desde a lactação até à fase de terminação (SERADJ et al., 2020). Diversas patologias têm sido associadas a estas mudanças no microbioma intestinal (CAMILLERI, 2019). A maioria das proteínas dietéticas é digerida e absorvida no intestino delgado. Quando a proteína não é digerida, chega ao intestino grosso sendo fermentada pela microbiota. É importante ressaltar que os microrganismos do intestino delgado também apresentam a capacidade de fermentar proteínas, entretanto, em bem menor proporção (DAVILA et al., 2013).

Portanto, a proteína consumida possibilita a determinação da microbiota intestinal e, de forma recíproca, os microrganismos intestinais modulam a fermentação e o perfil de metabólitos produzidos (FAN et al., 2015; SUNG et al., 2023). Além disso, o aumento da fermentação proteica afeta a sua disponibilidade para absorção, reduzindo a taxa de digestibilidade dos aminoácidos (DUARTE; KIM, 2022). Assim, estas associações determinam impactos ao sistema imune (CHEN et al., 2020; REN et al., 2020; FAN et al., 2021).

Estudos tem demonstrado que dietas com níveis mais baixos de proteína bruta melhoram a saúde intestinal. Estes achados são associados a redução de metabólitos prejudiciais e uma otimização dos componentes microbianos (ZHOU et al., 2020; ZHOU et al., 2022). E do mesmo modo, com o aumento do teor de proteína na dieta, o grau de dano intestinal também aumenta (PENG et al., 2017).

A fermentação de proteínas no intestino produz aminoácidos, ácidos graxos de cadeia curta, ácidos graxos de cadeia ramificada, amônia, aminas, sulfeto de hidrogênio, fenóis, indóis

e poliaminas (RAJILIC-STOJANOVIC et al., 2015; PIEPER et al., 2016). Estes componentes aumentam o pH da digesta (devido ao efeito tampão da proteína), alterando a abundância de diferentes filos ou gêneros bacterianos (XIA et al., 2022; FAN et al., 2023). De modo geral, favorece a proliferação de bactérias proteolíticas e potenciais patógenos, como *Escherichia coli* (KIM; CHEN; PARNSEN, 2019).

Zhou et al. (2016) observaram que o aumento no nível de proteína da dieta afetou significativamente a riqueza e diversidade microbiana (índices Ace, Chao, Shannon e Simpson) do ceco e cólon de leitões. Do mesmo modo, Hooda et al. (2013) descobriram que as concentrações de *Bifidobacterium* e *Lactobacillus* diminuíram significativamente enquanto houve aumento de *Clostridium* ao se elevar os níveis proteicos da dieta.

Os animais mantidos em sistemas de produção comercial, estão constantemente expostos a fatores estressantes que afetam a resposta imune. Além de que, desafios sanitários podem ser comuns nestes ambientes. Para agravar ainda mais a questão, os produtos da fermentação proteica têm efeitos tóxicos e pró-inflamatórios no epitélio intestinal (HEO et al. 2009; HEO et al., 2010b). Uma das classes de compostos produzidos a partir da atividade de microrganismos intestinais são as aminas biogênicas (BUŇKOVÁ et al., 2010; FEDDERN et al., 2019). Estes metabólitos são formados por meio da descarboxilação de aminoácidos livres pela microbiota intestinal, como *Bacteroides, Clostridium, Bifidobacterium, Enterobacterium* e *Streptococcus* (HART; GRIMBALDESTON; FINLAY-JONES, 2001; CAPOZZI et al., 2012; KABOURIDIS et al., 2015).

Altas concentrações de aminas biogênicas podem induzir reações adversas no organismo, como a capacidade de danificar a mucosa intestinal (LADERO et al., 2010). As aminas biogênicas são classificadas como monoaminas ou poliaminas. Putrescina, espermina e espermidina são poliaminas provenientes da ornitina e aminoácidos relacionados (RAMANI; DE BANDT; CYNOBER, 2014), enquanto a cadaverina vem da lisina (FAN et al., 2017).

Elevação na taxa de incidência de diarreia em leitões desmamados está associada ao aumento da produção de aminas biogênicas (MA et al., 2012; KOZAK et al., 2015). No entanto, as consequências destes componentes nos animais *in vivo*, ainda demandam maiores estudos (LIU et al., 2014). Portanto, é de suma importância fornecer uma concentração adequada de proteínas na dieta na busca por níveis aceitáveis das aminas biogênicas no intestino (FAN et al., 2017).

2.2.1.3 Impactos no sistema imune intestinal

Dietas com alto nível de proteina podem modular negativamente diferentes estruturas e componentes relacionados à ativação do sistema imune. Em acordo a esta afirmação, Anastasilakis et al. (2013) descreveram que estímulos provenientes da dieta no trato gastrointestinal resultam em alterações na imunidade, havendo mudanças na quantidade dos componentes celulares e desequilíbrio no perfil de citocinas. Esta é uma área oportuna para novos estudos, tendo em vista a compreensão dos impactos de nutrientes livres no intestino e a influência que promovem na imunidade e saúde do indivíduo (HUMPHREY; ZHAO; FARIS, 2019).

Níveis elevados de proteína na ração afetam significativamente tanto a imunidade celular, quanto a humoral (XIA et al., 2022). Dietas com elevada proteína desencadeiam a proliferação e diferenciação de linfócitos, assim como a produção de citocinas. Desta forma, há um agravamento na carga metabólica do organismo, impactando o sistema imune como um todo. Outro fator atrelado a este excesso de componentes proteicos na nutrição é a elevação nos níveis de imunoglobulinas (IgM, IgA e IgG) (MA et al., 2018).

Pieper et al. (2012) encontraram em seu estudo um aumento na expressão de mRNA das interleucinas 1β (IL-1β) e IL-6 em células epiteliais do cólon de leitões alimentados com dietas ricas em proteína. Do mesmo modo, Gao et al. (2020) descobriram que leitões alimentados com uma dieta com 29% de proteína bruta tinham concentrações mais altas de fator de necrose tumoral alfa (TNF-α), IL-6 e IL-8 no íleo quando comparados a leitões que consumiram uma dieta com 17% de proteína bruta.

Como descrito anteriormente, componentes antigênicos da proteína de origem vegetal podem desencadear reações imunológicas. Neste caso ocorre a proliferação e a diferenciação dos linfócitos. Mastócitos também podem liberar rapidamente mediadores ativos, como a histamina e citocinas. Como principais consequências deste mecanismo, pode ocorrer: infiltração de proteínas, indução ao edema da mucosa intestinal e má absorção de líquidos e eletrólitos. Por fim, resulta em quadros de diarreia (XIA et al., 2022).

Em resumo, após a ingestão da dieta com alto nível proteico, podem ocorrem modulações físicas, químicas e microbiológicas no intestino. Desta forma, ocorre a perda da capacidade de barreira, deixando-o mais permeável e susceptível a translocação bacteriana. Os componentes inatos e adaptativos do sistema imune intestinal reconhecem estas alterações e respondem frente ao desafio. A identificação deste processo danoso ao intestino ocorre pelos receptores de reconhecimento de padrões associados a patógenos (*pathogen-associated*

molecular patterns - PAMPs) e associados a danos celulares (DAMPs). Assim, o sistema imune é capaz de detectar tanto agentes infecciosos, quanto as consequências dos mesmos (constituintes intracelulares normais que são liberados após lise celular causada por infecção ou trauma) (MOSER; LEO, 2010). E, portanto, desencadeia os eventos inerentes ao processo inflamatório.

2.3 Enzimas exógenas como estratégia para reduzir o impacto de fatores antinutricionais

A produção de suínos representa uma parcela significativa da economia da agroindústria mundial, sendo a nutrição um dos fatores mais relevantes para o sucesso dessa cadeia produtiva (WU et al., 2020). Apesar dos avanços significativos nas últimas décadas nesta área, a busca pelo diagnóstico de elementos capazes de aumentar a biodisponibilidade de nutrientes deve ser constante. Um exemplo destes componentes é o caso dos fatores antinutricionais. Entre eles, destacam-se os polissacarídeos não amiláceos (PNAs), amplamente presentes em ingredientes comuns das rações (YAQOOB et al., 2022).

Os PNAs, principais componentes da fibra alimentar, são macromoléculas constituídas por polímeros de monossacarídeos, atuando como elementos estruturais das paredes celulares de grãos, como milho e soja. A fibra alimentar é a fração dos alimentos que não é degradada pelas enzimas endógenas do trato digestivo. Esta, pode interferir negativamente na digestão ao interagir fisicamente com a mucosa intestinal e a microbiota (KNUDSEN; HEDEMANN; LAERKE, 2012).

Os PNAs são classificados em solúveis e insolúveis, sendo os solúveis geralmente mais fermentáveis pela microbiota (CHOCT, 2010; TEJEDA; KIM, 2021). Os PNAs afetam negativamente o funcionamento do trato gastrointestinal. Estes componentes promovem o aumento da viscosidade do conteúdo intestinal, promove a redução da motilidade atrasando o esvaziamento gástrico, prejudica a difusão passiva e a absorção de nutrientes, e, consequentemente, diminui o consumo de ração (DONG et al., 2018; BAKER et al., 2021; RADZIKOWSKI; MILCZAREK, 2021). Além disso, criam uma barreira física que dificulta a digestão, por limitar a ação das enzimas digestivas e da bile (DONG et al., 2018).

Quanto maior a capacidade de retenção de água e viscosidade do conteúdo intestinal, maior será a dificuldade de absorção de nutrientes no intestino delgado (CHOCT, 2010; TEJEDA; KIM, 2021). Deste modo, esse cenário favorece alterações na microbiota intestinal,

pelo aumento de proteína não digerida que serve como substrato para proliferação microbiana (OWUSU-ASIEDU et al., 2006).

Dentre os PNAs com efeito antinutricional estão celulose, hemicelulose, arabinana, manano e xilana (DHAWAN; KAUR, 2007). Os β-mananos, uma classe específica de hemiceluloses presentes em diversos ingredientes vegetais, podem causar perdas de até 90 kcal/kg de energia metabolizável (VEUM; ODLE, 2001). Esses compostos podem estar presentes nas formas de glucomanano, galactomanano, glucogalactomanano e glucuronomanano, sendo o segundo polissacarídeo mais abundantes na natureza (MCCLEARY, 1988; CHAUHAN et al., 2012).

Os β-mananos são polissacarídeos lineares compostos por unidades repetidas de β-1,4-manose, α-1,6-galactose e glicose ligadas à cadeia de β-mananos (JACKSON et al., 2004; ZYL et al., 2010). Por não serem digeríveis por animais monogástricos, esses compostos representam um fator antinutricional importante na alimentação de suínos, já que esses animais não possuem enzimas para romper as ligações α-1,6-galactosil e β-1,4-manosil (VEUM; ODLE, 2001). Portanto, há um comprometimento na utilização dos nutrientes (VANGROENWEGHE; POULSEN; THAS, 2021).

Grande parte das dietas comerciais utiliza ingredientes vegetais com altos níveis de fatores antinutricionais. A concentração de β-mananos solúveis (%) varia entre os ingredientes, sendo mais elevada na casca de soja (6,67%), seguida por valores moderados no milho (0,14%) e no DDGS (Dried Distillers Grains with Solubles - Grãos secos de destilaria com solúveis, em português) (0,57%) (POULSEN, 2020). O farelo de soja, por exemplo, contém entre 17% e 27% de PNAs, incluindo aproximadamente 1,3% de β-mananos. É relatado que teores de β-mananos acima de 0,2% a 0,25% na dieta — o equivalente a cerca de 12% de farelo de soja — já podem causar uma redução de 3% na eficiência produtiva. Além disso, esses compostos são resistentes a processos térmicos como secagem, peletização e extrusão (BACH KNUDSEN, 2014; YAQOOB et al., 2022).

Diante disso, estratégias nutricionais vêm sendo adotadas para mitigar esses efeitos. Nesse contexto, a biotecnologia aplicada à nutrição animal possibilitou o desenvolvimento de enzimas exógenas que viabilizam a degradação dos PNAs, como fitases, celulases, xilanases, glucanases, pectinases, galactanases e mananases (MOURA et al., 2019). Resultando em melhorias no desempenho, na digestibilidade dos nutrientes e na resposta a desafios sanitários (YAQOOB et al., 2022). Contudo, os benefícios decorrentes do uso dessas enzimas estão diretamente relacionados ao tipo e à concentração de PNA nos ingredientes, o que influencia a dosagem apropriada (KIM et al., 2017).

As enzimas são proteínas globulares com estrutura terciária ou quaternária que atuam como catalisadores biológicos, otimizando reações químicas específicas e reduzindo o gasto energético nos processos metabólicos (OLIVEIRA, 2019). As enzimas exógenas podem ter origem bacteriana ou fúngica. A adição de preparações enzimáticas específicas à ração tem como objetivo complementar o processo digestivo natural, promovendo maior digestibilidade dos nutrientes (SILVA et al., 2024). A inclusão dessas enzimas melhora o desempenho dos suínos mesmo sob condições dietéticas semelhantes, reduz os custos de alimentação e aumenta a rentabilidade dos sistemas de produção (ZUO et al., 2015; PAULO et al., 2019; WU et al., 2020). Outros benefícios potenciais incluem maior flexibilidade na formulação de rações (SILVA et al., 2024), com uso de ingredientes alternativos e promovendo sistemas mais sustentáveis (SAMPATH et al., 2023).

2.3.1 Características da β-mananase

A β-mananase é uma endocarboidrase responsável pela degradação dos mananos (MOREIRA; FILHO, 2008; ZYL et al., 2010), resultando em compostos como manobiose, manotriose e manose (DHAWAN; KAUR, 2007). A maioria das β-mananases compartilha uma estrutura tridimensional conservada, com o motivo de dobramento em barril (β/α)\₈ — conhecido como "TIM barrel" (SRIVASTAVA; KAPOOR, 2017). Muitas dessas enzimas também apresentam domínios modulares, incluindo módulos de ligação a carboidratos (CBMs), que facilitam a interação com os substratos. De forma geral, as β-mananases apresentam uma estrutura modular composta por domínios catalíticos, CBMs e, em alguns casos, domínios funcionais adicionais (SUNNA, 2010).

Nos últimos anos, as β-mananases têm despertado crescente interesse tanto na academia quanto na indústria, devido ao seu amplo potencial de aplicação em diferentes setores, como perfuração de petróleo, detergentes, têxteis, alimentos, nutrição animal e produção de bioetanol (DAWOOD; MA, 2020). Quanto usada na nutrição animal, essa enzima pode potencializar a atividade das enzimas digestivas, e a melhora na digestibilidade dos nutrientes está associada à sua capacidade de romper a parede celular, facilitando a liberação dos nutrientes ao reduzir a integridade estrutural dos ingredientes (YAQOOB et al., 2022).

A β-mananase contribui ainda para a redução da viscosidade do conteúdo intestinal e promove a liberação de nutrientes associados, como a D-manose, que pode ser utilizada como fonte energética (LEE; BAILEY; CARTWRIGHT, 2003; YAQOOB et al., 2022). Além disso,

a β-mananase apresenta potencial imunomodulador, uma vez que pode reduzir os níveis de imunoglobulinas (LI et al., 2010) e leucócitos circulantes (MEHRI et al., 2010).

2.3.1.1 β-mananos na dieta e seus impactos negativos na saúde intestinal

As enzimas exógenas exercem um papel importante na promoção da saúde intestinal dos animais. Os efeitos negativos dos β-mananos nas dietas estão associados ao aumento da viscosidade do conteúdo intestinal e a alterações na composição da microbiota (SHASTAK et al., 2015; LIU et al., 2024). A maior viscosidade e a consequente redução na taxa de passagem, criam condições propícias pela disponibilidade de substratos para microrganismos patogênicos. Esses microrganismos podem colonizar o intestino delgado, onde competem por nutrientes e energia, prejudicando o desempenho (GOMES et al., 2000). Além disso, favorecem a ocorrência de processos fermentativos indesejáveis. Ademais, tais bactérias também degradam os sais biliares, comprometendo a digestibilidade de lipídios (AGYEKUM et al., 2012; KIM et al., 2024).

Desde modo, a adição de enzimas pode promover alterações favoráveis na composição da microbiota intestinal. Enzimas que degradam PNAs podem estimular o crescimento de microrganismos benéficos produtores de ácidos graxos de cadeia curta (AGCC), como acetato, propionato e butirato, com reconhecido potencial prebiótico (MASEY-O'NEILL; SINGH; COWIESON, 2014; AFTAB; BEDFORD, 2018).

O mecanismo pelo qual os β-mananos aumentam o gasto energético está relacionado à ativação do sistema imune inato, que os reconhece como estruturas estranhas ao organismo (DUNCAN et al., 2002; VANGROENWEGHE; POULSEN; THAS, 2021), Como consequência, ocorre um redirecionamento dos nutrientes para sustentar essa resposta imune, em detrimento de funções relacionadas à manutenção e crescimento (FERREIRA et al., 2016; NUSAIRAT et al., 2024).

A resposta imune é desencadeada pela detecção de componentes reconhecidos como estranhos pelo organismo, independentemente dos efeitos fisiológicos ou patológicos que possam gerar (ABBAS; LICHTMAN; PILLAI, 2012). Quando ocorre ativação exacerbada do sistema imunológico, há prejuízo ao desempenho animal, pois os nutrientes e a energia são desviados de funções produtivas para sustentar o processo inflamatório. Além disso, a inflamação pode induzir catabolismo tecidual (CORZO et al., 2007; JIANG et al., 2010), afetando negativamente o desempenho produtivo de suínos (BAILEY et al., 2005; PABST, 2020).

