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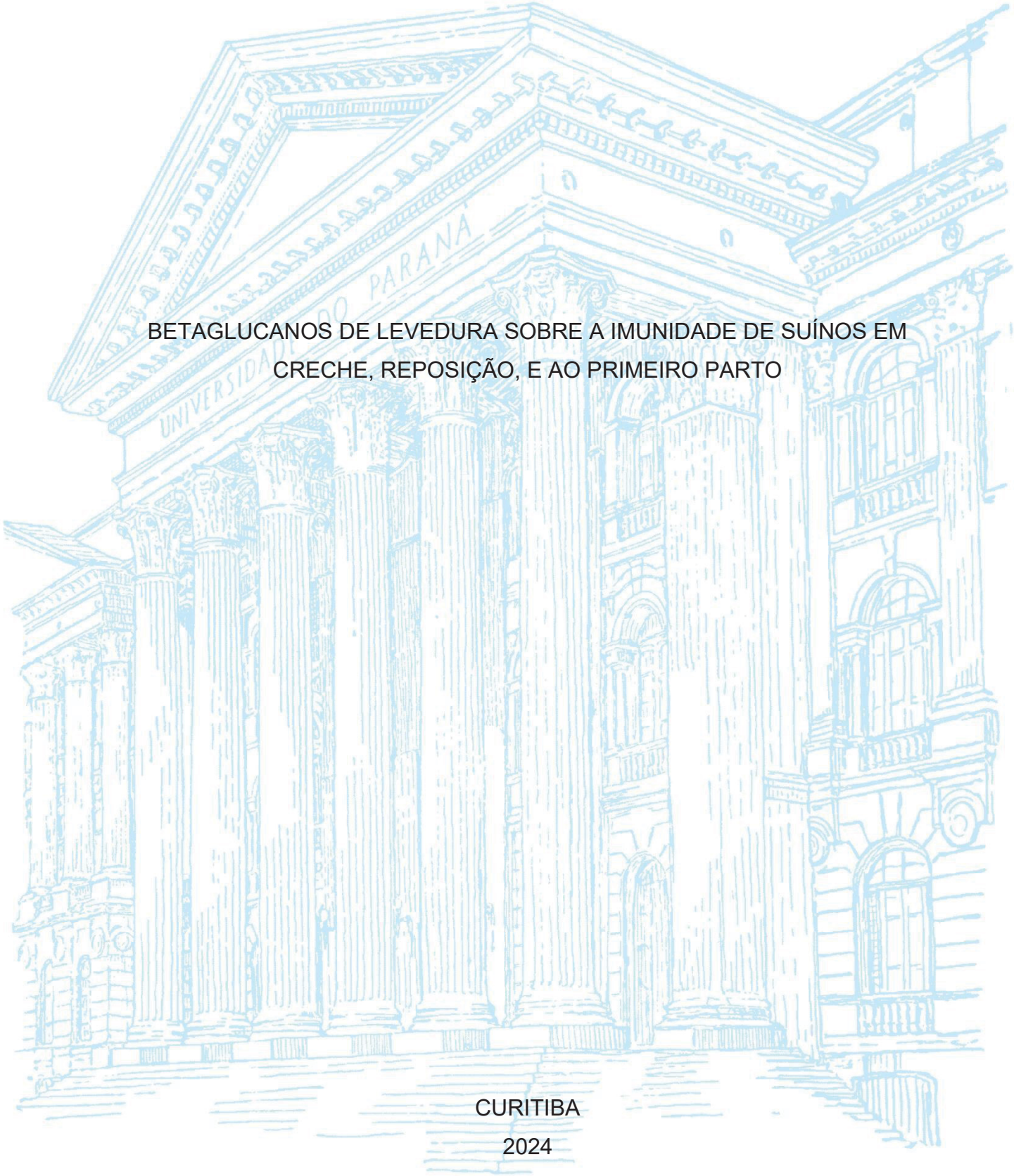
MARLEY CONCEIÇÃO DOS SANTOS



BETAGLUCANOS DE LEVEDURA SOBRE A IMUNIDADE DE SUÍNOS EM CRECHE, REPOSIÇÃO, E AO PRIMEIRO PARTO

CURITIBA

2024

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Tese apresentada ao curso de Pós-Graduação em Zootecnia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Zootecnia.

Orientadora: Profa. Dra. Simone Gisele de Oliveira

Coorientadora: Profa. Dra. Ananda Portella Félix

CURITIBA

2024

FICHA CATALOGRÁFICA

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Santos, Marley Conceição dos
Betaglucanos de levedura sobre a imunidade de suínos em creche, reposição, e ao primeiro parto/ Marley Conceição dos Santos. – Curitiba, 2024.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia.
Orientadora: Profª Drª Simone Gisele de Oliveira
Coorientadora: Profª Drª Ananda Portella Félix

1. Imunidade (Higiene). 2. Suínos. 3. Nutrição. 4. Intestinos - Doenças. I. Oliveira, Simone Gisele de. II. Félix, Ananda Portella. III. Universidade Federal do Paraná. Programa de Pós-Graduação em Zootecnia. IV. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

TERMO DE APROVAÇÃO

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ZOOTECNIA - 40001016082P0

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MARLEY CONCEIÇÃO DOS SANTOS** intitulada: **Betaglucanos de levedura sobre a imunidade de suínos em creche, reposição, e ao primeiro parto**, sob orientação da Profa. Dra. SIMONE GISELE DE OLIVEIRA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 06 de Março de 2024.

SIMONE GISELE DE OLIVEIRA
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Dedico esse trabalho a todos os animais, fundamentais para minha formação.

AGRADECIMENTOS

Após treze anos de UFPR, tenho muito a agradecer a essa instituição que me foi tão importante e me abriu as portas para o conhecimento e oportunidades únicas que eu nunca imaginei que poderia vivenciar. Espero cumprir com o meu papel como egressa de uma universidade pública, e retribuir o investimento que a sociedade fez em mim. Durante todos esses anos muitas pessoas me ajudaram para que eu chegasse até aqui, e sou grata a todas elas por cada contribuição à minha formação. Não serei capaz de nomear todas, mas as tenho em minha memória.

Mesmo antes de entrar na universidade, minha família foi minha base e meu suporte, me trouxeram até aqui sempre me dando a certeza de que estavam comigo e por mim em todos os momentos. Aos meus pais, Antônio e Marinaide, e minhas irmãs, Roberta e Tanes, tenho muito a agradecer e sei que também estarão do meu lado em meus passos futuros. Agradeço também à minha avó Jaci, sempre preocupada e tão importante em minha formação como mulher negra, meu exemplo, orgulho e motivação para ser alguém melhor.

Agradeço ao meu namorado, Rafael, que foi tão importante, principalmente nesse período de doutorado. Foi meu apoio, companheiro, amigo, confidente e parceiro nos bons e nos não tão bons momentos. E, ainda em minha família, agradeço ao meu fiel amigo e motivo de muitas alegrias, Ramirez, meu Ramz.

Desde o primeiro dia de Zootecnia até hoje eu tenho tanto a agradecer à minha melhor amiga, minha irmã, e a pessoa que me fazia ir feliz para a faculdade todos os dias, a Taís. Não tem nenhum momento marcante na minha vida nesses treze últimos anos que não tenha a presença dela. Nos formamos juntas em 2017, tivemos nossa colação de grau juntas em 2018, mesmo ano em que entramos juntas no mestrado, também entramos juntas no doutorado em 2020 e estamos agora defendendo esse mesmo doutorado juntas em 2024, agora com uma versão miniatura dela para estar presente em mais momentos importantes, o Apolo.

Outra melhor amiga que a UFPR me deu foi a Kariny, que me ensinou e ensina tanto todos os dias, não só sobre suinocultura, mas como ser uma pessoa melhor e mais positiva. Além de todo o companheirismo que ela sempre me ofereceu, ainda me deu o presente mais precioso, meu afilhado Arthur.

Também desde o início da graduação até esse final da pós-graduação tive o apoio dos meus grandes amigos Wlademir, Gislaine e Alina, que mesmo depois de

tantos anos continuam sendo as pessoas com quem eu mais amo conversar e passar tempo. E ao ingressar na pós-graduação tive a sorte de fazer mais amigos para a vida, em especial os meus amigos que me fizeram amar a suinocultura, Josiane e sua família tão cuidadosa e cheia de amor, que tanto me ajudou em todo o doutorado e especialmente nesse período final, e o Leopoldo que foi quem de fato me levou para os suínos. Cada um tem um lugar especial em minha vida, e sou muito grata por tudo que já fizeram por mim.

Agradeço também a todas as amigas e amigos do Lepnan, fundamentais para todo o trabalho que desenvolvi nesses anos e um alívio nos momentos de exaustão. Vocês fizeram tudo ser mais fácil. Um agradecimento especial à Isabella, Vivian e Renata, as amigas do cafezinho.

Meu agradecimento mais que especial vai para meus professores, orientadores, mentores, conselheiros e amigos, Simone, Alex e Ananda. Tive tantos momentos de incertezas e inseguranças e vocês me deram apoio em todos eles. Tenho muito a falar sobre vocês, mas de forma resumida, vocês realmente mudaram a minha vida e nem imaginam o quanto. Minha eterna gratidão a vocês.

Agradeço também aos membros da banca, que me deram o privilégio de estarem presentes nesse momento. Professora Ana Vitória, que me fez escolher a nutrição animal desde que eu fiz com ela a matéria de fisiologia animal. Professora Chayane, que me deu a oportunidade de conduzir meu primeiro experimento, com os Agapórnis no Lacrias. E a Tabyta, que eu conheci no Lenucan quando eu era uma estagiária lá, e me ensinou e ensina muito desde então.

Agradeço também a todos os profissionais com quem tive o prazer de trabalhar junto e aprender com eles nessa tese, em especial à toda a equipe do LNA, ao professor Scandolera, à equipe da Fazenda Canguiri, ao Lúcio e ao pessoal das granjas Pinheiros e Floresta da Copercampos, ao Fábio Catunda, à Melina Bonato e à Ana Paula Bastos.

Pelo período de experiência incrível, de muito aprendizado, acolhimento e boas surpresas, agradeço ao pessoal com quem pude conviver da University of Saskatchewan, em especial ao Dr. Rex Newkirk, e ao Prairie Swine Center. E mais uma vez à Chossinha, por ter me proporcionado essa oportunidade.

Finalmente, agradeço à CAPES pelo apoio à pesquisa.

Meu muito obrigada a todos, de coração.

Marley

É necessário sempre acreditar que o sonho é possível
Que o céu é o limite e você é imbatível
Que o tempo ruim vai passar, é só uma fase
Que o sofrimento alimenta mais a sua coragem.
(Afro-X e Racionais MC's, 2002)

RESUMO

Com as crescentes restrições ao uso de antibióticos como promotores de crescimento na produção animal, o estudo do sistema imune e saúde intestinal de suínos cresceu muito na última década. Nesse contexto, os aditivos a base de betaglucanos têm se mostrado uma boa alternativa para a substituição gradativa dos antibióticos. Com isso, essa tese teve como objetivo estudar os efeitos de aditivos a base de betaglucanos de levedura sobre o desempenho, imunidade e microbioma intestinal de suínos. Para isso, o capítulo I traz uma revisão da literatura com o que se sabe até hoje dos possíveis efeitos desses aditivos na suinocultura, seus principais alvos e mecanismos de ação. O capítulo II traz os resultados de dois experimentos que avaliaram a inclusão de um aditivo a base de betaglucanos de parede de levedura purificados, a 300g/ton, (I) para marrãs de reposição e (II) para matrizes de primeiro parto, sobre sua resposta vacinal e a resposta de suas leitegadas às vacinas para parvovírus e *Leptospira* spp, além de sua produção de imunoglobulinas, macrófagos, granulócitos e linfócitos T no colostro, imunoglobulinas no leite, atividade mitogênica do colostro sobre células do epitélio intestinal, e desempenho ao parto. A inclusão do aditivo na dieta de porcas primíparas durante os períodos de gestação e lactação aumentou a concentração de IgA no colostro e no leite. Além disso, o colostro das porcas suplementadas com o aditivo a base de betaglucanos foi capaz de estimular a proliferação de células epiteliais intestinais in vitro. Por outro lado, a suplementação no período de adaptação não foi capaz de modular a resposta vacinal contra os antígenos vacinais de parvovírus e *Leptospira* spp, nem alterar o desempenho reprodutivo destas porcas no primeiro parto. No capítulo III, outro aditivo proveniente da fermentação de leveduras, dessa vez com alta concentração de mananoligossacarídeos e betaglucanos, e com uma inclusão de 2kg/ton, foi suplementado para leitões em fase de creche, consumindo dietas simples (com alta inclusão de farelo de soja) ou complexas, sobre seu desempenho, permeabilidade intestinal, concentração de citocinas, perfil de leucócitos e fagocitário, e microbioma intestinal. Nesse capítulo, a inclusão de farelo de soja acima do limite recomendado (30%) não interferiu no desempenho dos animais durante a maior parte da fase de creche, tendo efeito positivo apenas no ganho de peso dos animais dos 22 aos 28 dias e na última semana da fase de creche. Além disso, dietas complexas com a inclusão do aditivo promoveram diminuição da inflamação intestinal. Dietas complexas reduziram a ocorrência de diarreia do 8º ao 21º dia do experimento, enquanto a inclusão do aditivo aumentou a ocorrência na primeira semana. Por fim, as dietas complexas e a adição do aditivo diminuíram o número de microrganismos patogênicos nas amostras de fezes ao final da fase de creche. Por fim, o capítulo IV traz algumas considerações sobre todo o trabalho exposto na tese, e a conclusão que foi possível obter, de que o efeito principal de aditivos baseados em betaglucanos é sobre a atividade mitogênica e a inflamação do epitélio intestinal de animais jovens.

Palavras-chave: Microbioma intestinal; Inflamação intestinal; Nutrição de Suínos; Saúde intestinal; Imunidade.

ABSTRACT

With growing restrictions on the use of antibiotics as growth promoters in animal production, the study of pigs' immune system and intestinal health has grown a lot in the last decade. In this context, beta-glucan-based additives are a good alternative for gradually replacing antibiotics. Therefore, this thesis aimed to study the effects of additives based on yeast beta-glucans on the performance, immunity, and intestinal microbiome of pigs. To this end, Chapter I reviews the literature with what is known to date about the possible effects of these additives in pig farming, their main targets, and mechanisms of action. Chapter II presents the results of two experiments that evaluated the inclusion of an additive based on purified yeast wall beta-glucans at 300g/ton, (I) for replacement gilts and (II) for first parity sows, on their vaccine response and the response of their litters to vaccines for parvovirus and *Leptospira* spp, in addition to their production of immunoglobulins, macrophages, granulocytes, and T lymphocytes in colostrum, immunoglobulins in milk, the mitogenic activity of colostrum on intestinal epithelial cells, and birth performance. Including the additive in the diet of primiparous sows during the gestation and lactation periods increased the concentration of IgA in colostrum and milk. Furthermore, colostrum from sows supplemented with the beta-glucan-based additive stimulated the proliferation of intestinal epithelial cells in vitro. On the other hand, supplementation during the adaptation period could not modulate the vaccine response against parvovirus and *Leptospira* spp vaccine antigens nor alter the reproductive performance of these sows in the first farrowing. In Chapter III, another additive from yeast fermentation, this time with a high concentration of mannan oligosaccharides and beta-glucans and with an inclusion of 2kg/ton, was supplemented for piglets in the nursery phase, consuming simple diets (with a high inclusion of soybean meal) or complex, on their performance, intestinal permeability, cytokine concentration, leukocyte and phagocytic profile, and intestinal microbiome. In this chapter, the inclusion of soybean meal above the recommended limit (30%) did not interfere with the performance of the animals during most of the nursery phase, having a positive effect only on the weight gain of the animals from 22 to 28 days and in the last week of the nursery phase. Furthermore, complex diets, including the additive, reduced intestinal inflammation. Complex diets reduced the occurrence of diarrhea from the 8th to the 21st day of the experiment, while the inclusion of the additive increased the occurrence in the first week. Finally, the complex diets and adding the additive reduced the number of pathogenic microorganisms in the fecal samples at the end of the nursery phase. Finally, chapter IV brings some considerations about all the work exposed in the thesis and the conclusion that it was possible to obtain that the main effect of additives based on beta-glucans is on the mitogenic activity and inflammation of the intestinal epithelium of young animals.

Keywords: Intestinal microbiome; Intestinal inflammation; Swine nutrition; GIT health; Immunity.

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LISTA DE ABREVIATURAS OU SIGLAS

ANOVA - Análise de Variância

ASVs - Amplicon Sequence Variants

BCG - Bacillus Calmette Guerin

CEDISA - Centro Diagnóstico para Saúde Animal

CONAB - Companhia Nacional de Abastecimento

CON - Tratamento controle

DNA - Ácido desoxirribonucleico

ELISA - Enzyme-Linked Immunosorbent Assay

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária

EXP1 - Experimento 1

EXP2 - Experimento 2

GIT - Gastrointestinal tract (Trato gastrointestinal)

HI - Hemagglutination inhibition

HRP - Horseradish peroxidase

HSCs - Células-tronco hematopoiéticas

IEC-6 - Células do epitélio intestinal linha 6

IFN - Interferon

IgA – Imunoglobulina A

IgG – Imunoglobulina G

IgM – Imunoglobulina M

IL - Interleucina

LDA - Linear discriminant analysis

LEfSe - Linear discriminant analysis (LDA) effect size

MFI - Mean fluorescence intensity

MOS - Mananoligossacarídeos

mRNA - Ácido ribonucleico mensageiro

PBS - Phosphate-Buffered Saline

PCR - Proteína C-reativa

PERMANOVA - Permutational Multivariate Analysis of Variance

QIIME2 - Quantitative Insights Into Microbial Ecology

Sars-CoV-2 - Síndrome respiratória aguda grave – Coronavírus 2

S. cerevisiae - *Saccharomyces cerevisiae*

SEM - Erro padrão da média

SWC - Swine Workshop Clusters

TBG - Dieta controle com adição de betaglucanos

TLR9 - Receptores Toll-like

TNF-α - Fator de necrose tumoral alfa

LISTA DE SÍMBOLOS

α – Alfa

β - Beta

γ – Gama

μ – Micro

SUMÁRIO

Introdução

Em virtude das restrições cada vez maiores ao uso de antibióticos como promotores de crescimento na produção animal, a busca por alternativas ao seu uso vem se intensificando. Nesse contexto, aditivos vêm sendo amplamente estudados como possíveis substitutos, dentre eles destacam-se: os acidificantes, probióticos, prebióticos e óleos essenciais. De acordo com a literatura, não há ainda um único produto que seja capaz de substituir os antibióticos proporcionando os mesmos resultados (GRESSE et al., 2017). Contudo, há uma ampla gama de estudos sobre estes aditivos, usados isoladamente ou em conjunto, que demonstram que a associação entre eles pode ser o caminho para a substituição dos antibióticos.

O estudo de betaglucanos como aditivos para suínos começou a partir desse contexto, mas eles já vinham sendo estudados há anos em pesquisas voltadas para a medicina humana, que mostravam seu potencial como agente imunomodulador (NETEA et al., 2016). A partir de estudos relativamente recentes em plantas e invertebrados, surgiu o conceito de imunidade inata treinada (KURTZ, 2005; NETEA et al., 2016). Antes disso, acreditava-se que apenas a imunidade adaptativa fosse capaz de produzir células imunes de memória, mas, a partir destes trabalhos, começou-se a investigar a capacidade do sistema imune inato produzir células de memória também.

