UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

CAROLINA HAUBERT FRANCESCHI

EFEITOS DE PROBIÓTICOS NO DESEMPENHO E SAÚDE DE MATRIZES SUÍNAS E SUAS LEITEGADAS

PORTO ALEGRE
2024

CAROLINA HAUBERT FRANCESCHI

EFEITOS DE PROBIÓTICOS NO DESEMPENHO E SAÚDE DE MATRIZES SUÍNAS E SUAS LEITEGADAS

Tese apresentada como requisito para obtenção do Grau de Doutora em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul.

ORIENTADORA: Prof. Dra. Ines Andretta

PORTO ALEGRE

2024

CIP - Catalogação na Publicação

Haubert Franceschi, Carolina
EFEITOS DE PROBIÓTICOS NO DESEMPENHO E SAÚDE DE MATRIZES SUÍNAS E SUAS LEITEGADAS / Carolina Haubert Franceschi. -- 2024.
90 f.
Orientadora: Ines Andretta.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2024.

1. Aditivos alternativos. 2. Bacillus. 3. Lactobacillus. 4. Probióticos. 5. Suínos. I. Andretta, Ines, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOUTORA EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 19.04.2024
Pela Banca Examinadora:

Homologado em: 22/05/2024
Por

Ines Andretta

Ines Andretta
PPG Zootecnia/UFRGS
Orientadora

Ines Andretta

INES ANDRETTA
Coordenadora do Programa de
Pós-Graduação em Zootecnia

JANSLLER LUIZ GENOVA

Jansller Luiz Genova
UFV

Luan Sousa dos Santos

Luan Sousa dos Santos
UFMS

Marcos Kipper

Marcos Kipper da Silva
ELANCO

gov.br
Documento assinado digitalmente
PAULO VITOR DUTRA DE SOUZA
Data: 24/05/2024 07:19:20-0300
Verifique em https://validar.iti.gov.br

PAULO VITOR DUTRA DE SOUZA
Vice- Diretor da Faculdade de
Agronomia

Aos meus pais, Ariane e Antoninho, que nunca deixaram de acreditar em mim.

AGRADECIMENTOS

Aos meus pais, Ariane e Antoninho, que nunca mediram esforços para me dar toda educação que tive. Muitas vezes colocaram as minhas necessidades em frente à deles e foi por causa disso que eu consegui chegar até onde eu cheguei. Mãe, Pai, vocês são os meus maiores apoiadores, eu sou uma pessoa de muita sorte por ter uma base tão sólida e presente. Obrigada por me apoiarem até aqui, amo vocês infinitamente. (desculpa por ter ido morar em SP)

Aos meus amigos de LEZO, carinhosamente chamados de lezados, que sempre levarei no meu coração. Eu certamente fiz amigos para a vida inteira dentro deste laboratório. Foram anos de experimentos, churrascos, viagens, estresse, mas muito, muito amor. E eu acredito que é amor o que fazemos por esse laboratório todos os dias, não existe outra explicação. Catiane, Thais, Bruna's, Alexandre, Carol, Jéssica, Ariane, Danrlei, Melody, Matheus, Nathi, Aires, Júlio e Gabriel obrigada por tudo!

À família Schenkel, que foi fundamental para a relização deste experimento. Eles foram a minha família por mais de 8 meses e me mostraram a paixão de lidar com a suinocultura diariamente. Raul, Claudete, Edu e Rosi Obrigada!

À Universidade Federal do Rio Grande do Sul que foi minha casa por 11 anos. Foi neste lugar que me tornei zootecnista, mestre e em breve doutora. Não foram anos fáceis, porém nunca me faltou ensino de qualidade e professores dedicados. Viva à universidade pública, Obrigada UFRGS.

À minha querida orientadora, onde eu não tenho palavras para explicar o sentimento que eu tenho por ela. Hoje eu tenho uma orientadora que também posso chamar de amiga. Prof, tu é uma das grandes responsáveis por nos fazer voar e procurar sucesso. Teus ensinamentos vão além da suinocultura, são eles: lealdade, companherismo, empatia e amor. Tu nos ensina amor todos os dias. Pelos suínos, pela pesquisa, pela vida. Eu sou muito grata por ter trabalho contigo por 8 anos. Escrevo esse agradecimento de coração e com um pouco de tristeza por estar encerrando esse lindo ciclo. Porém eu aprendi nessa vida que alguns ciclos devem se fechar para que outros possam se abrir. Te agradeço por todos esses anos de ensinamentos, obrigada, também, por sempre acreditar em mim.

À todos que nesses 4 anos de doutorado possam ter auxíliado de alguma forma.

EFEITOS DE PROBIÓTICOS NO DESEMPENHO E SAÚDE DE MATRIZES SUÍNAS E SUAS LEITEGADAS[1]

Autora: Carolina Haubert Franceschi
Orientadora: Ines Andretta

Existem inúmeros microrganismos que podem fazer a composição de um probiótico, dentre eles podemos citar *Bacillus* e *Lactobacillus* como os principais gêneros utilizados com esse propósito. Muitos estudos já foram realizados para testar os efeitos de probióticos para suínos, demonstrando a crescente procura por essa ferramenta na produção animal. Porém, estudos com matrizes suínas não são frequentemente encontrados nesse tema. Esta tese descreve dois estudos desenvolvidos para avaliar probióticos com diferentes composições nas fases de gestação e lactação de matrizes suínas e seus efeitos na progênie. Cada estudo foi desenvolvido comparando um tratamento controle (dietas e programas de alimentação convencionais nas fases, n = 100) e um tratamento cujos animais recebiam suplementação diária com probióticos (n = 100). Um probiótico a base de *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, e *Streptococcus thermophilus* foi testado no primeiro estudo (Trial 1), enquanto um produto a base de *Bacillus subtilis* e *Bacillus licheniformis* foi avaliado no segundo estudo (Trial 2). A suplementação ocorreu diariamente através do fornecimento dos aditivos em cápsulas gelatinosas (para minimizar o risco de contaminação cruzada) concomitantemente com a ração iniciando no primeiro dia de gestação (totalizando 114 dias) e perdurando até o último dia de lactação (21 dias). Os dados de desempenho e saúde das matrizes suínas e dos leitões foram coletados, avaliados através de análise de variância e interpretados considerando 5 e 10% de significância. Ambos os probióticos aumentaram ($p<0.05$) o peso dos leitões ao nascer (6% no Trial 1; 7% no Trial 2), o consumo de ração das porcas durante a lactação (1% no Trial 1; 0.25% no Trial 2) e o peso ao desmame dos leitões (7% no Trial 1; 6% no Trial 2). Em um contexto marginalmente significativo ($p<0.10$), foi também observada redução na frequência de diarreia nos leitões durante o período de lactação de ambos os estudos. O probióticos de *Lactobacillus spp.* ainda reduziu a umidade fecal após o parto ($p<0.10$) e aumentou o número de leitões no ciclo subsequente ao experimento ($p<0.10$). Matrizes que receberam *Bacillus spp.* como probióticos apresentaram menor número de leitões nascidos mumificados por leitegada ($p<0.10$); menor intervalo desmame-estro ($p<0.05$) e melhor qualidade do colostro ($p<0.10$). O uso de probióticos durante o período de gestação e lactação melhorou o desempenho das matrizes suínas e suas leitegadas, além de contribuir para melhor saúde destes animais.

Palavras-chave: Aditivos, *Bacillus*, *Lactobacillus*, Leitões, Microorganismos, Porcas.

[1] Tese de doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (90p) Abril, 2024.

EFFECT OF PROBIOTICS ON THE PERFORMANCE AND HEALTH OF SOWS AND ITS PROGENY[2]

Author: Carolina Haubert Franceschi
Supervisor: Ines Andretta

There are several microorganisms that can compose a probiotic, among them we can mention *Bacillus* and *Lactobacillus* as the main genera used for this purpose. Many studies have already been carried out to test the effects of probiotics for pigs, demonstrating a growing demand for this tool in animal industry. However, studies with sows are not frequently found on this topic. This thesis describes two studies developed to evaluate probiotics with different compositions in the gestation and lactation phases of swine sows and their effects on the progeny. Each study was developed comparing a control treatment (efficient diets and feeding programs in the phases, n = 100) and a treatment of animals that received daily supplementation with probiotics (n = 100). A probiotic based on *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, and Streptococcus thermophilus* was tested in the first study (Trial 1), while a product based on *Bacillus subtilis* and *Bacillus licheniformis* was evaluated in the second study (Trial 2). Supplementation occurred daily through the provision of additives in gelatin capsules (to minimize the risk of cross-contamination) concomitantly with the feed, starting on the first day of pregnancy (totaling 114 days) and lasting until the last day of lactation (21 days). Performance and health data from swine sows and piglets were collected, evaluated through analysis of variance and interpreted considering 5 and 10% significance. Both probiotics increased ($p<0.05$) the piglets' birth weight (6% in Trial 1; 7% in Trial 2), sows' feed intake during lactation (1% in Trial 1; 0.25% in Trial 2). and the weaning weight of the piglets (7% in Trial 1; 6% in Trial 2). In a marginally significant context ($p<0.10$), a reduction in the frequency of diarrhea in piglets was also observed during the lactation period in both studies. Probiotics from *Lactobacillus spp.* it also reduced fecal moisture after birth ($p<0.10$) and increased the number of piglets in the subsequent cycle to the experiment ($p<0.10$). Sows that received *Bacillus spp.* as probiotics showed a lower number of piglets born mummified per litter ($p<0.10$); shorter weaning-estrus interval ($p<0.05$) and better colostrum quality ($p<0.10$). The use of probiotics during the gestation and lactation period improved the performance of swine sows and their litters, in addition to contributing to better health of these animals.

Keywords: Additives, Bacillus, Lactobacillus, Microorganisms, Piglets, Sows

---

[2] PhD thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (90p) April, 2024.

SUMÁRIO

RELAÇÃO DE TABELAS

CAPÍTULO I **Página**

CAPÍTULO II

CAPÍTULO III

RELAÇÃO DE FIGURAS

RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

ALT – Alanine aminotransferase

AST – Aspartate aminotransferase

CA – Conversão alimentar

CDR – Consumo médio de ração

CFU – Colony forming unit

FAO – Food and Agriculture Organization

GPD – Ganho de peso diário

OMS – Organização Mundial da Saúde

CAPÍTULO I

INTRODUÇÃO

Assim como em todos os mamíferos, incluindo humanos, o intestino saudável de um suíno é habitado por centenas de espécies de microrganismos, que juntos formam uma comunidade microbiana que é frequentemente chamada de microflora ou, mais precisamente, microbiota (FOUHSE; ZIJLSTRA; WILLING, 2016). Essa microbiota varia com muitos fatores do animal e do ambiente, podendo ser modulada com o uso de aditivos alimentares como ácidos orgânicos e inorgânicos, enzimas, antibióticos, prebióticos e probióticos (DE LANGE; PLUSKE; GONG; NYACHOTI, 2010).

Probióticos são definidos como "microrganismos vivos que, quando administrado em quantidades adequadas, conferem um benefício na saúde do hospedeiro" (BAJAGAI; KLIEVE; DART; BRYDEN, 2016). O uso de probióticos para a saúde humana e em animais de produção têm sido muito reportado na literatura na última década. Estudos recentes têm demonstrado que humanos e animais alimentados com probióticos têm alteração na microbiota intestinal, aumentando a imunidade, melhorando a resistência às doenças, reduzindo patógenos e sintomas de doenças (KENNY; SMIDT; MENGHERI; MILLER, 2011; YIRGA, 2015).

Os resultados do uso de probióticos em suínos são ainda muito variados, pois os efeitos irão depender da condição dos indivíduos suplementados, das cepas utilizadas, das doses aplicadas, da duração do tratamento e das práticas de manejo. O uso de probióticos em suínos em crescimento pode beneficiar o ganho de peso (BAJAGAI; KLIEVE; DART; BRYDEN, 2016; MENG; YAN; AO; ZHOU et al., 2010), porém os resultados ainda são controversos quanto ao seu uso nas fases de gestação e lactação.

Estudos sobre o efeito dos probióticos no desempenho reprodutivo de suínos são relativamente limitados. Entretanto, alguns estudos nesta área foram reunidos por AHASAN; AGAZZI; INVERNIZZI; BONTEMPO et al. (2015), indicando que algumas espécies de probióticos utilizados em matrizes suínas melhoraram a qualidade do colostro, qualidade e quantidade de leite, tamanho e viabilidade da leitegada e peso dos leitões. ALEXOPOULOS; GEORGOULAKIS; TZIVARA; KRITAS et al. (2004) estudaram o efeito de um probiótico comercial em matrizes suínas e evidenciaram uma melhoria no desempenho da leitegada, com redução de casos de diarreia entre leitões, redução na mortalidade pré-desmame e aumento no peso dos leitões no desmame. A produção de leite com maiores quantidade de gordura e proteína foram as razões sugeridas para a melhoria da saúde e desempenho dos leitões (ALEXOPOULOS; GEORGOULAKIS; TZIVARA; KRITAS et al., 2004). Outra razão apontada pelo autor pode estar ligada à melhoria do ambiente microbiano das matrizes e dos leitões. Porém, mais estudos são necessários para entender essa relação com mais profundidade. Assim, os estudos descritos nesta tese foram propostos diante da escassez de informações a respeito da suplementação de dietas para matrizes suínas com aditivos probióticos a fim de responder as questões levantadas anteriormente.

REVISÃO BIBLIOGRÁFICA

1.1 Probióticos: definição e variedades

O termo probióticos tem se tornado muito popular nos últimos anos e foi descrito por LILLY e STILLWELL (1965) como a produção desconhecida de substâncias advindas de diversas espécies de protozoários durante sua fase de crescimento, as quais estimulavam o crescimento de outras espécies. Hoje, este termo é configurado como "para vida" e é utilizado para nomear associações de bactérias e outros organismos com efeitos benéficos na saúde humana e animal (BAGCHI, 2014). De acordo com a Organização das Nações Unidas para Alimentação e a Agricultura (FAO) e Organização Mundial da Saúde (OMS) definiram probióticos como "microrganismos vivos que quando administrado em quantidades adequadas conferem saúde benéfica para o hospedeiro" (FAO, 2006).

Um microrganismo para ser considerado um probiótico deve atender uma série de pré-requisitos: fazer parte da flora intestinal normal do indivíduo; sobreviver e colonizar o intestino do indivíduo; ser capaz de aderir ao epitélio intestinal do indivíduo; sobreviver às ações das enzimas digestivas; apresentar ação antagonista aos microrganismos patogênicos; não ser tóxico e/ou patogênico; ser cultivável em escala industrial; ser estável e viável na preparação comercial do mesmo e estimular a imunidade (FULLER, 2012).

De acordo com a Instrução Normativa nº 13, de 24 de novembro de 2004 do MAPA (BRASIL, 2004), os probióticos são definidos como aditivos zootécnicos, juntamente com prebióticos, simbióticos e promotores de crescimento. Existe uma abundância de microrganismos que podem ser classificados como probióticos, incluindo leveduras e bactérias. Os gêneros de bactérias mais conhecidos como probióticos são os *Lactobacillus*, *Bacillus*, *Bifidobacterium, Enterococcus, Streptococcus* e as leveduras (*Saccharomyces cerevisiae*) (SIMON; JADAMUS; VAHJEN, 2001). Na Tabela 1.1 é possível encontrar as variedades de probióticos mais conhecidas e estudadas pela ciência.

Tabela 1.1 Microrganismos utilizados como probióticos.