Como a β-manana não é digerida por enzimas endógenas, permanecem intactas e disponíveis para se ligarem aos domínios de reconhecimento de carboidratos presentes em receptores de reconhecimento padrão (PRRs) das células imunes inatas presentes no epitélio intestinal (DALE; ANDERSON; HSIAO, 2008; HUNTLEY; NYACHOTI; PATIENCE, 2018). Isso ocorre porque essas moléculas apresentam similaridade estrutural com componentes de patógenos como fungos, bactérias e vírus (HSIAO; ANDERSON; DALE, 2006). Essa semelhança impede o organismo de distinguir os β-mananos de verdadeiros patógenos, resultando na ativação do sistema imune inato. Tal ativação leva à proliferação de células como macrófagos, monócitos e células dendríticas, além do aumento na produção de citocinas inflamatórias (LEE et al., 2018; SCAPINI et al., 2019; LIU et al., 2024).

2.3.2 Efeitos da adição de β-mananase na nutrição de suínos

Na indústria de nutrição animal, a inclusão de β-mananase na dieta tem se mostrado altamente benéfica, especialmente em formulações com elevado teor de fibras ou baixo conteúdo energético (AGYEKUM et al., 2014; AGYEKUM et al., 2015). Essa enzima pode ser utilizada isoladamente ou em conjunto com outras carboidrases, promovendo ganhos no desempenho produtivo e no aproveitamento da energia ileal digestível (LATHAM et al., 2018).

As dietas destinadas a suínos geralmente contêm quantidades variáveis de PNAs, que podem impactar negativamente a fisiologia digestiva e o funcionamento intestinal. Suínos alimentados com β-mananase em uma dieta com redução de 1,6% na energia líquida apresentaram desempenho comparável ao dos animais do grupo controle, com maior eficiência no uso da energia e menor incidência de diarreia (SÁNCHEZ-URIBE et al., 2022). De acordo com Vangroenweghe, Poulsen e Thas (2021), os efeitos energéticos positivos de dietas contendo β-mananase estão associados à redução da ativação imune desnecessária. Os autores também relataram uma menor ocorrência de diarreia em leitões desmamados que receberam dietas suplementadas com essa enzima.

Evidências adicionais indicam que a adição de β-mananase pode diminuir os escores de lesões intestinais e reduzir a viscosidade da digesta, sugerindo melhor absorção dos nutrientes pela maior quebra de moléculas (CHO; KIM, 2013; BALASUBRAMANIAN et al., 2018). Além disso, pode contribuir para uma menor produção de mucina (ROMERO et al., 2013).

Kim et al. (2017) observaram que a administração de β-mananase em diferentes concentrações (400–1.600 U/kg) melhorou o desempenho de suínos em fase de crescimento. Da mesma forma, Pettey et al. (2002) mostraram que suínos alimentados com uma dieta

composta por milho e farelo de soja, suplementada com 0,05% de β-mananase, tiveram melhor desempenho do desmame ao abate.

Lv et al. (2013) identificaram que o nível ótimo de inclusão da β-mananase proveniente de *Aspergillus sulphureus* foi de 400 U/kg, promovendo um aumento de 14,6% no ganho de peso e de 17,7% na eficiência alimentar. Além disso, Jo et al. (2012) verificaram que a combinação de 0,05% de α-amilase, β-mananase e protease resultou em melhor desempenho de suínos em crescimento.

De acordo com Galli et al. (2024), a adição da dieta com 300 g/tonelada de β-mananase, combinada com uma redução de 90 kcal/kg na energia metabolizável, proporcionou melhorias significativas nos coeficientes de digestibilidade da matéria seca, da proteína e da energia, além de elevar os coeficientes de metabolização da proteína (2,87%) e da energia (2,61%) em comparação ao tratamento controle. Também foi verificado efeitos benéficos na morfologia intestinal, com aumento da área das vilosidades e da razão entre a altura das vilosidades e a profundidade das criptas em suínos em fase de crescimento.

Pettey et al. (2002) relataram ganhos de 3,4% no ganho de peso e de 3,9% na conversão alimentar em suínos em fase de crescimento e terminação alimentados com dietas contendo β-mananase. De forma semelhante, a adição de dietas para suínos em crescimento e terminação com enzimas que degradam mananos elevou o ganho de carne magra (KIM et al., 2017).

Jeon et al. (2019) observaram que a adição com 0,05% de β-mananase de *Bacillus subtilis* aumentou a digestibilidade ileal padronizada dos aminoácidos, bem como a digestibilidade total da proteína bruta em suínos em crescimento. Do mesmo modo, Upadhaya et al. (2016) reportaram que dietas à base de milho e farelo de soja suplementadas com β-mananase diminuíram a população de coliformes fecais e tenderam a reduzir a concentração de amônia (NH_3) na fração sólida das fezes após 24 horas de fermentação.

Yoon et al. (2010) verificaram que a adição de 400 U/kg de β-mananase a dietas contendo 15% de DDGS elevou o ganho de peso diário e os níveis séricos de triglicerídeos, glicose e colesterol, possivelmente devido ao aumento na digestibilidade da proteína bruta e da matéria seca. Quanto a seu impacto ao sistema imune, Ibuki et al. (2014) constataram que o uso de mananase associada à β-1,4-mannobiose reduziu tanto os níveis quanto a expressão de TNF-α em leitões, sugerindo uma melhora na integridade intestinal e menor inflamação.

3 CONSIDERAÇÃO FINAL

Garantir a saúde intestinal é crucial para otimizar a produtividade dos suínos. Dietas com elevado teor de proteína bruta podem comprometer a saúde intestinal de suínos, principalmente devido à ativação do sistema imune desencadeada pela disbiose e perda da integridade da barreira intestinal. Nessa condição, microrganismos proteolíticos proliferam e produzem metabólitos tóxicos que afetam negativamente o epitélio intestinal. De modo similar, os β-mananos são substratos para a proliferação bacteriana indesejada. Além disso, aumentam a viscosidade da digesta – com redução da digestibilidade – e levam a uma ativação exacerbada do sistema imune inato, que os reconhece como estruturas estranhas ao organismo. Dessa forma, a adoção de estratégias nutricionais, como a alimentação de precisão e o uso de β-mananase na dieta, podem proporcionar suínos em crescimento e terminação com menos processos inflamatórios, melhor aproveitamento dos nutrientes e maior desempenho.

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CAPÍTULO 2

ARTIGO 1 - PRECISION FEEDING AS A PROMISING APPROACH FOR INFLAMMATION REDUCTION AND GUT MICROBIOME MODULATION IN IMMUNOCASTRATED PIGS

Artigo formatado de acordo com o periódico *Journal of Animal Science*
https://academic.oup.com/jas/pages/General_Instructions#Manuscript%20preparation

Precision feeding as a promising approach for inflammation reduction and gut microbiome modulation in immunocastrated pigs

Short title: Precision feeding and gut health in pigs

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ABSTRACT

This study aimed to evaluate the effects of individual precision feeding compared to a phase feeding system on the immune response, gut microbiota composition, metabolic biomarkers, and oxidative stress status in immunocastrated pigs. A total of 50 entire male pigs from the same high-performance genotype (Landrace × Large White; HS Genetic – BRF S.A., Curitiba, Brazil), with an average initial body weight of 23.2 ± 3.12 kg, were randomly assigned to one of two feeding programs: a phase-feeding program (PF), provided all pigs with five phases in this group; and the daily feeding per individual (DFI), was designed to attempted 100% of the estimated nutrient requirements of single individual pig, using automatic and intelligent precision feeders. The trial lasted 104 days. All animals were immunocastrated (days 44 and 86 of the experiment). Blood samples were collected from seven pigs per treatment on days 1, 21, 26, 83, 98, and 104 to assess a panel of metabolic biomarkers, oxidative stress indicators, and inflammatory cytokines. Fecal samples from the same pigs were collected on days 20, 25, and 103 to evaluate fecal concentrations of fatty acid-binding proteins (FABP) and calprotectin, and for microbiota analysis. No major differences were observed between treatments for biochemical parameters or oxidative stress markers ($P<0.05$). However, levels of tumor necrosis factor-α and interleukin-10 were significantly higher in the PF group on day 104 ($P < 0.050$). There were no significant differences in FABP or calprotectin levels between groups ($P > 0.050$). Although no overall treatment effect was detected in alpha or beta diversity ($P > 0.050$), a significant reduction in alpha diversity was observed in the PF group from day 20 to day 103 ($P = 0.012$). Furthermore, age was a significant and consistent predictor of variation in beta diversity ($P < 0.0001$). Individual precision feeding influenced the abundance of specific bacterial genera. In conclusion, individual precision feeding reduced inflammatory activation compared to the multiphase feeding system. Microbial diversity shifted over time, particularly

after the second immunocastration dose, indicating a potential link between hormonal changes and enhanced immune activity in immunocastrated pigs.

TEASER TEXT: Precision feeding tailored to each pig's needs reduces inflammation and improves gut microbiota during growth and finishing. This study highlights how individualized diets promote stable immune response and better gut health, offering new strategies for swine production.

Keywords: Daily-phase feeding, immune response, immunocastration, intestinal health, swine.

INTRODUCTION

Precision feeding is a technique in which pigs receive continuously adjusted diets based on their individual intake and growth patterns (Hauschild et al., 2012, Kala et al., 2025). Improving protein utilization efficiency reduces the environmental impact per kilogram of pork produced, thereby enhancing the sustainability of the swine production chain (Millet et al., 2018; Aparicio-Arnay et al., 2025), without compromising performance (Andretta et al., 2014; Andretta et al., 2016; Andretta et al., 2018). In addition, reducing crude protein levels in diets has been shown to simultaneously improve gut health in pigs (Zhao et al., 2014; Shang, Pai and Patil, 2024). Studies have demonstrated that high dietary protein levels significantly affect both the immune system (Xia et al., 2022) and the gut microbiome of pigs (Sung et al., 2023).

Gut health has significant implications for overall health status. It influences nutrient digestion and absorption, mucin and immunoglobulin secretion, and ensures the barrier function against harmful antigens and pathogens (Yang and Liao, 2019). Research and commercial interest in swine nutrition have grown exponentially, particularly regarding the relationship between gastrointestinal function and immune response (Lauridsen, 2020). This is due to the

fact that the gastrointestinal tract is the body's largest immune organ (Denbow, 2015). Therefore, understanding the mechanisms of intestinal immune processes in pigs allows for the optimization of their overall health regulation.

Additionally, sex hormones play a key role in modulating feeding behavior and immune responses in pigs. Several studies have demonstrated the positive effects of immunocastration, particularly in enhancing male growth rate and improving feed efficiency, which in turn influence both the intestinal microbiome and immune function (Pesenti Rossi et al., 2022; Zoels et al., 2020). This effect occurs because immunocastration harnesses the animal's own immune system to reproduce the outcomes of surgical castration (Lealiifano et al., 2011).

The interaction between gut health and the microbiota has been increasingly studied and recognized for its influence on swine performance and the development of intestinal and immune functions (Kabat, Srinivasan and Maloy, 2014; Chen et al., 2018; Li et al., 2018; Duarte and Kim, 2022). Thus, by promoting animal health, precision nutrition emerges as an effective alternative to enable efficient and sustainable production (Gaillard, Brossard and Dourmad, 2020; Maher, Sweeney and O'Doherty, 2025).

We hypothesize that daily adjusting amino acid levels in the diets according to individual requirements positively modulates the gut microbial profile, while reducing inflammatory processes and oxidative stress. Therefore, the objective of this study was to evaluate the effects of individual precision feeding compared to a multiphase feeding system on the immune response, microbiome, metabolic biomarkers, and oxidative stress status in immunocastrated pigs.

MATERIALS AND METHODS

This experiment was conducted at the Swine Research Facilities at Unesp, Jaboticabal, SP, Brazil. The experimental procedures were reviewed and approved by the Ethics Animal

Care Committee of São Paulo State University (Unesp), Jaboticabal, SP, Brazil (protocol no. 62/23).

Animals, experimental design, and housing

A total of fifty entire male pigs of the same high-performance genotype (Landrace × Large White; HS Genetic - BRF S.A; Curitiba, Brazil) with an average initial body weight (BW) of 23.2 ± 3.12 kg were randomly allocated in a randomized block design based on body weight and rooms, with the following two feeding programs: a phase-feeding program (PF) that provided all pigs with five phases in this group. Each phase followed a commercial strategy developed to satisfy the requirements of the mean population and thus maximize the population gain to feed ratio. The second treatment, the daily feeding per individual (DFI), was designed to attempted 100% of the estimated nutrient requirements of single individual pig, using automatic and intelligent precision feeders (AIPF; Exafan, San Mateo de Gállego, Spain). Each treatment had 25 replicates of one pig each.

The pigs were identified and assigned to one of two similar rooms following a density of 1.13 m^2 per pig, each on a climatized and full concrete floor. The room temperature was progressively decreased from 22 to 18°C. The photoperiod was fixed at 14 hours of artificial fluorescent light (05h00min -19h00min).

Water was provided with low-pressure nipple drinkers, and feed was provided individually with four electronic feeding stations. The operation of the feeders has been previously described in detail (Pomar, López and Pomar, 2011; Andretta et al., 2014). In summary, the feeding stations recognize individual pigs as they insert their heads into the feeder and dispense a custom blend of feeds with high and low nutrient density level, formulated to meet the estimated lysine requirements of each animal, adjusted according to the assigned experimental treatment. Typically, pigs consume the entire portion or leave only minimal

residues, ensuring that the intended blend was effectively delivered to each animal. Feed portions were dispensed according to the animal's experimental age: 12 g from weeks 1 to 9, 15 g during weeks 10 and 11, 20 g in weeks 12 and 13, and 25 g from weeks 14 to 17. A 10-second delay between feedings was implemented to encourage full consumption before a new request could be made. Feeder calibration — ensuring consistency between dispensed and recorded feed amounts — was verified and adjusted daily. This automated system enabled individualized feed delivery while allowing all pigs to be housed within the same pen. All pigs had an exclusive electronic chip (plastic button tag containing passive transponders of radio frequency identification; Allflex®, Joinville, SC, Brazil) inserted in the right ear, previously registered in AIPF.

On day 45 of the trial, animals received a subcutaneous injection of ivermectin (Ivomec™, Boehringer Ingelheim, São Paulo, SP, Brazil) as part of the deworming protocol. The immunocastration procedure involved the administration of Improvac™ (Zoetis, São Paulo, SP, Brazil), with the first dose given on day 44 and the second on day 86 of the experimental period, maintaining a 42-day interval between applications.

Diets and feeding

The pigs were fed a standard diet for two weeks prior to the beginning of the trial, in accordance with their nutritional requirements, to allow adaptation to the AIPF.

Two experimental diets, referred to as feeds A and B, were formulated based on net energy (NE) and standardized ileal digestible (SID) amino acids, using a consistent ingredient composition database. No energy restrictions were applied, and neither formulation included antibiotic additives (Table 1). Feed A was designed with a high nutrient density to meet the requirements of the most demanding animals at the start of the first growing phase. In contrast, feed B was formulated with lower nutrient density, aligned with the estimated needs of less

demanding pigs at the end of the final growing phase, and followed standard commercial guidelines for all nutrients and formulation parameters. The digestible amino acid to lysine ratios (AA:Lys) adopted for feeds A and B were, respectively: Met+Cys at 60% / 61%, Thr at 65% / 68%, Trp at 21% / 21%, Val at 61% / 85%, and Ile at 53% / 60%. The levels of vitamins and microminerals were maintained constant across both diets. Feeds were presented in a steam-pelleted form (approximately 95% pellets).

An appropriate final feed composition was obtained by blending the two feeds at each pig visit to the feeder, thus creating a complete feed with the desired estimated Lys concentration. The SID Lys concentration of PF pigs corresponded to the requirement of the animal placed in the 80th percentile. The 80th percentile pig was chosen assuming that its SID Lys requirement represented the dietary concentration that maximizes population growth performance (Hauschild et al., 2010; Remus et al., 2020). To determine the 80th percentile for this trial, we used the average of the 80th percentile of all pigs during the 3 d before the beginning of each phase. The lysine concentration required for each pig was estimated on an individual basis across all treatments, utilizing a previously developed mathematical model (Hauschild et al., 2012). This model incorporated each animal's daily feed intake and weekly body weight records. It combines an empirical component, which predicts body weight, feed intake, and daily gain for the following day, with a mechanistic component that applies a factorial approach to determine the ideal amino acid concentration to be provided to each pig on that specific day, ensuring their nutritional requirements are met.

Within the mechanistic component of the model, daily lysine (Lys) requirements (expressed in g/day) were determined by summing the needs for maintenance and growth. Maintenance requirements were calculated based on three components: basal endogenous losses estimated as 0.313 g Lys per kg of dry matter intake multiplied by daily feed intake; desquamation-related losses in the digestive tract calculated as 0.0045 g Lys per kg^0.75 of

metabolic weight per day; and basal turnover of body proteins, estimated at 0.0239 g Lys per kg^0.75 per day (van Milgen et al., 2008). The SID Lys requirements for growth were estimated assuming that 16% of the DG is protein (De Lange et al., 2003) and that 7% of the protein deposition is composed of Lys (Mahan and Shields, 1998), and that the efficiency of Lys retention from dietary ileal digestible Lys is 72% (Möhn et al., 2000).

The data from this study related to growth performance, body composition, nutrient intake and efficiency, feeding costs, and environmental assessment were previously described by Kippert et al. (2025; unpublished data).

Blood sampling and analysis

Blood samples were obtained from the jugular vein of seven pigs per treatment on days 1, 21, 26, 83, 98, and 104 of the experiment, following a fasting period of six hours. Animals were selected based on body weight closest to the treatment mean on day 1 and were retained for sampling on subsequent days. The samples were collected into 8 mL serum tubes (BD Vacutainer; NJ, USA) and left to clot for one hour. Subsequently, samples were centrifuged at 3000 × g for 10 minutes at 4°C (Novatecnica, NT 835, Piracicaba, SP, Brazil), and the serum was separated and stored at –80°C until further analysis.