Estudos com ratos e alguns relatos de casos em humanos mostraram que fatores como vacinas, fragmentos de microrganismos, citocinas inflamatórias, e componentes das paredes de fungos e leveduras, como é o caso dos betaglucanos de levedura, podem induzir uma resposta imune inata de memória, denominada imunidade inata “treinada” (QUINTIN et al., 2014; NETEA et al., 2016). Os betaglucanos de levedura para suínos têm como principal intuito ser um estimulador do sistema imune inato treinado, fazendo com que o organismo desenvolva uma resposta imune não específica, de memória, e com duração que pode chegar a meses contra microrganismos indesejados para o animal, agindo assim até mesmo como um *booster*, ou melhorador de resposta, vacinal (CÓRDOVA-MARTÍNEZ et al., 2021; WANI et al., 2021; FATHIMA et al., 2023).

Ainda não são tantos os trabalhos que mostram os efeitos da inclusão de betaglucanos de levedura na dieta de suínos abrangendo aspectos de imunidade e saúde intestinal, mas esse número vem crescendo cada vez mais, acompanhando o crescimento no número de trabalhos sobre saúde intestinal (KIM; DUARTE, 2021). Os trabalhos disponíveis mostram que os betaglucanos possuem efeito positivo sobre células do sistema imune inato e, posteriormente, adaptativo, com consequente melhora no desempenho dos animais (WU et al., 2018; HE et al., 2022; DOS SANTOS et al., 2023). No entanto, os resultados, as fontes e formas de produção dos betaglucanos utilizados ainda são muito variáveis, tornando difícil a tomada de decisão em utilizar ou não esse aditivo.

Nesse contexto, o primeiro capítulo dessa tese apresenta uma revisão de literatura que visa contextualizar como os betaglucanos provenientes de levedura se propõem a atuar como aditivos para a suinocultura, seu mecanismo de ação e os principais resultados já encontrados com seu uso em diferentes fases de produção para suínos.

Já no segundo capítulo, o objetivo foi avaliar o efeito da inclusão de β-glucanos purificados na dieta de marrãs de reposição durante as fases de adaptação, gestação e lactação sobre a sua resposta e de suas ninhadas às vacinas contra Parvovirose e Leptospirose, bem como seu efeito no desempenho ao parto e nos parâmetros imunológicos do colostro e do leite.

No capítulo três o objetivo foi avaliar os efeitos da alimentação com dietas com altos níveis de farelo de soja e a inclusão de um aditivo à base β-glucanos e mananoligossacarídeos (MOS) de leveduras provenientes da extração de etanol da cana de açúcar no desempenho, imunidade e microbioma intestinal de leitões em fase de creche.

Finalmente, no capítulo quatro, são feitas as considerações finais do trabalho, trazendo os principais resultados encontrados nos capítulos anteriores, e uma contextualização com a prática.

CAPÍTULO I - REVISÃO DE LITERATURA

1. Sistema imunológico

O sistema animal de defesa contra patógenos é conhecido como sistema imunológico ou imune. Ao longo dos anos seu papel e mecanismos vêm sendo estudados, com novas descobertas que esclarecem o funcionamento desse sistema tão importante e especializado. Basicamente, o sistema imune abrange um conjunto de órgãos linfoides, células e fatores humorais como proteínas complementares e células imunes. Dentre esses componentes estão os anticorpos, citocinas e fatores de crescimento (YATIM; LAKKIS, 2015; SATTLER, 2017).

O sistema imunológico é responsável pela defesa do organismo contra agentes infecciosos, bem como desempenha papel importante no desenvolvimento, homeostase e reparação de tecidos (SATTLER, 2017).

Apesar de ser altamente interligado e agir em conjunto, o sistema imune é dividido em dois tipos, o inato e o adaptativo. De maneira geral, o sistema imune inato é caracterizado por ter uma resposta mais rápida, por ser composto pelos elementos que fornecem defesa imediata do hospedeiro, como é o caso de neutrófilos, monócitos, macrófagos e citocinas. Já o sistema imune adaptativo, consiste em reações antígeno-específicas por meio dos linfócitos T e B (PARKIN & COHEN, 2001). Apesar do sistema inato ser mais rápido, ele pode causar danos em tecidos saudáveis, uma vez que não são tão específicos. Diferente do sistema adaptativo, que é altamente específico, no entanto, pode levar dias ou semanas para se desenvolver (PARKIN & COHEN, 2001).

O sistema inato é a primeira barreira contra infecções e/ou danos de tecidos e limita a disseminação de infecções até que o sistema adaptativo comece a agir. Ele é formado por variados componentes e células e pode ter variações entre espécies. A lista de componentes desse sistema é formada por anticorpos naturais, pentraxinas (como a proteína C-reativa, PCR), sistema complementar e receptores. A lista de células se dá por células fagocíticas como os macrófagos, células de apresentação de antígeno como as células dendríticas, células *natural killers,* células T *natural killers*

invariantes, células B B-1 e, finalmente, células epiteliais, que, apesar de não serem reconhecidas como membros oficiais do sistema imune, funcionam como barreira e, no caso dos rins, até como indicador de problemas no sistema (HATO; DAGHER, 2015).

Já o sistema adaptativo é caracterizado por ter uma resposta mais tardia e antígeno-específica, com reconhecimento de detalhes de moléculas individuais dos agentes patogênicos do sistema. Os receptores das células desse sistema são formados por recombinação somática e se expandem por clonagem. Suas células são de memória e possuem ampla diversidade, e, assim como o sistema inato, o sistema adaptativo também é formado por componentes e células. Os componentes são os receptores de células B e T e os anticorpos, e as células são os linfócitos T e B (HATO; DAGHER, 2015).

Com o tempo, os organismos podem sofrer com uma desregulação do sistema imune, por conta do stress oxidativo e encurtamento dos telômeros, acompanhada de involução do timo, redução nos níveis de linfócitos T e aumento nos níveis de células de memória mesmo que algumas já sejam disfuncionais (MÜLLER; DI BENEDETTO; PAWELEC, 2019a). Esse processo é conhecido como imunossenescência. O estresse crônico oxidativo decorrente desse processo é responsável por afetar as células do sistema imune causando modificações nas interações entre elas, e, entre essas mudanças, está a substituição das células T e B por células de memória, base da imunidade adaptativa (MÜLLER; DI BENEDETTO; PAWELEC, 2019b).

1.1 Sistema imune em suínos

A imunidade em suínos é primeiramente adquirida via colostro, nas primeiras 24 horas após o parto (INOUE; TSUKAHARA, 2021), por conta de sua fisiologia, já que esses animais possuem uma placenta epitéliocorial impermeável para imunoglobulinas, característica essa que não permite a passagem de anticorpos da matriz para o feto através de suas seis camadas (NELSON, 1934; ELAHI et al., 2006; SALMON et al., 2009). Nesse sentido, o colostro é de extrema importância para o desenvolvimento dos leitões, pela ingestão principalmente das imunoglobulinas ao nascerem, e os trabalhos de Quesnel et al. (2012) e Devillers et al. (2011) mostram que os suínos que ingerem menos de 100g de colostro nas primeiras 24 horas de vida têm uma maior taxa de mortalidade (60%) em comparação aos que consomem mais

de 200g (10%) (QUESNEL; FARMER; DEVILLERS, 2012). Além disso, os animais que consomem menos de 290g de colostro apresentam um peso de desmame 15% mais baixo em relação aos que consomem mais que essa quantidade (DEVILLERS; LE DIVIDICH; PRUNIER, 2011). A quantidade de IgG e cortisol presentes no plasma dos leitões 24 horas após seu nascimento é altamente relacionada à ingestão de colostro, ou seja, quanto maior a ingestão de colostro, maior a concentração de IgG e cortisol no plasma dos leitões em seu primeiro dia de vida. No entanto, há um limite de absorção baseado na quantidade de IgG no colostro, sendo que a partir de 15g de IgG do colostro ingerida o ganho não é mais crescente, atinge o platô (DEVILLERS; LE DIVIDICH; PRUNIER, 2011).

O sistema imunológico tem como base principal órgãos linfoides primários (medula óssea e timo) e secundários (baço, linfonodos, amígdalas e tecidos linfoides associados ao intestino (*Peyer's patches*), cada um com uma função definida (ROTHKÖTTER, 2009). Sem do assim, de forma fisiológica e anatômica, o sistema imune de suínos é muito parecido ao dos demais mamíferos (ROTHKÖTTER, 2009; DAWSON et al., 2013; MAIR et al., 2014).

A medula óssea é um tecido com microambientes especiais, chamados de nichos, que fornecem suporte para as células do sistema imune e células-tronco hematopoiéticas (HSCs). Esses nichos controlam a quantidade dessas células, enviando sinais que regulam sua renovação, especialização e senescência. Esses sinais podem ser transmitidos por meio do contato direto entre as células, ação de fatores de crescimento e citocinas, ou elementos da matriz extracelular (MERCIER; RAGU; SCADDEN, 2012).

O timo tem seu desenvolvimento identificado já aos 22 dias de gestação em suínos e é completamente formado aos 36 dias de gestação, a partir disso se desenvolve de acordo com o crescimento do animal. Esse órgão é composto por timócitos, células epiteliais, macrófagos e células dendríticas maduros e imaturos. Seu papel central está baseado na imunidade mediada por células, atuando como local primário de proliferação, diferenciação e seleção das células T (WANG et al., 2020).

O baço é o maior órgão linfoide secundário no organismo suíno, contendo aproximadamente um quarto dos linfócitos presentes no corpo. Sua função primordial é iniciar as respostas imunes aos antígenos que circulam pelo sangue. Devido ao seu foco na circulação sistêmica, o baço não possui vasos linfáticos aferentes (BALOGH; HORVÁTH; SZAKAL, 2004; CESTA, 2006). Anatomicamente, o baço é composto por

dois compartimentos distintos: a polpa vermelha e a polpa branca, que se diferenciam por sua função e morfologia. A polpa vermelha atua como filtro sanguíneo, removendo materiais estranhos e eritrócitos danificados e fracos, e serve como local de armazenamento de ferro, eritrócitos e plaquetas. Já a polpa branca, tem como principal função a produção e o armazenamento de linfócitos, desempenhando um papel crucial na resposta imune. É nesse compartimento que ocorre a proliferação e a diferenciação dos linfócitos, fundamentais para combater agentes patogênicos e desenvolver uma imunidade específica (CESTA, 2006; ROTHKÖTTER, 2009).

Diferente de outros mamíferos, os linfonodos suínos são invertidos, uma vez que o tecido possui uma menor área medular e é composto majoritariamente por áreas corticais e paracórtex (ROTHKÖTTER, 2009). Já as amígdalas, são conglomerados de tecido linfoide que desempenham um papel fundamental como barreiras imunitárias nos tratos respiratório e gastrointestinal de mamíferos, visando proteger contra a ação invasiva de agentes patogênicos. A amígdala do palato mole assume destaque como a principal estrutura (FRIENDSHIP et al., 2011).

O tecido linfoide associado ao intestino é um importante componente do sistema imune, e consiste em massas linfoides localizadas no intestino delgado, as ditas placas de Peyer, no ceco (tecido da placa cecal), bem como no intestino grosso e reto. Por conta dessa distribuição, esse tecido é capaz de modular a composição da microbiota intestinal, mediando um sistema regulador dinâmico, e sua ação consiste basicamente em servir como ponto de entrada para alguns patógenos para que o organismo os reconheça e responda à infecção (ROTHKÖTTER, 2009; ABO-SHABAN et al., 2023).

1.2 Modulação do sistema imune inato

Até pouco tempo atrás acreditava-se que apenas a imunidade adaptativa era possível de ser modulada. Porém, trabalhos recentes mostram que a imunidade inata também possui a capacidade de adquirir algumas respostas não específicas de memória, com o estímulo de vacinas ou agentes derivados de fungos, por exemplo, sendo que essas adaptações duram de semanas a meses, e não por anos (NETEA et al., 2016; MITROULIS; HAJISHENGALLIS; CHAVAKIS, 2021; GECKIN et al., 2022). A imunidade inata treinada envolve alterações epigenéticas e metabólicas. As células progenitoras hematopoiéticas contribuem para isso ao sofrer alterações

funcionais duradouras em resposta à inflamação, levando ao aumento da produção de células imunes inatas treinadas (MITROULIS; HAJISHENGALLIS; CHAVAKIS, 2021).

O conceito de imunidade inata treinada, e sua comprovação, vêm de trabalhos com plantas e animais invertebrados, que não possuem sistema imune adaptativo, e, mesmo assim, apresentam proteção contra reinfecções (KURTZ, 2005). Em mamíferos esse conceito também passou a ser observado por conta de alguns trabalhos mostrando proteção contra patógenos diferentes dos que já foram apresentados a seu sistema imune, um tipo de proteção cruzada (QUINTIN et al., 2014; NETEA et al., 2016).

As vacinas são as principais formas de modular o sistema imune inato, como demonstrado em ratos (SHER et al., 1975; VAN "T WOUT; POELL; VAN FURTH, 1992) e humanos (GARLY et al., 2003; KLEINNIJENHUIS et al., 2014). Em ratos, a aplicação da vacina BCG (Bacillus Calmette Guerin), contra tuberculose, também foi capaz de imunizar os animais contra infecções por *Candida albicans* e *Schistosoma mansoni*, independente das células T (VAN "T WOUT; POELL; VAN FURTH, 1992; QUINTIN et al., 2014). Em humanos, vacinas como a BCG também se mostram capazes de induzir resposta imune inata contra outras doenças que não seriam o alvo da imunização (NETEA et al., 2016).

Apesar das vacinas serem as principais formas de modular o sistema imune inato, não são as únicas. Quintin et al. (2014) trouxeram evidências de que infecções não letais com patógenos como a *Candida albicans* podem levar à construção de uma resposta imune contra outros patógenos, como por exemplo o *Staphylococcus aureus,* e os macrófagos são apontados como principais responsáveis por essa resposta não específica da imunidade inata treinada.

Há também formas de induzir a imunidade inata treinada sem que haja contato do animal com patógenos ou partes deles. Algumas possibilidades são alguns componentes de bactérias, como o muramil dipeptídeo (componente do peptidoglicano das bactérias), agonistas de TLR9 (receptores Toll-like) como os oligodeoxinucleotídeos e a flagelina, além de citocinas inflamatórias e dos betaglucanos, componentes da parede celular de fungos e leveduras (QUINTIN et al., 2014; NETEA et al., 2016).

1.2.1 Uso de antibióticos na suinocultura

O uso excessivo e indiscriminado de antibióticos tem levado ao aumento da resistência microbiana, o que é uma grande ameaça para a saúde de humanos e animais. Estes antibióticos podem ser aplicados na saúde humana, e grande parte também ainda vem da produção animal, onde são usados também como promotores de crescimento (VAN DIJK et al., 2018).

Ainda não há consenso do quanto o uso de antibióticos na produção animal contribui para o desenvolvimento de microrganismos resistentes à ação de antibióticos. Porém, cada vez mais se aumenta a pressão pela redução do uso de antibióticos promotores de crescimento (ALLEN et al., 2014; VAN DIJK et al., 2018). Com isso, alternativas tanto para prevenir, quanto para tratamento de doenças e promoção de crescimento, estão sendo estudadas. Dentre elas destacam-se os moduladores de microbiota intestinal, prebióticos, probióticos, simbióticos, bacteriocinas, enzimas, acidificantes, extratos de plantas, nutracêuticos (cobre e zinco), vacinas, entre outros (THACKER, 2013; ALLEN et al., 2014).

Uma das formas de reduzir o uso de antibióticos como promotores de crescimento e como profiláticos para suínos, é potencializar a ação de vacinas nas diferentes fases de crescimento dos animais. Isso pode ser feito estimulando a imunidade inata treinada. Como esse tipo de imunidade se baseia em aumento de células imunes inatas treinadas (MITROULIS; HAJISHENGALLIS; CHAVAKIS, 2021), essas células, em sua maioria células de apresentação de antígenos, levam ao aumento na quantidade de células T imaturas, que irão, quando em contato com antígenos vacinais, favorecer o aumento na quantidade de células T efetivas específicas para aquele patógeno, função do sistema imune adaptativo. Portanto, a imunidade inata treinada pode potencializar a ação do sistema imune adaptativo, principal alvo das vacinas (QUINTIN et al., 2014; CASTRO; CALDER; ROCHE, 2021).

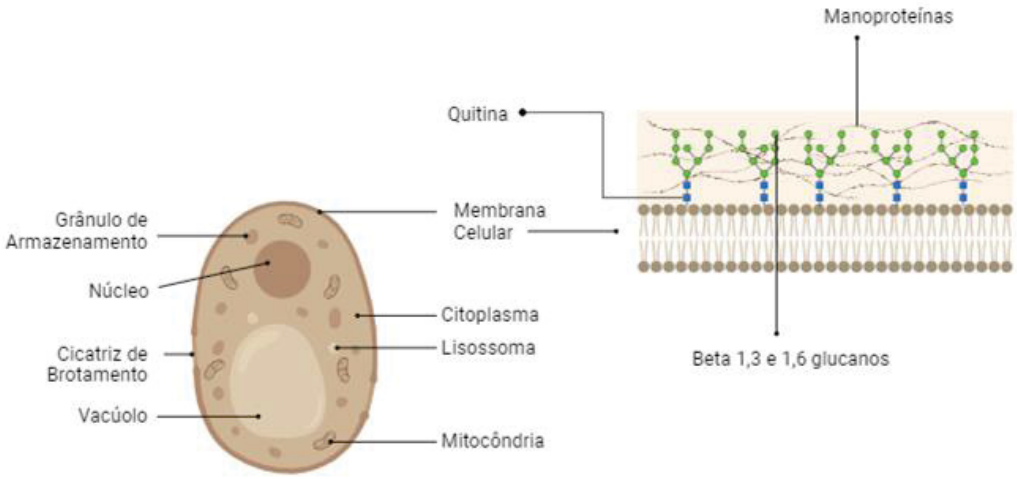
A partir desse cenário, o foco dessa revisão será o potencial dos betaglucanos provenientes de leveduras como estimuladores do sistema imune inato treinado em suínos, tendo em vista sua potencial eficiência, diante de respostas positivas em trabalhos publicados (THOMPSON; OYSTON; WILLIAMSON, 2010; NOVAKOVIC et al., 2016; KIM et al., 2019; DE VRIES et al., 2020a, 2020b).

1.2.1.1 Leveduras

As leveduras e seus coprodutos são normalmente classificados como alimentos funcionais (que promovem benefícios à saúde, além da nutrição básica). Elas podem atuar como probióticos (leveduras vivas), prebióticos (componentes da parede celular), e metabólitos bioativos por conta da ação de células inviáveis ou fragmentos celulares de leveduras, chamados paraprobióticos (FATHIMA et al., 2023).

Dentre as leveduras, a mais utilizada comercialmente é a *Saccharomyces cerevisiae (S. cerevisiae)* (CASTRO; CALDER; ROCHE, 2021), com estrutura básica representada na FIGURA 1.

FIGURA 1. Estruturas básicas da levedura *Saccharomyces cerevisiae* e de sua membrana celular.



FONTE: O autor (2023). Imagem criada em Biorender.com.

A *S. cerevisiae* é uma levedura de reprodução assexuada (levedura de brotamento), geralmente considerada segura para consumo, e é amplamente utilizada na produção de alimentos e bebidas como pães, vinho e cerveja, além de produtos farmacêuticos e bioquímicos, combustíveis, e aditivos para nutrição animal (BELDA et al., 2019). Cerca de 95% do etanol produzido no mundo vem da fermentação de açúcares pela *S. cerevisiae,* o que pode ser considerado expressivo, visto que, somente no Brasil foram produzidos aproximadamente 26 bilhões de litros de etanol na safra 2022/2023 (CONAB, 2023). Considerando a produção de vinho e cerveja no Brasil, é possível concluir que há uma grande quantidade de leveduras *S. cerevisiae* em uso no Brasil e no mundo.