Gênero Probiótico	Espécies envolvidas	Referência
Lactobacillus	*L. plantarum, L. paracasei, L. acidophilus, L. casei, L. rhamnosus, L. crispatus, L. gasseri, L. reuteri, L. bulgaricus, L. brevis, L. farciminis, L. fermentum, L. murinis, L. gallinarium, L. pentosus, L. plantarum, L. rhamnosus, L. salivarius.*	(DIXIT; WAGLE; VAKIL, 2016) (HOLZAPFEL; SCHILLINGER, 2002)
Propionibacterium	*P. jensenii, P. freudenreichii*	
Peptostreptococcus	*P. productus*	
Bacillus	*B. coagulans, B. subtilis, B. laterosporus, B. lincheniformis*	(NGUYEN; TRUONG; KOUHOUNDÉ; LY *et al.*, 2016)
Lactococcus	*L. lactis, L. reuteri, L. rhamnosus, L. casei, L. acidophilus, L. curvatus, L. plantarum*	(EID; JAKEE; RASHIDY; ASFOUR *et al.*, 2016)
Enterococcus	*E. faecium*	(ONYENWEAKU; OBEAGU; IFEDIORA; NWANDIKOR, 2016)
Pediococcus	*P. acidilactici, P. pentosaceus*	(SORNPLANG; PIYADEATSOONTORN; TECHNOLOGY, 2016)
Streptococcus	*S. sanguis, S. oralis, S. mitis, S. thermophilus, S. salivarius*	(ARORA; SINGH; SHARMA, 2013)
Bifidobacterium	*B. longum, B. catenulatum, B. breve, B. animalis, B. bifidum, B. pseudolongum, B. thermophilum*	(WESTERMANN; GLEINSER; CORR; RIEDEL, 2016)
Bacteroides	*B. uniformis*	(KOBYLIAK; CONTE; CAMMAROTA; HALEY *et al.*, 2016)
Akkermansia	*A. muciniphila*	
Saccharomyces	*S. boulardii*	(CHEN; YANG; SONG; XU *et al.*, 2013)

1.2 Mecanismos de ação dos probióticos

O uso de probióticos em substituição aos antibióticos promotores de crescimento têm sido proposto com bastante frequência nos últimos anos. Porém, é importante salientar que os mecanismos de ação dos probióticos são diferentes dos conhecidos antibióticos. Dentre os principais mecanismos de ação dos probióticos funções podemos citar:

- *Competição por nutrientes:* Os nutrientes que estão presentes no lúmen intestinal são providos dos ingredientes que foram clivados pelas enzimas digestivas dos animais e normalmente são substrato para a nutrição das bactérias. Assim, existe uma competição entre as bactérias pelos nutrientes disponíveis no lúmen intestinal. Os probióticos conseguem controlar ou até excluir populações de bactérias patogênicas em alguns contextos, preservando maior disponibilidade de nutrientes para microrganismos com efeitos positivos (LUTGENDORFF; AKKERMANS; SODERHOLM, 2008).
- *Competição por sítios de ligação:* Os microrganismos probióticos ocupam sítios de ligação (receptores ou pontos de ligação) presentes na mucosa intestinal. Isso inviabiliza a inserção de microrganismos patogênicos nesses locais. (MONTEAGUDO-MERA; RASTALL; GIBSON; CHARALAMPOPOULOS et al., 2019).
- *Antagonismo direto:* Algumas substâncias como bacteriocinas, ácidos orgânicos e peróxido de hidrogênio são produzidas pelas bactérias probióticas e têm ação antibacteriana. Assim, tais compostos inibem o crescimento e estabelecimento de populações microbianas indesejáveis.
- *Modulação do sistema imune pela ativação dos macrófagos:* O sistema imunológico está inteiramente relacionado com a microbiota intestinal. À nível de lúmen intestinal há a produção de anticorpos, ativação de macrófagos, proliferação de células T e produção de interferon por algumas bactérias probióticas, com isso há modulação da resposta imune pelo animal.

Os mecanismos citados acima protegem o organismo de apresentar uma disbiose intestinal. Probióticos suprimem os patógenos através de diversas ações, incluindo a diminuição do pH luminal, produção de proteínas antimicrobianas, inibição de adesão e translocação da flora, exclusão competitiva de patógenos, melhoria da barreira epitelial, aprimoramento da adesão de bactérias comensais na mucosa intestinal, e modulação do sistema imune da mucosa gastrointestinal (ZHANG; SUN; WU; YANG et al., 2017) (Figura 1.1). Combinadas, essas ações beneficiam o hospedeiro e auxiliam no contexto de saúde intestinal.

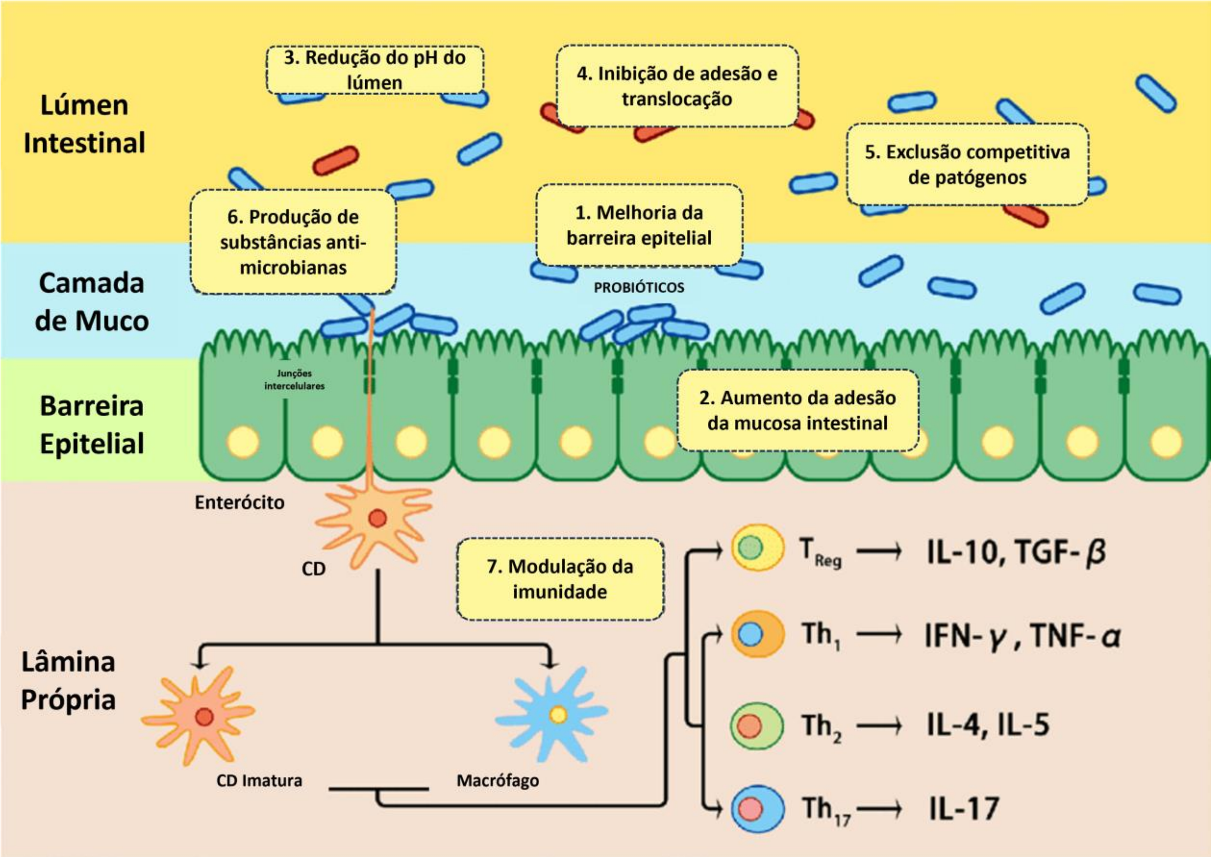

Figura 1.1 Mecanismos envolvidos na proteção induzida por probióticos contra disbiose intestinal (Adaptado de ZHANG; SUN; WU; YANG et al. (2017)).

Outras funções dos probióticos que podem ser benéficas para o organismo do hospedeiro já foram reportadas na literatura. Dentre essas funções, podem ser citadas:

- *Restauração da microbiota intestinal após antibioticoterapia:* A administração de antibióticos ocasiona distúrbios para a população microbiana presente no trato gastrointestinal. A administração de probióticos tem se mostrado benéfica no processo de recuperação da microbiota e da população normal daquele sistema (PLUMMER; GARAIOVA; SARVOTHAM; COTTRELL *et al.*, 2005).
- *Nutrição:* Em decorrência da diminuição do pH intestinal promovido pelas bactérias láticas, ocorre uma maior absorção de ácidos de cadeia curta e podem promover condições mais adequadas para ação de algumas enzimas digestórias. Em estudo realizado por JIN; HO; ABDULLAH e JALALUDIN (2000) foi demonstrado que algumas espécies de *Lactobacillus* secretavam uma maior quantidade de amilase, o que auxilia na absorção de nutrientes do trato.

- *Supressão da produção de amônia:* Em estudo realizado em suínos, foi observado uma redução na produção de amônia presente nas fezes quando houve suplementação de probióticos na dieta (TAN; LIM & BOONTIAM 2020). Altas concentrações de amônia na microbiota podem ser tóxicas para as células epiteliais (DOWARAH; VERMA; AGARWAL; PATEL *et al.*, 2017).
- *Redução de enterotoxinas:* A produção de enterotoxinas é afetada no momento em que o probiótico tem a capacidade de modular as populações microbianas negativas no ambiente intestinal.

Os probióticos podem apresentar ainda outros mecanismos de ação, sendo alguns facilmente associados com respostas de interesse zootécnico, principalmente: *Modulação das respostas imunes do hospedeiro; Redução de diarreia e efeitos antitoxinas; e Modulação das digestibilidades de nutrientes*. Na tabela 1.2 encontra-se um resumo dos mecanismos de ação dos probióticos que são de maior interesse para a área de nutrição e saúde animal.

Tabela 1.2. Resumo dos mecanismos de ação de vários probióticos sobre a saúde e função do intestino animal ou humano[1].

Item	Descrição
Modulação da microbiota intestinal	
Modulação das respostas imunes do hospedeiro	Melhorar a imunidade inata intestinal através da restituição da integridade e função da barreira intestinal Melhorando a imunidade inata intestinal através do aumento da produção de muco intestinal ou secreção de cloreto Estimular ou suprimir respostas imunes adquiridas por animais Influenciando o sistema imunológico animal por metabólitos semelhantes a produtos, componentes da parede celular e DNA
Redução de diarreia e efeitos antitoxinas	Inibindo a expressão de toxinas em bactérias patogênicas Neutralizando as enterotoxinas produzidas por bactérias patogênicas

Item	Descrição
Modulação das digestibilidades de nutrientes	Pela alta atividade fermentativa dos probióticos Aumento da produção enzimópica digestiva e atividades Afetando as atividades de absorção e secreção do intestino suíno Produzindo algumas vitaminas
Outros modos de ação	Atividade antioxidativa e alívio do estresse Alterando a expressão genética bacteriana e hospedeira

[1]Os dados foram resumidos dos seguintes estudos: (POLLMAN, 1986), (NG; HART; KAMM; STAGG *et al.*, 2009), (OELSCHLAEGER, 2010), (CHO; ZHAO; KIM, 2011) e (YIRGA, 2015).

1.3 Probióticos em suínos

O uso de probióticos para saúde humana e em animais de produção tem sido amplamente relatado na literatura. Muitos estudos recentes mostraram que humanos e animais alimentados com dietas contendo probióticos alteraram a microbiota intestinal, aumentaram a imunidade intestinal, melhoraram a resistência à doenças, reduziram a presença de patógenos e melhoraram seu estado de saude (YIRGA, 2015). Os probióticos também poderiam ser fornecidos aos animais que foram tratados terapeuticamente com antibióticos ou outras drogas, para recolonizar o intestino após o tratamento terapêutico (HUGHES; HERITAGE, 2004; PAMER, 2016).

Para melhorar a eficiência da produção, a indústria suína moderna adaptou algumas práticas tecnológicas avançadas, mas não naturais, que poderiam induzir certo estresse aos suínos, causando alterações na composição da microbiota intestinal natural e, assim, comprometendo a resistência dos suínos aos patógenos (FULLER, 2012). Os probióticos mais comuns para animais monogástricos são as leveduras (*Saccharomyces boulardii*), e bactérias (*Lactobacillus spp., Enterococcus spp., Pediococcus spp., Bacillus spp.*) visando porções do intestino como ceco e colón que abrigam uma população microbiana abundante e muito diversificada composta principalmente de bactérias e leveduras.

Existe uma grande variação nas cepas microbianas, doses aplicadas e duração do tratamento com o uso dos probióticos. Isso ocasiona uma grande variabilidade de manejos adotados para utilizar essa ferramenta na nutrição animal.

Muitos estudos mostraram que a administração de cepas probióticas, separadamente ou em combinação, melhorou significativamente o ganho de peso diário (GPD), a ingestão média diária de ração (CDR) e a conversão alimentar (CA) dos suínos (LIAO; NYACHOTI, 2017). Na Figura 1.2 é possível visualizar a alta demanda de estudos em probióticos para suínos nos últimos anos. Indicando que há uma busca cada vez maior para entender os efeitos deste aditivo nos últimos 20 anos.

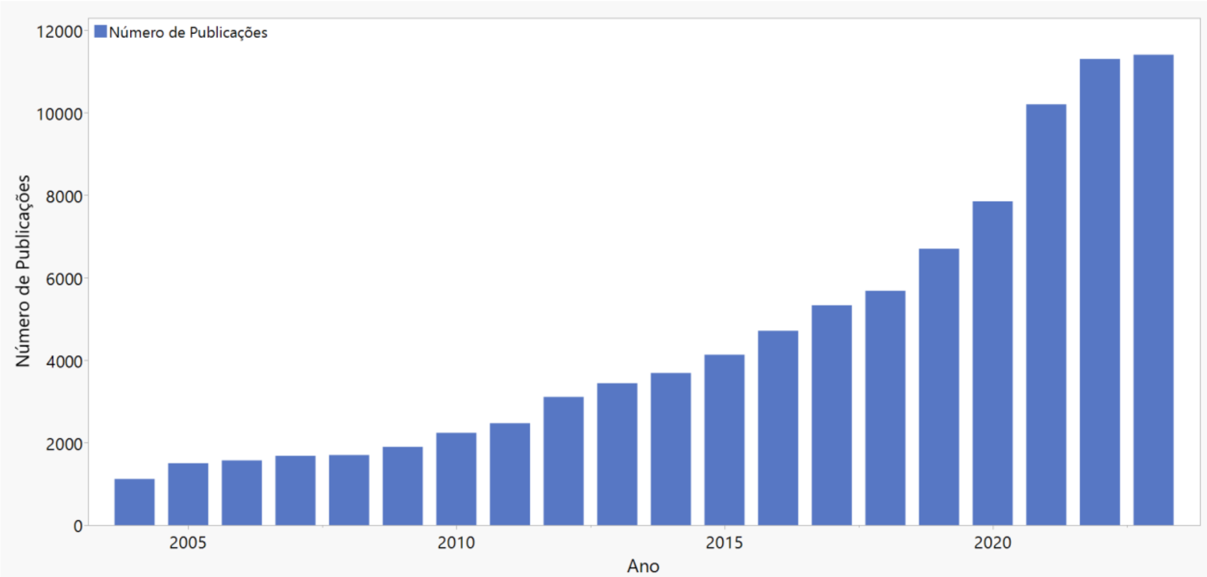

Figura 1.2. Número de publicações sobre probióticos para suínos entre os anos de 2004 à 2023.

1.4 Modulação da microbiota em suínos

Uma microbiota intestinal bem estabelecida é um completo microssistema composto em sua majoritária parte em bactérias, que coexistem com o suíno como seu hospedeiro (KIM; ISAACSON, 2015). Quando essa coexistência (simbiose) for equilibrada, o intestino do suíno será normal, saudável e funcional (WILLING; MALIK; VAN KESSEL, 2012). Animais que, de alguma forma, são criados na ausência de bactérias podem apresentar um retardo no desenvolvimento da morfologia intestinal adulta, fisiologia digestiva e função imunológica normal (KENNY; SMIDT; MENGHERI; MILLER, 2011).

Os microrganismos iniciam a colonização do intestino de um leitão recém-nascido logo após o parto, em um processo conhecido como sucessão microbiana. A microbiota deste leitão será estabelecida algumas semanas após seu nascimento. (BAUER; WILLIAMS; SMIDT; MOSENTHIN *et al.*, 2006; KIM; ISAACSON, 2015; TORTUERO; RIOPEREZ; FERNANDEZ; RODRIGUEZ, 1995). Na figura 1.2 podemos observar os resultados de uma meta-análise que analisou cerca de 939 amostras de intestinos de suínos com o intuito de conhecer a população microbiana nas diferentes porções do intestino (HOLMAN; BRUNELLE; TRACHSEL; ALLEN, 2017).