A panel of ten metabolic biomarkers was assessed, including total protein, albumin, urea, triglycerides, cholesterol, glucose, D-lactate, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT), using an automated biochemical analyzer (SINNOWA SX-300®), following the manufacturer's protocol.

For oxidative stress evaluation, glutathione peroxidase (GPx) activity was quantified by measuring the rate of NADPH oxidation in the presence of hydrogen peroxide (H_2O_2), with reduced glutathione and glutathione reductase (GR) as substrates, at an absorbance of 340 nm (Günzler et al., 1984). GR activity was determined through the decrease in NADPH absorbance

at 340 nm according to Carlberg and Mannervik (1975). Superoxide dismutase (SOD) activity was assessed by monitoring the auto-oxidation of pyrogallol at 570 nm, using the technique described by Madesh and Balasubramanian (1997). Catalase activity was evaluated by measuring the breakdown of H_2O_2 at 240 nm as per Aebi's method (1984). Lipid peroxidation levels were determined by quantifying thiobarbituric acid reactive substances (TBARS) at 530 nm (Buege and Aust, 1978). All assays were performed in triplicate using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA), with enzymatic activities expressed as specific activity units.

Additionally, serum concentrations of the inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) were measured using ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China), adhering strictly to the manufacturer's instructions.

Fecal sampling and analysis

On days 20, 25, and 103 of the study, fecal samples were obtained from the same seven animals per treatment through digital stimulation of the rectal ampulla. All samples were then stored at –80°C until they were processed.

For biomarker analysis, the procedures outlined by Galli et al. (2024) were followed. The concentrations of fatty acid-binding proteins (FABP) and calprotectin were measured using commercially available ELISA kits, following the protocols provided by the manufacturer (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Fecal microbiota analyses

Total bacterial DNA was extracted from the fecal sample contents using a commercial kit (High Pure PCR Template Preparation Kit, Roche, Germany) according to the manufacturer's instructions. Fifty milligrams of feces were diluted in 200 µL of nuclease-free

water and subsequently treated with lysozyme (Sigma-Aldrich Brasil Ltda, Cotia, SP, Brazil). The extracted DNA was quantified by spectrophotometry at 260 nm using a NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA).

Genomic libraries were prepared using the 16S Metagenomic Sequencing Library Preparation protocol (Illumina®) for amplification of the 16S rRNA V3-V4 region (460 bp). A negative control (molecular-grade water) was included for quality assurance. The Nextera XT Index Kit v2 (Illumina) was used for DNA library preparation, and PCR was performed using Phusion High-Fidelity PCR Master Mix with GC Buffer Enzyme (Invitrogen). All amplified PCR products were then purified using AMPure XP beads (Beckman Coulter). Library concentrations were quantified using a Qubit DNA HS assay (Invitrogen) and checked for fragment distribution with a 4200 TapeStation System capillary electrophoresis instrument (Agilent). Pooled libraries were loaded onto the MiSeq® platform using a v3 reagent kit (2x300 bp; ~200,000 reads/sample) (Illumina, Inc.).

The raw reads were processed following a previously described protocol (Callahan et al., 2016a), using the DADA2 algorithm (Callahan et al., 2016b). The reads were quality-checked, trimmed, filtered, and truncated. Subsequently, paired-end reads were merged, amplicon sequence variants (ASVs) were inferred, and chimeric sequences were removed. Taxonomic annotation was performed using the SILVA taxonomic training database formatted for DADA2 (Silva version 138.2) (Callahan, 2024). The ASVs, counts, taxonomic tables, and sample metadata were combined into a phyloseq object (McMurdie and Holmes, 2013) for downstream analyses. Taxonomic clustering was conducted to identify dominant taxa and their relative abundances at the phylum, family, and genus levels. For subsequent analyses, a Compositional Data Analysis (CODA) approach was applied (Aitchison, 1982), using centered log-ratio (CLR) and isometric log-ratio (ILR) transformations (Pawlowsky-Glahn et al., 2015;

Greenacre et al., 2021; Bars-Cortina, 2022). A total of 100,000 paired reads (200,000 reads in total) per sample were used.

Statistical analyses

All analyses were performed using SAS Studio - On Demand for Academics (SAS Institute, Inc., Cary, NC, USA). Each animal was considered as the experimental unit. Normality was assessed for all variables using the Shapiro–Wilk test (UNIVARIATE procedure), and the data were analyzed by analysis of variance (MIXED or GLIMMIX procedures). Adjusted means (LS-means) were used to describe the treatments, and potential differences were evaluated using Tukey's multiple comparison test. Differences were interpreted at $P \leq 0.05$.

Bioinformatics analyses were performed using the open-source software R version 4.4.3 (Trophy Case) (R Core Team, 2021), the RStudio development interface (version 2022.12.0-353) (RStudio Team, 2020), and packages from the Bioconductor project (version 3.21) (Huber et al., 2015). Alpha diversity (α-diversity) was assessed using the Shannon index, and statistical significance was evaluated through linear mixed-effects modeling. Beta diversity (β-diversity) was analyzed using Principal Component Analysis (PCA), Principal Coordinates Analysis (PCoA), and Non-metric Multidimensional Scaling (NMDS) based on Aitchison (Aitchison, 1982) and Jaccard distances (Jaccard, 1908). Statistical significance and the proportion of explained variance were determined by Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2017) and linear mixed-effects models. Differential abundance analyses were conducted using the Microbiome Multivariable Association with Linear Models 2 (Maaslin2) algorithm (Mallick et al., 2021). To correct for multiple testing, the Benjamini-Hochberg (BH) method (Benjamini and Hochberg, 1995) was applied to control the false

discovery rate (FDR), along with a mixed directional FDR (mdFDR) control using Holm's family-wise error rate procedure (Holm, 1979), with a significance level of 5%.

RESULTS

Biochemical and Oxidative Parameters

The metabolic biomarkers of the pigs during the experimental period are presented in Fig. 1. At 21 days of the experiment, pigs in the PF treatment had higher ALT levels ($P = 0.036$). The DFI pigs had a higher concentration of cholesterol at 83 days ($P = 0.012$), and more total protein at 98 days ($P = 0.034$). There were no differences in the other biomarker variables between treatments during the experiment ($P > 0.050$).

As shown in Fig. 2, pigs under individual precision feeding showed higher SOD activity on day 1 ($P = 0.007$) and higher TBARS activity at 21 days ($P = 0.027$). GPx activity was higher at 83 days in the PF treatment ($P = 0.034$). There were no significant differences in GSH and CAT activities ($P > 0.05$).

Immune parameters

Serum TNF-α and IL-10 concentrations were influenced by the treatments (Fig. 3). At 104 days of the experiment, pigs in the PF group showed higher concentrations of both cytokines ($P < 0.05$). There were no differences in fecal fatty acid-binding proteins (FABP) and calprotectin levels ($P > 0.050$).

Microbiome

A total of 1045 ASV were detected. The top six most abundant phyla in PF samples were Firmicutes (59.4%), Bacteroidota (27.4%), Euryarchaeota (9.5%), Proteobacteria (1.8%), Spirochaetota (0.8%), and Verrucomicrobiota (0.7%). The top six most abundant phyla in DFI

samples were Firmicutes (58.4%), Bacteroidota (27.5%), Euryarchaeota (9.5%), Proteobacteria (1.0%), Spirochaetota (0.9%), and Actinobacteriota (0.3%). The top six most abundant family in PF samples were Muribaculaceae (17.1%), Lachnospiraceae (12.4%), Oscillospiraceae (12.1%), Methanobacteriaceae (9.9%), Prevotellaceae (6.4%), and Christensenellaceae (5.6%). The top six most abundant family in PF samples were Muribaculaceae (16.4%), Lachnospiraceae (13.5%), Oscillospiraceae (12.2%), Methanobacteriaceae (9.8%), Prevotellaceae (7.8%), and Ruminococcaceae (5.8%). The composition per treatment and per time point at phylum and family level are shown at Fig. 4 and Fig. 5.

No significant differences in alpha diversity index (Shannon's index) were observed between treatments ($P > 0.05$; Fig. 6). However, there was a significant reduction in diversity from day 20 to day 103 in the PF group ($P = 0.012$). In the beta diversity analysis using ordination methods (Aitchison PCA and Jaccard PCoA), age was the only significant and consistent predictor for PC1 ($P < 0.0001$; Fig. 6) and Axis 1 ($P < 0.0001$; Fig. 7). Treatment effects and the Age × Treatment interaction were not significant in any of the models.

The differential abundance analysis revealed significant differences in microbial composition between treatment groups and age (Fig. 8).

DISCUSSION

Reducing crude protein levels in pig diets is a promising strategy to enhance intestinal health (Zhao et al., 2014). Until now, no previous studies have examined the effects of precision feeding on the intestinal health of immunocastrated pigs using the same approach described in this project. This study demonstrated that individual precision feeding influenced the immune response of finishing pigs following the second immunocastration dose. As a pioneering investigation in this area, the findings offer valuable insights that could guide decision-making in swine production.

Intestinal health can be defined as a state of overall homeostasis within the gastrointestinal tract, encompassing both its structure and function (Pluske, Turpin and Kim, 2018). When pigs are subjected to stressful events, their physiological regulation is altered, leading to behavioral, endocrine, metabolic, and immune responses (Yu, Chen and Chang, 2019). These responses are accompanied by a range of morphological, enzymatic, and inflammatory changes in the intestine (Boudry et al., 2004; Montagne et al., 2007; Wijtten, van der Meulen and Verstegen, 2011). Consequently, the animals become more susceptible to diseases, resulting in decreased production efficiency and economic losses (Yu, Chen and Chang, 2019).

Understanding the immunometabolic, cellular, and biochemical pathways of the intestine helps establish biomarkers that serve as indicators of normal or pathological processes in production animals (Soares et al., 2022; Sciascia and Metges, 2023). In this study, six fecal and blood samples were collected over the 104 day experimental period. The selected sampling days were strategically chosen to capture key moments: the beginning and end of the trial (days 1 and 104), before and after the greatest protein reduction in the control group (days 21 and 26), and before and after the second immunocastration dose (days 83 and 98). Fecal samples were collected in the afternoon of the day prior to blood collection, as the animals were fasted. Immunocastration is a management technique used to suppress testicular development and function, effectively converting entire males into immunologically castrated pigs. One major advantage of this method is that the animals retain the beneficial traits of entire males—such as superior feed efficiency and better carcass quality—through most of the growth and finishing phases (Bee et al., 2020).

Despite the strategically planned sample collections, no significant differences were observed between treatments in terms of biochemical or oxidative stress markers, contrary to the initial hypothesis. Antioxidant components may respond differently to varying production

conditions, as noted by Cui et al. (2017), Long et al. (2021), and Ma et al. (2021). However, the sensitivity and specificity of these analyses may be limited, and interference from other compounds could affect quantification (Ito, Sono and Ito, 2019), which might explain the results observed in this study. Moreover, there were period effects on GR, GPx, and TBARS, indicating that, regardless of treatment, pigs exhibit varying enzymatic activities throughout the production cycle.

Similarly, age emerged as the most influential factor for several biochemical parameters, including ALT, ALP, AST, triglycerides, urea, albumin, and total protein. Elevated ALP activity is associated with high osteoblastic activity during skeletal growth. In piglets and young pigs, ALP levels are naturally high and tend to decline as animals mature and bone growth slows (Makris et al., 2023). Additionally, urea is the primary end-product of amino acid metabolism and ammonia detoxification in the liver. Blood urea nitrogen levels reflect both hepatic urea synthesis and urinary nitrogen excretion (Berghaus et al., 2023). In this context, the abrupt increase in feed intake following the second immunocastration dose in both groups (Kippert et al., 2025; unpublished data) likely increased the pigs' metabolic demand. As the urea cycle requires energy to synthesize urea, diets that promote higher urea production can impair animal performance (Marín-García et al., 2022). Therefore, research focused on understanding amino acid requirements is crucial for optimizing swine nutrition.

Undigested protein becomes available for fermentation in the ileum and large intestine, which can negatively affect intestinal permeability and immune function (Heo et al., 2010), while also serving as a substrate for bacterial fermentation (Kim et al., 2012). This promotes a shift toward more proteolytic microbial populations and increased production of fermentation by-products (Williams et al., 2001). Excess dietary protein, therefore, may act as a precursor for intestinal pathologies and systemic inflammation (Camilleri, 2019). Consistent with this, by day 104 of the trial, after the second dose of immunocastration, pigs in PF had significantly

higher IL-10 and TNF-α levels, with average concentrations approximately three times greater than those in the DPI group. One of the main consequences of excessive immune activation is the substantial increase in the energy and nutrient demands of the immune system, which diverts essential resources away from productive functions (Kvidera et al., 2017). In growing pigs, immune system activation can raise maintenance requirements by approximately 25% (Huntley, Nyachoti and Patience, 2017).

The precision feeding program presents advantages over phase feeding. The greater availability of substrates in the intestine may have been the main factor responsible for microbiome modulation, resulting in a reduction in alpha diversity from day 20 to day 103 in the phase-fed group. Alpha diversity tends to increase with the age of pigs, especially from the end of the nursery phase until the pre-slaughter period (Zhao et al., 2015; Wang et al., 2019; Luo et al., 2022). At this stage, the microbiome is already fully developed and closely resembles that of sows (Wang et al., 2019). It is presumed that the higher protein supply to the control group had a negative effect on microbial diversity throughout the experiment. Thus, the nutrient oversupply resulting from the increased daily feed intake after the second immunocastration dose may have promoted the proliferation of specific substrate-dependent microorganisms. This selective proliferation of certain bacterial groups — although not detailed in this study — may have caused an imbalance in the intestinal microbiota, characterizing a state of dysbiosis (Weiss et al., 2021).

Commensal or pathogenic microorganisms can significantly influence host physiology (Clemente et al., 2012; Campbell, Crenshaw and Polo, 2013; Soares et al., 2022). Differences in the abundance of bacterial phyla and families were observed among the treatments. Notably, the Ruminococcaceae family showed higher abundance in the DFI group. This family is well known for its ability to produce butyrate through the fermentation of carbohydrates, particularly from resistant starch (RS) (Singh et al., 2023). The biosynthesis of butyrate begins with the

conversion of acetyl-CoA into butyryl-CoA, which serves as a primary energy source for colonic epithelial cells (Baxter et al., 2019). In addition to its energetic role, butyrate also contributes to the maturation of mucosa-associated lymphoid tissue (MALT) (Liu et al., 2021; Kim et al., 2024).

Some bacterial genera showed significant differences in abundance between treatments, reflecting changes in the intestinal ecosystem of pigs in response to diet and sampling time. *Prevotella* is one of the most predominant genera throughout the large intestine of pigs. Its presence has been associated with several host-beneficial traits, including feed intake, feed efficiency, weight gain, and incidence of diarrhea. These associations suggest that *Prevotella* may play an important role in mediating growth performance and disease resilience in pigs. *Prevotella* increased in the comparisons DFI.D20 vs PF.D20 and DFI.D25 vs PF.D20, whereas *Selenomonadaceae* decreased in the same contrasts, as well as in D103 vs D20. These patterns suggest that the expansion of *Prevotella* may exert a competitive effect on *Selenomonadaceae*, since both are involved in carbohydrate fermentation and short-chain fatty acid (SCFA) production. *Prevotella* possesses a broad enzymatic repertoire to degrade complex polysaccharides, conferring an advantage under conditions of lower protein availability, which may explain the concomitant reduction of *Selenomonadaceae* (Amat et al., 2020; Iljazovic et al., 2021). Thus, the interaction between intestinal maturation, dietary substrate availability, and physiological changes following immunocastration may influence microbial succession and SCFA production, impacting intestinal health and metabolic performance in pigs.

Analysis of members of the Lachnospiraceae family, including ASV1145.UCG-007, ASV992 (*Eubacterium nodatum* group), ASV279.*Colidextribacter*, ASV559.*Fusicatenibacter*, and ASV401.NK4B4.group, revealed distinct response patterns over time and between treatments. The increase of UCG-007 in D103 vs D20 and *Colidextribacter* in D25 vs D20 indicates that certain complex carbohydrate-fermenting groups may thrive with intestinal

maturation or under specific dietary regimes, contributing to SCFA production, particularly butyrate, which is essential for intestinal mucosal integrity and energy metabolism (Zhang et al., 2025). Furthermore, at D103, the animals had already received the second dose of immunocastration, resulting in hormonal changes that modulate metabolism, feed intake, and the inflammatory response. These physiological changes may create an intestinal environment favorable for the colonization of these microorganisms, enhancing the fermentation of available substrates and increasing the production of beneficial metabolites.

On the other hand, the reduction of *Eubacterium nodatum* in DFI.D20 vs PF.D20, as well as of *Fusicatenibacter* and NK4B4 in D25 vs D20, shows that not all groups within the same family respond in the same way, reflecting microbial competition and variations in the availability of fermentable substrates. Other microorganisms, such as ASV1166.*Paludicola* (Ruminococcaceae), ASV2613.*Cellulosilyticum*, and ASV324.*Acetitomaculum*, also showed reductions in DFI.D20 or DFI.D25 compared to PF.D20. All are involved in the degradation of complex carbohydrates, SCFA production, and maintenance of intestinal health, suggesting that dietary regimes favoring *Prevotella* and certain Lachnospiraceae members may simultaneously reduce the abundance of these groups, reflecting competitive interactions and functional adaptations within the microbiome (Xie et al., 2022).