A parede celular das leveduras é composta por aproximadamente 22% de lipídeos, 23% de proteínas e 50% de carboidratos, sendo que os carboidratos se dividem nos polissacarídeos mananos, glucanos e quitina (WANG et al., 2018). Seu interior, por sua vez, é composto por carboidratos, ácidos nucléicos, enzimas, aminoácidos, peptídeos, lipídeos, vitaminas, minerais e sais (HASSAN, 2011; FATHIMA et al., 2023).

As leveduras há muito tempo vêm sendo usadas como coprodutos da indústria de produção de etanol, bebidas alcoólicas, e alimentos, que, após o processo de produção desses produtos, não teriam outro destino. Elas podem ser benéficas nutricionalmente tanto para humanos quanto para nutrição animal, por possuir ácidos nucleicos, mas a recomendação para humanos é que leveduras não sejam consumidas em alta quantidade para não aumentar o nível de ácido úrico no sangue, sendo a dose máxima recomendada de 2g de ácidos nucleicos por dia, o que equivale a aproximadamente 35g de levedura seca por dia (RAKOWSKA et al., 2017). Os ácidos nucleicos são provenientes do metabolismo de purinas, que em seu processo de catabolismo têm como produto o ácido úrico, convertido em alantoína pela uricase na maioria dos mamíferos, mas não em humanos, por isso estão associados a doenças nesse último caso, principalmente cardiovasculares (MAIUOLO et al., 2016).

Pensando em leveduras, elas podem ser adicionadas às dietas de suínos de várias formas, incluindo células vivas (como probióticos), células tratadas termicamente, extratos e culturas. Os efeitos diferem de acordo com a forma utilizada, levando a diversas aplicações. Seu uso, de maneira geral, normalmente está relacionado à melhoria do desempenho dos suínos e da sua imunidade, auxiliando o desenvolvimento intestinal, a adsorção de micotoxinas, redução da diarreia pós-desmame e influência positiva da microbiota intestinal. A resistência da levedura no intestino permite que ela tenha impacto na colonização intestinal. Os benefícios vêm da composição das leveduras, principalmente dos tipos de açúcar em suas paredes celulares (β-D-glucanos e α-D-mananos) (LIU et al., 2018).

Os componentes da parede celular das leveduras são considerados prebióticos, que são carboidratos complexos indigestíveis para os organismos pluricelulares, mas que promovem o crescimento e a atividade de bactérias intestinais desejáveis, trazendo benefícios para o hospedeiro (FATHIMA et al., 2023).

2. Betaglucanos

Betaglucanos são polissacarídeos de ocorrência natural em plantas, algas, fungos e leveduras, compostos por monômeros de D-glicose unidos por ligações β-glicosídicas que atuam como reservas de energia e componentes estruturais (CÓRDOVA-MARTÍNEZ et al., 2021; WANI et al., 2021). Eles podem ter efeitos diversos quando consumidos, dependendo da sua fonte e estrutura (FIGURA 2), podendo atuar como fibras dietéticas (betaglucanos da aveia), melhorando parâmetros como dislipidemia e resistência à insulina, ou como imunomoduladores (betaglucanos de levedura), visando especificamente a resposta imune inata (CASTRO; CALDER; ROCHE, 2021). Apenas os betaglucanos com ligações β-1,3 unidas a ligações β-1,6, que são os betaglucanos insolúveis, são capazes de estimular e modificar a resposta imune (CASTRO; CALDER; ROCHE, 2021; CÓRDOVA-MARTÍNEZ et al., 2021).

FIGURA 2. Estrutura química de betaglucanos de acordo com sua fonte.

Tipo	Estrutura	Descrição
Bactéria		β-1,3-glucano linear
Fungo		β-1,3/1,6- glucano ramificado curto
Levedura		β-1,3/1,6- glucano ramificado longo
Cereal		β-1,3/1,4-glucano linear

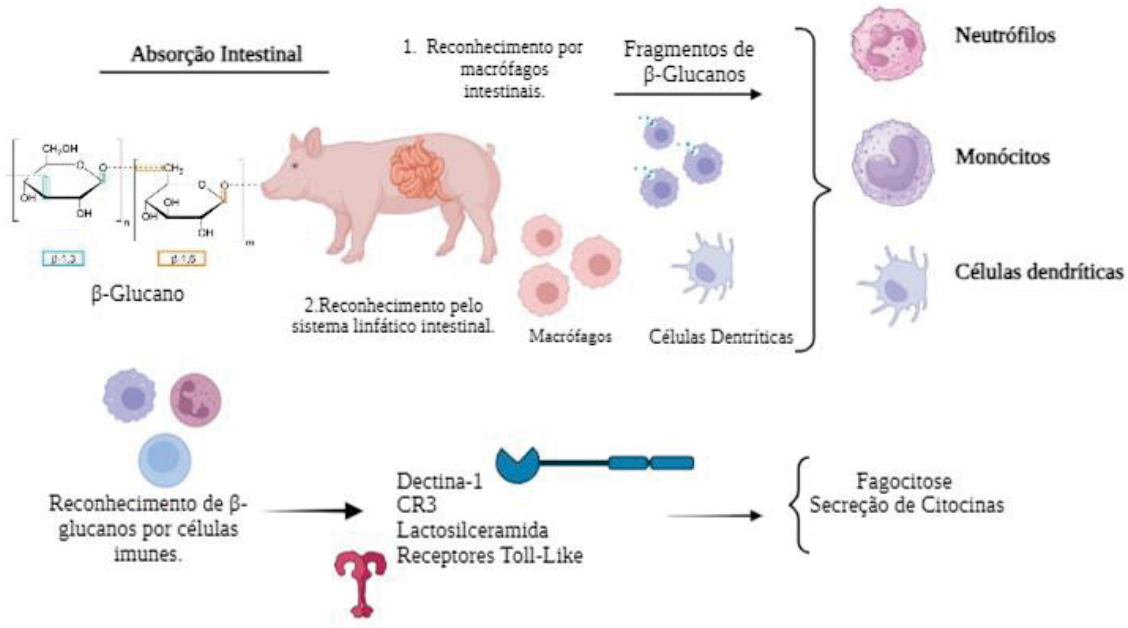
FONTE: Adaptado de Seo et al. (2019).

Os betaglucanos derivados de levedura possuem a capacidade de treinar monócitos para uma maior produção de citocinas pró-inflamatórias, o que pode lhe conferir um importante papel de proteção contra infecções secundárias por conta da

indução de alterações funcionais em células maduras da linhagem mieloide (QUINTIN et al., 2012; MITROULIS; HAJISHENGALLIS; CHAVAKIS, 2021). O processo de treinamento envolve o uso de um receptor chamado β-glucano dectina-1 e uma via específica, chamada via Raf-1 (FIGURA 3).

Após sua ingestão, os betaglucanos são absorvidos no intestino e reconhecidos por macrófagos intestinais que os degradam em fragmentos que serão levados à medula e ao sistema fagocítico. Após esse processo, os betaglucanos são liberados pelos macrófagos e capturados pelos neutrófilos, monócitos e células dendríticas, que desencadearão a resposta imune. Outra possibilidade, é este processo ser desencadeado por células epiteliais do tecido linfoide associado ao intestino (CÓRDOVA-MARTÍNEZ et al., 2021). Isso leva a mudanças duradouras na maneira como certas partes do DNA são marcadas, o que sugere que a programação epigenética pode estar desempenhando papel importante nesse mecanismo (QUINTIN et al., 2012; CÓRDOVA-MARTÍNEZ et al., 2021).

FIGURA 3. Mecanismo de ação dos betaglucanos após ingestão sobre o sistema imune de suínos.



FONTE: O autor (2023), adaptada de Córdova-Martinez et al. (2021) e Castro et al. (2021). Imagem criada em Biorender.com.

Embora todos os betaglucanos apresentem um esqueleto de β-1,3-glucano, há variações em relação à fonte e aos métodos de extração e purificação empregados em sua preparação comercial. Há diversos trabalhos com sua aplicação para humanos, inclusive como *boosters* para as vacinas contra o SARS-CoV-2 mais recentemente, avaliando betaglucanos de diferentes empresas e com diferentes formas de obtenção (SEO et al., 2019; CÓRDOVA-MARTÍNEZ et al., 2021; WANI et al., 2021). Para suínos os trabalhos são mais escassos e recentes.

2.1 Efeitos dos betaglucanos de levedura para matrizes suínas

Estudos recentes mostram que betaglucanos podem ter efeito positivo sobre a imunidade de matrizes suínas, com a suplementação realizada no terço final de gestação (DOS SANTOS et al., 2023; XU et al., 2023). Por conta da imunidade passiva que os leitões adquirem ao consumirem o colostro e leite das matrizes, por conta dos aspectos fisiológicos discutidos anteriormente, um tratamento que vise melhorar a imunidade das fêmeas nessa fase pode ter efeito não só sobre a imunidade das matrizes como também sobre a imunidade de seus leitões.

No trabalho de Santos et al. (2023) as matrizes nulíparas foram suplementadas do dia 75 de gestação ao dia 10 de lactação com um aditivo a base de betaglucanos de levedura *Saccharomyces cerevisiae* (50% de betaglucanos), e foi observada maior quantidade de imunoglobulina A (IgA) e granulócitos em seu colostro, além de uma quantidade de IgA mais elevada em seu leite. Também foi observado que o colostro dessas matrizes foi capaz de estimular uma atividade mitogênica de células do epitélio intestinal mais pronunciada. Isso sugere que o colostro com mais IgA e granulócitos pode promover melhor desenvolvimento do epitélio intestinal de leitões, o que faz com que esses animais consigam apresentar menor permeabilidade intestinal nessa fase em que ainda não são imunocompetentes, dificultando a passagem de organismos indesejados.

Em outro trabalho foi avaliada a suplementação de betaglucanos de levedura *Saccharomyces cerevisiae* (Biothera) associados com caseína hidrolisada para matrizes dos 83 dias de gestação até o desmame, e para os leitões por 10 dias pós desmame. Os leitões das matrizes suplementadas com a associação de betaglucanos com caseína hidrolisada, e que também consumiram o *blend* de aditivos,

apresentaram melhor escore fecal e menor incidência de diarreia em comparação aos leitões provenientes das fêmeas que não foram suplementadas. Além disso, os leitões das fêmeas suplementadas apresentaram maior concentração de butirato no intestino, melhor eficiência alimentar, maior abundância de *Lactobacillus* e menor de *Campylobacteraceae* (CONWAY et al., 2022).

2.2 Efeitos dos betaglucanos de levedura para leitões

Alguns trabalhos avaliando a suplementação de betaglucanos para leitões mostram que esse aditivo pode ser um importante aliado para melhorar a resposta imune destes animais, além do seu potencial de melhorar a digestibilidade da dieta, composição da microbiota intestinal e desempenho dos leitões. Esses achados são especialmente importantes quando se trata das fases de pré e pós desmame desses animais, visto que são fases extremamente desafiadoras imunologicamente (DE VRIES et al., 2020a).

Logo após o desmame o leitão passa por uma fase de profundo estresse social, ambiental e dietético, promovendo deterioração da barreira intestinal. Isso aumenta sua permeabilidade e facilita a passagem de organismos indesejados, o que afeta diretamente de maneira negativa seu sistema imune nesta fase (WIJTTEN; MEULEN; VERSTEGEN, 2011). Dessa forma, essa é a fase em que se focam grande parte dos trabalhos com betaglucanos, visando melhorar a imunidade do animal para que ele consiga passar pela creche da melhor maneira possível, reduzindo os impactos negativos no desempenho futuro.

Em trabalho avaliando um produto comercial a base de betaglucanos de leveduras *Saccharomyces cerevisiae* (Glucagen) ofertado a leitões em fase de creche foi observado que conforme se aumentava a dose ofertada do produto, maior era o ganho de peso dos leitões e a digestibilidade da dieta, além de terem apresentado maior quantidade de células CD4 e CD8 (linfócitos T auxiliares e citotóxicos, respectivamente) quando comparados aos leitões que não receberam os betaglucanos (HAHN et al., 2006).

Em leitões, também em fase de creche, desafiados com a inoculação de *Escherichia coli* também foi observado melhor desempenho dos animais suplementados com betaglucanos provenientes também de *Saccharomyces cerevisiae,* com 98,5% de pureza. Além disso, esses animais também apresentaram

uma maior quantidade de células do sistema imune, TNF-α (fator de necrose tumoral alfa), Complemento C3 e IL-1β (interleucina-1 beta) (WU et al., 2018).

Em leitões suplementados entre a fase de lactação e a fase de creche com betaglucanos de *Saccharomyces cerevisiae* (Energy Plus) misturados ao leite e à dieta, desafiados com lipopolissacarídeos, foi observado aumento em seu ganho de peso e aumento na expressão de mRNA TNF-α em comparação ao tratamento controle, sem adição de betaglucanos à dieta, mostrando um efeito semelhante à suplementação de vitamina C na dieta (EICHER et al., 2006).

2.3 Efeitos dos betaglucanos de levedura para suínos em crescimento e terminação

Apesar de nas fases de crescimento e terminação os suínos serem mais imunocompetentes por se tratar de animais mais maduros, ou seja, com seu sistema imune quase que por completo desenvolvido, já tendo tido acesso, e possivelmente desenvolvido imunidade, a diferentes patógenos, ainda é uma fase em que a aplicação de betaglucanos à dieta pode ter efeito muito positivo.

A maioria dos trabalhos com betaglucanos mostram seu efeito sobre a imunidade dos animais, mas, principalmente nessas fases, há outros efeitos desejáveis advindos desse aditivo. Com a suplementação de um aditivo a base de betaglucanos de *Saccharomyces cerevisiae,* com 95% de concentração (Biorigin), suínos em fase de terminação apresentaram melhor qualidade de carne e maior capacidade antioxidante, com maior pH e capacidade de retenção de água na carne fresca, por meio da redução da glicólise muscular post-mortem, além de alterar a composição da microbiota intestinal dos animais (HE et al., 2022).

Alguns trabalhos sugerem uma ligação entre a quantidade de gordura intramuscular e a proporção entre *Firmicutes* e *Bacteroidetes* através do transplante de microbiota fecal. Também já foi observada a ação positiva da *Prevotella copri* na deposição de gordura na carcaça de suínos. Descobriu-se que os ácidos graxos de cadeia curta, produzidos através da fermentação bacteriana de carboidratos, influenciam o desenvolvimento das miofibras e a qualidade da carne suína. Isto sugere que as bactérias intestinais afetam as características da carne (WU et al., 2021; HE et al., 2022).

Para suínos em crescimento há o entendimento que betaglucanos também têm maior efeito sobre a imunidade desses animais, sendo capazes de melhorar sua resposta por anticorpos e até mesmo prevenir doenças como o vírus da febre suína clássica (PORNANEK; PHOEMCHALARD, 2021).

3. Considerações Finais

Há uma grande variedade de trabalhos com betaglucanos de levedura disponíveis, porém, em sua maioria, voltados à medicina humana. Este não é um problema, pois, além de fornecer uma alta gama de informações para o desenvolvimento da medicina humana, também nos dá uma grande base de trabalhos para explorar os possíveis efeitos dos betaglucanos para os animais. Em especial os suínos, não só por conta de seu alto impacto na produção de proteína animal, mas também por serem tão utilizados em pesquisas para humanos, por conta das semelhanças anatômicas e fisiológicas.

Os trabalhos disponíveis avaliando suínos ainda são limitados, mas já mostram que esse aditivo pode ser uma boa estratégia nutricional com efeito significativo sobre a imunidade e saúde intestinal dos animais. Mas esses trabalhos também mostram uma gama de oportunidades para desenvolver melhor as pesquisas nesse sentido, visando ter o melhor aproveitamento desse composto, entendendo os benefícios desse aditivo em todas as fases de crescimento dos suínos.

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CHAPTER II - EFFECT OF YEAST EXTRACTED B-GLUCANS ON THE IMMUNE RESPONSE AND REPRODUCTIVE PERFORMANCE OF GILTS IN THE ADAPTATION, GESTATION, AND LACTATION PERIODS

This chapter is written per the guidelines for Livestock Science authors, and it is already published under: https://doi.org/10.1016/j.livsci.2023.105289

Effect of yeast extracted β-glucans on the immune response and reproductive performance of gilts in the adaptation, gestation, and lactation periods

M. C. dos Santos[1*], K. F. da Silva[1], A. P. Bastos[2], A. P. Félix[1], S. G. de Oliveira[1], A. Maiorka[1].

[1]Department of Animal Science, Federal University of Paraná, 80035-050, Curitiba, PR, Brazil

[2]EMBRAPA – Swine and Poultry, Concórdia, SC, Brazil

Primary Audience: Animal Nutritionists, Researchers, Swine Producers, Animal Scientists.

*Corresponding author: marleyconceicaos@gmail.com

Departament of Animal Science, Federal University of Paraná (UFPR), Curitiba 80035-060, PR, Brazil.

Phone: +55 41 99717-7361

A B S T R A C T

Yeast β-glucans may have beneficial effects on the immune response of gilts and their litter through immunomodulation from the gastrointestinal tract. Thus, the objective of this study was to evaluate the effect of the inclusion of β-glucans in the diet of replacement gilts during the adaptation, gestation and lactation phases on their response and the response of their litter to vaccines against Parvovirus and Leptospirosis, as well as its effect on parity performance and immune parameters of colostrum and milk. In two trials, gilts were randomly assigned to one of two treatments: with or without the inclusion of β-glucans at 300 g/ton in the basal diet. Gilts consumed the experimental diets during the adaptation period in the first experiment (EXP1) and from d 75 of gestation until d 10 of lactation in the second experiment (EXP2). Blood samples were collected from sows and from their piglets. In addition to blood collections, colostrum and milk were also collected in EXP2, and the reproductive performance of sows at the first farrowing was evaluated. Qualitative data were analyzed by chi-square, and quantitative data were evaluated using ANOVA or Kruskal-Wallis, according to their normality. There was an effect of treatment in EXP2 on the IgA and IgM concentrations and mitogenic activity in colostrum ($P<0.05$), and for IgA in milk from sows ($P<0.05$). For parvovirus and Leptospira spp. antibodies, in the two experiments, there was no difference between treatments ($P>0.05$), as well as for reproductive parameters. Thus, under the experimental conditions of this study, it is possible to conclude that including 300 g/ton β -glucans in the diet of first-parity sows can increase the concentration of IgA in their colostrum and milk, as well as the proliferation of intestinal epithelial cells, while decreasing the concentration of IgM in their colostrum, but not in milk.

Keywords: Parvovirus, Prebiotics, Sows, Colostrum, Leptospira spp.

Funding: This study was supported by CAPES.

1. Introduction

The immune system is one of the most complex in higher animal organisms, being divided into innate and adaptive immunity. The innate immune system is historically characterized by promoting a rapid response to pathogens through cytokines, mono- and polymorphonuclear phagocytes, and natural antibodies, among others, but without long-term memory. The adaptive system, on the other hand, promotes a slower, but more specific and memory-based immune response, with the help of T and B lymphocytes and specific antibodies (Hato and Dagher, 2015; Sattler, 2017).

New approaches suggest that the innate immune system can also develop an immune response with long-term memory, known as “trained immunity” (Saeed et al., 2014; Netea et al., 2016; Novakovic et al., 2016; Netea and Joosten, 2018). According to Netea et al. (2016), trained immunity is based on an altered functional state of innate immune system cells (myeloid, natural killer and innate lymphoid cells) that lasts for weeks to years after exposure to the initial stimulus, a pathogen or vaccine, for example, and that confers protection to the organism after a re-exposure to the same stimulus.