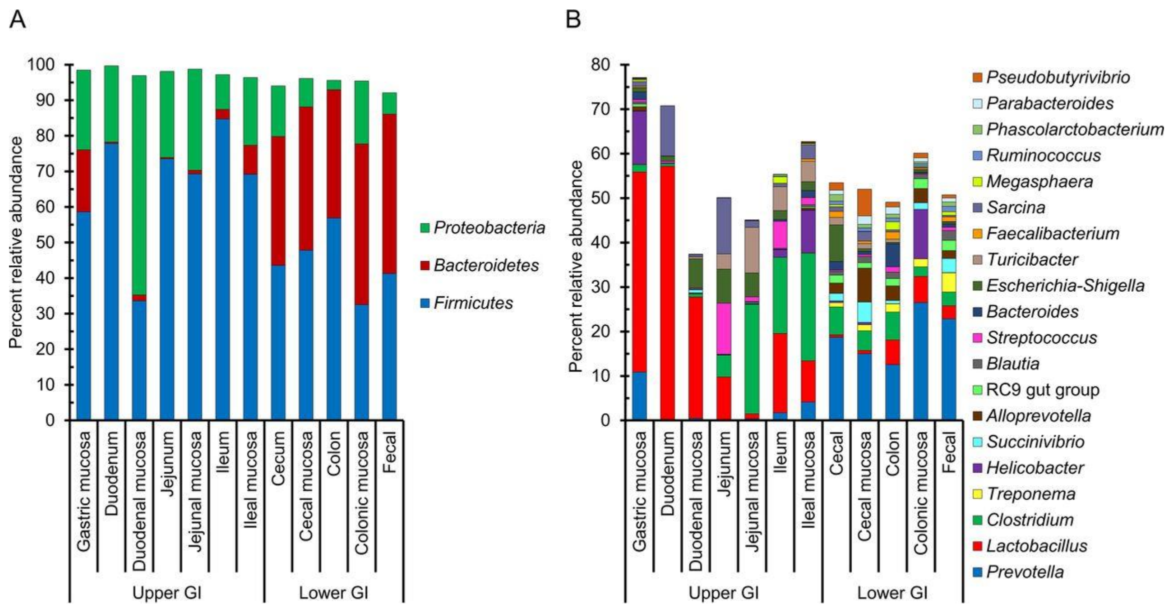

Figura 1.3. Abundâncias relativas dos três filos mais presentes (A) e 20 gêneros mais presentes (B) por tipo de amostra do trato gastrointestinal.

Acredita-se que os probióticos melhorem a saúde geral dos animais, prevenindo o desequilíbrio da microbiota intestinal e melhorando a saúde intestinal através da modificação da população microbiana intestinal (LESCHEID; DISEASE, 2014; VEIZAJ-DELIA; PIRUSHI, 2012), porque a introdução de microrganismos benéficos pode reparar as deficiências de microrganismos benignos no intestino, restaurar ou melhorar a resistência dos suínos às doenças. Esse efeito benéfico, por sua vez, pode levar os suínos com melhor capacidade de digestão e absorção de nutrientes, e melhor aproveitamento de nutrientes e desempenho de produção (KENNY; SMIDT; MENGHERI; MILLER, 2011; YIRGA, 2015).

BAJAGAI; KLIEVE; DART e BRYDEN (2016) resumiram vários estudos sobre leitões desmamados e relataram que os probióticos aumentaram a contagem de *Lactobacillus* e diminuíram bactérias patogênicas como *Clostridium*, *Escherichia Coli* e *Enterobacterium spp.* no intestino suíno. YANG; HUANG; QIN; WU *et al.* (2009) relataram que os probióticos enriquecidos com selênio *(Candida utilis, Lactobacillus acidophilus,* *Lactobacillus rhamnosus* e *Streptococcus thermophilus*) podem fortemente antagonizar *Escherichia coli patogênicas in vitro* ou *in vivo* (em camundongos). Os níveis *de Escherichia coli* em suínos desmamados foram reduzidos transitoriamente, mas dramaticamente após 4 semanas de suplementação com *Saccharomyces cerevisiae* e *Pediococcus acidilactici* em comparação com os suínos alimentados com dieta não suplementada (LE BON; DAVIES; GLYNN; THOMPSON *et al.*, 2010). POSPÍŠKOVÁ; ZORNÍKOVÁ; KOLÁŘOVÁ; SLÁDEK *et al.* (2013) relataram que as contagens de *Escherichia coli* e *Clostridium perfringens* nas fezes de porcas desmamadas alimentadas com uma monocultura de *E. faecium* foram significativamente reduzidas.

O manejo do microsistema intestinal é uma das estratégias comuns aplicadas para prevenir a diarreia, melhorar o estado de saúde e melhorar o desempenho de crescimento dos suínos nos modernos sistemas intensivos de produção (BAUER; WILLIAMS; SMIDT; MOSENTHIN *et al.*, 2006; WILLIAMS; VERSTEGEN; TAMMINGA, 2001; ZIMMERMANN; FUSARI; ROSSLER; BLAJMAN *et al.*, 2016) Então, torna-se interessante conhecer a modulação causada pelos probióticos para manter a saúde intestinal de suínos.

1.5 Matrizes suínas: o desafio

O período de gestação e lactação é bastante desafiador para qualquer espécie. Na suinocultura, a produção de leitões é uma fase cada vez mais crítica e requisitada para apresentar máxima eficiência em períodos curtos de tempo. Em outras palavras, o sistema de produção pretende gerar leitões de qualidade sem haver perdas reprodutivas para as fêmeas suínas.

Ao nascer, o leitão possui nível básico de competência imunológica, especialmente imunidade inata. Uma vez que a placenta não permite a transferência de anticorpos ou células imunes maternamente derivadas para o feto, os leitões dependem da proteção imunológica inata e da absorção da imunidade passiva materna (QUESNEL et al., 2012).

O fechamento intestinal do leitão de recém-nascido ocorre de 24 a 36 horas após o nascimento, impedindo a absorção de imunoglobulinas. A falha dos leitões em alcançar uma ingestão adequada de colostro é a principal causa para a maioria dos óbitos por leitões ocorridos nos primeiros dias do período pós-natal (QUESNEL et al., 2012). Há evidências claras de que o colostro e a ingestão de leite influenciam o desenvolvimento intestinal e o amadurecimento do sistema imunológico (TURFKRUYER E VERHASSELT, 2015).

Matrizes suínas durante o início da gestação são fisiologicamente comparavéis à leitões em crescimento e terminação. Isso pois os nutrientes são utilizados pelo corpo da fêmea (para mantença) e também para crescimento materno, enquanto uma quantidade limitada de nutrientes é utilizada para reprodução. (SOLA-ORIOL et al., 2017). Com o progresso da gestação, a necessidade de nutrientes é destinada ao crescimento fetal, placenta e da região mamária. (NRC., 2012; ROSTAGNO et al., 2017).

O uso de probióticos durante a gestação pode ser uma ferramenta para auxiliar na nutrição dessas fêmeas que estão em constante necessidades de nutrientes. O modo de ação de algumas cepas de probióticos podem auxiliar na produção de enzimas, vitaminas e peptídeos. (LUISE et al., 2023). Cepas de *Bacillus coagulan*, *Bacillus subtilis*, *Bacillus amyloliquefaciens* e *Bacillus cereus* foram identificados por ELSHAGHABEE et al (2017) e que apresentavam características de produzir enzimas que degradam carboidratos, peptídeos e também lipídios (GHANI et al., 2013).

A capacidade de absorver nutrientes e digerir melhor os alimentos também está indiretamente relacionado ao efeito imunoestimulatório do uso de probióticos (gênero *Bacillus spp.*). Um desses efeitos está ligado ao controle do estresse oxidativo, que pode melhorar a morfologia do trato gastrointestinal e, consequentemente, a capacidade absortiva (MOHAMMED et al., 2014; TANAKA et al., 2014)

Estudos sobre os efeitos dos probióticos no desempenho reprodutivo dos suínos são relativamente limitados. No entanto, alguns estudos, como resumido por AHASAN et al. (2015), mostraram que algumas espécies probióticas (nos gêneros de *Bacillus*, *Lactobacillus*e e *Streptococcus*, por exemplo) melhoraram a qualidade do colostro, a qualidade e a quantidade do leite, além do peso e da vitalidade do leitão.

ALEXOPOULOS et al. (2004) relataram que porcas gestantes alimentadas com o probiótico *Bacillus licheniformis* e *Bacillus subtilis* com suplementação iniciando duas semanas antes do parto e durante o período de lactação melhoraram o desempenho das leitegadas, com diarreia de leitões reduzida e também redução da mortalidade pré-desmame e aumento dos pesos ao desmame. A diminuição da perda de peso nas porcas durante a lactação e a produção de leite com maior teor de gordura e proteína foram as razões sugeridas para a melhoria da saúde e desempenho dos leitões (ALEXOPOULOS et al., 2004). Outra razão pode ser o melhor ambiente microbiano em torno das porcas e dos leitões. Na tabela 1.4 podem ser encontrados estudos sobre probióticos para matrizes suínas realizados nos últimos anos.

Tabela 1.3 – Compilado de estudos de probióticos para matrizes suínas.

Autor	**Número de Animais**	**Microrganismo**	**Período de administração**	**Resultado principal na leitegada**
ZHANG et al., 2020	32 Matrizes suínas	*Bacillus subtilis, Bacillus licheniformis*	Final da gestação até o desmame dos leitões	Aumento de peso no desmame e ganho de peso durante lactação
TSUKAHARA et al., 2018	20 Matrizes suínas	*Bacillus mesentericus, Clostridium butyricum, Enterococcus faecalis*	4 semanas pré-parto até uma semana após o parto	Aumento de peso ao nascimento e redução na porcentagem de mortalidade durante 21 dias de lactação
NGUYEN et al., 2018	26 Matrizes suínas	*Bacillus subtilis* + Óleo essencial	107º dia de gestação até o parto	Aumento no ganho de peso dos leitões durante a lactação
INNAMA, NGAMWONGSATIT & KAEOKET., 2023	34 Matrizes suínas	*Lactobacillus spp.* + *Enterococcus faecium* + *Pediococcus pentosaceus* + *Bacillus spp.* + *Saccharomycez cerevisiae*	84 dias de gestação até o desmame dos leitões	Aumento na produção de colostro e no peso de desmame dos leitões
MA et al., 2023	74 Matrizes suínas	*Bifidobacterium lactis* + *Lacticaseibacillus rhamnosus*	Terço final da gestação até o final da lactação	Redução da constipação das fêmeas no final da gestação e redução no número de leitões nascidos mumificados

É de conhecimento que os probióticos possuem inúmeras funções positivas, porém ainda há uma escassez de estudos em matrizes suínas nas fases de gestação e lactação. Assim, torna-se necessário, também, conhecer a real interação entre microbiota da mãe-filho e a imunidade que lhe é conferido após o parto através do colostro.

2. OBJETIVO

Este estudo foi desenvolvido para avaliar o uso de probióticos como aditivos para matrizes suínas nas fases de gestação e lactação e verificar seu efeito em matrizes suínas: a) performance reprodutiva; b) escore corporal; c) qualidade do colostro; d) indicadores bioquímicos de saúde e também em sua progênie: a) desempenho durante a lactação; b) incidência de diarreia; c) indicadores bioquímicos de saúde

CAPÍTULO II[1]

[1]Artigo escrito nas normas da revista *Journal of Animal Science*

Running title: Probiotic effects on sow and progeny

Supplying a multi-strain probiotic during the gestating and lactating enhances the performance and health status of sows and progeny

Abstract

Multi-strain probiotics are used as feed additives and may improve animal performance and health, particularly during challenging phases, such as gestation and lactation. Therefore, this study aimed to assess the effects of a multi-strain probiotic on the performance and health of sows and their progeny. Sows (Camborough, PIC) with parity order ranging from 2 to 9 were divided into two groups: one fed a control diet (control-fed sows; n = 100 and parity order = 4.3) and the other orally supplemented with a multi-strain probiotic (probiotic-fed sows; n = 100 and parity order = 4.2). The microorganisms that comprised the multi-strain probiotics were *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Streptococcus thermophilus*. Probiotic inclusion was 50 g/ton and ssupplementation occurred daily, starting from day zero of the gestation period (± 114 days) and lasting until the end of the lactation period (21 days). The performance and health responses of the sows and piglets were recorded. Data were subjected to analysis of variance and interpreted at a level of significance of $p<0.05$, and a marginal significance was considered at $0.05 \leq p<0.05$. Probiotics improved the birth weight by 6% and weaning weight by 7% ($p<0.05$). Probiotic-fed sows presented greater feed consumption related to body weight during lactation (12%; $p<0.01$) and a tendency toward a higher postpartum fecal humidity (12%; $p<0.10$). Probiotic supplementation reduced the presence of diarrhea in the litter (14%; $p<0.05$) and increased the total number of births in the subsequent cycle of the sow (7%; $p<0.10$). Additionally, probiotic supplementation decreased serum glucose, total cholesterol, total protein, and albumin concentrations in sows during the preweaning period ($p<0.05$). Thus, probiotics enhance the performance and health status of both sows and piglets.

Keywords: feed additive; *Lactobacillus*; gut health; microorganisms; pig; piglet.

Introduction

Swine industry urges for innovation in the animal nutrition field. More specifically, sow nutrition is a challenging phase that requires the use of technologies that can enhance performance and health. Some feed additives, such as probiotics, can play an important role in swine nutrition and also modulate the microbiota (De Lange et al., 2010). A simple definition of a probiotic is “live microorganisms that when administered in adequate amounts, confer a health benefit to the host” (FAO, 2006). Probiotics in animal nutrition have become increasingly popular tools for improving animal health and performance (Kenny et al., 2011).

Probiotics are known for: a) modulation of the gut microbiota, b) competitive exclusion against pathogenic microorganisms (e.g., nutrient competition and competition for binding sites), and c) strengthening the gut epithelial barrier and immune system stimulation through macrophage activation and key signaling pathways (Monteagudo-Mera et al., 2019). These mechanisms protect the host from intestinal dysbiosis (Lutgendorff et al., 2008; Zhang et al., 2017).

Lactobacillus and *Bifidobacterium* probiotics, that are commonly used in livestock, have their main characteristic to have adherence to the intestinal epithelium (Lee et al., 2023). In suckling and nursery piglets, the use of *Lactobacillus* as a probiotic improved weight gain and feed conversion, while reducing diarrhea (Afonso et al., 2013; Liu et al., 2014). Performance improvements have been reported in piglets supplemented with *Enterococcus*, *Streptococcus*, and *Bifidobacterium* strains (Laskowska et al., 2019; Satora et al., 2021).

Bacillus probiotic administration is commonly used in sows, resulting in a greater number of piglets born and higher birth weight (Barba-Vidal et al., 2019). However, few studies have been conducted on *Lactobacillus* and multi-train probiotics in sows. The interaction between multi-strain probiotics has been reported to be beneficial, as several different microorganisms act to develop health (Ouwehand et al., 2018). Therefore, this study was performed to investigate the effect of a multi-strain probiotic (*Lactobacillus strains* + *Enterococcus* + *Streptococcus* + *Bifidobacterium*) on reproductive performance, body condition score, serum biochemical, and fecal score in sows and its influence on the performance and health of their progeny.

Material and methods

The experimental trial was conducted at a commercial farm located in the state of Rio Grande do Sul, Southern Brazil. All procedures were performed with the approval of the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul (protocol number: 39736).

Animals, experimental design, and management

A total of 200 sows (PIC – Camborough, Agroceres-PIC, São Paulo, Brazil) with parity order ranging from 2 to 9 were used in this study and randomly assigned (within each parity order) to receive one of the two treatments: a control diet or a multi-strain probiotic diet during the gestation and lactation period (Table 1). Abortion and death of sows were recorded and excluded from the trial. Supplementation started on the first day after insemination and persisted during gestation (114 days) until the end of lactation (21 days) for a total of 135 days. The probiotic was a commercial product containing *Lactobacillus acidophilus* (2.06×10^8 CFU/g), *Lactobacillus bulgaricus* (2.08×10^8 CFU/g), *Lactobacillus plantarum* (1.26×10^8 CFU/g), *Lactobacillus rhamnosus* (2.06×10^8 CFU/g), *Bifidobacterium bifidum* (2.00×10^8

CFU/g), *Enterococcus faecium* (6.46 × 10^8 CFU/g), and *Streptococcus thermophilus* (4.10 × 10^8 CFU/g) (Protexin Concentrate, Elanco Animal Health, São Paulo, Brazil).

Sows were individually housed in crates during gestation (2.20 × 0.60 × 1.00 m) and lactation (2.20 × 0.60 × 2.20 m). The facilities were equipped with water ventilation cooling system and drinking fountain. In both feeding phases, sows from each treatment were randomly distributed throughout the barn to avoid any eventual bias effects. To prevent cross-contamination among diets, the amount of probiotic additive to be supplied to each sow was weighed daily and orally administered during feeding using gelatin capsules, following the label recommendations of 50 g/ton of feed. The daily individual supplementation was calculated by considering the daily feed intake of the sows.

Individual feeding was ensured during gestation using specially dispenser feeders. Feeding programs during gestation considered the body condition of the sow to provide from 1.5 to 1.9 kg of corn/soybean meal-based feed (Table 1) once a day, in the morning, for each animal (e.g., an average of 1.8 kg/day). Bump feeding management was not performed. Sows were transferred from gestation to farrowing rooms approximately one week before the predicted farrowing date. Lactating sows were fed *ad libitum* five times a day (i.e., an average of 7 kg/day). Feed was manually provided to the animals during this period. Creep-feeding was not used for the suckling piglets to avoid an eventual effect on the body weight at weaning. Water was supplied *ad libitum* by nipple drinkers throughout the trial.