Furthermore, ASV25.*Catenibacterium* (Erysipelotrichaceae) and ASV557.*Ligilactobacillus* (Lactobacillaceae) decreased in D103 vs D20, indicating that with intestinal maturation and potential dietary changes, both simple and complex carbohydrate-fermenting groups can be suppressed or displaced by more competitive groups, such as *Prevotella* and *Colidextribacter*. The simultaneous reduction of these groups and expansion of other fermenters reflects a functional restructuring of the microbiome, in which the relative composition of microorganisms determines overall SCFA production and host intestinal health (Duarte and Kim, 2022).

Furthermore, ASV25.*Catenibacterium* (Erysipelotrichaceae) and ASV557.*Ligilactobacillus* (Lactobacillaceae) decreased at D103 compared to D20, indicating that, with intestinal maturation, potential dietary changes, and hormonal alterations induced by immunocastration, both simple and complex carbohydrate-fermenting groups can be suppressed or displaced by more competitive groups, such as *Prevotella* and *Colidextribacter*. The simultaneous reduction of these groups and the expansion of other fermenters reflect a functional restructuring of the microbiome, in which the relative composition of microorganisms determines host intestinal health (Duarte and Kim, 2022; Zhang et al., 2025).

Overall, the results indicate that diet and time modulate the intestinal microbiome through competitive interactions among carbohydrate-fermenting groups, impacting substrate availability, SCFA production, and consequently energy metabolism and mucosal integrity (Pu et al., 2025). This scenario highlights the importance of microbial dynamics and functional relationships in the intestinal ecosystem's response to environmental and dietary changes in pigs.

CONCLUSIONS

Individual precision feeding resulted in lower immune responses compared to phase feeding. The microbiome was modulated according to age, with a progressive reduction in alpha diversity over time and a significantly distinct beta diversity at day 103 compared to earlier time points. These findings suggest that finishing pigs, following the second dose of immunocastration, exhibit a gut microbiota profile that is significantly different from previous phases, potentially contributing to increased immune activation.

ACKNOWLEDGMENTS

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support, and the Laboratório de Estudos em Suinocultura (LabSui) for providing the facilities and support that enabled the completion of this study.

CONFLICT OF INTEREST

The authors of the present study declare that they have no competing interests.

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Table 1 Ingredient formulas and chemical composition of experimental feeds.

	Feed A High nutrient density	Feed B Low nutrient density
Ingredient formulas, as feed basis (%)		
Sorghum	61.600	93.005
Soybean meal 46%	29.300	2.000
Corn acid oil	4.322	0.757
Limestone	1.157	1.263
Lysine sulphate 60%	0.717	0.450
Monobicalcium phosphate	0.692	0.861
Sodium chloride	0.580	0.580
Methionine hydroxy analogue 88%	0.312	-
L-threonine 98.5%	0.235	0.071
L-tryptophan 60%	0.121	0.049
L-valine 96.5%	0.032	0.032
Whey permeate[1]	0.625	0.625
Vitamin and mineral premix[2]	0.310	0.310
Chemical composition		
Dry matter (%)	89.70	89.50
CP (%)	19.69	9.03
Total lysine (%)	1.402	0.510
SID[2] lysine, calculated[3] (%)	1.302	0.470
Metabolizable energy[3] (Mcal/kg)	3.374	3.216
Net energy[3] (Mcal/kg)	2.607	2.606
Calcium (%)	0.69	0.71

Total phosphorus (%)	0.46	0.41
Digestible phosphorus, calculated[3] (%)	0.33	0.33
Crude fiber (%)	2.78	2.03
Ash (%)	5.23	4.27

[1]Whey permeate was added to improve pellet durability/quality[2]Premix provide the following nutrient amounts per kilogram: vitamin A 4400 IU; vitamin D3 880 IU; vitamin E 28 IU; vitamin K3 1.78 mg; vitamin B12 14 mcg; niacin 24.6 mg; pantothenic acid 10.67 mg; pyridoxin 1.78 mg; riboflavin 2.66 mg; thiamine 1.34 mg; folic acid 0.40; biotin 89 mcg; copper, 225mg; iodine 0.35 mg; iron 80mg; manganese 30mg; selenium 0.30 mg; zinc 80mg; fitase 500ftu; xilanase 10,000 U, antioxidant blend 50mg[2] Standardized ileal digestible.[3] Values for growing pigs were estimated from the gross composition of the ingredients according to EvaPig (Software Version 1.3.1.4, INRA, Saint-Gilles, France).

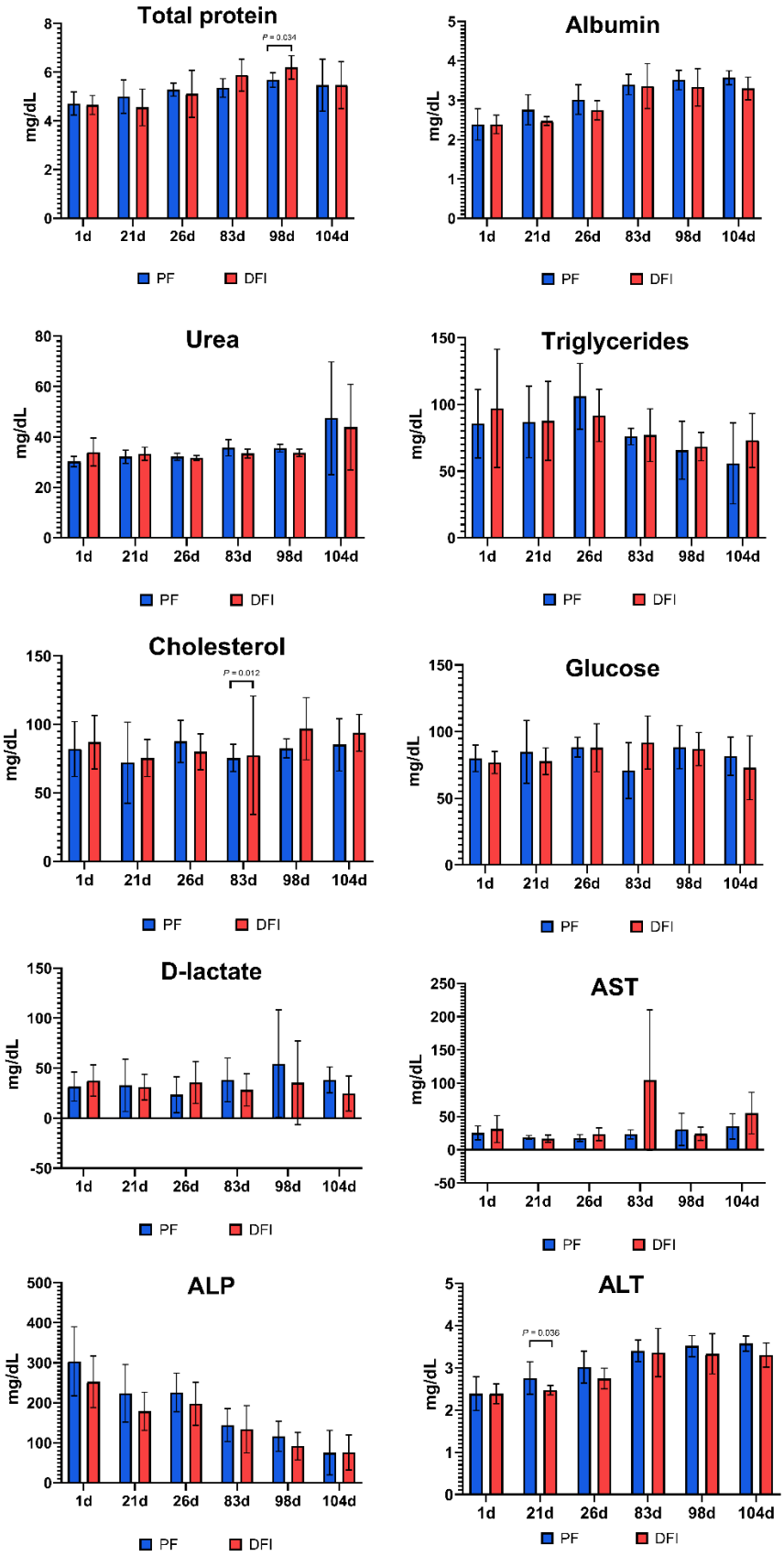

Figure 1 Metabolic biomarkers in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI). Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

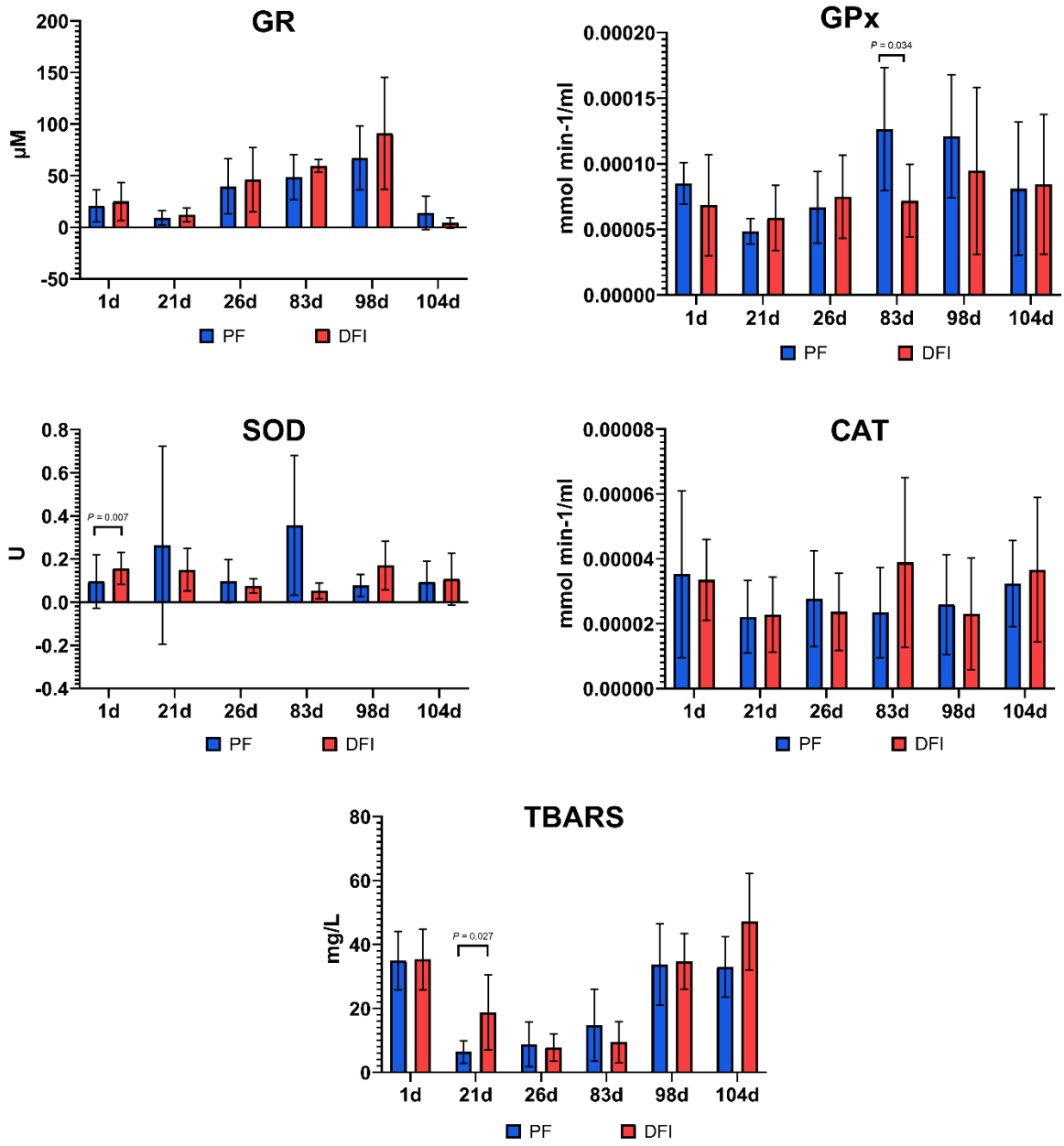

Figure 2 Serum glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid-reactive substances (TBARS) in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs.

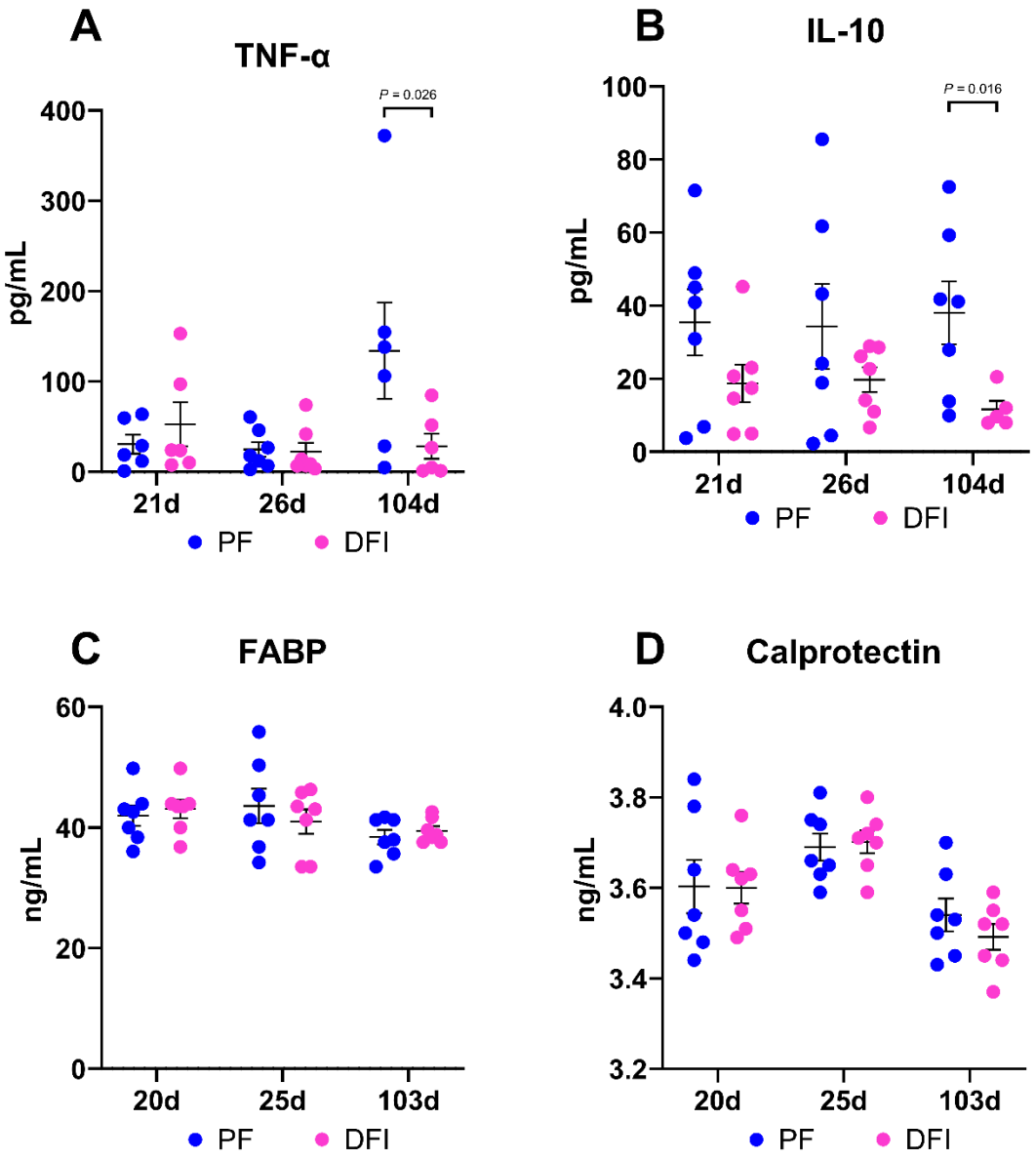

Figure 3 Serum tumor necrosis factor (TNF)-α (**A**), and interleukin (IL)-10 (**B**), and fecal fatty acid-binding proteins (FABP, **C**), and calprotectin (**D**) in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individual.