With this new evidence, β-glucans, glucose polymers naturally occurring in fungi, yeasts, algae, and cereal grains, have been further explored for having an immunomodulatory effect on the innate immune system in animal biosafety (Thompson et al., 2010; Kim et al., 2019). With increasing restrictions on the use of antibiotics in animal production (Boyd et al., 2018), the use of β-glucans can be a good ally to potentiate trained immunity, improve the effect of vaccines and

even mitigate the pathogenic effect of biological agents harmful to the animal (Thompson et al., 2010).

Some studies on the inclusion of β-glucans in the diet of suckling and weaning piglets found an effect on their immunity and vaccine response, indicating that this early period in these animals' lives is critical for their later development (Kim et al., 2019; Vries et al., 2020). These works do not explain the effect that β-glucans could have in other phases of swine production, such as in the adaptation period of gilts, gestation, and lactation phases of primiparous sows. These phases are of great importance because they are where the gilts will receive the greatest number of vaccines and will be exposed to the pathogens present in the new environment in which they will be held. In addition, the litter of these gilts tends to have a more weakened immunity due to the immunity passed by the gilt itself, which is also more weakened compared to multiparous sows (Piñeiro et al., 2019; Maciag et al., 2022).

Based on this information, the objective of this study was to evaluate the effect of supplementation of β-glucans, extracted from *saccharomyces cerevisiae* yeast during the periods of gilt adaptation, gestation and lactation of primiparous sows, on their response to vaccines against Parvovirus and Leptospirosis, pathogens that significantly interfere with reproductive parameters and that are achieved with the same vaccine, parameters of immunity of colostrum and milk from these sows, and its effect on performance at parturition as measured by the number of total births, live births, stillbirths and mummified piglets.

2. Material and Methods

All animal care and experimental procedures were approved by the Animal Ethics Committee of the Agricultural Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol 029 - 2019. Two experiments were conducted in the period between January 21, 2020, and August 8 of the same year, on a commercial farm.

Experiment 1 – Gilts treated during the adaptation period

Animals and facilities

96 females of the Camborough genetic lineage (PIC Camborough, Hendersonville, TN) were used, with an average weight of 98.45 kg ± 5.6 kg and 153 ± 1.4 days old. Gilts arrived at the farm in two batches, with a 15-day interval between batches. Each batch was divided into four groups of 12 gilts each, housed in collective pens, forming a total of eight pens of gilts per treatment until the beginning of pre-insemination flushing, when females are fed more nutritionally dense feed, so they are ready for first insemination, which took place at 185 days of life. From the beginning of flushing until two days before parturition, the gilts were housed in individual pens, after that they were transferred to the lactation pen.

For gilts in adaptation period, the collective pens were equipped with a collective funnel-type feeder with a tray, with a capacity of 100 kg of feed, divided between two pens. Each pen had dimensions of 6.0 m (L) x 2.5 m (L). The gestation pens were 2.0 m (L) x 0.6 m (L) and were equipped with ditch-type feeders fed with droppers and the feed was provided by a pipe traction system. The lactation pens were also individual with 2.2 m (L) x 0.6 m (L), equipped with

crates that aim to protect the piglets from possible crushing and with concealing boxes with 1.5 m (L) x 2, 0 m (L), heated by incandescent lamps and underfloor heating, and the ration was also provided through droppers in trough-type feeders.

There was no induction of parturition and during farrowing, the sows were monitored by the farm staff. Also, there was no induced health challenges, just the usual ones in a commercial farm.

The temperature of the facilities was controlled by opening and closing curtains and remained on average at 24.3ºC ± 6.9ºC in the experimental period.

Diets and Treatments

The gilts were divided into two treatment groups in a completely randomized design as: CON – control treatment that consisted only of the standard gestation diet of the farm based on corn and soybean meal (Table 1) and TBG – Standard diet of the farm with the addition of a product based on yeast β-glucans, containing 50% of 1,3/1,6 beta-glucans, at 300 g per ton of complete feed. The treatment period took place between the arrival of the gilts at the farm until their first insemination (50 days approximately).

To make the TBG, feed present the β-glucan in the proportion of 300 g/ton, a pre-mix was made as follows: 24 g of product was mixed with 76 g of feed to prepare a pre-mix of 100 g product. The 100 g premix was added to an additional 900 g of feed to form another 1 kg premix. The 1 kg pre-mix was mixed with 9 kg of feed to form 10 kg of pre-mix which was later mixed with 70 kg of feed to obtain the final mixture that was fed to the gilts, and this process was repeated three times to have the enough amount of feed to supply the four treatment pens.

The product for the first pre-mix was weighed on a digital scale and for the other mixtures buckets with the markings for the specific weights were used.

From the arrival of the gilts to the farm until the second dose of vaccines (vaccinal program is available in Figure 1) Farrowsure® Gold (MSD), *Streptococcus suis* (IPEVE) and *E. coli* (IPEVE), where the flushing period began, the feed was provided ad libitum through a traction system by pipes in the feeders for the gilts of the CON and, in the pens of the TBG, the system traction was locked and the feed was supplied in buckets with the β-glucan mixture in the amount of 80 kg per feeder, being filled in the afternoon, when necessary, with approximately 20 kg more so that in the TBG pens the feed was also offered ad libitum.

It is important to note that each feeder was responsible for serving two pens of 12 gilts, that is, each gilt consumed approximately 4 kg of feed per day.

From the beginning of flushing until the first insemination of the gilts, around 20 days, the gilts were transferred to individual pens, where they consumed 2.5 kg of feed per day. The TBG gilts received 0.8 g of β-glucan in the ration, equivalent to 300 g/ton. During the gestation and lactation periods, the gilts received the standard diet of the farm for gestation and lactation (Table 1). Water was provided ad libitum and fresh for pacifier-type drinkers.

Experiment 2 – Gilts treated during gestation and lactation periods

Animals and facilities

40 gilts with 290 ± 1.4 days of age were used. They were housed in individual gestation pens until two days before parturition, when they were transferred to the lactation pens where they remained until weaning

(approximately 21 days of lactation). The facilities, management and temperature were as described in experiment 1.

Diets and Treatments

The gilts were divided into two treatments in a completely randomized design with 20 gilts each: CON – control treatment (standard farm diet without addition of yeast β-glucans) and TBG – Standard farm diet with addition of yeast β-glucans to 300 g per ton.

During the gestation period, the gilts consumed 1.8 kg of feed until 90 days of gestation in a daily supply, and for this amount of feed, 0.6 g of β-glucan was placed in the droppers for the TBG gilts. From 90 days until the second day before farrowing they consumed 2.5 kg of feed, with the addition of 0.8 g of β-glucan for TBG gilts. In the period between the two days before farrowing until the third postpartum day they consumed 3 kg of feed per day, with the addition of 0.9 g of β-glucan for the TBG sows and about 6 kg per day on average until the tenth postpartum day, with the addition of 1.8 g of β-glucan for TBG sows. After the tenth day postpartum until weaning, all sows consumed only the standard farm diet, without the addition of β-glucan for any of the treatments. For TBG sows, an amount of β-glucan equivalent to the period of consumption was added to the feed at the time of supply.

During gestation, fresh water was provided ad libitum in trough-type drinkers and cup-type in lactation pens. The composition of gestation and lactation diets is described in Table 1.

Blood Collections

In the first trial (adaptation), 5 mL blood samples were collected from 8 gilts per treatment in each batch, totaling 32 gilts, 16 from the CON and 16 from the TBG. The samples were collected in four time points, as pointed in Figure 1. In the first collection, the TBG gilts were consuming the β-glucans for 5 days and in the two following collections they were being supplemented for an average of 32 and 52 days, respectively.

In the second trial (gestation and lactation) 5 mL of blood was collected from 10 gilts per treatment, totaling 20 gilts. The samples were collected in three time points, as pointed in Figure 1.

Blood collection was performed by puncturing the jugular vein, and the blood was stored in EDTA tubes immediately after collection. Subsequently, the samples were centrifuged at 3000 rpm for five minutes, to obtain the serum. The sera obtained were aliquoted into Eppendorfs® tubes and stored in a freezer at -80°C until use, to preserve the cytokines.

After collection and centrifugation, which took place on the farm, the serum samples were stored at -4°C.

Also, for the second trial, colostrum was manually collected in 50mL sterile conical tubes from all functional teats to a final volume of 10mL, soon after the birth of the first piglet. To minimize colostrum contamination, the teats were previously washed with water and detergent, then with iodized alcohol, the first jet was discarded, and handling was performed with disposable latex gloves. The samples were stored in flasks at -20ºC until analysis. On the tenth day of lactation, milk collection and storage were also performed by manual milking, following the pattern of colostrum collection.

Parvovirus and Leptospira spp analysis

Hemagglutination inhibition (HI) analysis was performed with the blood samples, which is the most widely used test for the serological diagnosis of parvovirus infection and is based on the property of parvovirus to agglutinate red blood cells of some species (Fujisaki et al., 1982). If the animal's serum contains antibodies against the virus, this hemagglutinating capacity is blocked, as the antibodies bind to the viral antigens and prevent them from adsorbing to the red blood cells.

For the serological diagnosis of leptospirosis infection, microserum agglutination analysis was performed at CEDISA (Diagnostic Center for Animal Health, internal methodology), also in Concórdia – SC. This analysis is based on the ability of the anti-leptospira antibodies produced by the animal to agglutinate specimens of leptospires belonging to various serovars in a liquid medium. The final titer obtained from each sample corresponds to the highest dilution of serum that causes 50% or more agglutination of leptospires. The samples were tested for nine strains of leptospires: *L. hardjo, L. canícola, L. gryppotyphosa, L. Bratislava, L. icterohaemorrhagiae, L. pomona, L. autumnalis, L. wolffi e L. tarassovi*.

Quantification of IgG and IgA

ELISA analyzes were performed to quantify the concentrations of IgG, IgM and IgA immunoglobulins in colostrum and milk. Before analysis, colostrum samples were centrifuged (1300 x g at 4°C for 20 minutes) to remove fat and diluted with a suitable diluent (50mM Tris buffer, 0.14M NaCl, 1% BSA and 0.05% Tween 20). ELISA reagents were obtained from Bethyl Laboratories (Montgomery, TX, USA). Briefly, 100µL of colostrum sample or standard solution

was added to each pit and incubated at room temperature for 1 hour, then washed 4 times with the wash buffer. The concentrations of IgG, IgM and IgA in the standard solutions were 333.3, 111.1, 37, 12.3, 4.1, 1.37 and 0 ng/mL. All samples were analyzed in duplicate.

After that, 100µL of anti-IgG, anti-IgM or IgA were added and incubated at room temperature for 1 hour and washed 4 times with the wash buffer. Then, 100µL of horseradish peroxidase (HRP) was added. The plates were incubated for 30 minutes at room temperature and washed 4 times with the wash buffer. TMB substrate (3, 30, 5, 50-tetramethylbenzidine) was added to the plates and the plates were incubated for 30 minutes in the dark. The reaction was terminated with the addition of 100µL of stop solution. Plates were read on a microplate reader (Thermolab System, MRX Revelation, Chantilly, VA) at 540 nm. Results were obtained in ng/ml but expressed in mg/ml after appropriate correction of the dilution factor.

Measurement of cell viability

Colostrum and milk cell suspensions were counted in an automated Coulter counter (Orflo Moxi Z, USA) and analyzed on a Neubauer hemocytometer by a single operator. Each sample was mixed with a 0.4% trypan blue solution (Sigma Chemical Co. Germany) in a ratio of 1:2 (V/V). Cell concentration corresponded to the average of all four sets of squares evaluated, considering the Neubauer chamber volume and dilution. Trypan blue-stained cell counts were used to determine the concentration of non-viable cells. The proportion of non-viable cells was calculated based on the number of cells stained with trypan blue (non-viable) compared to the total number of cells.

Cell preparation for flow cytometry

Antibodies produced against porcine leukocyte antigens were purchased from BioRad Serotec (Oxford, UK), and stabilizing fixer (FACSLyse) and compensation beads were purchased from BD (North Ryde, Australia). Flow cytometry buffer was prepared in PBS (Phosphate-Buffered Saline) supplemented with heat-inactivated FBS (Stain Buffer; 2% V/V), bovine albumin (2% W/V, Sigma-Aldrich), and sodium azide (0.01% W/V, Sigma-Aldrich). Antibodies were selected according to Forner et al. (2021). Despite the high homology for some orthologous proteins, there are still uncertainties about their nomenclature. For these clusters, we name them Swine Workshop Clusters (SWC) and their CD-marking orthology is in parentheses.

Colostrum and milk cells (suspended in a flow cytometry buffer at approximately 1 x 10^6 cells/mL) were incubated for 30 minutes at room temperature with a specific mAb cocktail. Our panels were designed for a four-color cytometer to assess populations of T and B lymphocytes, macrophages, and granulocytes. The following fluorochrome-conjugated mAbs were used: panel A): panel 7-AAD (BD Biosciences); panel B): FITC-granulocyte (clone 6D10), RPE-CD79a (clone MB-1), PE-Cy7-CD3 (clone PPT3), APC-macrophages (clone BA4D5). To evaluate the nonspecific fluorochrome staining, control isotypes for anti-IgG1, anti-IgG2a and anti-IgG2b were introduced in the preliminary procedure to configure the photomultiplier and technical parameters of the instrument. The antibody dilution for the experiment was established by previous titration.

For intracellular staining panels, cells were resuspended in Cytofix/Cytoperm solution (BD Biosciences) and incubated for 20 minutes. The samples were then washed twice with BD Perm/Wash (BD Biosciences) to keep

303 the cells permeabilized and make it easier to stain CD79a (the epitope recognized
304 by the mAb is located in the cytoplasmic domain) and CD3 (the PPT3 clone
305 recognizes an epitope extracellular and intracellular in CD3) in the subsequent
306 incubation.

307 After staining, cells were centrifuged (400 x g at 10°C for 5 minutes) and
308 the pellet was washed once with 1 mL of flow cytometry buffer followed by
309 centrifugation (400 x g at 10°C for 5 minutes). Cells were resuspended in 300 µL
310 of stabilizing fixative and transferred to a plate, and samples were analyzed by
311 flow cytometry within 2 hours.

312 Cytometry was performed on an Accuri C6 cytometer (BD Biosciences).
313 Fifty thousand events were analyzed (based on FSC and SSC) using Accuri C6
314 plus software (Becton Dickinson). Compensation beads were used for each
315 antibody in a different reading channel to establish compensation settings. The
316 side scatter (SSC) was set at 8000 gating.

317

318 *Mitogenic assay*

319 Mice cells of the IEC-6 cell line were suspended (1 x 10^6 cells/mL) in DPBS
320 and labeled with CFSE (2.5uM; Molecular Probes, USA) for 10 minutes at 37ºC.
321 The labeling process was stopped by adding five times the volume of RPMI 1640
322 containing 10% FBS (RPMI-SFB) followed by incubation for five minutes on ice,
323 protected from light. The cells were washed twice with 20 ml of RPMI-SFB and
324 then suspended in the same medium. IEC-6 cells were plated in 6-well plates (1
325 x 10^6 cells/well), allowed to adhere for 18 hours and then washed twice in Hank's
326 balanced salt solution (HBSS, Sigma-Aldrich).

327 The medium was then changed to 1mL of serum-free DMEM and cultured
328 with colostrum (100µL). As a positive control, two pits received the cells with FBS

(100µL), and the cell culture without stimulants represented the negative control (untreated). Cells were cultured for 48 hours at 37°C under 5% CO2. Cells (1 x 10^6) were transferred to 7-AAD labeled flow cytometry tubes. Samples were tested in triplicate. A total of 50,000 events per tube were acquired on the flow cytometer and analyzed. Cells were recovered after 48 hours of cultivation and evaluated for CFSE staining intensity.

The percentage of proliferated IEC-6 cells was determined by CFSE dilution and the geometric mean values of the triplicates stimulated with colostrum were calculated and divided with the geometric mean values of the medium control triplicates to obtain the stimulation index.

Reproductive and productive performance

In addition to blood analyses, birth data were collected from all sows, with total births, live births, stillbirths (animals with perfect external development, but stillborn) and mummified piglets (piglets whose lives were interrupted between the days 35 and 90 of gestation and are born with the appearance of a mummy), to evaluate the performance of the females at farrowing.

Statistical analysis

The collected data were initially divided into qualitative and quantitative. Qualitative data (titration) were submitted to chi-square analysis at 5% significance. Quantitative data were tested for normality using the Anderson-Darling test. IgA, IgG, IEC proliferation, macrophages, granulocytes and B lymphocytes from colostrum, IgM from milk, and total births were the parameters indicated as normal by the Anderson-Darling test ($P<0.05$). For normal data, analysis of variance was performed, and for the other non-normal quantitative

355 parameters, the Kruskal-Wallis analysis was performed, both at 5% significance.
356 All analyzes were performed in statistical software Minitab 18® (Minitab, Inc.
357 State College, PA).

358

359 **3. Results**

360 IEC 6 cells stimulated with colostrum from gilts that received β-glucans
361 supplementation showed significantly higher mitogenic activity ($P<0.008$) than
362 cells stimulated with colostrum from non-supplemented gilts (Figure 2).

363 The supplementation of β-glucans for sows did not affect the rates of sows
364 that presented antibodies against Parvovirus ($P>0.05$) in all blood collections
365 from both experiments, as well as the titers observed ($P>0.05$). Against
366 Leptospira spp., in the first experiment, the sows showed antibodies only after
367 the second dose of the vaccine, at insemination and at 85 days of gestation, while
368 in the second one, only the sows showed antibodies and only at the end of
369 lactation. Both the rates of positive and negative animals and the titers observed
370 were not affected by the treatment ($P>0.05$; Tables 2 and 3).

371 There was no negative effect of the β-glucans on the reproductive and
372 productive performance of the sows and their litters ($P>0,05$; Table 4).

373 Trypan blue exclusion staining, and microscopic assessment of viability
374 were performed immediately after colostrum collection and at subsequent times,
375 according to flow cytometry analysis. The colostrum samples evaluated were rich
376 in cells (5-6 x 10^6 for gilts), and their cell viability was greater than 92%. A
377 summary of the cellular components in colostrum from gilts and sows is shown in
378 Table 5. Supplementation of β-glucans for sows did not change the cellular
379 immune composition of their colostrum and milk ($P>0.05$), the main types of

immune cells were granulocytes (neutrophils 34-37%) predominantly followed by T lymphocytes (CD3+ 28-29%), B lymphocytes (CD79a+, 14-15%) and macrophages (9-10%). The population of phagocytic cells in mammary secretions consisted of neutrophils and macrophages.

There is an overall increase of immune cells in the colostrum and milk of sows from β-glucan supplemented group, however, the increase was statistically significant only in IgA concentration in colostrum, and there was a decrease in IgM concentration in colostrum of sows with the β-glucan supplementation in the diet ($P<0,05$). The level of IgA in the milk of the sows was also significantly higher in the β-glucan supplemented group ($P<0.05$; Table 5).

4. Discussion

The initial hypothesis of this study, based on the literature (Kim et al., 2019; Vetvicka et al., 2020; Vries et al., 2020), was that the inclusion of β-glucans in the diet of first parity sows would demonstrate an increasing effect on the vaccine response of these sows, which could also be passed on to their piglets through colostrum and milk. The results of this work showed that this inclusion in the gestation period increased the IgA concentration and the proliferation rate of intestinal epithelial cells (IEC) from colostrum and milk, but there was no effect on vaccine response, neither on sows nor their piglets to the pathogens Leptospira spp. and Parvovirus, with supplementation during gestation and lactation or during the adaptation phase.