Performance measurement

Sows were individually weighed, and their backfat thickness was measured (Renco Lean Meter, MS Schippers, São Paulo, Brazil), and their body condition was assessed using a caliper tool (PIC North America, Hendersonville, USA) at the beginning of the trial, on day 90 of gestation, during the transfer from gestation to the farrowing crate (day 107 of gestation for most sows), and at weaning (day 21 of lactation for most sows). Caliper measurements were evaluated in the last two ribs and then divided into three groups (thin, ideal, and fat), and transformed into numerical scores for statistical analysis.

Farrowing was individually monitored, and data regarding litter size and the number of piglets born alive, stillborn, and mummified fetuses were registered. Piglets were identified with earrings of different colors for each treatment and individually weighed up to 12 h after birth. Litters were homogenized preferably between sows of the same treatment until 24 hours postpartum. Any piglets that did not remain in the same treatment after being transferred from the litter were not considered in the subsequent assessments.

Piglets were classified according to birth weight into three categories: average, those that fell within the range between the mean (1.343 kg) plus or minus one standard deviation (372g); light, those weighing less than 971 g (equivalent to the mean minus 1 standard deviation); and heavy, those weighing more than 1.715 kg (equivalent to the mean plus 1 standard deviation). Piglets were individually weighed on day 20 (for most piglets, one day before weaning), and the average daily gain during lactation was calculated. Piglets were also classified according to their weaning weight into three categories: average, those that fell within the range between the mean (5.692 kg) plus or minus one standard deviation (1.290 kg); low, those weighing less than 4.402 kg (equivalent to the mean minus 1 standard deviation); and heavy, those weighing more than 6.982 kg (equivalent to the mean plus 1 standard deviation).

The feed intake of each sow during the lactation period was measured daily and later adjusted in relation to average body weight. The performance of all sows was evaluated in the subsequent cycle in terms of the total number of piglets born, born alive, stillborn, and mummified fetuses. The interval between weaning and estrus was recorded.

Animal health assessment

Feces of 20 sows (randomly selected) were collected to assess the dry matter content using the Weende method on days 3, 7, 14, and 21 postpartum. The fecal score of the sows was also evaluated daily with scores from 1 to 5 (1: hard, dry pellet in a small, hard mass; 2: hard, formed stool that remains firm and soft; 3: soft, formed, and moist stool that retains its shape; 4: soft, unformed stool that assumes the shape of the container; and 5: watery, liquid stool that can be poured). The presence or absence of diarrhea in piglets was evaluated daily in each crate during the lactation period. A litter was considered positive for diarrhea when there was the presence of the following aspects: a) signals of yellow and soft feces in the crate; b) signals of inflammation in the caudal region of the piglet; and c) yellow feces allocated in the caudal region of the piglet.

Blood samples were collected during the prepartum and weaning periods from 14 sows (randomly selected within the sow parity order in a range of 2 to 5). (At weaning, blood samples were collected from 14 piglets (randomly chosen within 14 liters). Collection was performed in the morning using a vacuum tube for each animal, and the jugular vein was assessed for both sows and piglets. Samples were centrifuged at 3500 rpm for 15 min, and the serum was removed and stored at -20 ºC for further analysis. The concentrations of total protein (g/dL), albumin (g/dL), total cholesterol (mg/dL), glucose (mg/dL), alanine aminotransferase (ALT; U/L), and aspartate aminotransferase (AST, U/L) were analyzed (Bio-Plus® Biochemical Analyzer, Bioplus, São Paulo, Brazil) using commercial kits (Wiener Lab Group, São Paulo, Brazil).

Statistical analysis

Data were analyzed using the SAS software (SAS Institute Inc., Cary, NC, USA). Responses were analyzed using the GLIMMIX procedure, and residuals were checked for normality using the Shapiro-Wilk test. The treatment means were separated using the PDIFF option and presented as a linear combination of the estimated effects from a linear model (least-squares means). The fixed effects of body condition score, parity order, and litter size were tested in each statistical model (e.g., for each response) and removed from the final analysis when not significant ($p>0.10$). Each sow or each piglet (grouped within the sow as a random effect) was considered the experimental unit. Differences between means were interpreted at $p\leq0.05$ (significant) and $p\leq 0.10$ (trend).

Results

Reproductive performance

A total of 95 sows remained in the control group and 89 in the supplement group. The parity order was similar between the treatments (Table 2). There was no difference between the control- and probiotic-fed sows in the total number of piglets born, born alive, stillborn, and mummified fetuses. Furthermore, no effect of treatment was observed on the body composition variables (Table 3).

Feed intake (adjusted as a % of body weight) during lactation was greater in probiotic-fed sows (3% of body weight) than in the control animals (2% of body weight; $p<0.05$). Despite removing probiotic supplementation after weaning, the performance responses of the sows were measured in the subsequent reproductive cycle (Table 4). The total number of piglets born tended to be greater for probiotic- than control-fed sows ($p=0.076$). However, no differences were found in the number of piglets born alive, stillborn, and mummified fetuses, or weaning-estrus intervals.

Progeny growth performance

Piglets born to probiotic-fed sows had greater birth weight (6%), live birth weight (5%), average daily gain (4%), and weight at weaning (7%) than piglets born to control-fed sows ($p<0.05$, Table 5). The benefits of probiotics were more relevant in light-birth-weight piglets, as the light animals born from probiotic-fed sows had greater average daily gain and weight at weaning than those born from control-fed sows (Table 5; $p<0.05$).

There was considerable variation in body weight among piglets at weaning (Figure 1). For instance, a small frequency of piglets with low weaning weight was observed in probiotic-fed sows compared with control-fed sows (15 versus 17%); whereas probiotic-fed sows had a greater frequency of the piglets with high weaning weight than control-fed sows (8 versus 6%).

Health indicators of sows and progeny

Control-fed sows tended to have lower fecal moisture content on the third day postpartum than probiotic-fed sows ($p<0.10$; Figure 2). Piglets born to control-fed sows also had greater diarrhea than piglets born to probiotic-fed sows during lactation ($p<0.05$; Table 7).

Biochemical responses of sows and progeny

Concentrations of AST, ALP, glucose, cholesterol, and total protein were similar between the treatments during the prepartum period (Table 8). However, the prepartum albumin concentration was higher in the probiotic-fed group than in the control group ($p<0.05$). At weaning, probiotic-fed sows had lower serum concentrations of glucose, cholesterol, total protein, and albumin than the control-fed sows ($p<0.05$). The biochemical responses of piglets were not influenced by the sow dietary treatments.

Discussion

Probiotics are feed additives used in swine production that may confer health benefits to animals. The microorganisms in the additive can colonize the gastrointestinal tract and can be used to restore or avoid gut dysbiosis (Martín and Langella, 2019). In addition to improving intestinal health, probiotics are commonly studied in swine diets for performance purposes (Cameron and McAllister, 2019). Some studies reported that using multi-strain probiotics (i.e., a complex of *Lactobacillus* with the addition of other microorganisms) may promote more effective results due to their synergistic effects when compared to single-strain probiotics (Liu et al., 2018). However, most of the data available on the use of probiotics in pig production comes from the nursery phase. Vertical sow-to-piglet transmission of microbiota has been well documented by several groups; thus, improving gut health during the gestation and lactation phases can be a useful strategy to support piglet development. Therefore, this study aimed to assess the potential benefits of feeding probiotics to sows, and their effects on piglets.

Probiotic supplementation for sows

Despite the improvement observed in piglet performance, which may indicate greater milk production in probiotic-fed sows, the body assessments (sow body weight, caliper, and backfat measurements) did not differ between treatments in all measured periods (beginning of gestation, farrowing, and weaning). In addition, feed consumption adjusted to body weight was greater in probiotic-fed sows than that in control-fed sows during farrowing. Improvements in feed intake during lactation have been previously observed in sows fed *Bacillus subtilis* and *Bacillus licheniformis* (Alexopoulos et al., 2004). A greater litter size and weight gain can directly affect feed intake (Eissen et al., 2000). In addition, in this study, there was greater weight gain for piglets during the lactation period. Milk production and feed intake during lactation are closely related to weight loss, which is a crucial variable for assessing modern sows. Studies have indicated that sows fed probiotic diets present lower weight loss than control-fed sows (Alexopoulos et al., 2004; Kritas et al., 2015). In the present study, no difference was observed in weight loss during lactation, which is a desirable response in the context of improved piglet performance.

Furthermore, the probiotic-fed sows tended to have an increased total number of piglets born per litter in the subsequent cycle compared to the control treatment. This result was observed after a long period without supplementation, which can be interpreted as a long-term effect, probably related to gut microbiota modulation. Probiotics are used to maintain gut homeostasis and stimulate innate immunity in pigs (Everaert et al., 2017). Therefore, a healthy gut status allows the sow to express its genetic potential through performance results, even in the next cycle of production.

Probiotic supplementation increased fecal moisture content in the immediate postpartum period, which can be interpreted as a reduced risk of constipation, possibly due to greater motility and activity in the intestine. Gut dysbiosis can result in the reduction of intestinal motility, consequently causing constipation and other metabolic disorders, which may be dangerous in peripartum sows, particularly in the context of high prolapse occurrence. Accordingly, the same effect was previously reported in probiotic-fed sows compared to control-fed sows (Tan et al., 2020). In addition, the increased presence of some microorganisms, such as *Bacteroidetes*, is related to lower constipation signals (Lu et al., 2022).

Probiotic supplementation for sows: effects on the piglets

Providing probiotics to sows during gestation and lactation resulted in piglets with better performance. For instance, piglets were born with greater body weight and had a higher weaning weight than the control group. Neonates begin gut colonization immediately after birth. The gut microbiota of piglets is established weeks after birth and is directly affected by sow gut microbiota (Kim and Isaacson, 2015). Thus, probiotics can modulate the gut of sows, and consequently, their neonates. Some genders in the gut are increased (e.g., *Bacteroides*) with the use of this additive and have the ability to degrade monosaccharides and oligosaccharides, which are components of sow milk (Lamendella et al., 2011). This may enhance the ability of piglets to absorb nutrients from the gut mucosa and support growth during lactation. In a study of *Lactobacillus* strains, Wang et al. (2014) reported greater litter weight at birth and weaning in piglets born from supplemented sows, which corroborates the findings of the current study.

Probiotics mainly enhance the growth performance of low-birth-weight piglets, resulting in body weight recuperation during lactation and a more consistent distribution of weaning weight among piglets. Low-birth-weight piglets may have a different gut microbiota composition than normal-birth-weight piglets, with a lower abundance of the *Lactobacillus* genus (Li et al., 2019). Considering the importance of this community in metabolism, feeding probiotics to sows during lactation could be an alternative to modulate the microbiota of piglets born with low birth weight through milk and colostrum consumption.

Piglets born from probiotic-fed sows also exhibited reduced diarrhea compared to the control. Some probiotics, such as *Lactobacillus* strains, can produce certain antimicrobials that are active against some pathogenic bacteria and some species of yeast, protozoa, and viruses (Cleusix et al., 2008). Probiotics can also inhibit pathogen replication by increasing short-chain fatty acid production (Oelschlaeger, 2010; Mori et al., 2011). In accordance with the current results, Hayakawa et al. (2016) administered *Bacillus mesentericus, Clostridium butyricum,* and *Enterococcus faecalis* strains as probiotics for sows and neonates, and highly decreased the piglet diarrheal score during the lactation period.

Animal health indicators: biochemical parameters

Glucose and cholesterol concentrations decreased in probiotic-fed sows at weaning. Glucose metabolism during gestation is altered to provide glucose to the uterus. Glucose is crucial for milk synthesis during lactation (Yang et al., 2021). In addition, lactation is a challenging phase in the life of sows and a high-energy consumption period due to milk production (Farmer, 2015). A greater feed intake of sows and weaning weight of the piglets can explain a higher glucose mobilization for milk synthesis. Probiotics can modify the intestinal microbiota and consequently influence several metabolic patterns. For instance, some *Lactobacillus* species have the ability to activate enzymes that are cholesterol-lowering agents through enzyme interactions with the host (Ishimwe et al., 2015).

An increase in the serum albumin concentration during gestation is necessary to support intrauterine nutrition. Albumin, also known as acute-phase protein, is associated with inflammation. Probiotics can increase intestinal integrity and absorption, which decreases bacterial translocation and inflammatory responses (Akbari Nargesi et al., 2020; Rashidi et al., 2020). Serum albumin levels at weaning decreased in probiotic-fed sows, which may be related to the higher milk production in this group. Albumin is involved in the transport of several milk components. Thus, the lower concentration of serum albumin may be related to the rapid extraction of immunoglobulin from plasma during colostrum synthesis in the mammary gland (Elwakiel et al., 2019).

Studies with probiotics are necessary to understand the mechanisms and the benefits of this feed additive. In this study, several variables were assessed to provide a complete overview of probiotics in swine nutrition.

Conclusion

In conclusion, supplementation of sows with multi-strain probiotics during gestation and lactation improved piglet performance. These findings highlight the potential of probiotics as an effective strategy for enhancing piglet growth, health, and overall productivity in swine production systems. The application of multi-strain probiotics during critical periods, such as gestation and lactation phases, presents a promising solution for optimizing piglet outcomes and further exploration in swine management practices.

Declaration of interest

Carolina Franceschi was employed by Danisco Animal Nutrition and Health (IFF), São Paulo, Brazil. Thais Stefanello was employed by JEFO Animal Nutrition. Marcos Kipper was employed by Elanco Animal Health, São Paulo, Brazil. The remaining authors declare no conflicts of interest.

References

Akbari Nargesi, E., Falahatkar, B., & Sajjadi, M. M. (2020). Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (Oncorhynchus mykiss) broodstock. Aquaculture Nutrition, 26(1), 98-108.

Afonso, E. R., Parazzi, L. J., Marino, C. T., Martins, S. M. M. K., Araújo, L. F., Araújo, C. S. D. S., ... & Moretti, A. D. S. A. (2013). Probiotics association in the suckling and nursery in piglets challenged with Salmonella typhimurium. Brazilian archives of biology and technology, 56, 249-258.

Alexopoulos, C., Georgoulakis, I. E., Tzivara, A., Kritas, S. K., Siochu, A., & Kyriakis, S. C. 2004. Field evaluation of the efficacy of a probiotic containing Bacillus licheniformis and Bacillus subtilis spores, on the health status and performance of sows and their litters. Journal of animal physiology and animal nutrition, 88(11-12), 381-392

Barba-Vidal, E., S. M. Martín-Orúe, and L. J. L. S. Castillejos. 2019. Practical aspects of the use of probiotics in pig production: A review. Livestock Science 223:84-96.

Cameron, A., & McAllister, T. A. (2019). Could probiotics be the panacea alternative to the use of antimicrobials in livestock diets?. Beneficial microbes, 10(7), 773-799.

Cleusix, V., C. Lacroix, S. Vollenweider, and G. J. F. m. e. Le Blay. 2008. Glycerol induces reuterin production and decreases *Escherichia coli* population in an in vitro model of colonic fermentation with immobilized human feces. FEMS Microbiology Ecology 63(1):56-64.

De Lange, C., J. Pluske, J. Gong, and C. J. L. S. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 134(1-3):124-134.

Eissen, J. J., Kanis, E., & Kemp, B. 2000. Sow factors affecting voluntary feed intake during lactation. Livestock Production Science, 64(2-3), 147-165.

Elwakiel, M., S. Boeren, J. A. Hageman, I. M. Szeto, H. A. Schols, and K. A. J. N. Hettinga. 2019. Variability of serum proteins in Chinese and Dutch human milk during lactation. 11(3):499.

Everaert, N., S. Van Cruchten, B. Weström, M. Bailey, C. Van Ginneken, T. Thymann, R. J. A. f. s. Pieper, and technology. 2017. A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. Animal Feed Science and Technology 233:89-103.

FAO, W. 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. 2006.

Farmer, C. 2015. The gestating and lactating sow. Wageningen Academic Publishers.

Hayakawa, T., Masuda, T., Kurosawa, D., & Tsukahara, T. 2016. Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. Animal Science Journal, 87(12), 1501-1510.

Ishimwe, N., E. B. Daliri, B. H. Lee, F. Fang, G. J. M. n. Du, and f. research. 2015. The perspective on cholesterol-lowering mechanisms of probiotics. Molecular nutrition & food research, 59(1):94-105.

Kenny, M., H. Smidt, E. Mengheri, and B. J. A. Miller. 2011. Probiotics–do they have a role in the pig industry? Animal 5(3):462-470.

Kim, H. B., and R. E. J. V. m. Isaacson. 2015. The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. Veterinary Microbiology 177(3-4):242-251.

Kritas, S. K., Marubashi, T., Filioussis, G., Petridou, E., Christodoulopoulos, G., Burriel, A. R., & Pískoriková, M. 2015. Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (Bacillus subtilis C-3102). Journal of animal science, 93(1), 405-413.