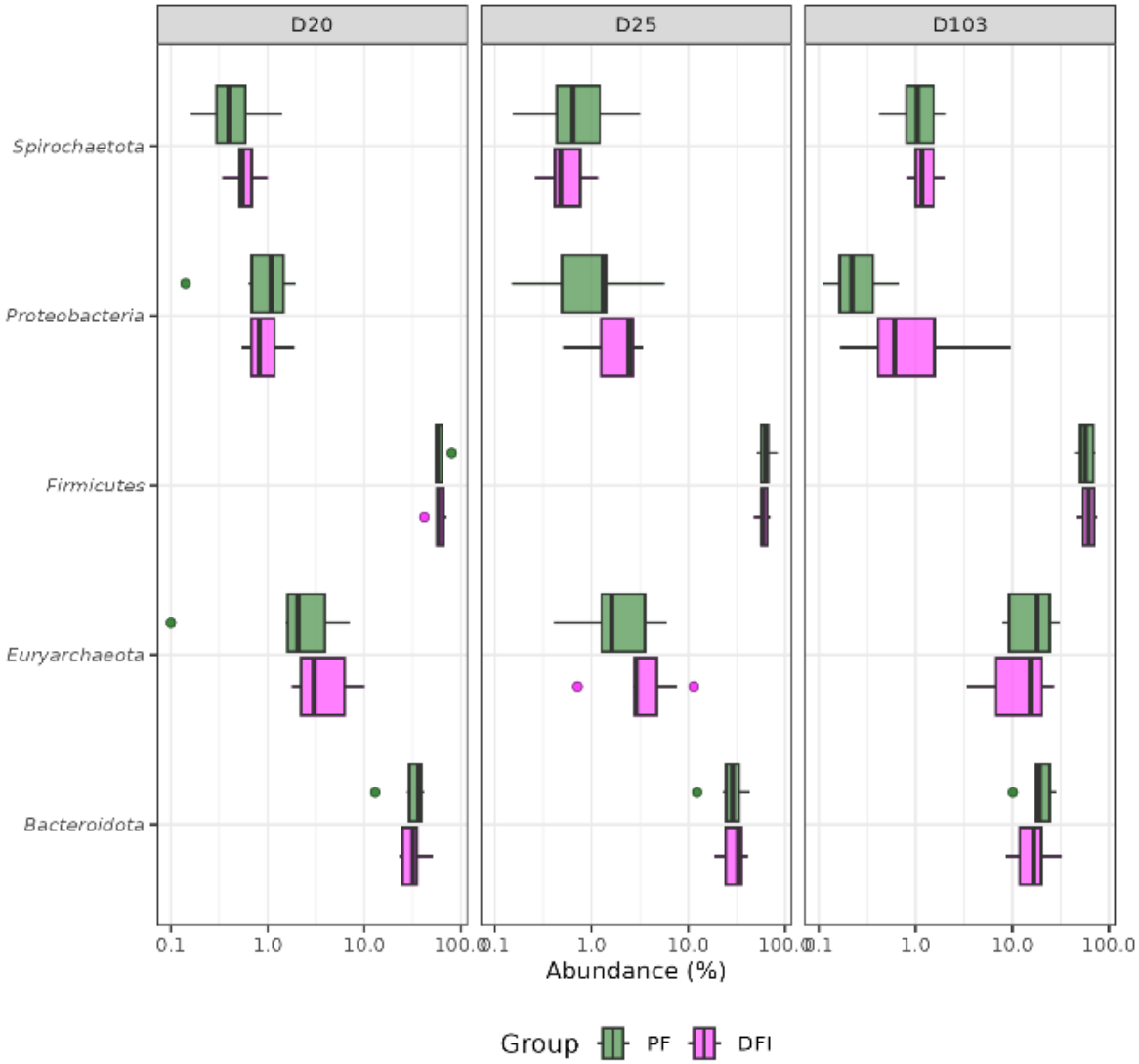

Figure 4 Composition per time point (days 20, 25 and 103 of experiment) at phylum level in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI).

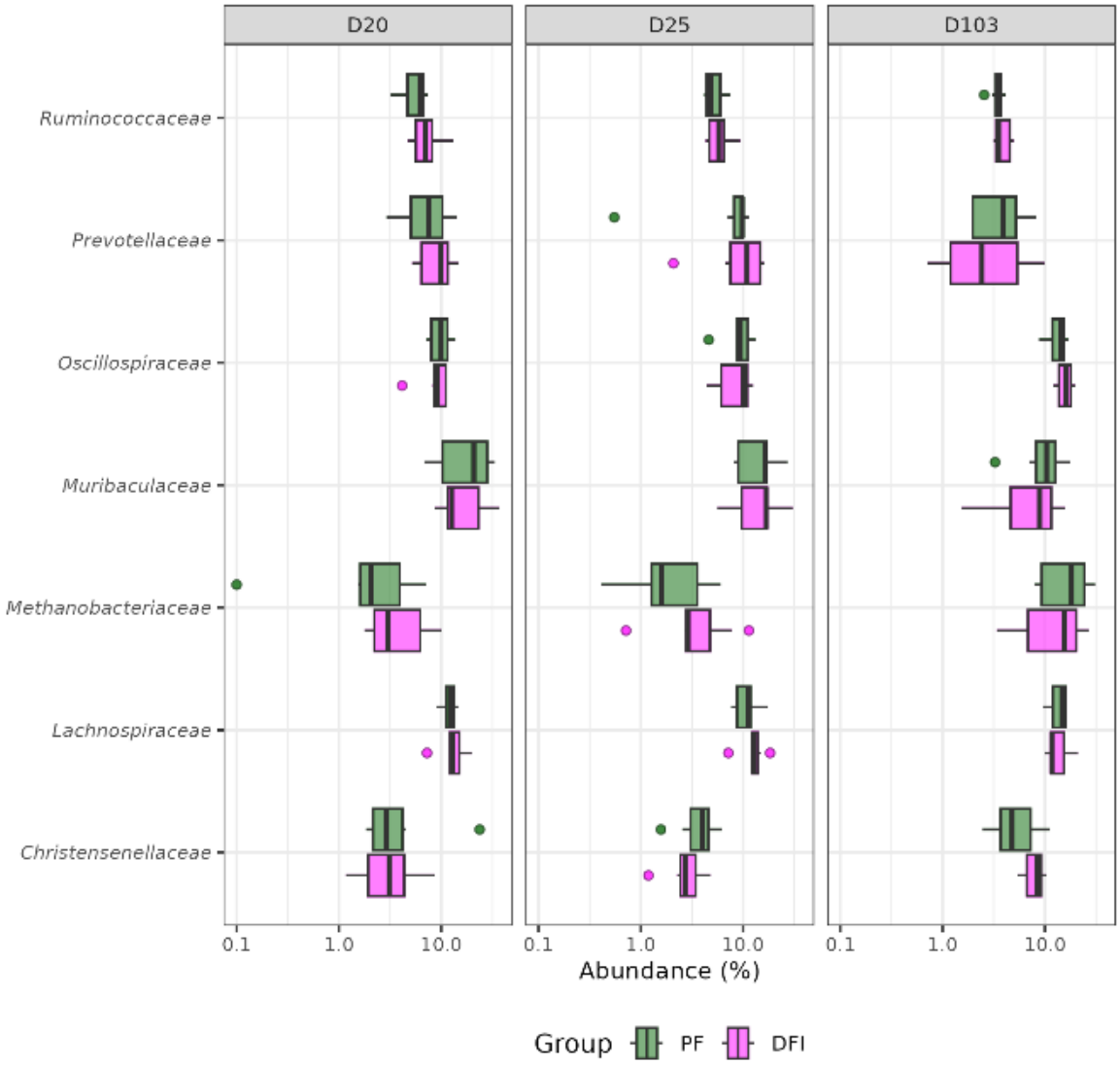

Figure 5 Composition per time point (days 20, 25 and 103 of experiment) at family level in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI).

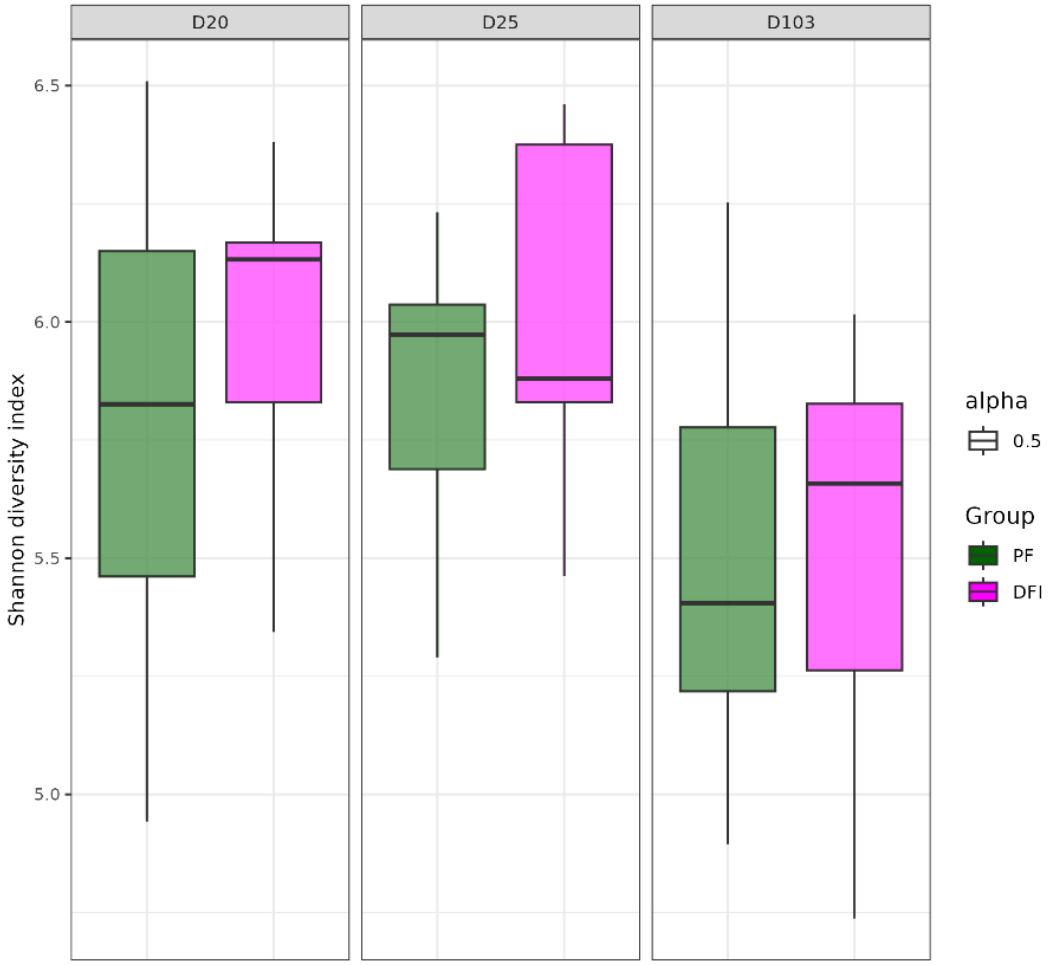

Figure 6 Mean Shannon diversity over age by group in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI).

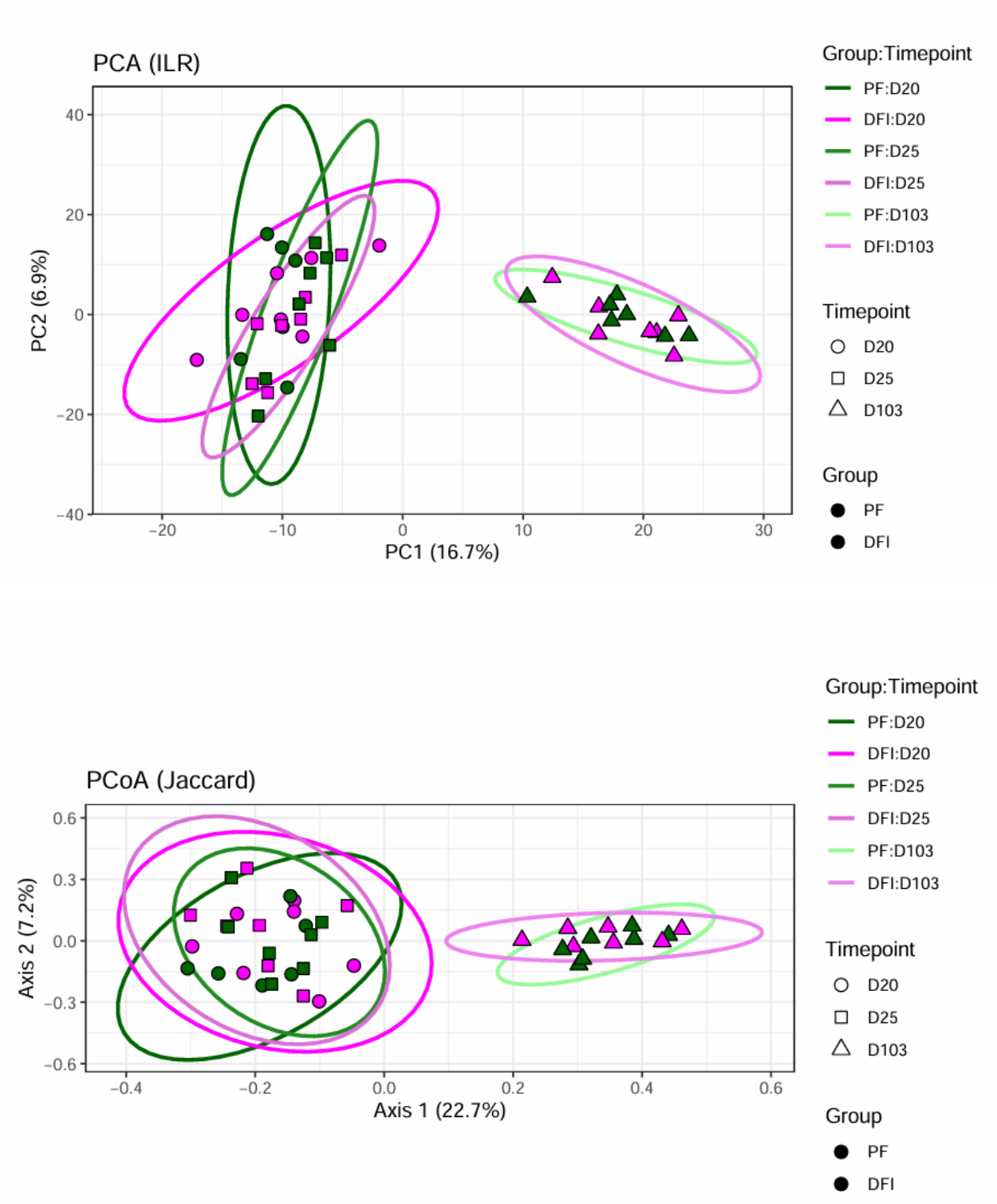

Figure 7 Beta diversity based on principal component analysis (PCA) and principal coordinates analysis (PCoA) in growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI).

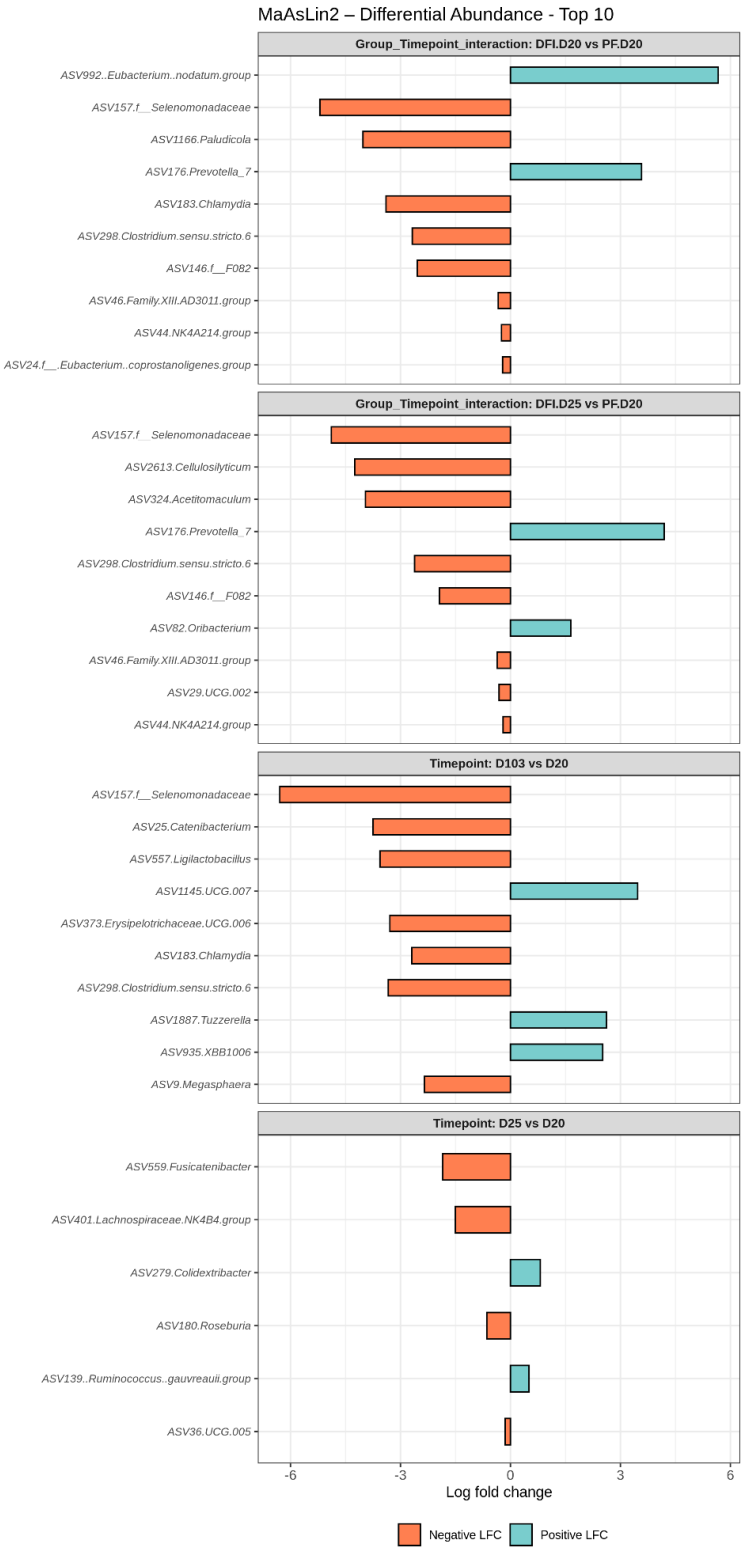

Figure 8 Differential abundance in fecal samples of growing-finishing pigs in the phase-feeding program (PF), or daily-phase feeding programs provided individually (DFI) on days 20, 25 and 103 of experiment. Log Fold Change (LFC). The comparisons shown are only those that are statistically significant and are always paired with the reference (baseline – PF.D20, control group, at the first sampling time).