Regarding the results for Parvovirus, which is responsible for reproductive disorders in sows, such as an increase in the stillbirths rate, mummifications, embryonic death and sow infertility (Streck et al., 2020), the animals inside the

405 farm probably will have contact with the field virus during their lifetime, generating
406 a greater and longer-lasting immune response, while vaccination generates a
407 milder response, which reduces clinical manifestations, but does not prevent
408 infection, and must be reinforced throughout the animal's life (Streck et al., 2020).
409 That's why β-glucans has been used as vaccine boosters, being able to reduce
410 the number of times the same vaccine must be applied and even the number of
411 vaccines (Vetvicka et al., 2020), but this effect was not evaluated in the present
412 study.

413 Leptospira spp., like parvoviruses, can cause reproductive problems in
414 sows and the infection can be passed to the litter in the case of pregnant sows
415 (Ellis, 2014). It is important to note that in this study, only a few sows and none
416 of the piglets tested positive for leptospirosis, and all sows were vaccinated.
417 Furthermore, among the positives, some had leptospira serovars not included in
418 the vaccine, and there were persistent post-vaccination agglutinin titers for four
419 months after the application of commercial vaccines (Dobson & Davos, 1975),
420 which may indicate that the positive ones were infected in the environment. The
421 vaccine applied against leptospirosis is the same used against parvovirus, and
422 the results indicate that it was efficient in inducing an immune response only in
423 the second case.

424 Vries et al. (2020) also evaluated the vaccine response of piglets
425 supplemented with β-glucans to the Salmonella vaccine. They found an increase
426 in the titer of antibodies against Salmonella, but the addition of β-glucans had no
427 effect on this parameter. The answer they found for this result is that the vaccine
428 was very efficient at inducing an immune response, thus, the inclusion of β-
429 glucans in the diet had no noticeable effects, which may also have happened in

the current work. The vaccine against parvovirus and leptospirosis may have induced a high immune response in sows to parvovirus and the effect of the inclusion of β-glucans may have been minimal. And for leptospirosis, as there was no effect of the vaccine, the diet had nothing to act on.

Antibody research in unvaccinated gilts (before the first vaccine) showed that most animals were negative, with HI > 8 titers. However, lower titers were also found, confirming the viral presence with a lower degree of infection in some gilts. Others had anti-parvovirus antibody titers of 256, 512 and 1024, showing that the virus is actively circulating in the farm. After vaccination, there was an increase in anti-parvovirus antibody titers in gilts, these titers were higher than those considered protective in the literature, 1:80 (Brown et al., 1987; Barcellos et al., 1998; Sobestiansky et al., 1998; Sobestiansky et al., 1999). Finally, it is known that the level of neutralizing antibodies to parvovirus increases with the number of vaccinations. However, the level of these antibodies can remain stable or decrease due to a possible saturation of the immune response due to repeated and short gaps between antigenic stimuli, which is also called “immunological paralysis” (Jin et al., 2019).

One of the factors that may have caused the vaccine response of the animals not to be affected by the treatments was the time of exposure to the product before the first vaccine of the gilts, which was five days because it was a commercial farm where the gilts could not go long without being vaccinated. The β-glucan product manufacturer recommends at least 15 days of supplementation before exposing animals to vaccination for greater product efficiency. At the beginning of the tests, this information was still not accurate and, also because of that, this time was not respected.

In addition to the time of inclusion of the product in the diet before the first vaccination, the concentration of the product may also have been insufficient, as despite being greater than the amount used for piglets (108 g/ton in the work by Kim et al. (2019) and 50 to 300 g/ton in de Vries et al. (2020), may have been insufficient for gilts, as there was no other works found by the authors that tested the same product to this specific production phase. Thus, this work is even more important for bringing findings that may guide other researchers in the search for the best use of beta-glucans.

Piglets do not receive antibodies from sows before birth because of the physiology of the swine reproductive system, therefore, colostrum is most responsible for providing the immunity that the newborn piglet needs. In first parity sows, the amount of B and T lymphocyte cells and IgG in colostrum is lower than in multiparous sows (Forner et al., 2021). Hence the interest in working with first-parity sows, an increase in the immune cells of colostrum and milk from these sows may have a more significant effect than it would have in multiparous sows, precisely because of their greater limitations.

IgA is the most abundant immunoglobulin in animals and its main role is to protect mucosal surfaces against infectious microorganisms. It is found in greater amounts following immunization of animals (Sousa-Pereira and Woof, 2019). The IgA concentration in the colostrum and milk of the sows from the second experiment was higher in those that were receiving β-glucans supplementation in the diet, indicating that this additive was able to induce a greater immune response in the sow, which was passed to her colostrum and milk. This is reinforced by the proliferation rate of IECs, which was also higher in the colostrum of sows treated with the addition of β-glucans in the diet.

The intestinal epithelium represents a tissue with rapid cell turnover. An increase in healthy cell proliferation rate in the crypts results in an overall increase in the population of epithelial cells and associated increases in the height of the intestinal villi. Thus, colostrum with higher mitogenic activity or a higher proliferation rate of IECs has the potential to accelerate the maturation of the newborn piglet's gastrointestinal tract and provide piglets with better protection while maintaining the integrity of its intestinal mucosa (Soderholm and Pedicord, 2019; Li et al., 2020).

IgM is the first immunoglobulin secreted after exposure of the organism to a health challenge. In addition to its ability to neutralize pathogens, it also acts as a cell and pathogen signaler for lysis by complementary cells (Keyt et al., 2020). Its concentration was higher in the colostrum of sows treated without the addition of β-glucans, and as it is the first immunoglobulin secreted in the face of a challenge, this may have happened because the sows must have been experiencing some sanitary challenges and their immune systems were less mature compared to the sows treated with β-glucans, causing them to depend more on IgM at that first moment (Walker et al. (2020).

Due to the reproductive disorders that can be caused by parvovirus and leptospirosis (Ellis, 2014; Streck et al., 2020), data on the performance of sows at parturition were also analyzed, including total births, live births, stillbirths, and mummifications. However, these was no effect on these parameters, which is consistent with the data on sow immune response in this study and with studies such as Vries et al. (2020), who found only modest responses when including β-glucans in the diet of lactating piglets.

β-glucans can have a very positive effect on the immunity of gilts, sows and their litters, as can be seen in this work. Even with the shorter supplementation time compared to that currently recommended by the manufacturer. Because this is a new area of research, adjustments such as β-glucans concentration in the diet and supplementation timing will be required in future work to clarify the role of β-glucans as health additives in swine nutrition.

5. Conclusion

Inclusion of β-glucans at 300 g/ton in the diet of primiparous sows during the gestation and lactation periods increases the concentration of IgA in their colostrum and milk. In addition to this, colostrum from β-glucan supplemented sows was able to stimulate proliferation of intestinal epithelial cells in vitro. On the other hand, supplementation of sows' diets with 300 g/ton β-glucans in the adaptation period was not able to modulate the vaccine response against the parvovirus and Leptospira vaccine antigens, nor do they alter the reproductive performance of these sows at the first parturition.

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Table 1

Composition of the diets used in the periods of adaptation, gestation, and lactation of the gilts and sows.

	Adaptation/Gestation	Lactation
Ingredients	Inclusion g/kg	Inclusion g/kg
Corn 7.86% CP	684.763	698.434
Soybean hull	180.000	-
Soybean meal 46% CP	86.941	200.900
Viscera meal	15.253	30.000
Poultry fat	-	28.232
Limestone 36% Ca	12.549	12.744
Monocalcium Phosphate	5.104	6.462
Salt	5.000	3.105
Lysine*	2.802	6.587
L-Threonine	0.746	1.661
DL-Methionine 99%	0.562	0.952
Vitamin and Mineral Premix[1]	6.280	10.923
Calculated Nutritional Composition (%)		
Crude Protein	13.800	18.000
Ether Extract	3.610	6.449
Crude Fiber	7.336	2.070
Ash (%)	4.235	4.087
Calcium (%)	0.930	0.970
Total Phosphorus (%)	0.582	0.702
Available Phosphorus (%)	0.420	0.500
Sodium (%)	0.230	0.260
Total lysine (%)	0.779	1.250
Total Methionine (%)	0.279	0.387
Total Met + Cys (%)	0.539	0.697
Total Threonine (%)	0.604	0.852
Total Tryptophan (%)	0.154	0.263
Swine Met. Energy (Kcal/kg)	3.104,56	3.470,00

*BioLys 60.

[1]Contains per kg of complet diet: Vit. A, 16.500 UI; Vit. D3, 3.500 UI; Vit. E, 99.000 UI; Vit. K3, 3.3 mg; Vit. B2, 8.500 mg; Vit. B12, 38.500 mcg; Pantothenic acid, 25.000 mg; Folic acid, 3.850 mg; Se, 0.44 mg; Mn, 60.001 mg; Cu, 22.0 mg; Fe, 121 mg; Zn, 110 mg; I, 1.65 mg.

Table 2

Rates of first parity sows, with (TBG) and without (CON) supplementation of β-glucans in the diet in the adaptation, gestation and lactation periods, which presented or not antibodies against parvovirus and Leptospira spp. in seven different periods, and of their piglets in experiment 2.

Periods	Treatments	[1]Negative %	[2]Positive %	P-value
Parvovirus				
Experiment 1 - Adaptation				
Before 1st vaccine	CON	56.25	43.75	0.719
	TBG	62.50	37.50	
After 2nd dose of the vaccine	CON	37.50	62.50	0.508
	TBG	37.50	62.50	
Insemination	CON	25.00	75.00	0.233
	TBG	46.15	53.85	
d 85 of gestation	CON	23.08	76.92	0.658
	TBG	30.77	69.23	
Experiment 2 – Gestation and Lactation				
Before 2nd vaccine	CON	20	80	0.528
	TBG	10	90	
Farrowing	CON	20	80	0.592
	TBG	11.10	88.90	
Weaning	CON	10	90	1.053
	TBG	0	100	
3 day-old Piglets	CON	5.88	94.12	0.669
	TBG	3.57	96.43	
Leptospira spp.				
Experiment 1 - Adaptation				
Before 1st vaccine	CON	100.00	0.00	-
	TBG	100.00	0.00	
After 2nd dose of the vaccine	CON	93.75	6.25	-
	TBG	100.00	0.00	
Insemination	CON	81.25	18.75	0.811
	TBG	84.62	15.39	
d 85 of gestation	CON	71.43	28.57	0.662
	TBG	78.57	21.43	
Experiment 2 – Gestation and Lactation				
Before 2nd vaccine	CON	100.00	0.00	-
	TBG	100.00	0.00	
Farrowing	CON	100.00	0.00	-
	TBG	100.00	0.00	
Weaning	CON	50.00	50.00	1
	TBG	50.00	50.00	
3 day-old Piglets	CON	100.00	0.00	-
	TBG	100.00	0.00	

[1] Animals that did not present a positive serological result for parvovirus or Leptospira spp. [2] Animals that tested positive for parvovirus or Leptospira spp.

Probability level at 5%

Table 3

Titers observed for the Hemagglutination Inhibition (HI) tests for parvovirus and microserum agglutination for Leptospira spp. from first parity sows, receiving (TBG) or not (CON) β-glucans in the diet in the adaptation, gestation, and lactation periods, in seven blood collections in different production periods and from the piglets of the experiment 2 sows.

	COM	TBG	P-value
Parvovirus			
Experiment 1 - Adaptation			
Before 1st vaccine	1024	2048	0.506
After 2nd dose of the vaccine	4096	4096	0.935
Insemination	2048	4096	0.305
d 85 Gestation	4096	4096	0.676
Experiment 2 – Gestation and Lactation			
Before 2nd vaccine	4096	4096	0.454
Farrowing	4096	4096	0.699
Weaning	4096	4096	0.698
3 day-old Piglets	4096	6144	0.702
Leptospira spp.			
Experiment 1 - Adaptation			
Insemination	100	150	0.221
d 85 Gestation	200	100	0.307
Experiment 2 - Gestation and Lactation			
Weaning	100	100	0.513

Probability level at 5%. 16 samples per treatment in experiment 1, and 10 samples per treatment in experiment 2.

Table 4

Reproductive performance of sows at first parity, receiving (TBG) or not (CON) β-glucans in the diet during the adaptation, gestation, and lactation periods.

	CON	TBG	P-value
Experiment 1 - Adaptation			
Total born, n	15.39	16.16	0.225
Born alive, n	14.02	14.84	0.234
Stillbirths, n	0.95	0.66	0.482
Deaths, n	2.02	1.95	0.972
Mummifieds, n	0.34	0.36	0.515
Experiment 2 – Gestation and Lactation			
Total born, n	14.85	15.90	0.274
Born alive, n	14.45	15.10	0.280
Stillbirths, n	0.15	0.45	0.207
Deaths, n	2.25	1.80	0.534
Mummifieds, n	0.25	0.29	0.941

Probability level at 5%. 16 samples per treatment in experiment 1, and 10 samples per treatment in experiment 2.

Table 5

Immunoglobulins and concentration of macrophages, granulocytes and B and T lymphocytes from colostrum and milk of first parity sows, receiving (TBG) or not (CON) β-glucans in the diet.

	CON	TBG	SEM	P
Colostrum				
IgA mg/mL	10.969	12.636	0.372	0.006
IgG mg/mL	79.360	82.890	3.300	0.461
IgM mg/mL	6.949	6.294	-	0.002
Macrophages mg/mL	9.066	10.760	0.792	0.162
Granulocytes mg/mL	34.171	37.000	1.042	0.073
Lymphocytes B mg/mL	14.574	15.083	0.539	0.513
Lymphocytes T mg/mL	28.245	29.455	-	0.450
Milk				
IgG mg/mL	15.192	11.796	-	0.806
IgA mg/mL	10.763	13.452	-	0.008
IgM mg/mL	4.409	4.458	0.168	0.839

SEM: Standard error of the mean.

Experimental Schedule				
Animals age (average)	**Time point**	**Vaccine**	**Blood collection**	**Diet program**
153 days	Gilts arrival at the farm		Gilts from 1st experiment	Adaptation diet ad libitum
160 days	First vaccine	Porcilis® PCV M HYO		Adaptation diet ad libitum
165 days	Vaccines	Farrowsure® B Gold		Adaptation diet ad libitum
		Oily Autogenous *E. coli* and *Clostridium*		
		Autogenous *Streptococcus suis*		
185 days	Flushing	Farrowsure® B Gold	Gilts from 1st experiment	2.5kg/day of adaptation diet
		Oily Autogenous *E. coli* and *Clostridium*		
		Autogenous *Streptococcus suis*		
205 days	First insemination		Gilts from 1st experiment	2.5kg/day of adaptation diet
75 days of gestation	Start of the 2nd experiment		Gilts from 2nd experiment	1.8kg/day of gestation diet
80 days of getation	Vaccine	Oily Autogenous *E. coli* and *Clostridium*	Gilts from 1st experiment	1.8kg/day of gestation diet
85 days of gestation	Blood collection			1.8kg/day of gestation diet
100 days of gestation	Vaccine	Oily Autogenous *E. coli* and *Clostridium*		2.5kg/day of gestation diet
114 days of gestation	Farrowing day		Gilts from 2nd experiment	3kg/day of lactation diet
3 days of lactation	Blood collection		Piglets from sows of the 2nd experiment	6kg/day of lactation diet
7 days of lactation	Piglets vaccine	Autogenous *Streptococcus suis*		6kg/day of lactation diet
11 days of lactation	Vaccine	Farrowsure® B Gold		7kg/day of lactation diet
21 days of lactation	Weaning		Gilts from 2nd experiment	7kg/day of lactation diet

Figure 1. Schedule for both experiments, with vaccination, blood collection and diet program. The vaccines were: Inactivated vaccine (Porcilis® PCV M HYO, MSD, Boxmeer, Netherlands)) against porcine circovirus type 2 and Mycoplasma hyopneumoniae; Inactivated swine parvovirus, *Erysipelothrix rhusiopathiae* and Leptospira (*L. bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae and L. pomona*) vaccine (Farrowsure® B *Gold,* Zoetis, Campinas, Brazil), named for this paper as parvovaccine; Oily autogenous vaccines *Escherichia coli* and *Clostridium perfringens* (IPEVE, Belo Horizonte, Brazil); Autogenous vaccine *Streptococcus suis* (IPEVE, Belo Horizonte, Brazil); and Inactivated vaccine (Porcillis® Glasser, MSD, Boxmeer, Netherlands).

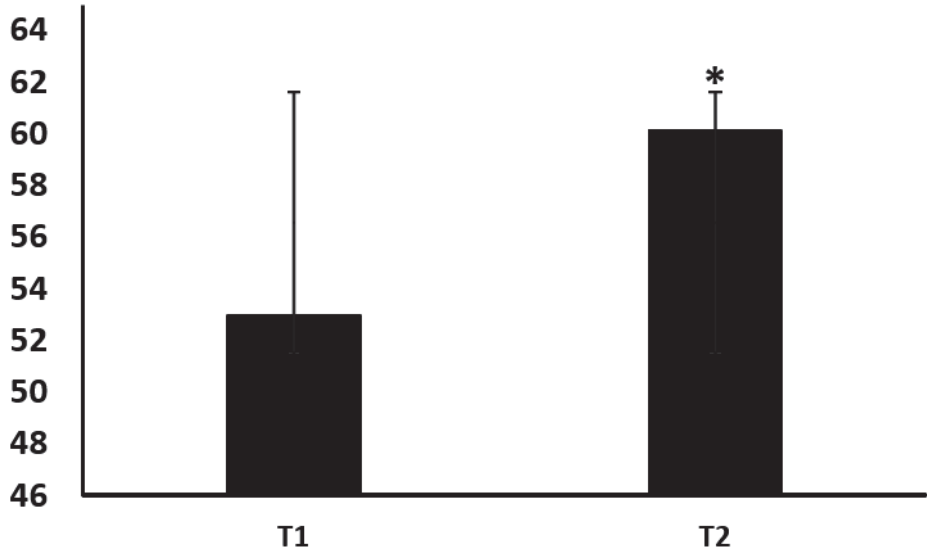

Figure 2. Mitogenic activity (%) of sow colostrum on intestinal epithelial cells (IEC-6). Data are expressed as mean percentage (± SEM) of cell viability calculated relative to untreated cells (n = 5 replicates per treatment). *p<0.05. T1: No Beta-glucan; T2: With Beta-glucan.

CHAPTER III - YEAST-DERIVED B-GLUCANS AND MANNAN OLIGOSACCHARIDES AS MODULATORS OF INTESTINAL INFLAMMATION AND MICROBIOME IN NURSERY PIGS

This chapter is written per the guidelines for Livestock Science authors.

Yeast-derived β-glucans and mannan oligosaccharides as modulators of intestinal inflammation and microbiome in nursery pigs

M. C. dos Santos[1*], A. P. Félix[1], A. Maiorka[1], S. G. de Oliveira[1]

[1]*Department of Animal Science, Federal University of Paraná, 80035-050, Curitiba, PR, Brazil*

Primary Audience: Animal Nutritionists, Researchers, Swine Producers, Animal Scientists.

*Corresponding author: marleyconceicaos@gmail.com

Departament of Animal Science, Federal University of Paraná (UFPR), Curitiba 80035-060, PR, Brazil.