Laskowska, E., Jarosz, Ł., Grądzki, Z., 2019. Effect of multi-microbial probiotic formulation bokashi on pro-and anti-inflammatory cytokines profile in the serum, colostrum and milk of sows, and in a culture of polymorphonuclear cells isolated from colostrum. Probiotics Antimicrob. Proteins 11 (1), 220–232

Lamendella, R., J. W. Santo Domingo, S. Ghosh, J. Martinson, and D. B. J. B. m. Oerther. 2011. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. BMC microbiology 11:1-17.

Lee, Choong-Gu et al. Exploring probiotic effector molecules and their mode of action in gut–immune interactions. 2023 FEMS Microbiology Reviews, v. 47, n. 4, p. fuad046.

Li, N., S. Huang, L. Jiang, Z. Dai, T. Li, D. Han, and J. J. F. i. m. Wang. 2019. Characterization of the early life microbiota development and predominant Lactobacillus species at distinct gut segments of low-and normal-birth-weight piglets. Frontier in Microbiology 10:797.

Liu, H., Zhang, J., Zhang, S., Yang, F., Thacker, P. A., Zhang, G., ... & Ma, X. 2014. Oral administration of Lactobacillus fermentum I5007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. Journal of agricultural and food chemistry, 62(4), 860-866.

Liu, W. C., Ye, M., Liao, J. H., Zhao, Z. H., Kim, I. H., & An, L. L. 2018. Application of complex probiotics in swine nutrition–a review. Annals of animal science, 18(2), 335-350.

Lu, D., Y. Pi, H. Ye, Y. Wu, Y. Bai, S. Lian, D. Han, D. Ni, X. Zou, and J. J. N. Zhao. 2022. Consumption of dietary fiber with different physicochemical properties during late pregnancy alters the gut microbiota and relieves constipation in sow model. Nutrients 14(12):2511.

Lutgendorff, F., L. Akkermans, and J. D. J. C. m. m. Soderholm. 2008. The role of microbiota and probiotics in stress-induced gastrointestinal damage. Current molecular medicine 8(4):282-298.

Martín, R; Langella, P. 2019. Emerging health concepts in the probiotics field: streamlining the definitions. Frontiers in microbiology, 10, 457799.

Monteagudo-Mera, A., R. A. Rastall, G. R. Gibson, D. Charalampopoulos, A. J. A. m. Chatzifragkou. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Applied microbiology and biotechnology 103:6463-6472.

Mori, K., T. Ito, H. Miyamoto, M. Ozawa, S. Wada, Y. Kumagai, J. Matsumoto, R. Naito, S. Nakamura, H. J. J. o. b. Kodama, and bioengineering. 2011. Oral administration of multispecies microbial supplements to sows influences the composition of gut microbiota and fecal organic acids in their post-weaned piglets. Journal of bioscience and bioengineering 112(2):145-150.

Oelschlaeger, T. A. 2010. Mechanisms of probiotic actions–a review. International journal of medical microbiology, 300(1):57-62.

Ouwehand, A. C., Invernici, M. M., Furlaneto, F. A., & Messora, M. R. (2018). Effectiveness of multi-strain versus single-strain probiotics: current status and recommendations for the future. Journal of clinical gastroenterology, 52, S35-S40.

Rashidi, N., A. Khatibjoo, K. Taherpour, M. Akbari-Gharaei, and H. J. P. s. Shirzadi. 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. Poultry Science 99(11):5896-5906.

Satora, M., Rząsa, A., Rypuła, K., Płoneczka-Janeczko, K., 2021. Field evaluation of the influence of garlic extract and probiotic cultures on sows and growing pigs. Medycyna weterynaryjna-veterinary medicine-science and practice 77 (1), 21–29.

Tan, B. F., Lim, T., & Boontiam, W. 2020. Effect of dietary supplementation with essential oils and a Bacillus probiotic on growth performance, diarrhoea and blood metabolites in weaned pigs. Animal Production Science, 61(1), 64-71.

Wang, J., H. Ji, C. Hou, S. Wang, D. Zhang, H. Liu, D. Shan, and Y. J. L. S. Wang. 2014. Effects of *Lactobacillus johnsonii* XS4 supplementation on reproductive performance, gut environment, and blood biochemical and immunological index in lactating sows. Livestock Science 164:96-101.

Yang, Y., M. Deng, J. Chen, X. Zhao, K. Xiao, W. He, X. Qiu, Y. Xu, Y. Yin, and C. J. A. N. Tan. 2021. Starch supplementation improves the reproductive performance of sows in different glucose tolerance status. Animal Nutrition 7(4):1231-1241.

Zhang, M., K. Sun, Y. Wu, Y. Yang, P. Tso, and Z. J. F. i. i. Wu. 2017. Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. Fro ntier in immunology 8:942.

Tables

Table 1. Chemical composition (as-fed) of the experimental diets to sows.

Ingredients, %	Sow diet[1]	
	Gestation	**Lactation**
Corn (8% CP)	81.20	66.42
Soybean meal (48% CP)	15.02	28.05
Soybean oil	1.425	1.425
Dicalcium phosphate	0.878	1.184
Calcitic limestone	1.637	1.104
Salt	0.421	0.502
Premix[1]	0.500	0.500
L-Lysine (65%)	0.168	
L-Valine		0.158
L-Threonine	0.100	0.128
DL- Methionine	0.049	0.100
Tryptophan	0.005	0.050
Phytase[2]	0.005	0.005
Carbohydrase[3]		0.005

[1]The requirements of each phase were estimated using the Brazilian Tables of Poultry and Swine (Rostagno, 2017).
[2] Premix with vitamins and minerals. Vitamin A:10.000.000,0 IU/kg; vitamin B_1: 1.600,00 mg/kg; vitamin B_2:6 mg/kg; vitamin K_3 6.600,00 mg/kg; vitamin B_6: 1.600,00 mg/kg; vitamin B_{12} 24.000,00 mcg/kg; vitamin D_3:1.800.00,00 IU/kg; vitamin E: 20.200,00 IU/kg; folic acid 1.2 mg/kg; niacin: 80.00g/kg; biotin 200.000 mg/kg; pantothenic acid: 12.00 g/kg; Iron 60 mg/kg; Zinc 70 mg/kg; Copper 140 mg/kg; Calcium 273 mg/kg; Phosphorus 150 mg/kg.
[3] Axtra PHY GOLD, Danisco Nutrition.
[4] Rovabio, Adisseo.

Table 2. Performance responses of sows supplemented with multi-strain probiotics.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotics**	
Sows, n	95	89	-
Piglets, n	1341	1255	-
Parity order, mean	4.3	4.2	-
Gestation period, days	114.3 (0.153)	114.1 (0.158)	0.209
Total born, n/litter	14.11 (0.417)	14.10 (0.430)	0.985
Total born alive, n/litter	13.13 (0.367)	13.21 (0.380)	0.885
Stillborn, n/litter	0.600 (0.106)	0.584 (0.110)	0.918
Mummified, n/litter	0.383 (0.084)	0.287 (0.085)	0.428

[1] Means with standard errors in the parentheses.
[2] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 3. Body condition of sows supplemented with multi-strain probiotics.

Variables[1]	**Sow treatment[2]**		***P*-value[3]**
	Control	**Probiotics**	
Sows, n	95	89	
Body weight			
Beginning of gestation, kg	213.0 (2.522)	214.2 (2.568)	0.730
Farrowing, kg	243.4 (2.144)	245.8 (2.180)	0.442
Weaning, kg	229.4 (2.345)	231.1 (2.371)	0.612
Variation during gestation, kg	30.87 (1.921)	33.32 (1.946)	0.372
Variation during lactation, kg	-13.56 (1.705)	-13.91 (1.715)	0.884
Caliper measurement			
Beginning of gestation	1.576 (0.086)	1.712 (0.085)	0.614
Day 90 of gestation	2.516 (0.066)	2.540 (0.067)	0.782
Farrowing	2.158 (0.085)	2.301 (0.083)	0.899
Weaning	1.761 (0.082)	1.937 (0.080)	0.937
Variation during gestation	0.581 (0.086)	0.581 (0.086)	0.999
Variation during lactation	-0.384 (0.089)	-0.232 (0.086)	0.223
Backfat thickness			
Beginning of gestation, mm	9.236 (0.423)	9.455 (0.436)	0.719
Day 90 of gestation, mm	10.09 (0.474)	10.42 (0.468)	0.624
Farrowing, mm	9.881 (0.370)	10.17 (0.370)	0.575
Weaning, mm	8.113 (0.283)	8.706 (0.290)	0.146
Variation during gestation, mm	0.560 (0.429)	0.193 (0.462)	0.646
Variation during lactation, mm	-1.807 (0.393)	-1.438 (0.376)	0.499

[1] Means with standard errors in the parentheses.
[2] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 4. Performance responses in the subsequent cycle of sows supplemented with multi-strain probiotics[1].

Variables[2]	Sow treatment[3]		*P*-value[4]
	Control	**Probiotics**	
Total born, n/litter	14.88 (0.438)	15.94 (0.462)	0.076
Total born alive, n/litter	14.16 (0.418)	14.76 (0.435)	0.292
Stillborn, n/litter	0.622 (0.110)	0.681 (0.114)	0.688
Mummified, n/litter	0.232 (0.063)	0.220 (0.066)	0.888
Weaning-estrus interval, days	7.257 (0.886)	6.460 (0.934)	0.537

[1]Probiotic supplementation was not provided in the subsequent gestation phase.
[2] Means with standard errors in the parentheses.
[3] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[4] Probability of treatment effect.

Table 5. Effect of multi-strain probiotic supplementation to gestating-lactating sows on the overall performance of piglets (from 0 to 21 days).

Variables[1]	Sow treatment[2]		*P*-value[3]
	Control	Probiotics	
Piglets, n	1306	1228	
Birth weight, kg	1.329 (0.013)	1.404 (0.014)	0.002
Birth weight alive, kg	1.342 (0.016)	1.406 (0.018)	0.009
Average daily gain, g/day	193.5 (2.918)	202.0 (2.600)	0.023
Weight at 21 days, kg	5.329 (0.076)	5.725 (0.072)	<0.001
Mortality, %	4.389 (0.792)	4.250 (0.843)	0.908

[1] Means with standard errors in the parentheses.
[2] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 6. Effect of multi-strain probiotic supplementation to gestating-lactating sows on performance of piglets according to their birth weight category.

Variables[1]	Sow treatment[2]		*P*-value[3]
	Control	Probiotics	
Low birth-weight, n			
Frequency, n/litter	2.178 (0.280)	2.303 (0.290)	0.758
Birth weight alive, g	768.8 (12.66)	740.8 (13.86)	0.066
Average daily gain, g	154.6 (5.324)	174.3 (5.921)	0.005
Weight at 21 days, kg	4.071 (0.113)	4.458 (0.126)	0.010
Average birth-weight, n			
Frequency, n/litter	8.578 (0.421)	9.089 (0.435)	0.399
Birth weight alive, kg	1.343 (0.013)	1.355 (0.010)	0.491
Average daily gain, g	200.6 (3.925)	205.6 (2.672)	0.286
Weight at 21 days, kg	5.556 (0.087)	5.684 (0.059)	0.226
High birth-weight, n			
Frequency, n/litter	2.410 (0.246)	1.842 (0.254)	0.110
Birth weight alive, kg	1.874 (0.012)	1.886 (0.013)	0.413
Average daily gain, g	216.7 (4.967)	219.5 (5.159)	0.646
Weight at 21 days, kg	6.431 (0.104)	6.500 (0.108)	0.595

[1] Means with standard errors in the parentheses.
[2] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 7. Effect of multi-strain probiotic supplementation to gestating-lactating sows on the diarrhea presence[1] of piglets.

Variables[2]	Sow treatments[3]		*P*-value[4]
	Control	Probiotics	
n	95	89	
Total period, %	34.46 (0.057)	29.68 (0.061)	0.008
First week, %	21.41 (0.095)	16.47 (0.108)	0.026
Ten days pre-weaning, %	47.09 (0.077)	41.90 (0.078)	0.057

[1]Diarrheal presence was estimated in percentage of daily observation (litters showing clinical signals over the total number of litters evaluated).
[2] Means with standard errors in the parentheses.
[3] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[4] Probability of treatment effect.

Table 8. Effect of multi-strain probiotic supplementation to gestating-lactating sows on the biochemical responses.

Variables[1]	Sow treatment[2]		*P*-value[3]
	Control	Probiotics	
Sows - pre-partum, day 110			
AST, U/L	37.75 (7.587)	24.25 (7.587)	0.255
ALP, U/L	398.2 (75.72)	483.0 (87.44)	0.496
Glucose, mg/dL	125.3 (5.169)	119.8 (5.525)	0.478
Total cholesterol, mg/dL	60.26 (7.075)	49.01 (7.564)	0.297
Total protein, g/dL	7.400 (0.538)	7.775 (0.762)	0.696
Albumin, g/dL	3.250 (0.219)	4.175 (0.310)	0.035
Sows - weaning, day 20			
AST, U/L	70.28 (11.34)	74.33 (12.25)	0.813
ALP, U/L	1983 (235.7)	1922 (267.2)	0.963
Glucose, mg/dL	242.6 (12.24)	178.1 (13.08)	0.003
Total cholesterol, mg/dL	120.8 (13.81)	72.32 (15.66)	0.035
Total protein, g/dL	4.777 (0.200)	3.657 (0.227)	0.002
Albumin, g/dL	4.544 (0.177)	3.885 (0.201)	0.027
Piglets - pre- weaning, day 20			
AST, U/L	33.50 (5.572)	43.44 (5.253)	0.213
ALP, U/L	439.4 (81.83)	309.5 (100.2)	0.333
Glucose, mg/dL	260.8 (20.54)	262.2 (20.54)	0.964
Total cholesterol, mg/dL	81.84 (5.109)	88.60 (5.419)	0.378
Total protein, g/dL	5.988 (0.143)	5.866 (0.143)	0.554
Albumin, g/dL	5.466 (0.156)	5.255 (0.156)	0.354

[1] Means with standard errors in the parentheses.
[2] Supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Figures

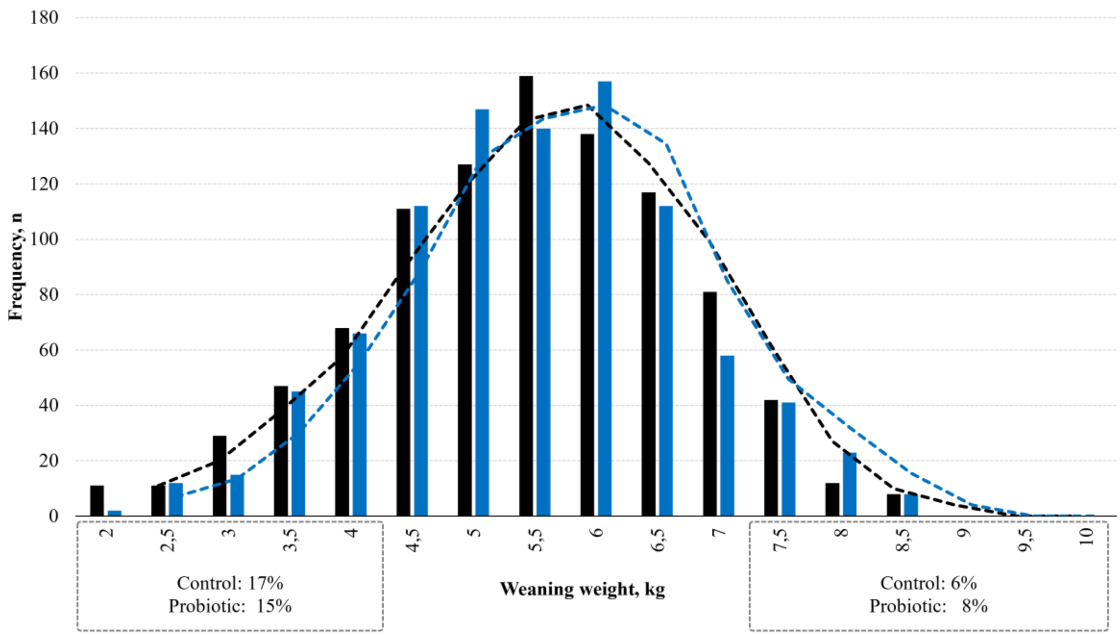

Figure 1. Effect of multi-strain probiotic supplementation[1] to gestating-lactating sows on the distribution of piglet weight at 21 days (means in the bars and moving averages in the lines). Black and blue lines represent control and probiotic, respectively.

[1] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.