CAPÍTULO 3

ARTIGO 2 - MODULATION OF NITROGEN METABOLISM, PERFORMANCE, PROTEIN GAIN AND INFLAMMATORY RESPONSES IN IMMUNOCASTRATED GROWING-FINISHING PIGS THROUGH DIETARY INCLUSION OF β-MANNANASE

Artigo formatado de acordo com o periódico *Animal*

https://www.sciencedirect.com/journal/animal/publish/guide-for-authors

Modulation of nitrogen metabolism, performance, protein gain and inflammatory responses in immunocastrated growing-finishing pigs through dietary inclusion of β-mannanase

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Short title: β-mannanase improves nitrogen efficiency and immune response in pigs

Abstract

This study was developed to assess the impact of β-mannanase (**BM**) on growth performance, nutrient balance, metabolic biomarkers, oxidative stress status, and inflammatory responses of growing-finishing pigs. Fifty uncastrated male pigs (23.02 ± 0.42 kg) were fed control diets (no addition) or a diet with BM (300 g/ton) for 104 days divided into five feeding phases. Performance and body composition were evaluated per at each feeding phase. Blood samples (n = 7 per treatment) were collected on days 1, 21, 26, 83, 98, and 104 of experiment, to analyse

metabolic biomarkers, oxidative stress status and cytokines. Fecal samples (n = 7 per treatment) were collected in the same animals on days 20, 25 and 103 to evaluate calprotectin and fatty acid-binding proteins (**FABP**). The addition of BM resulted in greater average daily gain (**ADG**, P=0.085), and feed efficiency (**G:F**, P=0.048) on days 63 to 82, and on days 83 to 104 (P=0.070, and P=0.025, respectively). In addition, improved backfat thickness at 104 days (P=0.003), but without differences to loin muscle depth and loin area (P>0.05). On day 104, BM tended to improve the protein mass (P=0.076) and protein + water mass (P=0.072). Greater lipid mass and ash mass were observed with BM (P<0.05). In addition, on days 83 to 104, BM pigs had increased lipid, protein, and ash gains (P<0.05). Overall, from 1 to 104 days, BM pigs had more protein gain (P=0.049), but no difference in ash and lipid gain (P>0.10). On days 83 to 104, BM pigs had greater nitrogen retention (P=0.015) and phosphorus retention (P=0.024). Overall, 1 to 104 days greater nitrogen retention was observed in BM pigs (P=0.035), and tended to improve the nitrogen efficiency (P=0.097). No differences were found between the treatments for metabolic biomarkers (P>0.05). BM pigs had higher serum concentration of glutathione reductase on day 98, (P=0.058), of glutathione peroxidase on day 83 (P=0.016), and lower serum concentration of TNF-α on day 104 (P=0.003), and of IL-10 on days 21 (P=0.005), 26 (P=0.011), and 104 (P=0.044). Lower fecal concentration of calprotectin was observed at 103 days (P=0.053) for BM pigs. No differences were found for FABP (P>0.05). In conclusion, BM improves growth performance and body composition by promoting muscle mass gain, enhancing nutrient utilization, and reducing both intestinal and systemic inflammatory responses.

Keywords: enzyme, immune response, immunocastration, nutrition, swine.

Implications

The present study investigated the effect of β-mannanase (**BM**) on performance, nutrient balance, metabolic biomarkers, oxidative stress status and inflammatory responses of growing-finishing pigs. Results clearly indicate that this additive helped reduce inflammation, allowing more nutrients to be used for protein gain and better nutrient utilization of the diet.

Introduction

Dietary levels of non-starch polysaccharides (**NSP**) exceeding 5% can negatively impact pig performance by increasing digesta viscosity and decreasing digestibility in the gastrointestinal tract (Wu *et al*., 2018). Therefore, higher amounts of NSP increase endogenous amino acid loss (Chen *et al*., 2017), the mucosal cell turnover rate, mucin secretion, and undigested content in pigs (Dong *et al*., 2018). As a result, numerous enzymatic addition programs have been developed to help animals cope with these deleterious effects.

Dietary β-mannanase (**BM**) can hydrolyze β-mannans, which represent approximately 15–35% of total soy NSP (Kim *et al*., 2017). This enzyme can reduce the innate immune response induced by feeding and save 85–100 kcal of metabolizable energy (**ME**)/kg, improving nutrient digestibility (Genova *et al*., 2023). Hence, BM can release mannan-oligosaccharides, which act as prebiotics to support the intestinal health of pigs (Kiarie, Steelman and Martinez, 2021). This enzyme has the potential to improve animal performance (Ferreira *et al*., 2016) and reduce post-weaning diarrhea and antibiotic use in pigs (Vangroenweghe, Poulsen and Thas, 2021). It may also have anti-inflammatory properties that reduce the cost of engaging the immune system (Li *et al*., 2010).

The mannan molecules have β-1, 4-mannosyl links, and α-1, 6-galactosyl bonds, which cannot be broken by endogenous enzymes in pigs. Therefore, the function of BM s is to save energy that would be spent in the immune response, as well as to release nutritional components for absorption. Thus, it is possible to hypothesize that this enzyme has an additive effect on

animal metabolism nutrients, and intestinal health. This study aimed to evaluate whether the addition of BM can improve the performance, nutrient balance, metabolic biomarkers, oxidative stress status, and inflammatory responses of growing-finishing pigs.

Materials and methods

Enzymes

The BM tested was derived from the fermentation of *Paenibacillus lentus* (Hemicell HT®, Elanco Animal Health, São Paulo, Brazil; registration number: SP626-2; minimum activity: 160,000 unit/g (1 unit is the amount of enzyme that releases 0.72 mcg of reducing sugars (equivalent to D-mannose) per min from goma locust (mannan concentration of 88%) at pH 7.5 and 40 °C)).

Animals, housing, and management

A total of 50 uncastrated male pigs of the same high-performance genotype (Landrace × Large White; HS Genetic-BRF S.A; Curitiba, Brazil) in the absence of clinical signs of diseases were shipped to Swine Research Facilities at São Paulo State University (**UNESP**), Jaboticabal, SP, Brazil. The pigs were fed the experimental diets for 2 weeks before the experiment, and then randomly assigned to the experimental treatments at 23.02 ± 0.42 kg body weight (**BW**) in two rooms with a solid concrete floor, following a density of 1.13 m^2 per pig.

Both rooms were equipped with an evaporative pad cooling system and exhaust fans to keep temperature progressively decreased from 22 to 18°C when the pigs reached approximately 100 kg BW, thus ensuring thermoneutral conditions. Also, the rooms had artificial fluorescent lighting with a 14-hour photoperiod (0500–1900 h).

The pigs were allocated in a randomized block design based on body weight and rooms, with the following treatments: the control consisted of a basal diet with no enzyme addition

(n=25), and the β-mannanase consisted of the addition of 300 g/ton of β-mannanase (n=25) with reduction of 35 kcal of net energy. Each treatment had 25 replicates of one pig each. All pigs had an exclusive electronic chip (plastic button tag containing passive transponders of radio frequency identification; Allflex®, Joinville, SC, Brazil) inserted in the right ear, which had already been registered in automatic and intelligent precision feeders (**AIPF**; Exafan, San Mateo de Gállego, Spain).

The pigs had free access to feed and fresh water throughout the experiment. Water was provided to nipple drinkers, and feed was provided individually at four AIPF in each room. The functioning of these feeders has been previously described (Pomar, López and Pomar, 2011; Andretta *et al.*, 2014). Pigs tend to empty the feeder hopper or leave only very small amounts of feed behind at each visit, providing assurance that each pig received the assigned amount of blended feeds. The feeder calibration (matching between registered and provided amounts of feed) was checked daily. Thus, the service was set to deliver the feed according to the experimental age. A time lag of 10 seconds was imposed between services to ensure that the pigs consumed each serving before requesting a new one. The AIPFs were designed to provide each pig with the required feed blend, and this feature allowed all pigs in the trial to be housed in the same pen.

A deworming protocol using ivermectin (Ivomec™, Boehringer Ingelheim, São Paulo, SP, Brazil) was performed via subcutaneous administration on day 45 of the trial. The immunocastration vaccine (Improvac™, Zoetis, São Paulo, SP, Brazil) had its first dose applied on day 44 of the experiment and the second dose on day 86, thus representing an interval of 42 days between doses.

Diets and feeding

Four experimental feeds (named A, B, C, and D) were independently formulated based on net energy (**NE**) and standardized ileal digestible (**SID**) amino acids, using the same ingredient composition database, without any energy constraints, and with no growth promoters or any other additives. Feed A and C were formulated with a high nutrient density level, given that it was determined for the most demanding pigs at the beginning of the first growing period, while feed B and D had a low nutrient density level, given that it was estimated for the less demanding pigs at the end of the last growing period following a standard commercial recommendation for all nutrients and other formulation parameters. The digestible AA:Lys ratios used for this study was Met+Cys: 60 / 61%; Thr: 65 / 68%; Trp: 21 / 21%; Val: 61 / 85%, and Ile:53 / 60% for feed A and B, respectively. Vitamin and micromineral additions were kept equal between the feeds. An appropriate final feed composition was obtained by blending the feeds at each pig visit to the feeder, thus creating a complete feed with the desired estimated Lys concentration (Table 1).

The treatments consisted of a phase feeding program that provided all pigs with five phases, each 21 days long, with a fixed blend of feeds A and B to control group, and feeds C and D to β-mannanase treatment within each feeding phase. The blend for each phase followed a commercial strategy developed to satisfy the requirements of the mean population and thus maximize the population gain to feed ratio.

Performance and body composition

The pigs were weighed individually on conventional scales at arrival and the beginning of each feeding phase (on days 1, 21, 42, 63, and 83), and at the end of the performance trial (on day 104). Feed intake was registered automatically by the feeders during all experiment.

All performance data (average daily feed intake – **ADFI**, average daily gain – **ADG**, and feed efficiency – **G:F**) were analyzed by feeding period.

The backfat thickness, loin muscle depth and *L. dorsi* area were evaluated on days 1, 42, 83, and 104. Pigs (n = 12 per treatment) were measured using an ultrasound device (KX5200V, 7.5Mhz linear transducer probe, Keebomed Inc. Mount Prospect, IL, USA) between the third and fourth ribs at 5 cm from the midline.

Total body fat, lean mass, bone mineral content, and bone mineral density were measured using dual-energy X-ray- absorptiometry (**DXA**) on days 1, 42, 83, and 104 with a densitometry device (GE Lunar Prodigy Advance; GE Healthcare, Madison, WI, USA). The pigs (n = 12 per treatment) were scanned in prone position using the total body scanning mode (GE Lunar enCORE, version 8.10.027). The pigs were sedated to prevent movement by intramuscular injection of Acepromazine maleate (0.1 mg/kg; Acepran™, Vetnil, SP, Brazil), xylazine (1.5 mg/kg; Sedanew™, Vetnil, SP, Brazil), and ketamine (15 mg/kg; Ketalex™, Dechra Veterinary Products, São Paulo, SP, Brazil) The DXA body lean, fat, and bone masses were converted to protein, lipid, and ash chemical equivalents (Kipper *et al*., 2019). Total body phosphorus was estimated assuming that 18% of bone mineral content is phosphorus and that DXA bone mineral content represents 80% of the total body phosphorus (Nielsen *et al*., 1973; Merkatoris, Rortvedt and Crenshaw, 2012). Nutrient efficiencies were calculated by dividing the gain of protein (estimated using the values obtained by DXA) or Lys (estimated assuming that 7% of body protein is Lys) by crude protein (**CP**) or SID Lys intake, respectively. Nitrogen and phosphorus excretion values were obtained for each pig by subtracting the respective nutrient retention from the respective nutrient intake values. Nitrogen and phosphorus efficiencies were calculated by dividing the retention by the intake.

Blood sampling and analysis

On days 1, 21, 26, 83, 98, and 104 of the experiment, after a 6-hour fasting period, blood samples were collected from the jugular vein of 7 animals per treatment group for the quantification of inflammatory cytokines, metabolic biomarkers, and oxidative stress markers. Animals were selected based on body weight closest to the treatment mean on day 1 and were retained for sampling on subsequent days. Blood samples were collected in 8 mL serum tubes (BD Vacutainer; NJ, USA) and allowed to clot for 1 hour. After centrifugation at 3000 × g for 10 minutes at 4°C (Novatecnica, NT 835, Piracicaba, SP, Brazil), serum samples were collected and stored at -80°C for later analysis. A total of 10 metabolic biomarkers, including total protein, albumin, urea, triglycerides, cholesterol, glucose, D-lactate, aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**), and alanine aminotransferase (**ALT**) were analyzed using an automated biochemical analyzer (SINNOWA SX-300®) according to the manufacturer's instructions.

For oxidative stress analysis, glutathione peroxidase (**GPx**) activity was measured by monitoring the oxidation rate of nicotinamide adenine dinucleotide phosphate (**NADPH**) in the presence of H_2O_2, using reduced glutathione and glutathione reductase (**GR**) as substrates at 340 nm (Günzler *et al*., 1984). GR activity was determined by measuring NADPH oxidation at 340 nm, as described by Carlberg and Mannervik (1975). Superoxide dismutase activity was determined based on the auto-oxidation of pyrogallol using the method of Madesh and Balasubramanian (1997), with absorbance read at 570 nm. Catalase activity was measured by assessing H_2O_2 decomposition at 240 nm, according to the method of Aebi (1984). Lipid peroxidation was assessed by measuring the production of thiobarbituric acid reactive substances (**TBARS**) at 530 nm (Buege and Aust, 1978). All samples were analyzed in triplicate using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, Massachusetts, USA). Enzymatic activities were expressed as specific activities.

Serum levels of the inflammatory cytokines tumor necrosis factor alpha (**TNF-α**), and interleukin 10 (**IL-10**) measured using ELISA test kits, according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Fecal sampling and analysis

Fecal samples were collected on days 20, 25, and 103 of the experiment, by digital stimulation of rectal ampoule of 7 animals per treatment. All samples were stored at -80˚C until processing for biomarkers assessment, as described by Galli *et al.* (2024). The detection of fatty acid-binding proteins (**FABP**), and calprotectin were performed using commercial ELISA test kits, according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Statistical analysis

Each pig was considered an experimental unit. Variables that did not provide normally distributed residuals with the Shapiro–Wilk test were transformed logarithmically. By means of SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), data were submitted to variance analysis to evaluate the treatment effect over time using the MIXED procedure. Interactions (treatment × period) were also included in the model. Eventual differences among the treatments were assessed with the Tukey multiple comparison test and then interpreted at $P \leq 0.05$ (significant differences) and $0.05 < P \leq 0.10$ (a trend for difference).

Results

On days 63 to 82, the β-mannanase had a greater G:F (P=0.048) and a trend to higher ADG (P=0.085) compared to the control (Table 2). On days 83 to 104, β-mannanase had a greater G:F (P=0.025) and a trend to higher ADG (P=0.070) compared to the control. No

differences were found between the treatments for ADFI, ADG, and G:F on days 1 to 20, 21 to 41, 42 to 62, and 1 to 104. β-mannanase improved backfat thickness at 104 days (P=0.003), but without differences to loin muscle depth and loin area (Table 3). No differences were also found on days 1, 42, and 83.

On day 104, β-mannanase tended to improve the protein mass (P=0.076) and also protein + water mass (P=0.072) relative to the control (Table 4). Greater lipid mass and ash mass were observed in β-mannanase than in the control (P<0.05). In addition, on days 83 to 104, β-mannanase had greater lipid, protein, and ash gains in relation to the control (P<0.05). Overall, from 1 to 104 days, β-mannanase had greater protein gain compared to the control (P=0.049), but no difference in ash and lipid gain (P>0.05). No differences were found between the treatments for protein mass, protein + water mass, lipid mass, ash mass, and bone mineral density on days 1, 42, and 83 (P>0.05). No differences were found between the treatments for lipid gain, protein gain, and ash gain on days 1 to 41, and 42 to 82 (P>0.05).

No differences were found between the treatments for net energy intake, SID lysine intake, CP intake, and phosphorus intake in any of the periods analyzed (P>0.05; Table 5).

On days 83 to 104, β-mannanase had greater nitrogen retention (P=0.015) and phosphorus retention (P=0.024) relative to the control (P>0.05; Table 6). Overall, 1 to 104 days greater nitrogen retention was observed in β-mannanase compared to the control (P=0.035), and the β-mannanase tended to improve the nitrogen efficiency in relation to the control (P=0.097). No differences were found between the treatments in nitrogen retention, nitrogen excretion, nitrogen efficiency, phosphorus retention, phosphorus excretion, and phosphorus efficiency on days 1 to 41 and 42 to 82.

No differences were found between the treatments for metabolic biomarkers: total protein, albumin, urea, triglycerides, cholesterol, glucose, D-lactate, AST, ALP, and ALT (Figure 1).

On day 98, β-mannanase tended a higher serum GR concentration when compared to control (P=0.058, Figure 2). Higher GPx serum concentration was observed in β-mannanase than in the control on day 21 (P=0.010), and on day 83 (P =0.016). No difference to SOD, CAT and TBARS were observed during this trial.

β-mannanase had lower serum concentration of TNF-α on day 104 (P=0.003), and of IL-10 on days 21 (P=0.005), 26 (P=0.011), and 104 (P=0.044, Figure 3). In addition, β-mannanase tended lower fecal concentration of calprotectin at 103 days (P=0.053). No differences were found between the treatments for FABP concentration on feces.

Discussion

This study was conducted to evaluate whether the addition of BM could improve the performance, nutrient balance, and intestinal health of growing-finishing pigs. To the best of our knowledge, this study represents the first effort to assess the carryover impact of BM on nutrient balance and body composition.

β-mannans can cause a feed-induced immune response (**FIIR**) because they have a similar molecular structure to some pathogens called pathogen-associated molecular patterns (PAMPs), which are expressed on the surface of pathogens (Saeed *et al*., 2019) such as fungi, bacteria, and viruses (Hsiao, Anderson and Dale, 2006). On the other hand, the innate immune system has no memory and is therefore unable to distinguish pathogens from β-mannans. Consequently, the innate immune system generates unnecessary expenditure of ATP and nutrients, creating a false signal for the presence of pathogens in the body. This false signal is associated with intestinal inflammation and reduced animal growth (Kiarie, Steelman and Martinez, 2022).

β-mannanase could improve nutrient utilization and ultimately the growth of pigs (Kiarie, Steelman and Martinez, 2021; Jang *et al.*, 2023). Therefore, as observed in our study,

pigs in the final stages of production, especially in the last weeks before slaughter, show an exponential increase in feed intake. Consequently, there will be an improvement in intake of β-mannans, which, as previously mentioned, generate signals that activate inflammatory processes.

Previous studies have shown that β-mannans can trigger innate immune responses by promoting macrophage proliferation and cytokine production, ultimately contributing to systemic inflammation (Zhang and Tizard, 1996; Duncan *et al*., 2002). On the other hand, the addition of BM attenuated the FIIR by hydrolyzing native β-mannans into smaller fragments with a reduced capacity to stimulate the innate immune system (Kiarie, Steelman and Martinez, 2022). According to this study, the inclusion of 300 g/ton of BM in the diet led to a reduction in serum concentrations of TNF-α and IL-10, as well as fecal calprotectin levels. Calprotectin is released upon neutrophil activation and exhibits antimicrobial, antiproliferative, and pro-apoptotic properties (Stříž; Trebichavský, 2004; Bressler *et al.*, 2015).

Namely, BM resulted in reduced activation of both the systemic and intestinal immune systems. Huntley, Nyachoti and Patience (2018) and Sánchez-Uribe *et al.* (2022) reported that this enzyme can decrease blood concentrations of acute-phase proteins and IL-6, while enhancing tight junction integrity and increasing IFN-γ and IL-8 levels.

This immune modulation was particularly evident in the period following the second dose of immunocastration. Immunocastrated pigs resemble entire males until the immunocastration becomes effective — shortly after the second dose — after which they begin to exhibit physiological and behavioral characteristics such as surgically castrated males (Dunshea *et al.*, 2013) Therefore, as the animals began to exhibit castrated male behavior, there was an increase in feed intake. Consequently, the greater availability of β-mannans in the gastrointestinal tract led to increased activation of the immune system, which was mitigated using BM. Thus, BM addition may reduce the stimulation of the immune system and provide

more nutrients and energy for growth performance and better body conditions, as noticed at this study.

Genova *et al*. (2023) observed no difference in growth performance for grower pigs fed with ME reduced and with the addition of BM which permits saving 85 to 100 kcal of ME/kg without compromising the growth performance. Yoon *et al*. (2010) found that the addition of BM improved the ADG and the apparent total tract digestibility of dry matter and CP compared to the control in growing-finishing pigs. Galli *et al*. (2024) reported that the addition of 300 g/ton of BM with a reduction of 90 kcal/kg of ME resulted in greater dry matter, protein, and energy digestibility coefficients, and protein (2.87%) and energy (2.61%) metabolizability coefficients compared to control. Hence, the authors observed that the addition of BM resulted in a greater villus area and villus height-to-crypt depth ratio compared to the control in growing pigs.

Thus, β-mannan is an NSP with deleterious effects on growth performance and decreased feed digestibility. Thus, the greater G:P, lipid, protein, and ash gain, as well as the nitrogen and phosphorus retention observed in this study, may be attributed to the enzyme having better access to the substrates, allowing for a greater amount of nutrients to be absorbed and/or increased access of endogenous proteolytic, amylolytic, and lipolytic enzymes to nutrients. In addition, greater villus height and villus area may have increased the surface area available for nutrient absorption, thus improving growth performance and nutrient retention.

There were no major differences in biochemical markers or antioxidant enzymes with the use of BM. The variations observed appear to be more related to the animals' daily conditions at the time of sampling, as the data trends were not consistent in subsequent collections. Jang *et al*. (2020) also found no differences in their study, reporting that the concentrations of superoxide dismutase and glutathione peroxidase in nursery pigs were not affected by BM supplementation. Furthermore, the absence of differences between treatments

may indicate that the dose used was safe. Acosta *et al.* (2025) stated that when fed to weanling pigs at the recommended level, the enzyme had no detrimental effects on serum biochemical and hematological parameters, or on growth performance. Moreover, supplementing diets with elevated doses of BM can result in an excessive generation of mannan oligosaccharides, which may interact with functional receptors on enterocytes involved in immune regulation and cell proliferation. This interaction can overstimulate the immune system and contribute to oxidative stress in the organism (Nochta *et al.,* 2009; Zhou *et al.,* 2019).

Galli *et al*. (2024) observed greater nitrogen retention and an improved ratio of retained to absorbed nitrogen in pigs that fed the BM. We found similar results for nitrogen. Thus, these results are attributed to the pigs' greater muscle growth, as we found a greater protein mass and gain, which explains the greater nitrogen retention and efficiency in pigs fed with BM. Lv *et al*. (2013) observed a linear effect with BM addition for phosphorus digestibility for weaned pigs (0, 200, 400, or 600 U/kg BM), the authors relate this improvement is because of the reduction of digesta viscosity. Kipper *et al*. (2020) in a meta-analysis verified that BM increased by 6% the phosphorus digestibility. We found greater phosphorus retention with the addition of BM as well as greater ash mass and gain, which can be related. However, better digestibility releases more nutrients, including minerals, that are trapped by plant cells. Modern pig production is moving towards more precise and sustainable practices. The use of exogenous enzymes in feed formulations is certainly a part of this context. Therefore, this enzyme is related to the hydrolysis of non-starch polysaccharides, breaking the cell walls that encapsulate them, degrading anti-nutritional factors (β-mannans), cleaving glycolytic bonds that are not hydrolyzed by endogenous enzymatic activity, and reducing the activation of the innate immune system.

Conclusion

BM supplementation in diets in growing-finishing pigs enhances growth performance by improving feed efficiency and promoting muscle mass gain. It also increases lipid and mineral mass. In addition, pigs fed with BM have lower inflammatory responses. The immune modulation was particularly evident in the period following the second dose of immunocastration.

Ethics approval

The experimental procedures were reviewed and approved by the Ethics Committee on Animal Use (CEUA) of the Faculty of Agricultural and Veterinary Sciences, UNESP – Jaboticabal Campus, São Paulo, Brazil, protocol no. 62/23.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

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Declaration of interest

Marcos Kipper da Silva is an employee of Elanco Animal Health that provided the β-mannanase tested in the current study. The other authors do not have any real or perceived conflicts of interest.

Acknowledgements

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support, Elanco Animal Health for provided the β-mannanase tested in the current study, and the Laboratório de Estudos em Suinocultura (LabSui) for providing the facilities and support that enabled the completion of this study.

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Table 1 *Ingredient formulas and chemical composition of experimental feeds used in control (C) or β-mannanase (β) treatments (%, as feed basis).*

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5	
	C	*β*	C	*β*	C	*β*	C	*β*	C	*β*
Ingredient formulas, %										
Sorghum	69.69	70.10	73.22	73.63	74.99	75.40	76.75	77.16	79.40	79.81
Soybean meal 46%	22.63	22.90	19.57	19.81	18.04	18.27	16.51	16.72	14.21	14.41
Corn acid oil	3.202	2.525	2.807	2.156	2.609	1.971	2.411	1.786	2.115	1.509
Limestone	1.161	1.161	1.181	1.181	1.191	1.191	1.201	1.201	1.217	1.217
Lysine sulphate 60%	0.739	0.721	0.698	0.681	0.678	0.661	0.657	0.641	0.627	0.612
Monocalcium phosphate	0.727	0.724	0.746	0.743	0.756	0.753	0.765	0.763	0.779	0.777
Sodium chloride	0.580	0.580	0.580	0.580	0.580	0.580	0.580	0.580	0.580	0.580
Methionine hydroxy-analogue 88%	0.273	0.267	0.233	0.228	0.213	0.209	0.193	0.189	0.163	0.160
L-threonine 98.5%	0.226	0.221	0.203	0.199	0.192	0.188	0.181	0.176	0.164	0.160
L-tryptophan 60%	0.119	0.117	0.109	0.106	0.104	0.101	0.098	0.096	0.091	0.088
Whey permeate[1]	0.313	0.313	0.313	0.313	0.313	0.313	0.313	0.313	0.313	0.313
Vitamin and mineral premix[2]	0.340	0.340	0.340	0.340	0.340	0.340	0.340	0.340	0.340	0.340
β-mannanase[3]	-	0.030	-	0.030	-	0.030	-	0.030	-	0.030
Chemical composition										
Dry matter (%)	88.56	88.47	88.54	88.46	88.53	88.45	88.52	88.44	88.50	88.43
Crude protein (%)	17.31	17.45	16.11	16.23	15.51	15.63	14.90	15.02	14.00	14.11
Total lysine (%)	1.238	1.236	1.132	1.129	1.079	1.076	1.025	1.023	0.946	0.943

SID[4] lysine, calculated[5] (%)	1.152	1.150	1.053	1.050	1.003	1.000	0.953	0.950	0.878	0.876
Net energy[5] (Mcal/kg)	2.566	2.531	2.570	2.535	2.572	2.537	2.573	2.538	2.576	2.541
Calcium (%)	0.787	0.787	0.792	0.792	0.794	0.794	0.796	0.796	0.800	0.800
Total phosphorus (%)	0.450	0.452	0.444	0.446	0.441	0.443	0.438	0.440	0.434	0.435
Digestible phosphorus, calculated[5] (%)	0.330	0.330	0.330	0.330	0.330	0.330	0.330	0.330	0.330	0.330
Crude fiber (%)	2.603	2.625	2.519	2.539	2.477	2.497	2.434	2.454	2.371	2.390
Ash (%)	4.965	4.983	4.866	4.882	4.816	4.831	4.766	4.781	4.692	4.706

[1] Whey permeate was added to improve pellet durability/quality.

[2] Premix providing the following nutrient amounts per kilogram of feed: vitamin A 4400 IU; vitamin D3 880 IU; vitamin E 28 IU; vitamin K3 1.78 mg; vitamin B12 14 mcg; niacin 24.6 mg; pantothenic acid 10.67 mg; pyridoxin 1.78 mg; riboflavin 2.66 mg; thiamine 1.34 mg; folic acid 0.40; biotin 89 mcg; copper, 225mg; iodine 0.35 mg; iron 80mg; manganese 30 mg; selenium 0.30 mg; zinc 80 mg; fitase 500 ftu; xilanase 10,000 U, antioxidant blend 50 mg

[3] Hemicell HT (Elanco Animal Health, São Paulo, Brazil) providing 160,000 U per kilogram of feed.

[4] Standardized ileal digestible.

[5] Values for growing pigs were estimated from the gross composition of the ingredients according to EvaPig (Software Version 1.3.1.4, INRA, Saint-Gilles, France).

Table 2 *Performance of growing-finishing pigs fed diets containing β-mannanase.*

	Treatments		SD[1]	*P-value*[2]
	Control	β-mannanase		
Period 1 - days 1 to 20				
Initial BW, kg	23.25	22.78	0.415	0.856
ADFI[3] (kg/day)	1.162	1.132	0.040	0.756
ADG[4] (kg/day)	0.769	0.741	0.024	0.602
G:F[5] (kg/kg)	0.518	0.527	0.022	0.799
Period 2 - days 21 to 41				
Initial BW, kg	38.56	37.47	0.735	0.673
ADFI (kg/day)	1.590	1.508	0.041	0.399
ADG (kg/day)	0.887	0.860	0.023	0.621
G:F (kg/kg)	0.521	0.473	0.015	0.799
Period 3 - days 42 to 62				
Initial BW, kg	58.08	55.53	1.187	0.326
ADFI (kg/day)	2.010	2.111	0.050	0.295
ADG (kg/day)	1.039	1.094	0.033	0.300
G:F (kg/kg)	0.569	0.573	0.018	0.901
Period 4 - days 63 to 82				
Initial BW, kg	79.88	79.42	1.441	0.860
ADFI (kg/day)	2.352	2.573	0.051	0.125
ADG (kg/day)	1.068	1.161	0.030	0.085
G:F (kg/kg)	0.482	0.554	0.018	0.048
Period 5 - days 83 to 104				
Initial BW, kg	101.2	102.8	1.703	0.544
Final BW, kg	126.2	129.6	1.826	0.187
ADFI (kg/day)	2.903	3.056	0.059	0.114
ADG (kg/day)	1.190	1.291	0.023	0.070
G:F (kg/kg)	0.493	0.536	0.016	0.025
Overall performance - days 1 to 104				
ADFI (kg/day)	2.003	2.076	0.047	0.317

ADG (kg/day)	0.990	1.029	0.016	0.277
G:F (kg/kg)	0.517	0.533	0.008	0.382

[1] Standard deviation.

[2] Treatment effect. Statistical models also included the period and interaction treatment × period.

ADFI: period, $P < 0.001$, interaction, $P = 0.018$.

ADG: period, $P < 0.001$, interaction, $P = 0.113$.

G:F: period, $P < 0.001$, interaction, $P = 0.161$.

[3] Average daily feed intake.

[4] Average daily weight gain (kg).

[5] Feed efficiency.

Table 3 *Backfat thickness, loin muscle depth, and loin area in growing-finishing pigs feeding diets containing β-mannanase.*

	Treatments		SD[1]	*P-value*[2]
	Control	β-mannanase		
Day 1				
Backfat thickness (mm)	5.062	4.820	0.461	0.794
Loin muscle depth (mm)	21.80	20.93	0.945	0.649
Loin area (mm^3)	11.14	9.423	0.857	0.321
Day 42				
Backfat thickness (mm)	7.361	7.192	0.454	0.853
Loin muscle depth (mm)	32.78	30.30	1.360	0.194
Loin area (mm^3)	22.88	20.89	1.234	0.249
Day 83				
Backfat thickness (mm)	9.523	11.06	0.462	0.101
Loin muscle depth (mm)	46.38	43.71	1.362	0.162
Loin area (mm^3)	39.90	37.24	1.284	0.133
Day 104				
Backfat thickness (mm)	13.53	16.45	0.479	0.003
Loin muscle depth (mm)	41.97	43.46	1.479	0.452
Loin area (mm^3)	39.24	41.89	1.340	0.142

[1] Standard deviation.

[2] Treatment effect. Statistical models also included period and interaction treatment × period.

Body weight: period, $P < 0.001$; interaction, $P = 0.132$.

Backfat thickness: period, $P < 0.001$; interaction, $P = 0.010$.

Loin muscle depth: period, $P < 0.001$; interaction, $P = 0.304$.

Loin area: period, $P < 0.001$; interaction, $P = 0.075$.

Table 4 *Body composition of growing-finishing pigs fed diets containing β-mannanase.*

	Treatments		SD[1]	*P-value*[2]
	Control	β-mannanase		
Period 1 and 2 - days 1 to 41				
Initial protein mass (kg)	3.791	3.679	0.063	0.853
Final protein mass (kg)	10.60	9.838	0.311	0.210
Protein gain (g/day)	167.5	150.2	7.267	0.271
Initial protein + water mass (kg)	20.87	20.44	0.260	0.864
Final protein + water mass (kg)	49.25	46.10	1.292	0.217
Initial lipid mass (kg)	5.362	5.460	0.046	0.946
Final lipid mass (kg)	10.12	9.755	0.317	0.799
Lipid gain (g/day)	131.2	104.7	11.02	0.434
Initial ash mass (g)	436.7	452.4	9.911	0.885
Final ash mass (g)	1243	1181	36.11	0.570
Ash gain (g/day)	20.62	17.78	0.967	0.261
Initial BMD[3] (g/cm^2)	0.430	0.438	0.006	0.776
Final BMD (g/cm^2)	0.685	0.662	0.011	0.415
Period 3 and 4 - days 42 to 82				
Final protein mass (kg)	18.21	18.20	0.369	0.993
Protein gain (g/day)	185.6	204.0	6.431	0.241
Final protein + water mass (kg)	81.01	81.03	1.544	0.993
Final lipid mass (kg)	18.21	19.39	0.784	0.415
Lipid gain (g/day)	197.1	235.1	15.34	0.264
Final ash mass (g)	2229	2279	59.79	0.643
Ash gain (g/day)	24.04	26.78	0.997	0.277
Final BMD (g/cm^2)	0.975	0.976	0.017	0.972
Period 5 - days 83 to 104				
Final protein mass (kg)	21.78	22.60	0.346	0.076
Protein gain (g/day)	170.2	209.6	10.07	0.015
Final protein + water mass (kg)	96.08	99.56	1.463	0.072
Final lipid mass (kg)	24.92	27.87	1.173	0.046

Lipid gain (g/day)	319.7	403.7	23.38	0.016
Final ash mass (g)	2888	3061	80.97	0.042
Ash gain (g/day)	31.41	37.23	1.746	0.024
Final BMD (g/cm^2)	1.137	1.152	0.018	0.586
Overall performance - days 1 to 104				
Lipid gain (g/day)	216.0	247.8	15.70	0.255
Protein gain (g/day)	174.4	187.9	4.953	0.049
Ash gain (g/day)	25.35	27.26	1.050	0.319

[1] Standard deviation.

[2] Treatment effect. Statistical models also included the period and interaction treatment period × period.

Protein mass: period, $P < 0.001$; interaction, $P = 0.068$.

Protein gain: period, $P = 0.011$; interaction, $P = 0.076$.

Protein + water mass: period, $P < 0.001$; interaction, $P = 0.068$.

Lipid mass: period, $P < 0.001$; interaction, $P = 0.163$.

Lipid gain: period, $P = 0.035$; interaction, $P = 0.008$.

Ash mass: period, $P < 0.001$; interaction, $P = 0.187$.

Ash gain: period, $P < 0.001$; interaction, $P = 0.014$.

BMD: treatment: period: $P < 0.001$; interaction, $P = 0.509$.

[3] Bone mineral density.

Table 5 *Energy and nutrient intake of growing-finishing pigs fed diets containing β-mannanase.*

	Treatments		SD[1]	*P-value*[2]
	Control	β-mannanase		
Period 1 - days 1 to 20				
Net energy intake (Mcal/day)	3018	2904	144.8	0.739
SID[3] lysine intake (g/day)	13.38	12.88	0.642	0.706
Crude protein intake (g/day)	206.6	199.1	9.920	0.719
Phosphorus intake (g/day)	5.243	5.139	0.253	0.859
Period 2 - days 21 to 41				
Net energy intake (Mcal/day)	4164	3756	148.2	0.246
SID lysine intake (g/day)	16.86	15.22	0.601	0.235
Crude protein intake (g/day)	264.6	239.3	9.436	0.245
Phosphorus intake (g/day)	7.147	6.548	0.256	0.323
Period 3 - days 42 to 62				
Net energy intake (Mcal/day)	5247	5477	144.1	0.516
SID lysine intake (g/day)	20.25	21.12	0.557	0.526
Crude protein intake (g/day)	320.6	335.3	8.883	0.502
Phosphorus intake (g/day)	8.951	9.477	0.249	0.388
Period 4 - days 63 to 82				
Net energy intake (Mcal/day)	6141	6535	199.9	0.266
SID lysine intake (g/day)	22.53	23.96	0.734	0.302
Crude protein intake (g/day)	360.2	384.1	11.77	0.273
Phosphorus intake (g/day)	10.41	11.22	0.344	0.183
Period 5 - days 83 to 104				
Net energy intake (Mcal/day)	7489	7408	206.6	0.820
SID lysine intake (g/day)	25.34	24.98	0.700	0.795
Crude protein intake (g/day)	411.8	407.7	11.41	0.852
Phosphorus intake (g/day)	12.58	12.57	0.350	0.998
Overall performance - days 1 to 104				
Net energy intake (Mcal/day)	5212	5216	127.2	0.987

SID lysine intake (g/day)	19.67	19.63	0.408	0.970
Crude protein intake (g/day)	312.8	313.1	6.705	0.984
Phosphorus intake (g/day)	8.865	8.992	0.213	0.775

[1] Standard deviation.

[2] Treatment effect. Statistical models also included the period and interaction treatment period × period.

Net energy intake: period, $P < 0.001$; interaction, $P = 0.249$.

SID lysine intake: period, $P < 0.001$; interaction, $P = 0.211$.

Crude protein intake: period, $P < 0.001$; interaction, $P = 0.209$.

Phosphorus intake: period, $P < 0.001$; interaction, $P = 0.222$.

[3] Standardized ileal digestible.

Table 6 *Nitrogen and phosphorus balance of growing-finishing pigs fed diets containing β-mannanase.*

	Treatments		SD[1]	*P-value*[2]
	Control	β-mannanase		
Period 1 and 2 - days 1 to 41				
Nitrogen retention (g/pig)	26.79	24.03	1.163	0.271
Nitrogen excretion (g/pig)	12.26	9.598	0.865	0.427
Nitrogen efficiency (%)	69.60	69.77	2.137	0.968
Phosphorus retention (g/pig)	3.712	3.201	0.174	0.262
Phosphorus excretion (g/pig)	2.690	2.407	0.160	0.545
Phosphorus efficiency (%)	58.59	56.33	1.768	0.512
Period 3 and 4 - days 42 to 82				
Nitrogen retention (g/pig)	29.69	32.64	1.029	0.241
Nitrogen excretion (g/pig)	24.13	26.03	1.305	0.570
Nitrogen efficiency (%)	55.18	56.22	1.617	0.809
Phosphorus retention (g/pig)	4.325	4.821	0.179	0.275
Phosphorus excretion (g/pig)	5.229	5.714	0.196	0.302
Phosphorus efficiency (%)	45.00	45.96	1.239	0.780
Period 5 - days 83 to 104				
Nitrogen retention (g/pig)	27.24	33.53	1.612	0.015
Nitrogen excretion (g/pig)	37.61	37.86	2.344	0.941
Nitrogen efficiency (%)	42.20	48.00	2.504	0.184
Phosphorus retention (g/pig)	5.653	6.702	0.314	0.024
Phosphorus excretion (g/pig)	6.711	7.013	0.305	0.518
Phosphorus efficiency (%)	45.40	49.19	1.967	0.275
Overall performance - days 1 to 104				
Nitrogen retention (g/pig)	27.91	30.07	0.792	0.035
Nitrogen excretion (g/pig)	24.67	24.50	1.642	0.934
Nitrogen efficiency (%)	55.66	58.00	1.040	0.097
Phosphorus retention (g/pig)	4.563	4.908	0.189	0.318
Phosphorus excretion (g/pig)	4.877	5.045	0.257	0.552

Phosphorus efficiency (%)	49.66	50.49	1.173	0.708

[1] Standard deviation.

[2] Treatment effect. Statistical models also included the period and interaction treatment period × period.

Nitrogen retention: period, P = 0.011; interaction, P = 0.075.

Nitrogen excretion: period, P < 0.001; interaction, P = 0.599.

Nitrogen efficiency: period, P < 0.001; interaction, P = 0.674.

Phosphorus retention: period, P < 0.001; interaction, P = 0.014.

Phosphorus excretion: period, P < 0.001; interaction, P = 0.467.

Nitrogen efficiency: period, P < 0.001; interaction, P = 0.428.

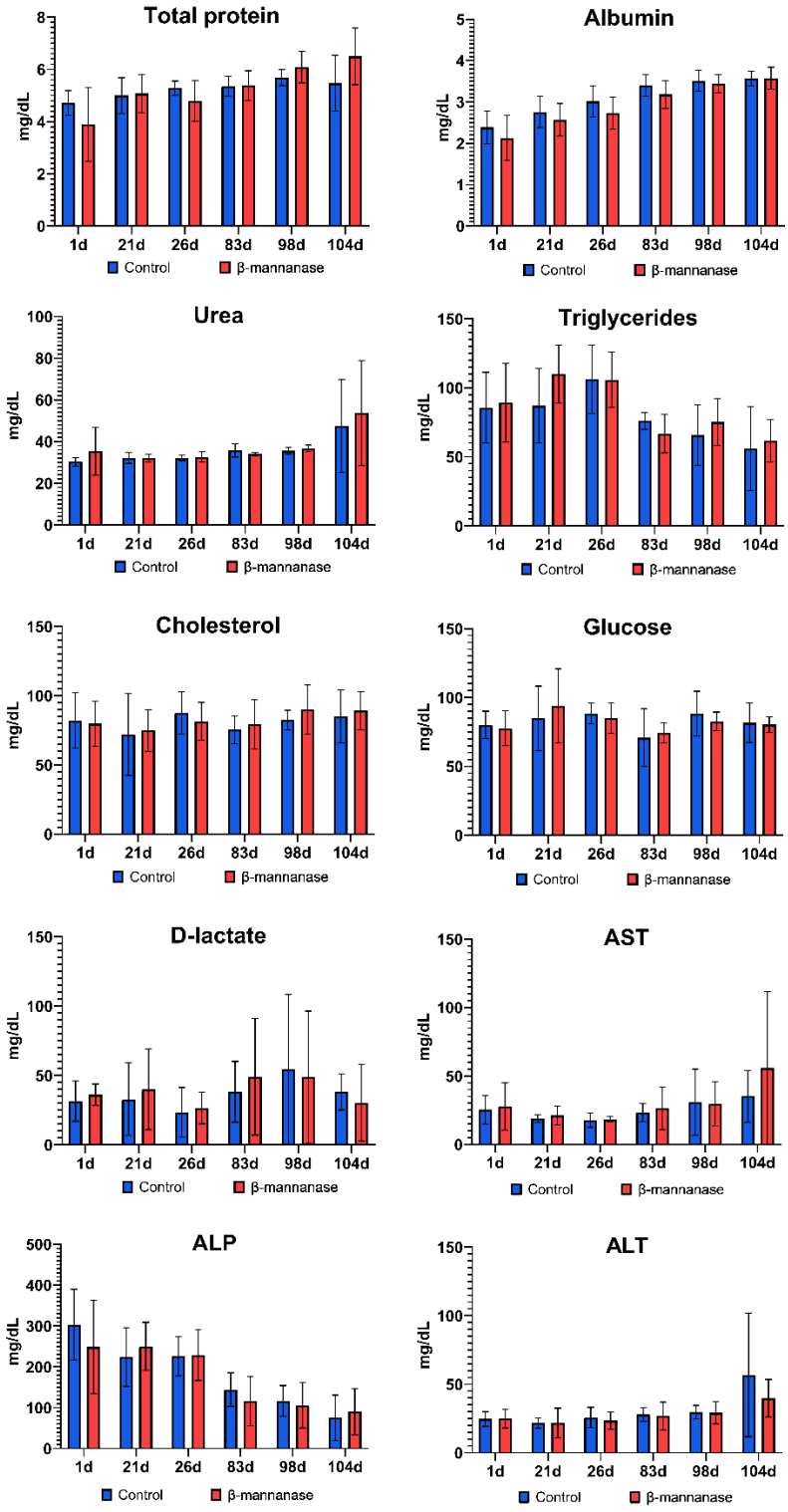

Fig. 1. Metabolic biomarkers in growing-finishing pigs feeding diets containing β-mannanase. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

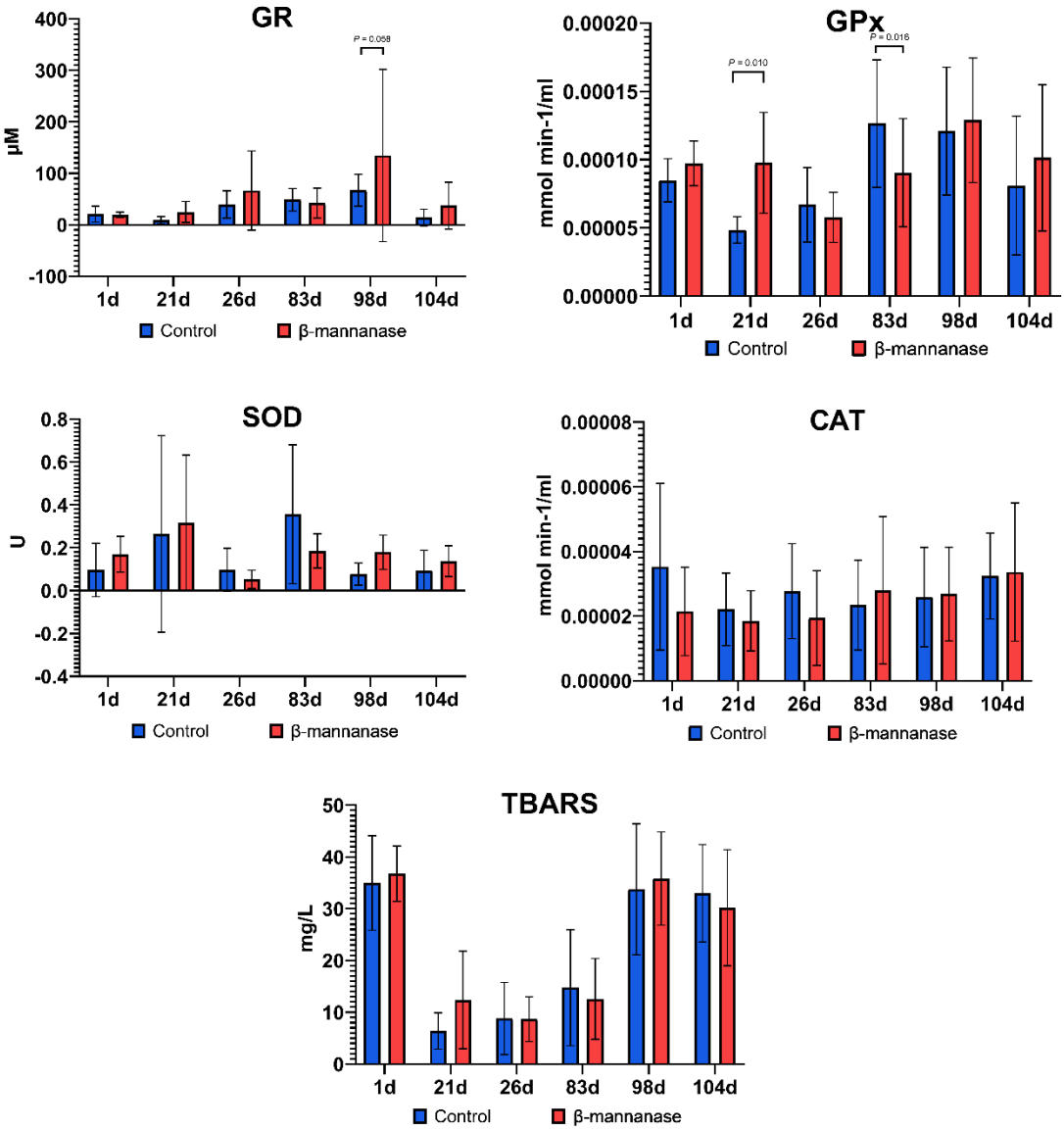

Fig. 2. Serum glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid-reactive substances (TBARS) in growing-finishing pigs feeding diets containing β-mannanase.

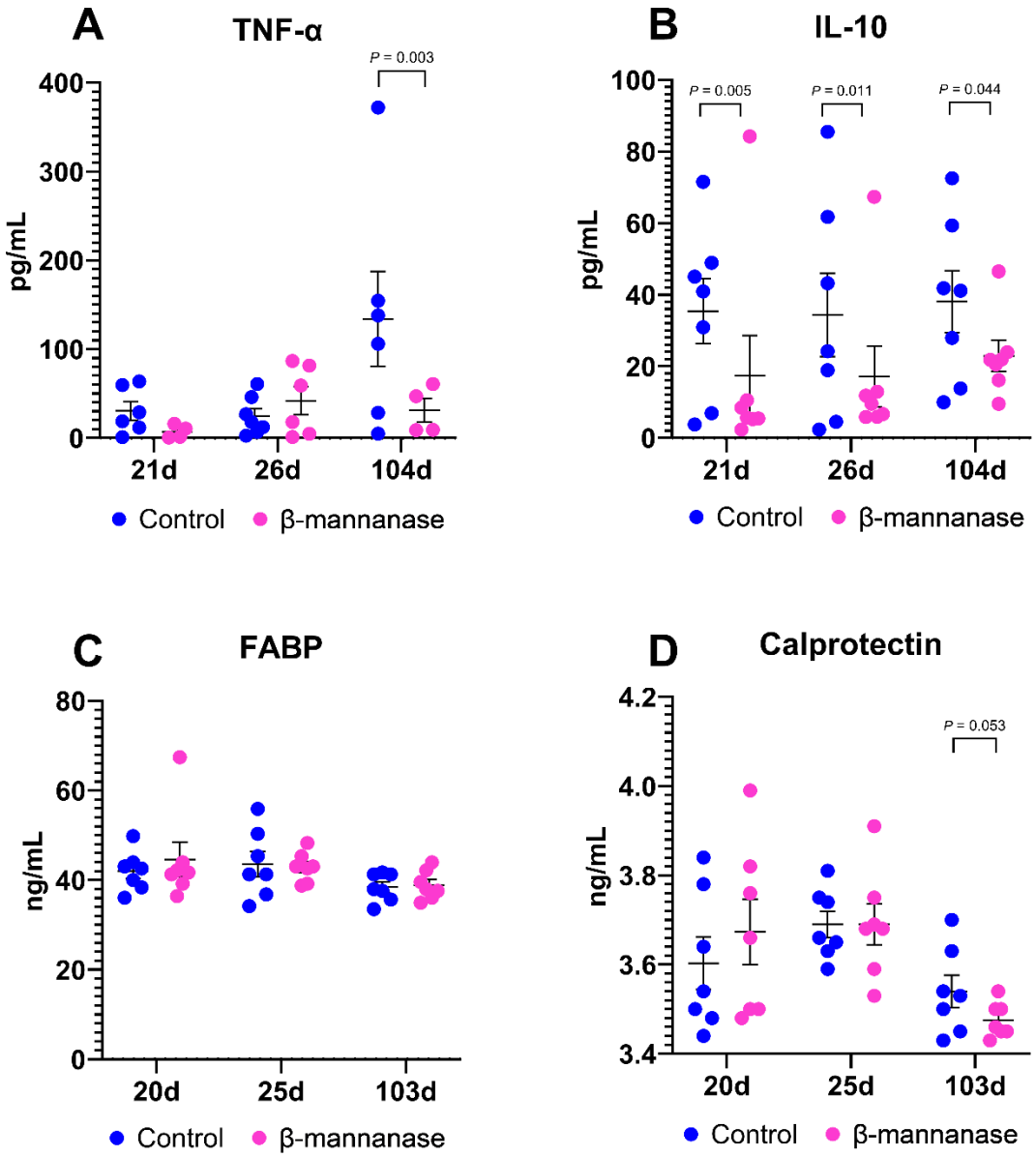

Fig. 3. Serum tumor necrosis factor (TNF)-α (**A**), and interleukin (IL)-10 (**B**), and fecal fatty acid-binding proteins (FABP, **C**), and calprotectin (**D**) in growing-finishing pigs feeding diets containing β-mannanase.