Phone: +55 41 99717-7361

A B S T R A C T

The objective of this study was to evaluate the effect of feeding diets with high levels of soybean meal and the inclusion of a yeast-based β-glucan and mannan oligosaccharide (MOS) additive on the performance, immunity, and intestinal microbiome of weanling pigs. 80 mixed-sex nursery piglets (2 piglets/pen) with an initial body weight of 5.9 ± 0.8 kg were randomly assigned to 1 of 4 dietary treatments (n=10 pens/treatment) in a completely randomized block design for a 42-d feeding trial. Dietary treatments were a complex diet (CD; standard diet with greater inclusion of highly digestible ingredients) and a simple diet (SD; with more than 30% soybean meal), with or without adding the yeast-based β-glucan and MOS additive. Pigs were fed ad libitum and weighed weekly. Blood samples were taken on day 7 and 21. Intestinal permeability, cytokines, and phagocytosis profiles were measured from the blood collected. On day 42, fecal samples were collected from the same animals. A greater weight gain was reported for pigs fed simple diets without the additive from day 22 to 28 and on the last week of the experiment ($P<0.05$). The CD with the additive showed the lowest mean fluorescence intensity of phagocytic granulocytes and IFN-α concentration at seven days ($P<0.05$). SD down-regulated the concentration of IL-6 cytokines and up-regulated IL-8 in the same period ($P<0.05$). Adding the additive to the diet showed a down-regulation in IL-8 and phagocytic monocytes at 7 days and a higher concentration of phagocytic monocytes and granulocytes at 21 days ($P<0.05$). Finally, complex diets and adding the additive decreased the number of pathological microorganisms at the end of the nursery phase ($P<0.05$). These results show that a diet with a higher soybean meal concentration may increase inflammation in the gastrointestinal tract of nursery pigs in the first week of the nursery phase. At the same time, adding the yeast-based β-glucan and MOS additive may reduce this inflammatory response. Furthermore, the interaction between a CD and the β-glucan-based additive

reduces intestinal inflammation and the number of pathological bacterial genera in the nursery phase.

Keywords: Leukocytes, Prebiotics, Dectin-1, GIT Microbiota.

Funding: This study was supported by CAPES.

1. Introduction

The challenges pigs face in the nursery phase are mainly associated with their immune system and gastrointestinal tract (GIT) health (Pluske et al., 2018). The intestinal immaturity and vulnerability of these animals can negatively alter the intestinal structure, immunity, and physiology (Jang and Kim, 2022), which can lead to poorer growth performance driven by lower feed consumption and, consequently, lower weight gain, often found along with pathological symptoms (Christensen et al., 2022).

To improve feed intake and nutrient absorption in these animals, highly digestible and highly palatable ingredients, such as milk coproducts (Jang and Kim, 2022; Tran et al., 2014), are included in the diet during at least the first days of the nursery phase. Milk coproducts are mainly used to replace plant-based proteins and carbohydrates, the primary example being soy, which has many allergic substances (Taliercio and Kim, 2014).

These plant-based ingredients are associated with a drop in performance and changes in the intestinal mucosa in recently weaned piglets, likely due to allergenic, antigenic, toxic, and pathogenic compounds (Kim and Duarte, 2021; Taliercio and Kim, 2014). According to Rostagno et al. (2017), the limit soybean meal inclusion

recommended for the nursery phase is 30% and 25% for extruded and micronized whole soybeans. Some other works show that up to 30%, higher levels of soybean meal can be beneficial to health-challenged nursery pigs due to bioactive compounds, such as isoflavones and saponins (Rochell et al., 2015; Cemin et al., 2021).

Additives are also an alternative to minimize the effects of post-weaning on nursery pigs, and among them, mannan oligosaccharides (MOS) and β-glucan-based ones have been extensively studied aiming at the intestinal and general health of these animals (Sun et al., 2015; Loving et al., 2023), and the association between them was reported to have a positive effect on their performance (Sun et al., 2015; Choi and Kim, 2023) and intestinal health (Kogan and Kocher, 2007; Choi and Kim, 2023).

However, the way these additives behave in the organism may be changed by health or nutritional challenges. MOS have shown growth-promoting effects, but that is in pigs held in unhygienic environments, compared to the ones raised in hygienic environments (Halas and Nochta, 2012). Moreover, β-glucans, in addition to having different effects depending on their source (vegetable or microbial; Choi & Kim, 2023), also present different responses according to the composition of the diet, with a more discreet effect in complex diets (de Vries et al., 2020).

Testing a yeast-based additive, with mainly MOS and β-glucans in its composition, this study investigated the correlation between the additive and diet composition on nursery pigs' performance, intestinal microbiota and permeability, leukocytes, cytokines, and diarrhea.

2. Material and Methods

The Animal Ethics Committee of the Agricultural Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, approved all animal care and experimental procedures under protocol 017 - 2023. The experiment was conducted between April 20th, 2023, and July 14th of the same year on an experimental farm in Pinhais, Paraná, Brazil.

Animals and facilities

A total of 80 mixed-sex nursery piglets (2 piglets/pen; PIC Camborough x Landrace and Large White sows) with an initial body weight of 5.9 ± 0.8 kg were randomly assigned to 1 of 4 dietary treatments, with ten replicates per treatment, in a completely randomized block design for a 42-d feeding trial. The blocking factor was the period, as the piglets were housed in three different periods. The piglets came from sows raised on the same experimental farm, and they were housed in 2m² pens with partially leveled floors, equipped with nipple drinkers and through feeders. Each pen was considered the experimental unit. Room temperature was controlled by curtain handling and infrared heating lamps, and it was measured and recorded daily, remaining at 20,8ºC ± 2,7ºC.

Experimental diets

Four mash diets were tested in a 2x2 factorial arrangement: complex diet, which was a standard diet with greater inclusion of more easily digestible ingredients, or simple diet, with fewer highly digestible ingredients and greater inclusion of soybean meal (>30%), above that recommended by Rostagno et al. (2017) for nursery phase.

Both with or without the inclusion of an additive based on autolyzed yeast (*Saccharomyces cerevisiae*) from the sugar cane ethanol production process, composed mainly of beta-glucans and mannan-oligosaccharides (MOS; ImmunoWall, ICC) at 2 kg/ton (Tables 1 and 2). Diets were formulated for four phases: pre-initial 1 (0 to 7 days), pre-initial 2 (8 to 14 days), initial 1 (15 to 21 days), and initial 2 (22 to 42 days), and throughout the experimental period it was provided in mash form ad libitum, as well as water.

Experimental analyses

For performance analyses, all feed provided and leftovers were weighed, as were all animals in housing at 7, 14, 21, 28, 35, and 42 days of the experiment.

From eight animals per treatment, 10ml of blood was collected on the 7th day of the experiment for leukocyte and phagocytic profile, intestinal permeability, and cytokine panel analysis, and 5ml on the 21st day was collected for phagocytic profile analysis. Blood collection was performed by puncturing the jugular vein, and the blood was stored in EDTA tubes immediately after collection for subsequent analysis.

For the leukocyte profile, whole blood samples with anticoagulant were collected to determine the proportion of CD4+ and CD8+ T lymphocytes, monocytes, and B lymphocytes by flow cytometry, according to the methodology described by (Stabel et al., 2000), the same reference used to analyze the phagocytic profile, in which samples of whole blood with anticoagulant were also collected for determination by flow cytometry of the concentration and mean fluorescence intensity (MFI) of phagocytic monocytes and granulocytes, on the 7th and 21st days of the experiment.

Regarding the intestinal permeability analysis, for its evaluation, 1ml per animal of a non-absorbable fluorescent marker (Dextran-FITC, 3000 to 4000 kDa) was

administered orally six hours before blood collection, which can be identified in plasma/serum of the animal according to the level of injury in the intestinal epithelial monolayer. The higher the concentration of the marker in the plasma/serum, the greater the permeability degree and intestinal injury (Vicuña et al., 2015). Whole blood samples were also collected with an anticoagulant to evaluate this parameter.

The cytokine panel was analyzed in plasma samples, following the protocol indicated in the user manual of the commercial kit (Invitrogen, EPX090-60829-901), with the Luminex xMAP technique, which determines the concentration of interleukins (IL) 1β, 4, 6, 8, 10 and 12/IL-23p40, interferons alpha (IFN-α) and gama (IFN-γ), and tumor necrosis factor-alpha (TNF-α).

The occurrence of diarrhea was monitored daily in all pens, and animals with diarrhea were treated with Flumegan (2.2 ml/50kg; CEVA, Campinas/SP, Brazil) for three days and Spectomix (0,1 ml/kg; Farmabase, Jaguariúna/SP, Brazil) for five days. Diarrhea incidence was calculated weekly per pen as the percentage of days with a fecal score of 2 and 3, according to the scoring system of (van der Wolf et al., 2017), in which 0 = normally shaped feces, 1 = shapeless feces, 2 = thick, liquid feces (diarrhea), 3 = thin, liquid feces (watery diarrhea), and 9 = no score possible, following the procedures described by (Fabà et al., 2020).

Microbiome

At 42 days of the study, 250µl of fresh fecal samples were taken via rectal stimulation from the same animals that had previously collected blood samples for microbiome bioinformatics analysis.

Following the protocol recommended by the manufacturer, the standard commercial kit for the specific matrix was used to extract DNA from the samples.

Quality and quantification were evaluated by spectrophotometry in Nanodrop (Thermo Fisher Scientific™). A segment of approximately 460 bases from the V3V4 hypervariable region of the 16S rRNA gene was amplified using primers 375F/805R for bacterial analysis, following these PCR conditions: 95°C for 3 min; 25 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s, followed by a step at 72°C for 5 min.

The amplicons were linked to Illumina® Nextera dual index barcodes under the following conditions: 95°C for 3 min; 8 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s, followed by a step at 72°C for 5 min. The products were then purified, pooled, and sequenced on the Illumina® "NextSeq" sequencer (Degnan and Ochman, 2012) in a 300-base paired-end.

The readings, or "reads," obtained from the sequencer were analyzed on the QIIME2 (Quantitative Insights Into Microbial Ecology) platform (Caporaso et al., 2011, 2010), following a workflow of using forward and reverse sequences (R1 and R2) in bacterial analysis, continuing with removal of low-quality sequences, filtration, removal of chimeras and taxonomic classification. Sequences were classified into bacterial genera through the recognition of Amplicon Sequence Variants (ASVs), in this case, the homology between sequences when compared against a database. To compare the amplicons of regions of the 16s rRNA gene, the 2022 update (version GTDB 207) of the bacterial genome taxonomy database GTDB (Parks et al., 2022) was used, extracting in silico reads from the same amplified regions. To generate classifications of bacterial communities by identifying ASVs, 27,233 reads per sample were used for bacterial analysis, with all samples being analyzed. The minimum use of readings per sample is intended to normalize the data and not to statistically compare samples with different numbers of readings in each type of microbiome.

Statistical Analysis

All data were analyzed for normality using the Kruskal-Wallis test. Then, the data underwent factorial analysis of variance using a mixed linear model, including factors (feed and additive) as the main effects and block (period) as the random effect. Tukey test was the post hoc analysis. P values greater than 0.05 were considered significant, and between 0.05 and 0.10, they were considered tendency. All these analyses were performed using the statistical software Minitab 18® (Minitab, Inc. State College, PA).

ASVs count and Chao1 and Shannon's indexes were analyzed for alpha diversity. The effect of treatments on beta diversity was evaluated among groups by PERMANOVA (Permutational Multivariate Analysis of Variance; Anderson, 2001). The differential abundance of bacterial genera between treatments was analyzed using linear discriminant analysis (LDA) effect size (LEfSe) on MicrobiomeAnalyst, according to Chong et al. (2020). Bacterial genera with log LDA score higher than 2 and adjusted ($P<0.05$) for false discovery rate were considered significant.

3. Results

From day 21 to 28, the pigs' feed/gain ratio was better when fed the simple diet ($P=0.044$), and for the total period, feed/gain was better when the additive was included ($P=0,039$). There was no difference between treatments for the evaluated performance parameters for all other periods ($P>0.05$; Table 3).

On day 7, animals fed complex diets with the additive had the lowest MFI of phagocytic granulocytes compared to those without the additive ($P=0.01$). Also, animals fed diets with the additive had a lower MFI of phagocytic monocytes compared to pigs fed diets without the additive ($P<0.01$). Comparing animals fed complex or

simple diets, the complex diet tended to decrease the MFI of phagocytic monocytes (P=0.06). On day 21, the addition of the additive increased the MFI of phagocytic monocytes (P=0.02) and the concentration of phagocytic granulocytes (P=0.02), besides tending to decrease the concentration of phagocytic monocytes (P=0.06). Furthermore, animals fed complex diets had a higher concentration of B lymphocytes (P=0.04). The treatments did not influence the other parameters of the phagocytic and leukocyte profiles (P>0.05; Table 4).

Animals fed the simple diet had more diarrhea than those fed the complex diet in the second (d8-14; P<0.01) and third (d15-21; P=0.03) weeks. Pigs fed diets with the additive had a higher occurrence of diarrhea in the first week (d0-7; P=0.04), while in the last week (d36-42), diarrhea tended to occur more in pens where the pigs were fed without the additive (P=0.06). The treatments did not influence intestinal permeability (P>0.05; Table 5).

The complex diet with the additive promoted a lower IFN-α concentration than the complex diet without the additive (P=0.03). Diets that include the additive compared to those without the additive could decrease the concentration of IL-8 (P=0.02). Moreover, the complex diet increased IL-6 (P=0.04) and decreased IL-8 (P=0.04). Other cytokines evaluated did not differ between treatments (P>0.05; Table 6).

Regarding alpha diversity parameters, treatments did not differ for either ASVs or Chao1 (richness) and Shannon (diversity) indexes (P>0.05; Table 7), and no difference was observed for beta diversity (Figures 1 and 2).

Including the additive in the diets decreased the number of bacterial genera found in the samples, most of them associated with pathological and inflammatory processes (P<0.05; Figure 3), as well as feeding the animals with complex diets instead of the simple diet, that also decreased pathogenic bacterial genera found

(P<0.05; Figure 4). Likewise, when comparing simple diets with or without the inclusion of an additive (Figure 5) and complex diets with or without the inclusion of an additive (Figure 6), both had a greater quantity of bacteria with pathogenic potential when the additive was not included (P<0.05).

4. Discussion

The effect of additives based on MOS and β-glucans on pig performance is inconsistent. In some studies, such as that of Berto et al. (2020) and Christensen et al. (2022), an improvement in the performance of the animals is observed, with better feed conversion, mainly. However, some studies show that these additives are not capable of interfering with the performance of animals, such as that of Price et al. (2010), who also worked with an additive of similar composition to the current work and, different from this work, did not found any difference between the treatments in this parameter.

The difference in performance in this work was observed in the feed/gain ratio in two periods, 22-28 days, and the total period. From 22 to 28 days, animals fed simple diets showed better feed/gain, which can be explained by the tendency for a greater feed intake in the same animals. The inevitable difference in the metabolizable energy of the diets at this stage, due to the greater inclusion of soybean meal in the simple diet and brewer rice in the complex diet, may have stimulated greater feed consumption.

Although there is a consensus that lower calorie diets encourage greater feed intake to adjust energy consumption (Schinckel et al., 2012), it was expected that soybean meal would worsen animal performance as it was above the level recommended by Rostagno et al. (2017). This limit is based on antinutritional factors

that remain even after heat treatment, such as glycinin and β-conglycinin, which can cause abnormal morphology of the gastrointestinal tract (GIT) of weaned piglets, decreasing their absorption capacity; a hypersensitive immune response to these antinutritional factors triggers all of this (Li et al., 1990; Ruckman et al., 2020). Moreover, soybean oligosaccharide stachyose can cause diarrhea and decrease growth performance (Liying et al., 2003).

The animals had more diarrhea with the simple diet, but not enough to cause a drop in performance. Some studies show that soybean meal has some bio compounds with a positive effect on animals suffering from infection, one of which is isoflavones and their flavonoid compounds, mainly genistein, with antiviral, antioxidant, and anti-inflammatory effects (Andres et al., 2009). The work published by Rochell et al. (2015) confirmed this theory, as higher doses (29%) of soybean meal for nursery pigs infected with porcine reproductive and respiratory syndrome (PRRS) performed better. These studies may indicate that the same thing happened in this research, although they do not entirely explain it.

In general, the effects of diet and additive supplementation were few and modest for immunity parameters. The parameters that differed between the main effects and the interaction between them (IL-6, IL-8, IFN-α, phagocytic monocytes and granulocytes, and B lymphocytes) indicated less intestinal inflammation with the complex diet and the inclusion of the additive in the diet. This result may be a good indication of intestinal health. However, it also indicates that the additive could not promote a more significant immune stimulus, which was expected based on previous works (Sun et al., 2015; Choi and Kim, 2023).

For instance, cytokines can be divided into pro- and anti-inflammatory, although some do not have this classification as well defined. Interferons (IFN), TNF-α, and

interleukins (IL) 1β, 6, and 8 are pro-inflammatory, while IL-4, IL-10, and IL-13 are anti-inflammatory (Kim and Duarte, 2021; Choi and Kim, 2023). The complex diet in this study increased the concentration of IL-6 and decreased that of IL-8, which makes it difficult to conclude the inflammatory potential of the diet. Comparing the complex diet with or without the inclusion of the additive, the additive reduced the concentration of this IFN and the phagocytic granulocytes (MFI) at day 7, indicating less intestinal inflammation in this case, as phagocytic activity is directly related to intestinal inflammation as well as cytokines (Choi and Kim, 2023), similar to what was found by Upadhaya et al. (2019) and de Vries et al. (2020), also working with yeast-derived β-glucans for nursery pigs.

Most bacterial genera that differed between groups are not well studied in swine production, as interest in intestinal health is relatively new in animal science. Up to 2020, 90% of published papers focused on intestinal health and microbiota in pigs are from 2010 onwards (Kim and Duarte, 2021). Therefore, finding correlations between bacterial genera and effects on pigs in the literature is very challenging. However, it is known that some of them are associated with pathological processes, while others have beneficial effects.

Escherichia coli, Prevotella, Campylobacter, Mitsuokella, and *Clostridium* spp are well-known as dangerous microorganisms associated with infections and pathological processes and present both in the intestinal lumen and mucosa (Kogan and Kocher, 2007; Adhikari et al., 2019). These bacterial genera were all found in this work and differed between treatments, but they were in all treatments. The difference was the number of these microorganisms in each treatment. Complex diets and adding the additive decreased the number of pathological microorganisms in the fecal samples, which aligns with previous findings (Liying et al., 2003; de Vries et al., 2020).

The genera of the phylum Proteobacteria, such as *Escherichia coli* and *Campylobacter*, are considered markers of intestinal dysbiosis, usually found along with diarrhea in animals (Shin et al., 2015). In this work, these genera were found in more significant quantities in diets without including the additive, regardless of whether it was simple or complex diets. At the same time, the incidence of diarrhea was higher in simple diets, regardless of the inclusion of the additive, from 8 to 21 days of the experiment.

These findings indicate that the presence of microorganisms associated with pathogenicity does not necessarily impact the animals' performance, as, despite the differences found in the nursery pigs' microbiome, their performance did not differ in the same intensity and direction.

One of the limitations of this work was not having compared the change in the microbiota at each nutritional phase during the experiment, or at least at each blood collection so that the results could be compared with the immunity results and an association could be made between both. Despite this, this work can be added to published data to clarify the role of intestinal health in swine production.

5. Conclusion

Under the conditions of this study, it is possible to conclude that the inclusion of soybean meal above the recommended limit (30%) does not interfere with the performance of the animals during most of the nursery phase, with a positive effect only on the feed/gain ratio of the animals from 22 to 28 days. The inclusion of a yeast-based additive with a high concentration of mannan oligosaccharides and β-glucans

(additive) improves the feed/gain ratio of nursery pigs in the total period. Furthermore, complex diets and the additive promote decreased intestinal inflammation. Complex diets reduce the occurrence of diarrhea from 8 to 21 days of the experiment while including the yeast-based additive increases the occurrence in the first week. Finally, complex diets and adding the additive decreased the number of pathological microorganisms at the end of the nursery phase.