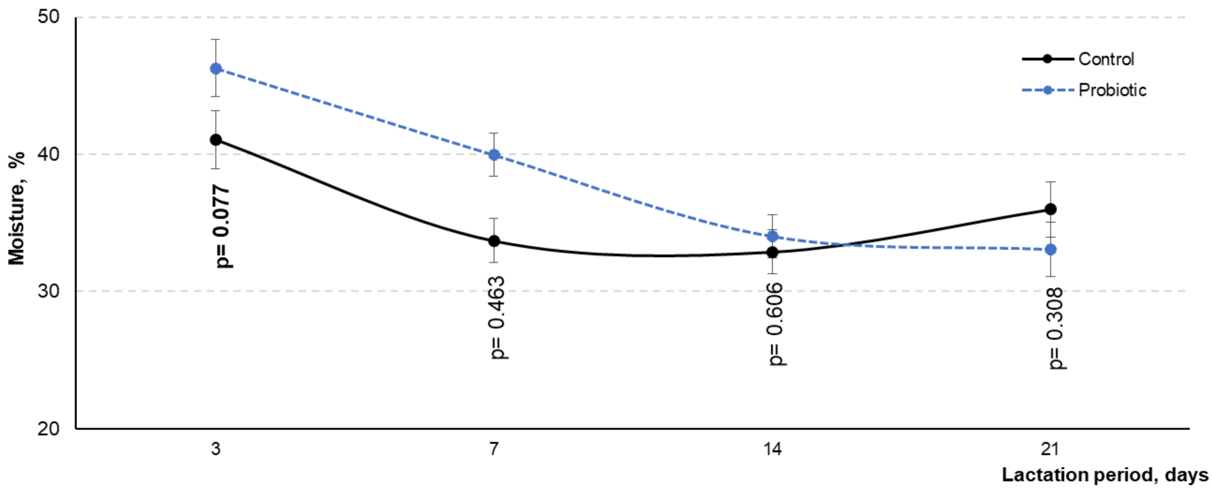

Figure 2. Effect of multi-strain probiotic supplementation[1] to gestating-lactating sows on the fecal moisture of sows during lactation period*.

[1] Sow dietary treatment consisted of providing a control diet or a probiotic diet supplemented with Protexin Concentrate (50 g/ton of feed) during gestation and lactation.
* P-values are presented as treatment effects for each period.

CAPÍTULO III[1]

[1]Artigo escrito nas normas da revista *Animal*

Bacillus spp. probiotic effect for sows and its progeny: performance, health, and colostrum quality

Abstract

As feed additives, *Bacillus spp.* probiotics are known for their ability to modify gut health, which could be especially important during critical phases such as gestation and lactation. Each strain of a probiotic could perform differently, which brings the necessity to study more about this topic. Thus, the purpose of this study was to evaluate whether *Bacillus spp.* probiotics affect the health and productivity of sows and their progenies. Two groups of sows with parity orders ranging from 2 to 9 were used. The control group (n = 100 and parity order = 4.3) was fed a control diet, whereas the probiotic-fed sows (n = 100 and parity order = 4.2) received an oral supplement based of *Bacillus subtilis* and *Bacillus licheniformis* (400 g/ton) from the first day of gestation until the end of lactation (21 days postpartum). Performance and health data were recorded for both the sows and piglets. All data were subjected to analysis of variance. A marginal significance was considered when $0.05 \leq p<0.10$ and significant when $p<0.05$. Probiotics tended to reduce the number of mummified piglets per litter by 37% ($p<0.10$). The probiotics increased the piglet weight at birth by 7%, and weight at weaning by 6% ($p<0.05$). The presence of diarrhea in piglets was reduced by 11% during the lactation phase ($p<0.05$). Probiotic supplementation increased serum ALP levels in the prepartum period ($p<0.05$) and a slightly difference was found in the glucose levels that decreased in the pre-weaning period ($p<0.10$). Colostrum quality was improved in sows that were supplemented with *Bacillus spp.* ($p<0.05$). Finally, the *Bacillus spp.* reduced return-to-estrus interval by 3 days ($p<0.05$). Probiotics improve the performance of sows and their progeny and are valuable tools to enhance the health status of these animal categories.

Keywords: feed additives, colostrum, farrowing, microorganisms, piglets.

Implications

Probiotics are tools used to enhance animal performance and health. Using probiotics for sows can enhance litter quality by improving birth weight and weaning weight. Also, sows can produce a colostrum with greater quality and reduce the interval between weaning and estrus, which increases productivity in a swine industry.

Introduction

The concept of probiotics is well known in the animal nutrition and health industries. Defined by FAO (2006) as “live microorganisms that, when provided in greater amounts, confer a health benefit to the host”, these feed additives have gained considerable attention for swine production. Probiotics can have various positive effects in pigs, including modulation of the intestinal microbiota, enhancement of the immune system, reduction of inflammatory reactions, and prevention of pathogen colonization (Wang et al., 2021).

The use of probiotics as alternatives to antibiotic growth promoters in pigs from weaning to finishing phases is probably a significant driving force behind their growing popularity in the swine industry (Lambo et al., 2021). However, the use of probiotics is not limited to growing animals. Studies have shown that probiotics, particularly those containing *Bacillus* spp., have the potential to improve gut health and performance in sows (Alexopoulos et al., 2004; Kritas et al., 2015; Tian et al., 2021).

Although the frequency of attention given to gut health management in sows is not as high as it is in nursery or growing pigs, maintaining intestinal integrity is nonetheless a crucial aspect also for the sows. For instance, hyperprolific sows are highly productive animals that often experience increased oxidative stress and inflammatory status (Li et al., 2022). The use of probiotics could help sows overcome these obstacles, thereby benefiting both the mother and the growing offspring (Pereira et al., 2022). Therefore, this study aimed to assess the effects of *Bacillus spp.* probiotics (*Bacillus subtilis* and *Bacillus licheniformis*) on sows during gestation and lactation.

Material and methods

The experimental trial was conducted at a commercial farm in Rio Grande do Sul, Brazil. All the experimental procedures were approved by the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul.

Animals, housing, and experimental design

Two hundred sows (PIC – Camborough, Agroceres-PIC, São Paulo, Brazil) with parity order ranging from 2 to 9 were assigned (randomly within each parity order) to two treatments: control diet and probiotic diet containing *Bacillus subtillis* and *Bacillus licheniformis* (3.2 x 10^6 CFU/g) (BioPlus® 2B, Chr Hansen, São Paulo, Brazil).

Probiotic supplementation started on the first day of pregnancy and continued until the end of the lactation period. To mitigate the risk of cross-contamination among animals and in the environment, pharmaceutical gelatin capsules were precisely filled with the designated quantity of the probiotic daily. The capsules were positioned atop the morning ration provided to each sow, and they were ingested swiftly along with the feed.

A feed based on corn and soybean meal was used in both phases (Table 1). During the gestation phase, 1.9 kg of feed was provided once a day (morning) per sow. Adjustments of this amount were performed for sows with inadequate body condition scores, with the daily feed intake varying from 1.8 to 2.0 kg. During the lactation phase, the feed was supplied *ad libitum*, with the daily feed intake expected to reach 7 to 8 kg per animal. Despite being supplied in the gelatin capsules, the daily amount of probiotics was weighed individually to represent an inclusion of 400 g of the additive per ton of feed, which is the concentration recommended by the supplier.

During the experimental period, sows were housed in individual pens (2.20 X 0.60 X 1.00 m). Approximately one week before the expected farrowing date, sows were transferred from the gestation sector to the farrowing room (2.20 × 0.60 × 2.20 m). During gestation and lactation, the treatments were distributed through the barn to avoid the eventual effect of the microclimate on the results. Both facilities were equipped with a water ventilation-cooling system and a drinking fountain.

Performance measurements - Sows

The sow body condition was evaluated using body weight, backfat thickness, and caliper measurements. Body weight was assessed at three periods: entrance of gestation, beginning, and end of farrowing. The backfat thickness of the animals was measured (Renco Lean Meter, MS Schippers, São Paulo, Brazil) and their body condition was assessed using the Caliper tool (PIC North America, Hendersonville, USA) before pregnancy, at day 90 of gestation, during the transfer from gestation to farrowing crate, and piglet weaning (day 21 of lactation for most sows).

Farrowing was monitored individually, and data regarding litter size, number of live-born piglets, stillbirths, and mummified fetuses were collected. The litters were preferentially homogenized between sows of the same treatment until 24 hours postpartum, and piglets that did not remain in the same treatment after being managed were not considered in the subsequent evaluations. Feed intake during lactation was evaluated daily and presented in relation to sow weight.

The performance of all sows was evaluated in the subsequent cycle, including total piglets born, piglets born alive, stillborn, and mummified fetuses. The interval between weaning and estrus was recorded. In addition, colostrum from the sows was collected until 12 h postpartum to evaluate the quality using a digital Brix refractometer (Milwaukee Electronics, Szeged, Hungary).

Performance measurements - Piglets

Birth weight was measured of all piglets and classified according to birth weight into three categories: medium, those in the middle range (1,343 kg ± 372 kg); light, weighing less than 971 g (equivalent to the mean minus 1 standard deviation); and heavy, those weighing more than 1.715 kg (equivalent to the mean plus 1 standard deviation). Piglets were weighed individually on day 20 (one day before weaning), and weight gain during lactation was calculated.

Animal health measurements

The dry matter content of sow feces was collected at 3, 7, 14, and 21 d post-partum and evaluated using the Weende method. The fecal score of the sows was evaluated daily with the following scores: 1 - hard, dry pellet in a small, hard mass; 2 - hard, formed stool that remains firm and soft; 3 – soft, formed, and moist stool that retains its shape; 4–soft, unformed stool that assumes the shape of the container; 5 – watery, liquid stool that can be poured.

Bacillus species count was assessed in the feces of sows and piglets. First, feces were collected directly from the rectum, placed in sterile tubes, and stored at room temperature until further analysis. Spores and viable *Bacillus* bacteria were enumerated using the plate count method.

The presence of diarrhea was assessed daily in each crate during lactation. A litter was considered positive for diarrhea when there was the presence of the following aspects: a) signals of yellow and soft feces in the crate; b) signals of inflammation in the caudal region of the piglet; and c) yellow feces allocated in the caudal region of the piglet. Blood samples were collected during two periods: prepartum and weaning. Fourteen sows and 14 piglets were randomly selected for sampling. The collection was performed in the morning, using a vacuum tube for each animal and the jugular vein was assessed for both sows and piglets. Samples were centrifuged at 3500 rpm for 15 min and serum was removed and stored at -20 ºC for further analysis. The samples were analyzed for biochemical responses (Bio-Plus®, Biochemical Analyzer, Bioplus, São Paulo, Brazil). Biochemical analyses, including total protein (g/dL), albumin (g/dL), total cholesterol (mg/dL), glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were performed using commercial kits (Wiener Lab Group, São Paulo, Brazil).

Statistical analysis

Data were analyzed using the SAS software (SAS Institute Inc., Cary, NC, USA). Responses were analyzed using the GLIMMIX procedure, and residuals were checked for normality using the Shapiro-Wilk test. Treatment means were separated using the PDIFF option and presented as a linear combination of the estimated effects from a linear model (least-squares means). The fixed effects of body condition score, parity order, and litter size were tested in each statistical model (e.g., for each response) and removed from the final analysis when not significant ($p>0.10$). Each sow or each piglet (grouped within the sow as a random effect) was considered the experimental unit. Differences between means were interpreted at $p \leq 0.05$ (significant) and $p \leq 0.10$ (trend).

Results

Performance measurements: Sows

There were no differences between the control and probiotic groups in parity, gestation period, total born, total born alive, and stillborn (Table 2). Mummified piglets tended to slightly decrease in the supplemented group (37% of reduction) ($p<0.10$).

Sows supplemented with *Bacillus spp*. probiotics tended to have a lower body weight at the beginning of farrowing when compared to the control group ($p<0.10$; Table 3). All other measurements related to the body condition of the sows (caliper measurement and backfat thickness) did not differentiate between the groups. Feed intake (adjusted as a % of body weight) during lactation was greater in *Bacillus spp.* sows (2.48% of body weight) than in the control animals (2.23% of body weight; $p<0.05$).

The performance of the sows in the subsequent cycle was also measured (Table 4). There was no difference in total born, total born alive, stillborn, and mummified fetuses when comparing control sows to supplemented ones. However, sows supplemented with *Bacillus* probiotics decreased the return-to-estrus interval when compared to control sows ($p<0.05$).

Performance measurements: Piglets

Piglet performance was enhanced when the sows were supplemented with *Bacillus spp.* probiotics (Table 5). The birth weight of the piglets increased by approximately 100 g when the sows were fed probiotics ($p<0.05$). Also, piglet birth weight alive was higher in 6.5% in the probiotic group than in the control group ($p<0.10$). Sows fed diets with *Bacillus spp*. also weaned 6.8% heavier piglets than control sows ($p<0.05$). Daily weight gain and weight gain during the lactation period were 7.4% greater for piglets the piglets that were born from sows fed with *Bacillus spp.*($p<0.05$). Also, mortality tended to be greater in piglets from the probiotic group (probiotic = 3.34 % against control = 4.04%) of sows. ($p<0.10$).

Animal health measurements

Diarrheal presence of the piglets was reduced in the litters that sows were supplemented with *Bacillus spp.* ($p<0.10$ and $p<0.05$) (Table 6). In the last 10 days of lactation period, there was a tendency to reduce the presence of piglets diarrheal in the litter ($p<0.10$). Total period of lactation presented a greater reduction in the presence of diarrheal in the litters that sows received the probiotic ($p<0.05$).

Sow faeces humidity during lactation period was not affected by the presence of probiotics in the diet (Table 7). Total *Bacillus spp.* count in the faeces were greater in the probiotic group for both of categories: sows and piglets ($p<0.05$) (Table 8). In table 9 all results from serum biochemical can be found. During the pre-partum of sows, there was an increase in the ALP in the probiotic group ($p<0.05$). In the pre-weaning phase, sows fed with probiotics tended to decrease glucose levels ($p<0.10$). The supplementation of probiotics for sows did not influence the serum biochemical parameters of the piglets. Colostrum quality tended to present greater values in the sows that were supplemented during gestation ($p<0.10$) (Table 10). Milk assessments did not present any differences in the quality when comparing treated sows to the non-treated ones.

Discussion

Bacillus probiotics are known for several benefits in swine industry, especially in sows. There were 41 studies using *Bacillus* strains for sows, which may corroborate the findings of the present study (Luise et al., 2022).

More than 100 species and subspecies of the genus *Bacillus* were already characterized as gram-positive, aerobic or facultative anaerobic, endospore-forming bacteria (Mingmongkolchai and Panbangred, 2018). The strains used in the composition of probiotics are key factors in the performance of this feed additive in the animal industry. The recombination of different *Bacillus* strains, such as *Licheniformis* and *Subtilis*, is a great tool for creating a specific environment for lactic acid production, which facilitates the exclusion of pH-sensitive pathogens (Baruzzi et al., 2011; Song et al., 2014). The mechanisms behind the effects from strains of Bacillus licheniformis were related to some enzyme production (i.e amylase, lipase, cylanase) and stimulation of innate immunity (i.e production of resveratrol and antioxidants) (Muras et al., 2021)

Performance measurements - Sows

In the present study, *Bacillus* supplementation affected the number of mummified piglets per litter. None of the other performance measurements were affected by the supplementation. A study conducted by Alexopoulos et al. (2004) using the same strains of probiotic of these study. *Bacillus spp.* did not show any differences in the total number of born, stillborn, and mummified piglets. Fetal mummification is a disorder that should be avoided in domestic animals and is common in multiparous animals, such as sows. These abnormalities are primarily caused by genetic and placental defects, infectious agents, and drugs (Vikram et al., 2020). Infectious diseases are the main cause of abortions during gestation in swine, and *Bacillus* strains could modify these responses by the capability of producing antimicrobial peptides that could enhance the health status of the sows (Martirani et al., 2002). However, this was not observed in the current study probably because the high-sanitary-standards of the farm did not impose health challenges to the animals. Other outcomes could be found in more challenges scenarios.

The body condition of the sows was not influenced by the supplementation. There was just a slight decrease in body weight at farrowing, which may be related to the greater piglet birth weight in the sows supplemented with probiotics.

The return-to-estrus interval was reduced by approximately 3 days in the group of sows that were supplemented with *Bacillus spp.* These results corroborate the findings of Gu et al. (2021), where supplementation with *Bacillus* in addition to oligosaccharides shortened the return to estrus interval. Gut and vaginal microbiota diversities play an important role in the return to estrus interval by coordinating sexual hormones, especially estrogens (Zhang et al., 2021). In this study, *Bacillus spp.* were abundant in the feces of sows, indicating a greater abundance of microorganisms in the gut, as reported by Tian et al. (2021). *Bacillus spp* in feces could be positively correlated with prostaglandin concentration and short chain fatty acids at farrowing' sows (Gu et al., 2021). Also, greater concentrations of prostaglandin could improve colostrum intake of piglets and increase litter weight of piglets (Maneetong et al., 2021).

Performance measurements - Piglets

Piglet birth weight increased by approximately 99 g when the sows were supplemented with *Bacillus spp.*, which could be related to greater nutrient absorption by the sows and bioavailability of nutrients for the piglets (Stamati et al., 2006; Mazur-Kuśnirek et al., 2023). Intestine health of sows is not commonly studied and how this could affect the piglets. When *Bacillus spp* were used as additive in weaned pigs, there was a tendency to improve protein digestibility (Mun et al., 2021). And, providing feeding probiotics only for the sow could show an improvement on the piglets' digestibility of dry matter and gross energy (Galli et al., 2024)

The ingestion of higher-quality milk is the most plausible reason for the indirect positive effects of probiotics on piglets at weaning. In fact, the current study showed that colostrum and milk from *Bacillus spp.* treated sows had higher levels of Brix index, indicating a greater sugar content and a more nutritive feed. This theory is also supported by Alexopoulos et al., 2004. A greater Brix index in sow colostrum is positively correlated with antibody concentration in the serum of piglets, which could categorize the immunity status of each litter (Schoos et al., 2021). Also, higher indexes of Brix were related to greater concentrations of immunoglobulin G content of colostrum of sows (Hasan et al., 2016).

Animal health measurements

During the lactation period, piglets that were born from supplemented sows presented a lower frequency of diarrhea. The administration of probiotics is described to be effective in reducing pathogen adhesion to the small intestinal mucosa (Daudelin et al., 2011; Hayakawa et al., 2016). In our study, improvements were found even in the piglets, which suggests that the effect of the probiotics were not exclusive for the sows. This indicates the ability to colonize the gut through mother-son relation, which can be beneficial to reduce number of Enterobacteriaceae and increase the amount of beneficial bacteria (Veljović et al., 2017).

The health status of the sows was changed by probiotics only for the ALT serum parameters in the prepartum period. Better amino acid metabolism has been linked to elevated plasma ALT activity (Liu et al., 2015). This also explains why the total protein in the serum was slightly decreased due to its enhanced ability to degrade amino acids. The enzymes AST and ALT are also involved in several metabolic biochemical events that are related to the conversion of amino acids with other metabolic intermediates. An elevation in their levels may indicate tissue damage, such as in cases of chronic liver disorders (Babazadeh et al., 2011).

Bacillus spp. is a great tool to enhance performance in the swine industry. Feed the sow could be an alternative to have positive effects in the litter. Also, the reduction of the return to estrus interval it’s a great asset for the industry to have more productivity days.

Ethics approval

This study was approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul (CEUA).

References

Alexopoulos, C., Georgoulakis, I. E., Tzivara, A., Kritas, S. K., Siochu, A., & Kyriakis, S. C. (2004). Field evaluation of the efficacy of a probiotic containing Bacillus licheniformis and Bacillus subtilis spores, on the health status and performance of sows and their litters. Journal of animal physiology and animal nutrition, 88(11-12), 381-392.

Babazadeh, D., Vahdatpour, T., Nikpiran, H., Jafargholipour, M. A., & Vahdatpour, S. (2011). Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (Coturnix japonica). The Indian Journal of Animal Sciences, 81(8).

Baruzzi, F., Quintieri, L., Morea, M., & Caputo, L. (2011). Antimicrobial compounds produced by Bacillus spp. and applications in food. Science against microbial pathogens: communicating current research and technological advances, 2(1), 1102-1111.

Daudelin, J. F., Lessard, M., Beaudoin, F., Nadeau, É., Bissonnette, N., Boutin, Y., ... & Fairbrother, J. M. (2011). Administration of probiotics influences F4 (K88)-positive enterotoxigenic Escherichia coli attachment and intestinal cytokine expression in weaned pigs. Veterinary research, 42, 1-11.

FAO, W., 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. 2006. In.

Galli, G. M., Andretta, I., Levesque, C., Stefanello, T., Carvalho, C. L., Perez Pelencia, J. Y., ... & Kipper, M. (2024). Using probiotics to improve nutrient digestibility and gut-health of weaned pigs: a comparison of maternal and nursery supplementation strategies. Frontiers in Veterinary Science, 11, 1356455.

Gu, X., Chen, J., Li, H., Song, Z., Chang, L., He, X., & Fan, Z. (2021). Isomaltooligosaccharide and Bacillus regulate the duration of farrowing and weaning-estrous interval in sows during the perinatal period by changing the gut microbiota of sows. Animal Nutrition, 7(1), 72-83.

Hasan, S. M. K., Junnikkala, S., Valros, A., Peltoniemi, O., & Oliviero, C. (2016). Validation of Brix refractometer to estimate colostrum immunoglobulin G content and composition in the sow. Animal, 10(10), 1728-1733.

Hayakawa, T., Masuda, T., Kurosawa, D., & Tsukahara, T. (2016). Dietary administration of probiotics to sows and/or their neonates improves the reproductive performance, incidence of post-weaning diarrhea and histopathological parameters in the intestine of weaned piglets. Animal Science Journal, 87(12), 1501-1510.

Kritas, S. K., Marubashi, T., Filioussis, G., Petridou, E., Christodoulopoulos, G., Burriel, A. R., ... & Pískoriková, M. (2015). Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (Bacillus subtilis C-3102). Journal of animal science, 93(1), 405-413.

Lambo, M. T., Chang, X., & Liu, D. 2021. The recent trend in the use of multistrain probiotics in livestock production: An overview. Animals, 11(10), 2805.

Li, Qihui et al. Nutritional strategies to alleviate oxidative stress in sows. Animal Nutrition, v. 9, p. 60-73, 2022.

Liu, Y., Kong, X., Jiang, G., Tan, B. E., Deng, J., Yang, X., ... & Yin, Y. (2015). Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. Journal of Animal Science and Biotechnology, 6, 1-10.

Luise, D., Bosi, P., Raff, L., Amatucci, L., Virdis, S., & Trevisi, P. (2022). Bacillus spp. probiotic strains as a potential tool for limiting the use of antibiotics, and improving the growth and health of pigs and chickens. Frontiers in Microbiology, 13, 801827.

Maneetong, P., Srisang, C., Sunanta, N., Muchalintamolee, P., Pearodwong, P., Suwimonteerabutr, J., ... & Tummaruk, P. (2021). Postpartum prostaglandin F2α administration affects colostrum yield, immunoglobulin G, and piglet performance. Animal bioscience, 34(5), 833.

Martirani, L., Varcamonti, M., Naclerio, G., & De Felice, M. (2002). Purification and partial characterization of bacillocin 490, a novel bacteriocin produced by a thermophilic strain of Bacillus licheniformi s. Microbial cell factories, 1, 1-5.

Mazur-Kuśnirek, M., Lipiński, K., Jørgensen, J. N., Hansen, L. H. B., Antoszkiewicz, Z., Zabielski, R., & Konieczka, P. (2023). The Effect of a Bacillus-Based Probiotic on Sow and Piglet Performance in Two Production Cycles. Animals, 13(20), 3163.

Mingmongkolchai, S., & Panbangred, W. (2018). Bacillus probiotics: an alternative to antibiotics for livestock production. Journal of applied microbiology, 124(6), 1334-1346.

Mun, D., Kyoung, H., Kong, M., Ryu, S., Jang, K. B., Baek, J., ... & Kim, Y. (2021). Effects of Bacillus-based probiotics on growth performance, nutrient digestibility, and intestinal health of weaned pigs. Journal of Animal Science and Technology, 63(6), 1314.

Muras, A., Romero, M., Mayer, C., & Otero, A. (2021). Biotechnological applications of Bacillus licheniformis. Critical reviews in biotechnology, 41(4), 609-627.

Pereira, W. A., Franco, S. M., Reis, I. L., Mendonça, C. M., Piazentin, A. C., Azevedo, P. O., ... & Oliveira, R. P. (2022). Beneficial effects of probiotics on the pig production cycle: An overview of clinical impacts and performance. Veterinary Microbiology, 269, 109431.

Schoos, A., De Spiegelaere, W., Cools, A., Pardon, B., Van Audenhove, E., Bernaerdt, E., ... & Maes, D. (2021). Evaluation of the agreement between Brix refractometry and serum immunoglobulin concentration in neonatal piglets. Animal, 15(1), 100041.

Song, J., Xiao, K., Ke, Y. L., Jiao, L. F., Hu, C. H., Diao, Q. Y., ... & Zou, X. T. (2014). Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poultry science, 93(3), 581-588.

Stamati, S., Alexopoulos, C., Siochu, A., Saoulidis, K., & Kyriakis, S. C. (2006). Probiosis in sows by administration of Bacillus toyoi spores during late pregnancy and lactation: effect on their health status/performance and on litter characteristics. International Journal of Probiotics and Prebiotics, 1(1), 33.

Tian, Z., Wang, X., Duan, Y., Zhao, Y., Zhang, W., Azad, M. A. K., ... & Kong, X. (2021). Dietary supplementation with Bacillus subtilis promotes growth and gut health of weaned piglets. Frontiers in veterinary science, 7, 600772.

Veljović, K., Dinić, M., Lukić, J., Mihajlović, S., Tolinački, M., Živković, M., ... & Terzić-Vidojević, A. (2017). Promotion of early gut colonization by probiotic intervention on microbiota diversity in pregnant sows. Frontiers in Microbiology, 8, 276630.

Vikram, R., Joshi, V., Khatti, A., Babu, M., Biam, K. P., & Barman, D. (2020). Fetal mummification in domestic animals: A critical review.

Wang, C., Wei, S., Xu, B., Hao, L., Su, W., Jin, M., & Wang, Y. (2021). Bacillus subtilis and Enterococcus faecium co-fermented feed regulates lactating sow's performance, immune status and gut microbiota. Microbial Biotechnology, 14(2), 614-627.

Zhang, J., Liu, M., Ke, S., Huang, X., Fang, S., He, M., ... & Huang, L. (2021). Gut and vagina microbiota associated with estrus return of weaning sows and its correlation with the changes in serum metabolites. Frontiers in Microbiology, 12, 690091.

TABLES

Table 1. Chemical composition (as-fed) of the experimental diets to sows.

Ingredients, %	Sow diet[1]	
	Gestation	**Lactation**
Corn (8% CP)	81.20	66.42
Soybean meal (48% CP)	15.02	28.05
Soybean oil	1.425	1.425
Dicalcium phosphate	0.878	1.184
Calcitic limestone	1.637	1.104
Salt	0.421	0.502
Premix[1]	0.500	0.500
L-Lysine (65%)	0.168	-
L-Valine	-	0.158
L-Threonine	0.100	0.128
DL- Methionine	0.049	0.100
Tryptophan	0.005	0.050
Phytase[2]	0.005	0.005
Carbohydrase[3]	-	0.005

[1]The requirements of each phase were estimated using the Brazilian Tables of Poultry and Swine (Rostagno, 2017).
[2] Premix with vitamins and minerals. Vitamin A:10.000.000,0 IU/kg; vitamin B_1: 1.600,00 mg/kg; vitamin B_2:6 mg/kg; vitamin K_3 6.600,00 mg/kg; vitamin B_6: 1.600,00 mg/kg; vitamin B_{12} 24.000,00 mcg/kg; vitamin D_3:1.800.00,00 IU/kg; vitamin E: 20.200,00 IU/kg; folic acid 1.2 mg/kg; niacin: 80.00g/kg; biotin 200.000 mg/kg; pantothenic acid: 12.00 g/kg; Iron 60 mg/kg; Zinc 70 mg/kg; Copper 140 mg/kg; Calcium 273 mg/kg; Phosphorus 150 mg/kg.
[3] Axtra PHY GOLD, Danisco Nutrition.
[4] Rovabio, Adisseo.

Table 2. Performance responses of sows supplemented with *Bacillus spp.* probiotic.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotic**	
Sows, n	95	95	-
Piglets, n	1341	1293	-
Parity order	4.3	4.1	-
Gestation period, days	114.3 (0.151)	114.1 (0.151)	0.260
Total born, n/litter	14.11 (0.435)	13.61 (0.435)	0.411
Total born alive, n/litter	13.13 (0.392)	12.91 (0.392)	0.690
Stillborn, n/litter	0.600 (0.097)	0.489 (0.097)	0.423
Mummified, n/litter	0.378 (0.082)	0.236 (0.083)	0.073

[1] Means with standard errors in the parentheses.
[2] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 3. Body condition of sows supplemented with *Bacillus spp.* probiotic.

Variables[1]	Sow treatment[2]		*P*-value[3]
	Control	Probiotic	
Body weight			
Beginning of gestation, kg	213.0 (2.557)	208.7 (2.484)	0.229
Farrowing, kg	243.4 (2.148)	238.0 (2.125)	0.078
Weaning, kg	229.4 (2.304)	226.2 (2.304)	0.336
Variation during gestation, kg	30.87 (1.715)	32.23 (1.694)	0.574
Variation during lactation, kg	-13.56 (1.750)	-11.65 (1.730)	0.438
Caliper measurement			
Beginning of gestation	1.576 (0.086)	1.674 (0.081)	0.614
Day 90 of gestation	2.516 (0.066)	2.452 (0.065)	0.782
Farrowing	2.158 (0.085)	2.126 (0.081)	0.899
Weaning	1.761 (0.082)	1.777 (0.079)	0.937
Variation during gestation	0.581 (0.083)	0.523 (0.077)	0.607
Variation during lactation	-0.384 (0.091)	-0.328 (0.080)	0.645
Backfat thickness			
Beginning of gestation, mm	9.236 (0.407)	8.768 (0.381)	0.404
Day 90 of gestation, mm	10.09 (0.432)	9.509 (0.393)	0.320
Farrowing, mm	9.881 (0.369)	9.542 (0.350)	0.507
Weaning, mm	8.113 (0.262)	8.272 (0.253)	0.663
Variation during gestation, mm	0.560 (0.444)	0.412 (0.416)	0.768
Variation during lactation, mm	-1.807 (0.327)	-1.310 (0.290)	0.257

[1] Means with standard errors in the parentheses.
[2] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 4. Performance responses of sows on the subsequent cycle to the supplementation with *Bacillus spp*[1].

Variables[2]	Sow treatment[3]		*P*-value[4]
	Control	Probiotic	
Total born, n/litter	15.04 (0.502)	14.18 (0.522)	0.191
Total born alive, n/litter	14.25 (0.466)	13.33 (0.484)	0.134
Stillborn, n/litter	0.650 (0.104)	0.655 (0.109)	0.972
Mummified, n/litter	0.217 (0.063)	0.188 (0.066)	0.730
Return to estrus interval, days	7.257 (0.674)	4.114 (0.674)	0.001

[1]Probiotic supplementation was not provided in the subsequent gestation phase.
[2] Means with standard errors in the parentheses.
[3] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[4] Probability of treatment effect.

Table 5. Effect of *Bacillus spp.* supplementation to gestating-lactating sows on the piglet overall performance.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotic**	
Birth weight, kg	1.339 (0.039)	1.438 (0.038)	0.044
Birth weight alive, kg	1.356 (0.039)	1.445 (0.039)	0.076
Daily weight gain, g/day	194.1 (5.683)	208.5 (5.065)	0.003
Weight gain, kg	4.078 (0.119)	4.379 (0.106)	0.030
Weight at 21 days, kg	5.477 (0.134)	5.853 (0.119)	0.002
Mortality, %	4.045 (0.630)	3.341 (0.626)	0.094

[1] Means with standard errors in the parentheses.
[2] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 6. Effect of *Bacillus* spp. supplementation to gestating-lactating sows on the diarrheal presence[1] of piglets.

Variables[2]	**Sow treatment[3]**		***P*-value[4]**
	Control	**Probiotic**	
1 to 7 days, %	21.53 (0.095)	18.66 (0.102)	0.198
7 to 21 days, %	47.17 (0.077)	40.92 (0.079)	0.064
Total period, %	34.39 (0.057)	30.54 (0.060)	0.031

[1]Diarrheal presence was estimated in percentage of daily observation (litters showing clinical signals over the total number of litters evaluated).
[2] Means with standard errors in the parentheses.
[3] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[4] Probability of treatment effect

Table 7. Effect of *Bacillus spp.* supplementation to gestating-lactating sows on the feces humidity of sows during lactation period.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotic**	
3 days post-partum	41.06 (1.596)	39.64 (1.666)	0.546
7 days post-partum	33.69 (2.043)	34.07 (1.560)	0.882
14 days post-partum	32.88 (1.336)	32.06 (1.118)	0.643
21 days post-partum	35.97 (1.960)	34.81 (1.773)	0.664
Total period	36.64 (0.997)	35.19 (0.889)	0.281

[1] Means with standard errors in the parentheses.
[2] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 8. Total *Bacillus* count in the feces of sows and piglets[1] due to the supplementation with *Bacillus spp.*

Variables[1]	**Sow treatment[2]**		**P-value[3]**
	Control	**Probiotic**	
Sows, UFC/g	6.95×10^3 (0.196)	7.98×10^5 (0.196)	<0.001
Piglets, UFC/g	4.45×10^3 (0.366)	2.71×10^5 (0.366)	<0.001

[1]Samples collected to represent the entire litter.
[2] Supplemented with BioPlus 2B (400 g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 9. Effect of *Bacillus spp.* on the serum biochemical values of gestating-lactating sows and piglets.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotic**	
Sows pre-partum			
AST, U/L	37.75 (7.141)	36.40 (6.387)	0.891
ALP, U/L	398.2 (75.72)	699.8 (77.63)	0.036
Glucose, mg/dL	125.3 (4.928)	119.0 (6.234)	0.439
Cholesterol, mg/dL	60.26 (8.467)	48.11 (9.777)	0.366
Total protein, g/dL	7.400 (0.550)	6.466 (0.636)	0.289
Albumin, g/dL	3.250 (0.219)	3.933 (0.308)	0.119
Sows pre-weaning			
AST, U/L	70.28 (7.917)	77.83 (8.551)	0.530
ALP, U/L	1983 (237.2)	1920 (318.2)	0.965
Glucose, mg/dL	242.6 (10.82)	212.0 (12.50)	0.088
Cholesterol, mg/dL	120.8 (15.53)	122.20 (19.02)	0.956
Total protein, g/dL	4.777 (0.186)	4.466 (0.229)	0.311
Albumin, g/dL	4.544 (0.181)	4.200 (0.222)	0.251
Piglets pre- weaning			
AST, U/L	33.50 (3.821)	38.25 (3.821)	0.394
ALP, U/L	439.4 (68.75)	274.8 (77.95)	0.135
Glucose, mg/dL	260.8 (6.522)	253.8 (6.918)	0.472
Cholesterol, mg/dL	81.84 (4.876)	74.97 (5.529)	0.367
Total protein, g/dL	5.988 (0.101)	5.987 (0.107)	0.992
Albumin, g/dL	5.466 (0.140)	5.400 (0.159)	0.757

[1] Means with standard errors in the parentheses.
[2] Supplemented with BioPlus 2B (400g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.

Table 10. Effect of *Bacillus spp.* supplementation to gestating-lactating sows on the total soluble solids (Brix content) of colostrum and milk.

Variables[1]	**Sow treatment**[2]		***P*-value**[3]
	Control	**Probiotic**	
Postpartum, %	21.81 (0.98)	24.94 (1.18)	0.091
Seven days postpartum, %	12.83 (0.46)	13.90 (0.39)	0.310
Fourteen days postpartum, %	11.17 (0.74)	12.55 (0.46)	0.402

[1] Means with standard errors in the parentheses. Means represent 52 samples for postpartum and 15 samples for 7 and 14 days postpartum.
[2] Supplemented with BioPlus 2B (400g/ton of feed) during gestation and lactation.
[3] Probability of treatment effect.
.

CONSIDERAÇÕES FINAIS

Aditivos alimentares são ferramentas de extrema importância para a produção animal. São eles que encontram alternativas para métodos já obsoletos e nem tão aceitos pela sociedade, tais como os antibióticos promotores de crescimento.

Estre trabalho trouxe respostas para um dos aditivos alimentares que vem ganhando espaço na indústria, que são os probióticos. Ainda, este estudo foi realizado em uma categoria animal pouco explorada na suinocultura, que é fase de gestação e lactação das matrizes suínas. O uso de ambos os probióticos mostrou-se eficaz na qualidade do leitão gerado por essas matrizes suínas, aumentando seu peso ao nascer e consequentemente ao desmame. A qualidade do colostro também foi positiva para o uso dos aditivos.

O uso de probióticos é uma excelente ferramenta para ser utilizado em uma fase tão delicada como a gestação e lactação de matrizes suínas. Seu uso é benéfico para ambas as partes: matriz suína e leitão.

REFERÊNCIAS

AHASAN, A. *et al*. The beneficial role of probiotics in monogastric animal nutrition and health. **Journal of Dairy, Veterinary & Animal Research**, Budapest, v. 2, n. 4, p. 116-132, 2015.

ALEXOPOULOS, C. *et al*. Field evaluation of the efficacy of a probiotic containing Bacillus licheniformis and Bacillus subtilis spores, on the health status and performance of sows and their litters. **Journal of Animal Physiology and Animal Nutrition**, Berlin, v. 88, n. 11/12, p. 381-392, 2004.

ARORA, T.; SINGH, S.; SHARMA, R. K. J. N. Probiotics: interaction with gut microbiome and antiobesity potential. **Nutrition**, Tarrytown, v. 29, n. 4, p. 591-596, 2013.

BAGCHI, T. J. T. Traditional food & modern lifestyle: impact of probiotics. **The Indian Journal of Medical Research**, Mumbai, v. 140, n. 3, p. 333-335, 2014.

BAJAGAI, Y. S. *et al*. **Probiotics in animal nutrition:** production, impact and regulation. Rome: FAO, 2016. (FAO Animal Production and Health Paper, no. 179).

BAUER, E. *et al*. Influence of dietary components on development of the microbiota in single-stomached species. **Nutrition Research Reviews**, Cambridge, v. 19, n. 1, p. 63-78, 2006.

CHEN, X. *et al*. Probiotic yeast inhibits VEGFR signaling and angiogenesis in intestinal inflammation. **PLoS One**, San Francisco, v. 8, n. 5, [art.] e64227, 2013.

CHO, J. H.; ZHAO, P. Y.; KIM, I.-S. Probiotics as a dietary additive for pigs: a review. **Journal of Animal and Veterinary Advances**, Faisalabad, v. 10, p. 2127-2134, 2011.

DIXIT, Y.; WAGLE, A.; VAKIL, B. Patents in the field of probiotics, prebiotics, synbiotics: a review. **Journal of Food: Microbiology, Safety & Hygiene**, Tokyo, v. 1, [art.] 2, [p. 1-13], 2016.

DOWARAH, R. *et al.* Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. **Livestock Science**, Amsterdam, v. 195, p. 74-79, 2017.

EID, R. *et al*. Potential antimicrobial activities of probiotic Lactobacillus strains isolated from raw milk. **Journal of Probiotics & Health**, Los Angeles, v. 4, n. 2, [art.] 1000138, 2016.

ELSHAGHABEE, Fouad M. F. *et al.* Bacillus as potential probiotics: status, concerns, and future perspectives. **Frontiers in Microbiology**, Lausanne, v. 8, [art.] 271541, 2017.

FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **Probiotics in food:** health and nutritional properties and guidelines for evaluation. Rome: FAO, 2006. (Food and nutrition paper, 85).

FOUHSE, J.; ZIJLSTRA, R.; WILLING, B. P. The role of gut microbiota in the health and disease of pigs. **Animal Frontiers**, Lausanne, v. 6, n. 3, p. 30-36, 2016.

FULLER, R. **Probiotics:** the scientific basis. Dordrecht: Springer, 2012.

GHANI, Maria *et al*. Isolation and characterization of different strains of Bacillus licheniformis for the production of commercially significant enzymes. **Pakistan Journal of Pharmaceutical Sciences**, Karachi, v. 26, n. 4, p. 691-697, 2013.

HOLMAN, D. B. *et al*. Meta-analysis to define a core microbiota in the swine gut. **Microbial Systems**, Washington, DC, v. 2, n. 3, [art.] e00004-17, 2017.

HOLZAPFEL, W. H.; SCHILLINGER, U. Introduction to pre-and probiotics. **Food Research International**, Ottawa, v. 35, n. 2/3, p. 109-116, 2002.

HUGHES, P.; HERITAGE, J. **Antibiotic growth-promoters in food animals**. Rome: FAO, 2004. (FAO Animal Production and Health paper, 160).

INNAMMA, N.; NGAMWONGSATIT, N.; KAEOKET, K. The effects of using multi-species probiotics in late-pregnant and lactating sows on milk quality and quantity, fecal microflora, and performance of their offspring. **Veterinary World**, Rajkot, v. 16, n. 10, p. 2055-2062, 2023.

JIN, L. *et al.* Digestive and bacterial enzyme activities in broilers fed diets supplemented with Lactobacillus cultures. **Poultry Science**, Champaign, v. 79, n. 6, p. 886-891, 2000.

KENNY, M. *et al*. Probiotics–do they have a role in the pig industry? **Animal**, Cambridge, v. 5, n. 3, p. 462-470, 2011.

KIM, H. B.; ISAACSON, R. E. The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. **Veterinary Microbiology**, Amsterdam, v. 177, n. 3/4, p. 242-251, 2015.

KOBYLIAK, N. *et al*. Probiotics in prevention and treatment of obesity: a critical view. **Nutrition & Metabolism**, London, v. 13, [art.] 14, [p. 1-13], 2016.

LANGE, C. *et al*. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. **Livestock Science**, Amsterdam, v. 134, n. 1/3, p. 124-134, 2010.

LE BON, M. *et al*. Influence of probiotics on gut health in the weaned pig. **Livestock Science**, Amsterdam, v. 133, n. 1/3, p. 179-181, 2010.

LESCHEID, David W. Probiotics as regulators of inflammation: a review. **Functional Foods in Health and Disease**, Dallas, v. 4, n. 7, p. 299-311, 2014.

LI, Qihui *et al*. Nutritional strategies to alleviate oxidative stress in sows. **Animal Nutrition**, Beijing, v. 9, p. 60-73, 2022.

LIAO, S. F.; NYACHOTI, M. Using probiotics to improve swine gut health and nutrient utilization. **Animal Nutrition**, Beijing, v. 3, n. 4, p. 331-343, 2017.

LILLY, D. M.; STILLWELL, R. H. Probiotics: growth-promoting factors produced by microorganisms. **Science**, Washington, DC, v. 147, n. 3659, p. 747-748, 1965.

LUISE, D. *et al*. A systematic review of the effects of functional amino acids on small intestine barrier function and immunity in piglets. **Animal**, Cambridge, [art.] 100771, 2023. Supl. 2.

LUTGENDORFF, F.; AKKERMANS, L. M. A.; SODERHOLM, J. D. The role of microbiota and probiotics in stress-induced gastrointestinal damage. **Current Molecular Medicine**, Boca Raton, v. 8, n. 4, p. 282-298, 2008.

MA, T. *et al.* Targeting gut microbiota and metabolism as the major probiotic mechanism-an evidence-based review. **Trends in Food Science & Technology**, Kidlington, v. 138, p. 178-198, 2023.

MENG, Q. W. *et al*. Influence of probiotics in different energy and nutrient density diets on growth performance, nutrient digestibility, meat quality, and blood characteristics in growing-finishing pigs. **Journal of Animal Science**, Champaign, v. 88, n. 10, p. 3320-3326, 2010.

MOHAMMED, Yousef *et al*. Development of a two-step cultivation strategy for the production of vitamin B12 by Bacillus megaterium. **Microbial Cell Factories**, London, v. 13, p. 1-10, 2014.

MONTEAGUDO-MERA, A. *et al*. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. **Applied Microbiology and Biotechnology**, Berlin, v. 103, n. 16, p. 6463-6472, 2019.

NG, S. *et al.* Mechanisms of action of probiotics: recent advances. **Inflammatory Bowel Diseases**, New York, v. 15, n. 2, p. 300-310, 2009.

NGUYEN, H.-T. *et al*. Biochemical engineering approaches for increasing viability and functionality of probiotic bacteria. **International Journal of Molecular Sciences**, Basel, v. 17, n. 6, [art.] 867, 2016.

NGUYEN, D. H.; LAN, R. X.; KIM, I. H. Bacillus subtilis, essential oil, chromium and glucose as sow pack alters the performance, immune and stress on pregnant sows and piglets. **Indian Journal of Animal Research**, Karnal, v. 52, n. 8, p. 1174-1179, 2018.

NRC - NATIONAL RESEARCH COUNCIL **Nutrient requirements of swine**. Washington, DC: NRC, 2012.

OELSCHLAEGER, T. A. Mechanisms of probiotic actions–a review. **International Journal of Medical Microbiology**, Jena, v. 300, n. 1, p. 57-62, 2010.

ONYENWEAKU, F. *et al.* Health benefits of probiotics. **International Journal of Innovation and Applied Studies**, Morocco, v. 4, n. 3, p. 21-30, 2016.

PAMER, E. G. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. **Science**, Washington, DC, v. 352, n. 6285, p. 535-538, 2016.

PLUMMER, S. F. *et al*. Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. **International Journal of Antimicrobial Agents**, Amsterdam, v. 26, n. 1, p. 69-74, 2005.

POLLMANN, D. S. Probiotics in pig diets. *In*: HARESIGN, W.; COLE, D. J. A. (ed.). **Recent advances in animal nutrition**. London: Butterworth-Heinemann, 1986. p. 193–205.

POSPÍŠKOVÁ, P. *et al*. Effect of probiotics in the pig nutrition on the pathogenic bacteria counts in the gut. **Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis**, Brünn, v. 61, n. 6, p. 1839-1843, 2013.

QUESNEL, Hélène; FARMER, Chantal; DEVILLERS, Nicolas. Colostrum intake: Influence on piglet performance and factors of variation. **Livestock Science**, Amsterdam, v. 146, n. 2/3, p. 105-114, 2012.

ROSTAGNO, H. S. *et al.* **Tabelas brasileiras para aves e suínos:** composição de alimentos e exigências nutricionais. 4. ed. Viçosa, MG: Departamento de Zootecnia, Universidade Federal de Viçosa, 2017. v. 488.

SIMON, O.; JADAMUS, A.; VAHJEN, W. Probiotic feed additives - effectiveness and expected modes of action. **Journal of Animal and Feed Sciences**, Jabłonna, v. 10, p. 51-67, 2001. Supl. 1.

SOLÀ-ORIOL, D.; GASA, J. Feeding strategies in pig production: Sows and their piglets. **Animal feed Science and Technology**, New York, v. 233, p. 34-52, 2017.

SORNPLANG, P.; PIYADEATSOONTORN, S. Probiotic isolates from unconventional sources: a review. **Journal of Animal Science and Technology**, Seoul, v. 58, [art.] 26, [p. 1-11], 2016.

TAN, B. F.; LIM, T.; BOONTIAM, W. Effect of dietary supplementation with essential oils and a Bacillus probiotic on growth performance, diarrhoea and blood metabolites in weaned pigs. **Animal Production Science**, Melbourne, v. 61, n. 1, p. 64-71, 2020.

TANAKA, K.; TAKANAKA, S.; YOSHIDA, K. A second-generation Bacillus cell factory for rare inositol production. **Bioengineered**, Philadelphia, v. 5, n. 5, p. 331-334, 2014.

TORTUERO, F. *et al.* Response of piglets to oral administration of lactic acid bacteria. **Journal of Food Protection**, New York, v. 58, n. 12, p. 1369-1374, 1995.

TSUKAHARA, T. *et al*. Probiotic supplementation improves reproductive performance of unvaccinated farmed sows infected with porcine epidemic diarrhea virus. **Animal Science Journal**, Tokyo, v. 89, n. 8, p. 1144-1151, 2018.

TURFKRUYER, M.; VERHASSELT, V. Breast milk and its impact on maturation of the neonatal immune system. **Current Opinion in Infectious Diseases**, London, v. 28, n. 3, p. 199-206, 2015.

VEIZAJ-DELIA, E.; PIRUSHI, R. The utilization of probiotics as a way to improve human and animal gut health. **Macedonian Journal of Animal Science**, Skopje, v. 2, p. 175-181, 2012.

WESTERMANN, C. *et al*. A critical evaluation of bifidobacterial adhesion to the host tissue. **Frontiers in Microbiology**, Lausanne, v. 7, [art.] 1220, 2016.

WILLIAMS, B. A.; VERSTEGEN, M. W. A; TAMMINGA, S. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. **Nutrition Research Reviews**, Cambridge, v. 14, n. 2, p. 207-228, 2001.

WILLING, B. P.; MALIK, G.; VAN KESSEL, A. G. Nutrition and gut health in swine. *In*: CHIBA, L. I. (ed.). **Sustainable swine nutrition**. New York: John Wiley, 2012. cap. 8, p. 197-213.

YANG, J. *et al*. Antibacterial action of selenium-enriched probiotics against pathogenic *Escherichia coli*. **Digestive Diseases and Sciences**, New York, v. 54, n. 2, p. 246-254, 2009.

YIRGA, H. The use of probiotics in animal nutrition. **Journal of Probiotics & Health**, Los Angeles, v. 3, n. 2, p. 1-10, 2015.

ZHANG, M. *et al*. Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. **Frontiers in Immunology**, Lausanne, v. 8, [art.] 942, 2017.

ZHANG, Qianqian *et al*. Dietary supplementation of Bacillus subtilis PB6 improves sow reproductive performance and reduces piglet birth intervals. **Animal Nutrition**, Beijing, v. 6, n. 3, p. 278-287, 2020.

ZIMMERMANN, J. A. *et al*. Effects of probiotics in swine growth performance: a meta-analysis of randomized controlled trials. **Animal Feed Science and Technology**, New York, v. 219, p. 280-293, 2016.