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Table 1.
Ingredients and calculated nutritional composition of complex experimental diets for nursery pigs with and without including mannan oligosaccharides and β-glucans-based additive.

		Inclusion (g/kg)			
Ingredients		Pre-starter 1	Pre-starter 2	Starter 1	Starter 2
Corn		403.66	437.38	515.45	545.64
Brewers rice		30.00	30.00	30.00	40.00
Soybean meal 46%		175.00	240.00	265.00	295.00
Extruded semi-whole soy		30.00	30.00	30.00	30.00
Whey powder		175.00	120.00	37.00	
Spray-dried swine plasma		35.00	20.00	5.00	
Biscuit residue		80.00	50.00	50.00	30.00
Sugar		20.00	20.00	20.00	10.00
Soybean oil		15.00	15.00	10.00	10.00
Limestone		5.00	6.50	6.00	6.00
Dicalcium phosphate19.5		12.00	12.00	12.00	10.80
Salt		4.00	4.00	4.00	5.00
Cl.-choline 60%		0.80	0.80	0.80	0.60
DL-Methionine		1.69	2.28	1.20	1.52
L - Lisine 79%		3.25	2.95	3.87	5.19
L-Threonine		0.93	0.85	0.88	1.51
L-Tryptophan		0.84	0.44	0.35	0.55
L-Valine		1.00	1.00	1.50	1.50
Phitase (10.000 FTU)		0.20	0.20	0.20	0.40
Antioxidant (B.H.T.)		0.15	0.15	0.15	0.15
Lactose		0.20	0.20	0.30	0.15
Palatable		0.28	0.25	0.30	0.20
Mycotoxin adsorbent		2.00	2.00	2.00	2.00
Premix[1]		2.00	2.00	2.00	2.00
Kaolin*		2.00	2.00	2.00	2.00
Additive*					
Calculated nutritional composition					
Crude protein	%	20.00	20.00	20.00	20.00
Crude fiber	%	2.16	2.68	2.89	3.11
EE	%	4.78	5.20	4.73	3.62
Ash	%	4.89	4.81	4.43	4.63
Lactose	%	12.00	9.00	3.00	
Swine M.E.	kcal/kg	3584	3546	3499	3386
Calcium	%	0.73	0.76	0.71	0.71
P total	%	0.72	0.64	0.67	0.65
P available	%	0.50	0.45	0.45	0.40

*A diet including kaolin and one including the tested additive at 2kg per ton.
Pre-starter 1 – 0-7 days; Pre-starter 2 – 8-14 days; Starter 1 – 15-21 days; Starter 2 – 22-42 days.
EE: Ether extract in acid hydrolysis; ME: Metabolizable energy
[1]Provide the following amounts per kg of diet: Fe, 77.6 mg; Cu, 11.6mg; Mn, 67.9 mg; I, 0.97 mg; Selenium, 0.31 mg; vitamin A, 11,250 IU; vitamin D3, 2250 IU; vitamin E, 22.5 IU; vitamin K3, 2.0 mg; vitamin B1, 1.75 mg; vitamin B2, 5.0 mg; vitamin B6, 1.75 mg; vitamin B12, 22.5 mcg; niacin, 37.5 mg; Pantothenic acid, 20.0 mg; Folic acid, 0.5 mg; biotin, 0.125 mg.

Table 2.
Ingredients and calculated nutritional composition of simple experimental diets for nursery pigs with and without including mannan oligosaccharides and β-glucans-based additive.

		Inclusion (g/kg)			
Ingredients		Pre-starter 1	Pre-starter 2	Starter 1	Starter 2
Corn		428.65	468.35	529.25	565.25
Soybean meal 46%		315.00	320.00	350.00	360.00
Whey powder		175.00	130.00	40.00	
Sugar		30.00	30.00	30.00	20.00
Soybean oil		15.00	15.00	15.00	15.00
Limestone		5.00	5.50	6.00	9.00
Dicalcium phosphate 19.5		12.00	12.00	12.00	12.00
Salt		4.00	4.00	4.00	5.00
Cl.-Choline 60%		0.80	0.80	0.80	0.60
DL-Methionine		2.10	2.00	1.50	1.50
L - Lisine 79%		3.50	3.50	2.90	2.90
L-Threonine		1.70	1.60	1.40	1.40
L-Tryptophan		0.40	0.40	0.30	0.30
Phitase (10.000 FTU)		0.20	0.20	0.20	0.40
Antioxidant (B.H.T.)		0.15	0.15	0.15	0.15
Lactose		0.20	0.20	0.20	0.20
Palatable		0.30	0.30	0.30	0.30
Mycotoxin adsorbent		2.00	2.00	2.00	2.00
Premix[1]		2.00	2.00	2.00	2.00
Kaolin*		2.00	2.00	2.00	2.00
Additive*					
Calculated nutritional composition					
Crude protein	%	20.50	20.00	21.00	20.00
Crude fiber	%	2.16	2.68	2.89	3.11
EE	%	4.78	5.20	4.73	3.62
Ash	%	4.89	4.81	4.43	4.63
Lactose	%	12.00	9.00	3.00	
Swine M.E.	kcal/kg	3388	3381	3385	3367
Calcium	%	0.73	0.76	0.71	0.71
P total	%	0.72	0.64	0.67	0.65
P available	%	0.50	0.45	0.45	0.40

*A diet including kaolin and one including the tested additive at 2kg per ton.
Pre-starter 1 – 0-7 days; Pre-starter 2 – 8-14 days; Starter 1 – 15-21 days; Starter 2 – 22-42 days.
EE: Ether extract in acid hydrolysis; ME: Metabolizable energy
[1]Provide the following amounts per kg of diet: Fe, 77.6 mg; Cu, 11.6mg; Mn, 67.9 mg; I, 0.97 mg; Selenium, 0.31 mg; vitamin A, 11,250 IU; vitamin D3, 2250 IU; vitamin E, 22.5 IU; vitamin K3, 2.0 mg; vitamin B1, 1.75 mg; vitamin B2, 5.0 mg; vitamin B6, 1.75 mg; vitamin B12, 22.5 mcg; niacin, 37.5 mg; Pantothenic acid, 20.0 mg; Folic acid, 0.5 mg; biotin, 0.125 mg.

Table 3.

Growth performance of nursery pigs fed with simple or complex diets (F), with or without including mannan oligosaccharides and β-glucans-based additive (A).

	Simple No Additive	Complex No aditive	Simple Additive	Complex Additive	SEM	P-value		
						F	A	F*A
0-21 days								
Body weight d0, kg	6,03	5,91	6,11	5,98	0,1	0,576	0,730	0,974
Feed intake, g/day	287	306	273	306	10,9	0,253	0,744	0,742
Weight gain, g/day	164	183	156	180	9,5	0,269	0,798	0,911
Feed/Gain, g/g	1,713	1,635	1,875	1,759	0,05	0,347	0,167	0,855
Body weight d21, kg	9,49	9,50	9,51	9,76	0,2	0,775	0,759	0,792
22-28 days								
Feed intake, g/day	697	659	646	640	27,3	0,696	0,543	0,790
Weight gain, g/day	488	404	452	412	28,9	0,304	0,821	0,713
Feed/Gain, g/g	1,431	1,801	1,461	1,735	0,08	0,044	0,907	0,756
Body weight d28, kg	13,73	13,03	12,25	12,36	0,4	0,716	0,191	0,620
29-35 days								
Feed intake, g/day	940	1004	911	877	36,4	0,837	0,300	0,514
Weight gain, g/day	614	659	647	586	25,0	0,875	0,705	0,311
Feed/Gain, g/g	1,649	1,583	1,421	1,555	0,04	0,690	0,138	0,244
Body weight d35, kg	17,47	17,64	16,78	16,73	0,5	0,956	0,455	0,916
36-42 days								
Feed intake, g/day	1258	1249	1119	1139	41,0	0,949	0,142	0,860
Weight gain, g/day	831	758	706	699	27,2	0,467	0,099	0,546
Feed/Gain, g/g	1,520	1,668	1,595	1,578	0,03	0,227	0,882	0,128
Body weight d42, kg	23,72	22,95	21,73	20,54	0,7	0,451	0,096	0,872
Total Period								
Feed intake, g/day	373	337	302	308	14,6	0,590	0,091	0,471
Weight gain, g/day	377	384	363	339	14,6	0,776	0,328	0,600
Feed/Gain, g/g	1,026	1,029	0,787	0,804	0,06	0,925	0,039	0,950

SEM: Standard error of the mean.

Table 4.

Leukocyte profile, with T (CD4+ and CD8+) and B lymphocytes and monocytes, and phagocytic profile, with phagocytic monocytes (P. mono) and granulocytes (P. granule) expressed in concentration percent (%) and mean fluorescence intensity (MFI) at day 7 and 21, of nursery pigs fed simple or complex diets (F), with or without including mannan oligosaccharides and β-glucans-based additive (A).

	Simple No Additive	Complex No additive	Simple Additive	Complex Additive	SEM	P-value		
						F	A	F*A
Day 7								
CD4+ (%)	30.41	25.41	27.32	32.94	4.08	0.97	0.79	0.93
CD8+ (%)	2.20	4.87	3.14	1.96	0.51	0.43	0.31	0.15
CD4+CD8+ (%)	2.92	3.18	3.07	3.32	0.39	0.76	0.85	0.99
CD4/CD8 (%)	6.41	3.89	4.67	6.34	0.53	0.70	0.74	0.25
Monocytes^{+} (%)	13.09	7.59	12.08	15.25	2.00	0.82	0.44	0.63
B^{+} Lymphocytes (%)	9.71	4.38	6.46	8.10	1.15	0.46	0.98	0.43
P. mono (%)	74.09	77.90	76.34	66.99	2.60	0.60	0.42	0.22
P. granule (%)	0.56	0.67	0.48	0.69	0.06	0.17	0.81	0.68
P. mono (MFI)	32.29	32.18	31.83	31.09	0.13	0.06	<0.01	0.16
P. granule (MFI)	53.64ab	69.93a	63.15ab	49.50b	2.82	0.80	0.29	0.01
Day 21								
CD4+ (%)	19.08	19.38	18.99	14.95	1.21	0.45	0.36	0.54
CD8+ (%)	10.90	7.81	6.35	8.34	0.79	0.74	0.18	0.22
CD4+CD8+ (%)	11.40	13.41	9.92	11.44	0.91	0.31	0.32	0.60
CD4/CD8 (%)	1.36	1.53	2.10	1.46	0.12	0.35	0.17	0.13
Monocytes^{+} (%)	9.35	12.87	11.88	12.27	0.87	0.27	0.59	0.52
B^{+} Lymphocytes (%)	5.34	8.44	6.26	9.63	0.80	0.04	0.52	0.22
P. mono (%)	70.90	72.21	61.96	60.89	2.59	0.98	0.06	0.82
P. granule (%)	0.99	1.25	1.37	1.43	0.06	0.17	0.02	0.40
P. mono (MFI)	33.93	33.93	35.12	35.09	0.24	0.98	0.02	0.98
P. granule (MFI)	73.29	65.20	62.45	68.73	4.12	0.92	0.67	0.41

Means followed by different lowercase letters on the same line differ by Tukey test (5%).

SEM: Standard error of the mean.

Table 5.

Intestinal permeability accessed by the concentration of the marker (FITC-Dextran) in the sample and weekly diarrhea occurrence per pen of nursery pigs fed with simple or complex diets (F), with or without including mannan oligosaccharides and β-glucans-based additive (A).

	Simple No Additive	Complex No additive	Simple Additive	Complex Additive	SEM	P-value		
						F	A	F*A
FITC - Dextran, µg/ml	0.80	0.63	0.63	0.62	0.03	0.20	0.16	0.24
Diarrhea d0-7 (%)	22.86	20.00	37.14	34.29	3.43	0.68	0.04	1.00
Diarrhea d8-14 (%)	57.14	32.86	51.43	31.43	4.11	<0.01	0.56	0.73
Diarrhea d15-21 (%)	21.43	12.86	27.14	11.11	2.77	0.03	0.73	0.49
Diarrhea d22-28 (%)	9.52	1.59	6.35	8.57	1.80	0.44	0.61	0.18
Diarrhea d29-35 (%)	6.35	5.71	11.43	4.76	1.92	0.38	0.57	0.47
Diarrhea d36-42 (%)	7.14	10.00	0.00	3.17	1.82	0.37	0.06	0.96

SEM: Standard error of the mean.

Table 6.

Cytokine panel with interleukins (IL), interferons (IFN) alpha (α) and gamma (γ), and tumor necrosis factor-alpha (TNF-α) at day 7 of nursery pigs fed with simple or complex diets (F), with or without including mannan oligosaccharides and β-glucans-based additive (A).

	Simple No Additive	Complex No additive	Simple Additive	Complex Additive	SEM	P-value		
						F	A	F*A
Concentration (pg/ml)								
IL-10	66.79	56.32	40.84	60.71	5.04	0.64	0.29	0.14
IL-1 β	14.19	17.24	17.99	12.36	1.49	0.67	0.86	0.16
IL-4	12.42	10.20	10.50	11.57	1.72	0.87	0.94	0.65
IL-6	35.37	76.52	28.72	86.69	11.97	0.04	0.94	0.72
IL-8 (CXCL8)	30.27	22.18	20.86	13.82	1.97	0.04	0.02	0.88
IL-12/IL-23p40	510.93	510.26	563.63	690.44	45.09	0.49	0.21	0.49
IFN-α	5.50ab	10.16a	8.47ab	2.76b	1.21	0.82	0.35	0.03
IFN-γ	9.57	9.07	6.65	6.94	0.80	0.95	0.13	0.81
TNF-α	140.38	121.72	92.50	140.45	20.10	0.73	0.73	0.43

Means followed by different lowercase letters on the same line differ by Tukey test (5%).

SEM: Standard error of the mean.

Table 7.

Alpha diversity expressed in the number of amplicon sequence variants (ASVs) and richness (Chao1) and diversity (Shannon) indexes at day 42 of nursery pigs fed with simple or complex diets (F), with or without including mannan oligosaccharides and β-glucans-based additive (A).

	Simple No Additive	Complex No additive	Simple Additive	Complex Additive	SEM	P-value		
						F	A	F*A
ASVs	983.0	855.9	926.3	866.0	89.5	0.71	0.82	0.74
Chao1	1109.0	961.0	1026.5	955.0	110.7	0.66	0.72	0.74
Shannon	5.366	5.236	5.262	5.229	0.108	0.71	0.82	0.74

SEM: Standard error of the mean.

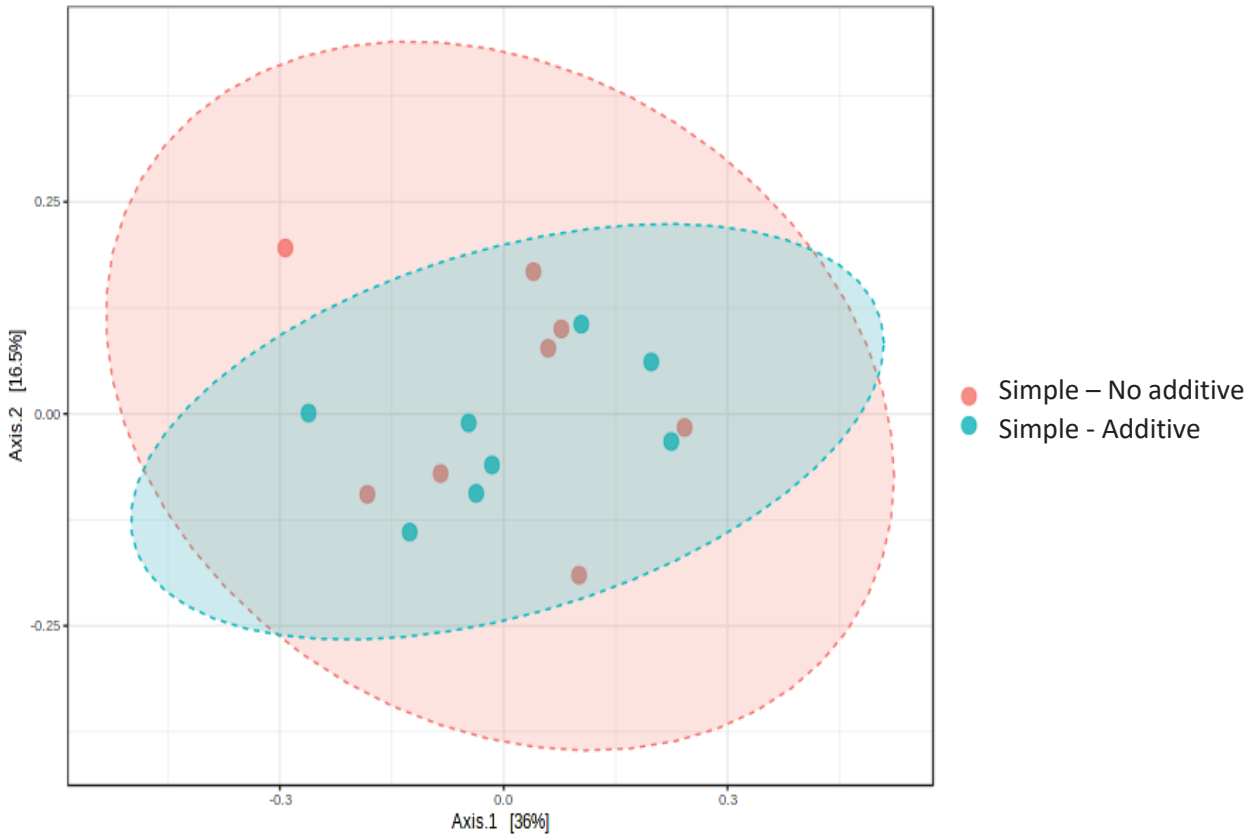

Figure 1. Beta diversity of animals fed the simple diet with or without including the mannan oligosaccharides and β-glucans-based additive (P>0.05).

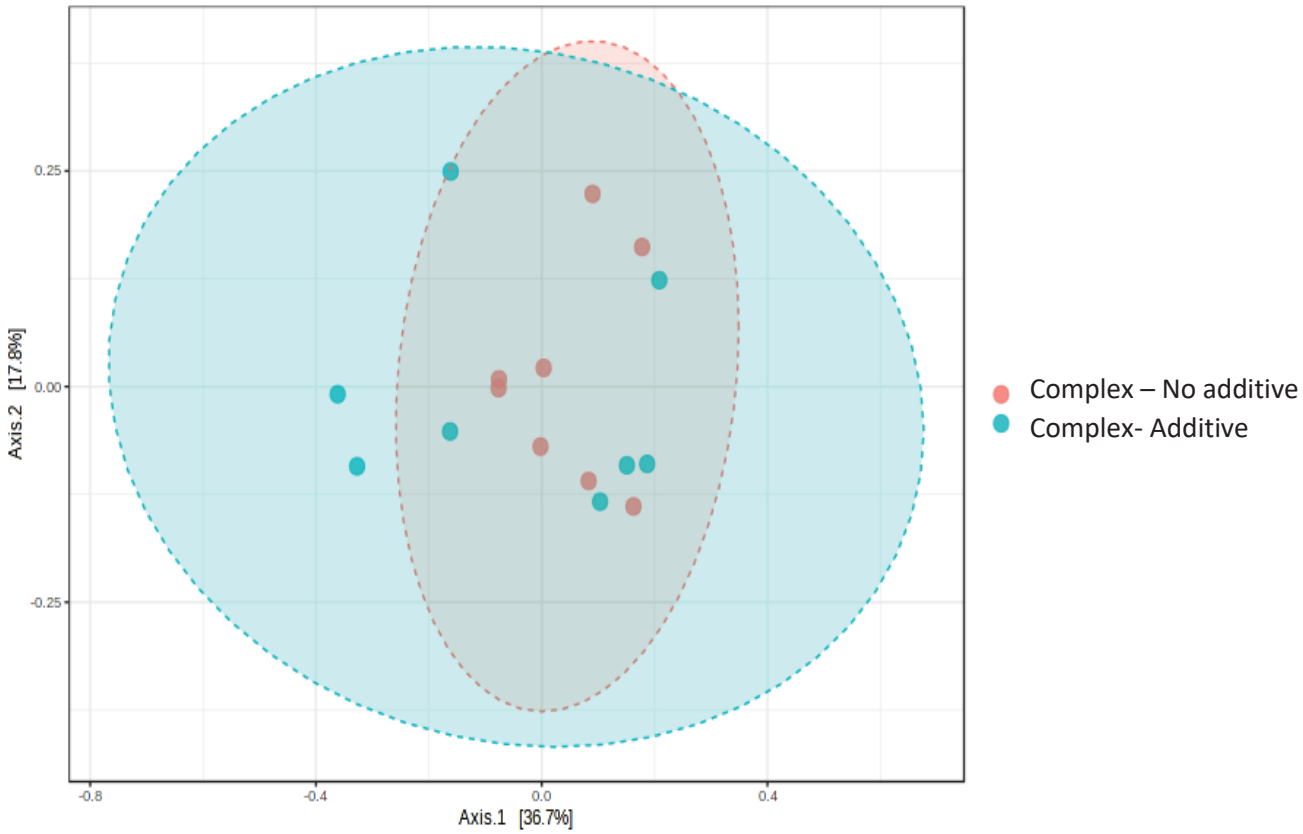

Figure 2. Beta diversity of animals fed the complex diet with or without including the mannan oligosaccharides and β-glucans-based additive (P>0.05).

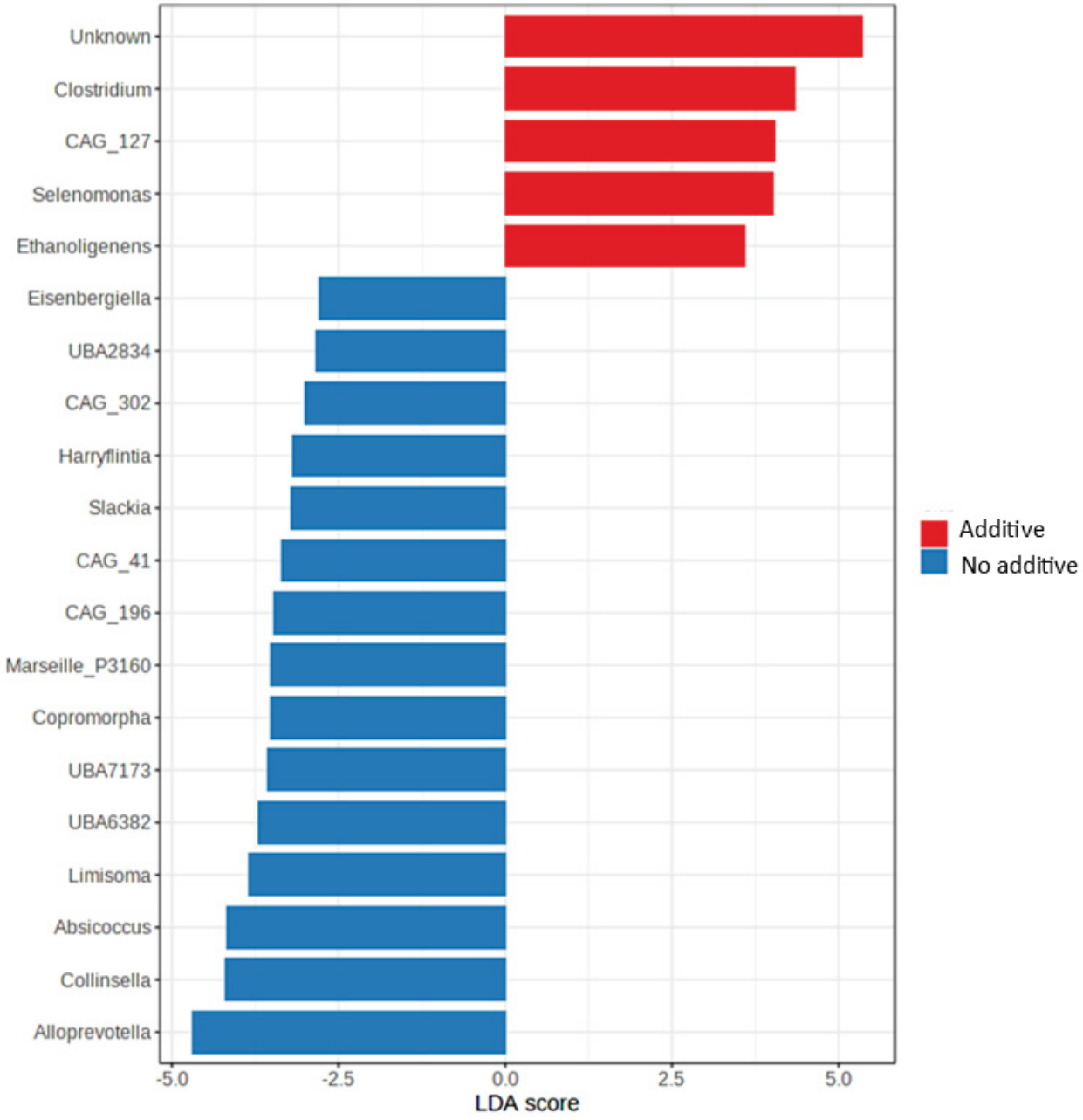

Figure 2. LDA score of genera that statistically differ (P<0.05) between samples from animals fed with or without including the mannan oligosaccharides and β-glucans-based additive.

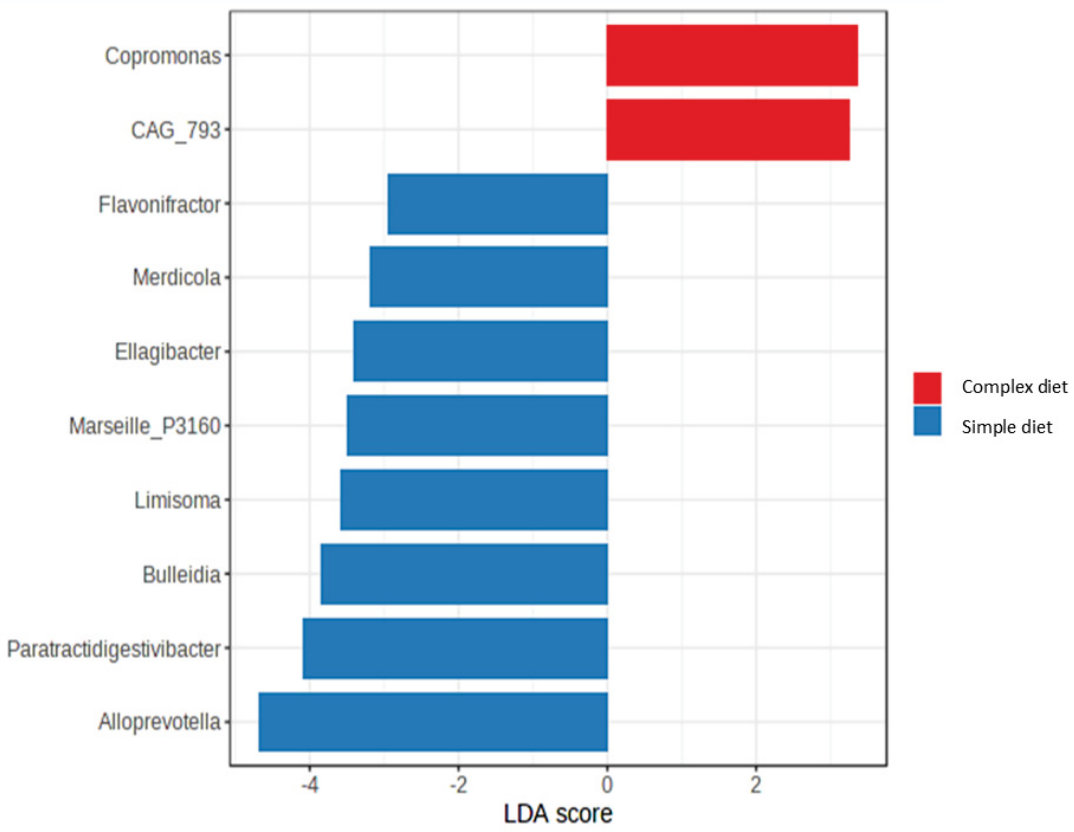

Figure 3. LDA score of genera that statistically differ (P<0.05) between samples from animals fed complex or simple diets.

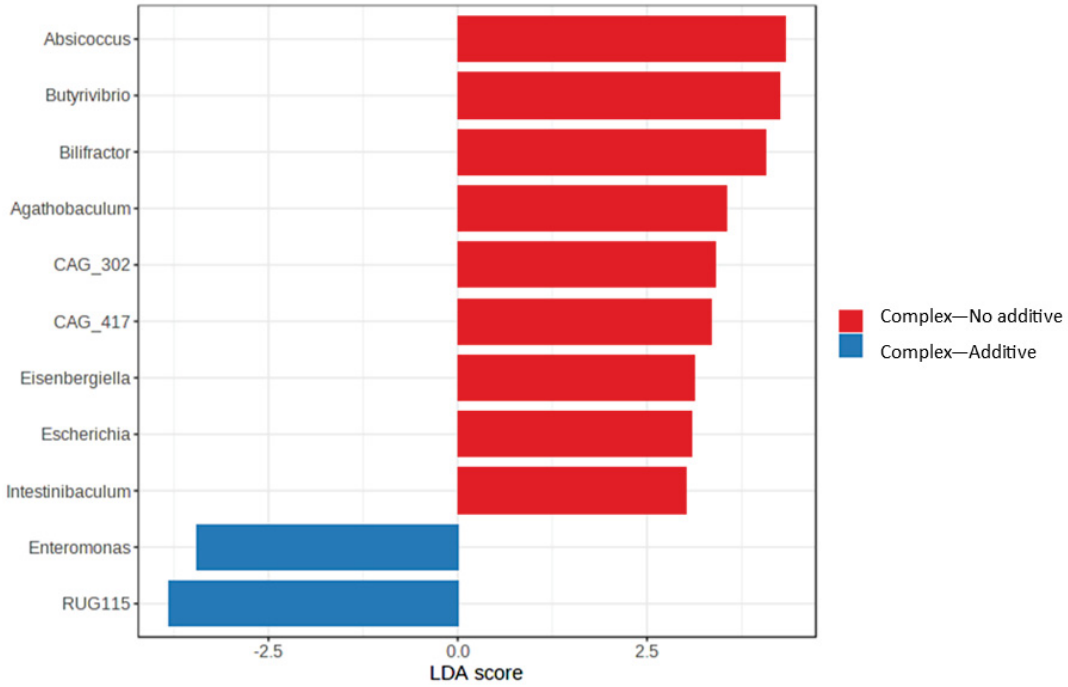

Figure 4. LDA score of genera that statistically differ (P<0.05) between samples from animals fed complex diets with or without including the mannan oligosaccharides and β-glucans-based additive.

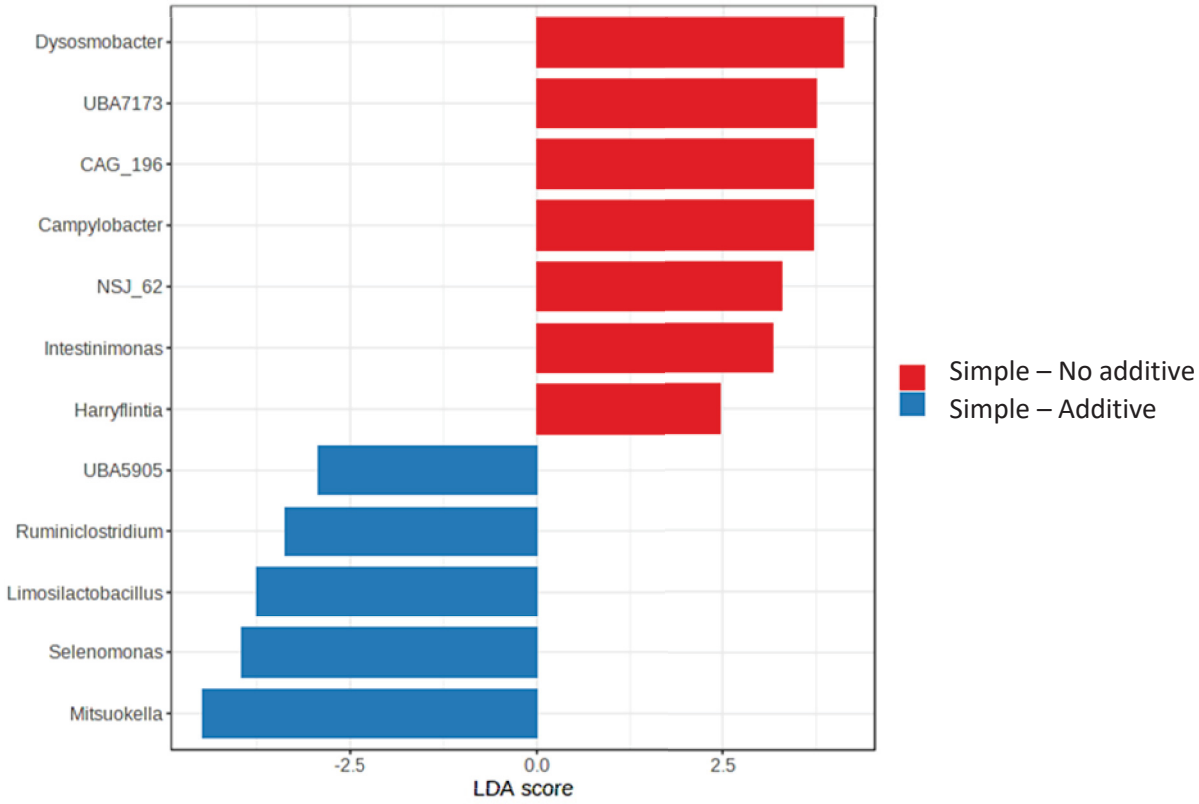

Figure 6. LDA score of genera that statistically differ (P<0.05) between samples from animals fed simple diets with or without including the mannan oligosaccharides and β-glucans-based additive.

CAPÍTULO IV – CONSIDERAÇÕES FINAIS

É preciso olhar com cuidado para os resultados dos trabalhos disponíveis que tratam da suplementação de betaglucanos para suínos. Além da baixa quantidade de artigos disponíveis para consulta, as condições em que os trabalhos foram feitos são muito variáveis, inclusive, as fontes de betaglucanos. É inegável que, em sua maioria, os resultados são promissores e sugerem que o uso de betaglucanos podem trazer efeitos benéficos para a suinocultura, sem efeitos adversos. Mas é preciso avaliar caso a caso se o custo/benefício vale a pena. Em muitos trabalhos os efeitos são discretos, e podem não ser suficientes para substituir o uso de antibióticos como profiláticos ou promotores de crescimento, funções às quais os betaglucanos se propõem em um primeiro momento.

O que pode ser observado dos trabalhos analisados é que os betaglucanos de levedura, especialmente os oriundos da *Saccharomyces cerevisiae*, possuem um grande potencial para atuar como *boosters* vacinais, mas isso também deve ser mais profundamente estudado, para entender com quais vacinas o aditivo pode ter um melhor efeito, e se não pode agir como um melhorador para umas, mas piorando outras. Nessa tese os betaglucanos não foram eficientes em induzir uma resposta vacinal mais acentuada em marrãs e porcas de primeiro parto, mas melhorou parâmetros de imunidade no colostro, como a concentração de imunoglobulinas e o maior estímulo à proliferação de células do epitélio intestinal, um indicativo de que pode ser um bom aditivo para melhorar a resposta imune de leitões.

Outro uso com grande potencial benéfico para os betaglucanos, e que tem sido pesquisado em extensão, é seu potencial como moduladores da microbiota intestinal. Nesse caso, trata-se os betaglucanos como prebióticos, e sendo carboidratos estruturais eles realmente podem agir como tal. Nessa tese leitões alimentados com dietas suplementadas com betaglucanos apresentaram uma menor quantidade de gêneros de bactérias patogênicas na fase de creche.

Para entender como e quando usar betaglucanos de levedura na produção animal, é primordial entender a fundo sobre o sistema imune, e, para isso, ainda serão necessários mais estudos na área. Mas o que pode ser concluído com os experimentos expostos nessa tese é que o efeito maior dos betaglucanos se dá sobre o epitélio intestinal, tanto aumentando sua atividade mitogênica quanto diminuindo inflamação, ambos em animais jovens.

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APÊNDICE – DIRETRIZES PARA AUTORES

O capítulo II foi apresentado de acordo com as diretrizes para autores do jornal ao qual foi submetido e aceito, que podem ser acessadas pelo link: https://www.sciencedirect.com/journal/livestock-science/publish/guide-for-authors.

As mesmas diretrizes foram seguidas para apresentação do capítulo III, que será submetido à mesma revista.

ANEXO I – CERTIFICADO DE COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 017/2023, referente ao projeto de pesquisa **"Suplementação de betaglucanos para leitões em fase de creche sobre sua imunidade e microbiota intestinal",** sob a responsabilidade de **Simone Gisele de Oliveira** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 08/05/2023.

Finalidade	Pesquisa
Vigência da autorização	Maio/2023 a Julho/2023
Espécie/Linhagem	*Sus domesticus* (suíno)
Número de animais	80
Peso/Idade	5kg a 25kg/28 a 70 dias
Sexo	Macho e fêmea
Origem	Fazenda Experimental do Canguiri da UFPR, em Pinhais, Paraná, Brasil

*A autorização para início da aula se torna válida a partir da data de emissão deste certificado.

CERTIFICATE

We certify that the protocol number 017/2023, regarding the research program "**Beta-glucan supplementation for nursery pigs on their immunity and intestinal microbiota**" under **Simone Gisele de Oliveira** – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 1 of invasiveness, on 2023, May 8th.

Purpose	Research
Validity	From May 2023 to July 2023
Specie/Line	*Sus domesticus* (swine)
Number of animals	80
Weight/Age	From 11,023lb to 55,116lb/From 28 days old to 70 days old
Sex	Male and female
Origin	Fazenda Experimental do Canguiri of the UFPR, in Pinhais, Paraná, Brazill

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 08 de maio de 2023

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Maity Zopollatto
Vice-coordenadora
Comissão de Ética no Uso de Animais
AG - UFPR

ANEXO II – CERTIFICADO DE COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 029/2019, referente ao projeto **"Safglucan – Mensuração de resposta em titulação vacinal em marrãs",** sob a responsabilidade **Alex Maiorka** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009,e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 08/05/2019.

Vigência do projeto	Junho/2019 até Outubro/2019
Espécie/Linhagem	*Sus domesticus* (suíno)/Toppigs
Número de animais	96
Peso/Idade	100 – 250 kg/135 – 350 dias
Sexo	Fêmea
Origem	Granja comercial em Campos Novos, Santa Catarina, Brasil.

CERTIFICATE

We certify that the protocol number 029/2019, regarding the project **"Safglucan – Measuring gilts a vaccine antibody titer response"** under **Alex Maiorka** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 08/05/2019.

Duration of the project	June/2019 until October/2019
Specie/Line	*Sus domesticus* (swine)/Toppigs
Number of animals	96
Wheight/Age	100 - 250 kg/135 – 350 days
Sex	Female
Origin	Commercial farm in Campos Novos, Santa Catarina, Brazil.

Curitiba, 08 de maio de 2019

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA