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MELODY MARTINS CAVALCANTE PEREIRA

EFEITOS DE BACTÉRIAS PROBIÓTICAS SOBRE O BEM-ESTAR DE MATRIZES SUÍNAS E DE LEITÕES LACTENTES

Porto Alegre
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EFEITOS DE BACTÉRIAS PROBIÓTICAS SOBRE O BEM-ESTAR DE MATRIZES SUÍNAS E DE LEITÕES LACTENTES

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

Orientadora: Prof.ª Dr.ª Andréa Machado Leal Ribeiro

Coorientadora: Prof.ª Dr.ª Ines Andretta

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RESUMO

Os benefícios dos probióticos para o bem-estar animal estão sendo cada vez mais estudados. Porém, sabe-se que a gestação e a lactação são períodos críticos de grande demanda para as fêmeas, o que pode levar ao aumento do estresse. Portanto, este estudo investigou os efeitos de diferentes aditivos probióticos em dois experimentos diferentes sobre aspectos multifatoriais do bem-estar das porcas e da vitalidade dos leitões. Em ambos os estudos, os dados foram analisados por ANOVA utilizando PROC GLIMMIX (software SAS 9.3). Primeiramente, 147 porcas multíparas foram utilizadas em um estudo contendo probióticos com múltiplas cepas *(Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium* e *Streptococcus thermophilus*), fornecido via capsulas de gelatina industriais. Comportamentos estereotipados (mastigação simulada, rolar a língua, morder a barra e lamber o chão), postura corporal (em pé, deitado, inclinado para frente e sentado) e o teste observacional de relacionamento humano-animal foram avaliados durante a gestação. Um subgrupo de 35 porcas foi avaliado quanto à duração do parto e vitalidade dos leitões. A ingestão de colostro foi estimada com base no peso do leitão ao nascimento e 24 horas após o nascimento. O consumo de ração e os escores fecais das porcas também foram analisados. Os níveis de cortisol salivar das porcas foram avaliados cinco dias após do parto e foram coletadas amostras de sangue para quantificação de serotonina nos mesmos animais após a coleta de saliva. Aos 14 dias de idade, foi realizada análise comportamental back-test em todos os leitões da ninhada. Ao final do período experimental, 11 leitões foram eutanasiados e analisados os pesos dos órgãos (baço, timo, coração e glândulas adrenais). As porcas suplementadas exibiram melhor índice de relação humano-animal (P=0,017). A frequência das estereotipias não foi afetada pelos tratamentos. Contudo, as porcas suplementadas passaram mais tempo em pé (P=0,054) e menos tempo sentadas (P=0,008). O nível de cortisol daquelas suplementadas foi 50% menor (P=0,047) e de serotonina 11% maior (P=0,034). Fêmeas suplementadas tiveram partos mais curtos (P=0,004). As leitegadas de porcas suplementadas tiveram maior consumo de colostro (P=0,036) e, consequentemente, maior peso 24 horas e 14 dias pós-parto. O escore fecal das porcas lactantes não foi afetado pelos tratamentos. O consumo de ração das porcas suplementadas foi maior (10,09%; P=0,067). No back-test, leitões nascidos de fêmeas suplementadas foram menos resistentes (P=0,076). Finalmente, os pesos dos órgãos dos leitões não foram afetados. Os probióticos provaram serem benéficos na melhoria do bem-estar das porcas e do colostro. No estudo que testou um produto à base de cepas de Bacillus (*Bacillus subtilis* e *Bacillus licheniformis*), 35 fêmeas multíparas foram utilizadas para avaliar a duração do parto, o consumo de ração, o escore fecal e a vitalidade, bem como o consumo de colostro dos seus leitões. Houve tendência na redução do tempo de expulsão dos leitões (P=0,065) e do tempo total de parto (P=0,095), quando comparado ao grupo controle sem suplementação. A ingestão estimada de colostro dos leitões de porcas suplementadas foi maior (P=0,014). As porcas suplementadas também tenderam a menor frequência de fezes úmidas (P=0,078). Porém, os tratamentos não afetaram a vitalidade dos leitões. As cepas de Bacillus mostram efeito na melhoria da eficiência do parto e aumentam indiretamente a ingestão de colostro. Portanto, a aplicação dos probióticos abrange não apenas variáveis de produção, mas também aquelas indiretamente relacionadas ao sucesso da indústria, como o bem-estar animal.

Palavras-chave: porcas, microbiota, eixo microbiota-intestino-cérebro, parto, consumo de colostro, comportamento.

ABSTRACT

The benefits of probiotics for animal welfare are being increasingly studied. However, it is known that pregnancy and lactation are critical periods of great demand for females, which can lead to increased stress.Therefore, this study investigated the effects of different probiotic additives in two different experiments on multifactorial aspects of sow welfare and piglet vitality. In both studies, the data were analyzed by ANOVA using PROC GLIMMIX (SAS 9.3 software). First, 147 multiparous sows were used in a study containing probiotics with multiple strains (*Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium, and Streptococcus thermophilus*). Stereotypical behaviors (sham chewing, tongue rolling, bar biting, and floor licking), body posture (standing, lying down, lean forward and sitting) and the observational human-animal relationship test were evaluated during pregnancy. A subgroup of 35 sows was evaluated for farrowing duration and piglet vitality. Colostrum intake was estimated based on piglet weight at birth and 24 hours after birth. Feed intake and fecal scores of the sows were also analyzed. Sow salivary cortisol levels were assessed five days after farrowing, and blood samples were collected for the serotonin quantification in the same animals after the saliva sampling. At 14 days of age, back-test behavioral analysis was performed on all piglets in the litter. At the end of the experimental period, 11 piglets were euthanized, and the weights of the organs (spleen, thymus, heart, and adrenal glands) were analyzed. Supplemented sows exhibited a better human-animal relationship index (P=0.017). The frequency of stereotypies was not affected by the treatments. However, the supplemented sows spent more time standing (P=0.054) and less time sitting (P=0.008). The cortisol level of those supplemented was 50% lower (P=0.047) and serotonin 11% higher (P=0.034). Supplemented females had shorter birth times (P=0.004). Litters from supplemented sows had greater colostrum intake (P=0.036) and, consequently, greater weight 24 hours and 14 days postpartum. The fecal score of lactating sows was not affected by the treatments. The feed intake of supplemented sows was higher (10.09%; P=0.067). In the back-test, piglets born to supplemented females were less resistant (P=0.076). Finally, the weights of the piglet organs were not affected. Probiotics have proven beneficial in improving sow welfare and colostrum welfare. In a study testing a product based on Bacillus strains (*Bacillus subtilis* and *Bacillus licheniformis*), 35 multiparous females were used to evaluate the duration of parturition, feed intake, fecal score, and vitality, as well as colostrum intake of their piglets. There was a tendency towards a reduction in piglet expulsion time (P=0.065) and total farrowing time (P=0.095), when compared to the control group without supplementation. The estimated colostrum intake of piglets from supplemented sows was higher (P=0.014). Supplemented sows also tended to have a lower frequency of wet feces (P=0.078). However, the treatments did not affect the vitality of the piglets. Bacillus strains show an effect on improving farrowing efficiency and indirectly increase colostrum intake. Therefore, probiotics applications covers not only production variables but also those indirectly related to the success of the industry, such as animal welfare.

Keywords: sows, microbiota, microbiota-gut-brain axis, farrowing, colostrum intake, behavior.

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LISTA DE ABREVIATURAS E SIGLAS

EROs - Espécies reativas de oxigênio

HPA - Eixo hipotálamo-pituitária-adrenal

AGCC - Ácidos graxos de cadeia-curta

SAM - Eixo simpático adrenal medular

GABA - Ácido gama-aminobutírico

IMC - Eixo intestino-microbiota-cérebro

GMB - Gut-microbiota-brain axis

CNS - Central nervous system

FI - Feed intake

ACTH - Adrenocorticotropic hormone

CRF - Corticotropin-releasing hormone

HAR - Human-animal relationship

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1. INTRODUÇÃO

Os probióticos são definidos como microrganismos vivos e são amplamente utilizados na suinocultura (WANG et al., 2014). O número de estudos que vêm sendo realizados sobre o efeito de probióticos para esta espécie é crescente, tendo em vista as vantagens proporcionadas ao setor (MATHIPA; THANTSHA, 2017). Probióticos podem promover benefícios ao organismo do hospedeiro, por meio da modulação da microbiota entérica e da resposta imune (FAO, 2002). Além disso, estudos mostram que bactérias probióticas são capazes de agir sobre o sistema neuroendócrino (HMAR., 2024). Essa influência dos probióticos pode se dar através do eixo intestino-microbiota-cérebro e essa é uma das questões mais discutidas da atualidade (KIERNAN et al., 2023).

A preocupação em torno da utilização excessiva de antibióticos, frente ao desenvolvimento da resistência antimicrobiana de patógenos, também é um dos pilares de interesse geral quando se inclui probióticos na dieta de suínos (LIU et al., 2024). Mas em adição, o compromisso com o bem-estar de suínos é outro alvo crescente da cadeia produtiva (KELLS, 2022). Portanto, vários estudos são realizados utilizando probióticos como psicobióticos, visando avaliar seus efeitos e de seus metabólitos na melhoria de condições de estresse, depressão ou ansiedade (SIQUEIRA., 2022).

A categoria de matrizes reprodutoras sofre prejuízo no bem-estar devido a fatores estressores advindos de fontes intrínsecas ou extrínsecas ao animal. Sobre estes primeiros, pode-se citar a hiperprolificidade de genéticas modernas e seu reflexo sobre o maior tempo de parto, além da produção de espécies reativas de oxigênio (EROs), devido a energia requerida por matrizes hiperprolíficas, podendo resultar no aumento do estresse oxidativo de porcas (LEE et al., 2023; LAGOLA, 2022). O próprio periparto também é considerado uma fonte intrínseca de estresse devido a ocorrência de desordens metabólicas e inflamações sistêmicas, que podem causar disbioses (BJORKMAN et al., 2023). Alterações drásticas na microbiota e o estresse crônico podem impactar negativamente porcas em relação a índices reprodutivos, padrões cognitivos e comportamentais, assim como a vitalidade de suas progênies (BARBA-VIDAL et al., 2019).

Como exemplo de fatores extrínsecos, o controle da ambiência nos alojamentos, assim como as próprias instalações (alojamento individual, coletivo) afetam positiva ou negativamente a saúde mental de porcas (PELTONIENI et al., 2021). Quando inadequados, tais fatores podem afetam o consumo de ração ou água, causar distúrbios comportamentais como estereotipias, além de prejuízos reprodutivos (TUMMARUK et al., 2023; MARTÍNEZ-MIRÓ et al., 2016; SPOOLDER et al., 2015).

Assim, a inclusão de aditivos alimentares como os probióticos poderiam influenciar a modulação da microbiota presente no intestino, capaz de estimular a produção de substâncias neuroquímicas que poderiam contribuir para a melhoria de bem-estar de matrizes (LYTE, LYTE et al., 2019).

Portanto, este trabalho foi desenvolvido para avaliar os efeitos da inclusão de aditivos probióticos na dieta de matrizes gestantes e lactantes, sobre aspectos comportamentais, de bem-estar e vitalidade de leitões neonatos.

2. REVISÃO BIBLIOGRÁFICA

2.1 Impacto do estresse em matrizes suínas gestantes e lactantes

Fêmeas suínas passam por inúmeros momentos estressores ao longo de suas vidas, principalmente nos períodos de gestação até o desmame, onde elevam o estresse oxidativo sistêmico (ISON et al., 2018). O estresse pode ser definido de diferentes formas. De acordo com a ciência do comportamento, está envolvido com a percepção de um indivíduo sobre seu tratamento, podendo resultar em ansiedade, desconforto e tensão emocional (MCEWEN., 2017). Em termos neuroendócrinos, o estresse pode ser entendido como qualquer estímulo que provoca liberação de hormônios adrenocorticotrópicos e glicocorticóides adrenais (MARTÍNEZ-MIRÓ ET AL., 2016). Do ponto de vista sociológico, estresse quando exacerbado, trata-se de qualquer influência negativa na estrutura social dentro de uma população (SILVA et al., 2023). Independentemente dos conceitos, sabe-se que estresse é um acontecimento fisiológico natural com origens adaptativas. Porém o resultado fisiológico da exposição ao estresse depende da fase de vida do animal, do tipo do estresse (físico, psicológico) e também da duração do estímulo estressor (estresse agudo ou crônico) (LAUFFER et al., 2015). O estresse de curto prazo e repetitivo é considerado agudo, podendo ou não causar prejuízos graves ao animal. Já o crônico é resultado de estressores que permanecem por longo período de tempo e com muita frequência, seus efeitos desgastam seriamente o organismo (MUSAZZI et al., 2017).

Fatores estressores podem advir de causas sociais, ambientais, metabólicas, imunológicas ou devido a práticas de manejo inadequadas (ZHANG et al., 2017; LUCY et al; SAFRANSKI., 2017; LIPPI et al., 2023). Portanto, podemos dizer que tais causas podem ser intrínsecas ou extrínsecas ao animal.

A respeito dos fatores intrínsecos de estresse para fêmeas suínas nas categorias gestantes e lactantes pode-se citar a hiperprolificidade de genéticas modernas, pois pesquisas mostram que grandes leitegadas podem afetar negativamente a reprodutora, quando considerado o maior tempo de parto (LEE et al., 2023). Além disto, a superprodução de EROs - incluindo superóxido e peróxido de hidrogênio - associadas ao aumento de energia requerida por matrizes hiperprolíficas, pode resultar no aumento do estresse oxidativo de porcas, afetando negativamente o desenvolvimento fetal, glândulas mamárias e bem-estar de matriz e sua prole (LEE et al., 2023; BERCHIERI-RONCHI et al., 2011).

No periparto as fêmeas suínas passam por desordens metabólicas e inflamações sistêmicas que podem acarretar aumento da condição de estresse, do ponto de vista fisiológico e comportamental (BJORKMAN et al., 2023). Como cita Kaiser et al. (2018), é normal que a fêmea passe por certo nível de estresse e inflamação no período periparto. Porém, caso a fêmea não seja capaz de se adaptar a esta condição fisiológica normal ou a intensidade desse desafio seja superior à capacidade de reação do organismo, todo o sistema fica desafiado, do ponto de vista fisiológico, metabólico, endócrino ou imunológico (KAISER et al., 2018; CHENG et al., 2018).

A composição da comunidade microbiana também é utilizada como um indicativo fisiológico de estresse em porcas. Estudos mostram que a dieta, o ambiente, a genética e *status* fisiológico do hospedeiro agem sobre a composição da microbiota (LIU et al., 2019). Já foi observado que alterações na microbiota intestinal são diretamente e naturalmente influenciadas pela gestação e lactação devido a mudanças metabólicas nestes períodos (LIU et al., 2019; CHENG et al., 2018). Alterações na função da microbiota são conhecidas como disbioses e podem ser afetadas por condições exacerbadas de estresse (KRAIME et al., 2019). Drásticas mudanças na diversidade e riqueza da microbiota ao longo da gestação e lactação estão relacionadas a distúrbios de ordem metabólica, como baixo grau de inflamação durante o período perinatal, que prejudica a ocorrência de contrações uterinas, expulsão de leitões e placenta (níveis de citoquinas pró-inflamatórias são geralmente estudados para medir o grau de inflamação do organismo no último terço da gestação) e/ou redução da sensibilidade à insulina no início da gestação (leva à redução do consumo alimentar de porcas, reduzindo o volume de leite na lactação e afetando negativamente a leitegada) (CHENG et al., 2018).

Dentre as consequências do estresse crônico em matrizes gestantes deve-se compreender mais profundamente o impacto sobre o desenvolvimento cognitivo de leitões. Estudos mostram que porcas em distresse demonstram mais medo por humanos e tendem a originar leitões com maior tendência à aversão (ROONEY et al., 2021). Além disso, o estresse materno na gestação e durante o parto tem implicações sobre a vitalidade de leitões e seu consequente desempenho futuro (OTTEN et al., 2015). Partos longos, acima de 300 minutos, podem afetar negativamente a vitalidade de leitões (ISLA-FABILA et al., 2018). A duração do parto pode ser afetada pela alta prolificidade, distocias, ordem de parto e status emocional da fêmea (HALES et al., 2015; EGLI et al., 2022). Sucessivas contrações uterinas podem promover a hipóxia de leitões ainda no ambiente intrauterino (GOURLEY., 2020). Altos níveis de hormônios do estresse, aumentam o comportamento estressado de porcas, resultando em agressividade contra os leitões e reduz a habilidade materna (foram observadas matrizes deitadas em posição lateral por menos tempo e maior inquietação) (RUTHERFORD et al., 2014).

A respeito dos fatores extrínsecos causadores de estresse para fêmeas suínas gestantes e lactantes, o conforto térmico deve ser buscado e mantido, sendo que para porcas gestantes a zona de neutralidade térmica encontra-se entre 15 e 18ºC e para as lactantes, próximo a 16°C (MARTÍNEZ-MIRÓ et al., 2016). Quando submetidas a temperatura e umidade relativa muito distantes dos valores mencionados, consumo de ração e de água, o desenvolvimento embrionário, taxa de parição e por consequência o número de leitões nascidos são afetados negativamente (TUMMARUK et al., 2023). A redução do consumo alimentar devido ao estresse por calor leva ao aumento da produção de EROs causando estresse oxidativo. Alto estresse oxidativo em fêmeas gestantes impacta a produção de leite e eficiência reprodutiva (KIM et al., 2013).

Modelos de instalações são também exemplo de fatores extrínsecos do animal que impactam nos níveis de estresse (BAXTER et al., 2018). Mesmo aparentemente estabelecido, a pauta é significativa atentando-se para os diversos estudos atuais com o intuito de descobrir ou melhor entender os efeitos de diferentes tipos de instalações para fêmeas gestantes e lactantes, além de opções alternativas (GOUMON et al., 2022).

Uma série de problemas são frequentemente observados em fêmeas gestantes alojadas em gaiolas individuais. A restrição de movimentos está associada a problemas de bem-estar, sendo ainda pior considerando que o tamanho corporal de fêmeas hiperprolíficas aumentou (PELTONIENI et al., 2021). Além disto, problemas na integridade dos aprumos e síndrome de descarga-vulvar também estão dentre os prejuízos oriundos da instalação individual (HALLOWELL., 2022). Como consequência, alterações endócrinas e comportamentais são notadas nestes animais, como por exemplo expressão de estereotipias e aumento da agressividade com outras fêmeas ou mesmo tratadores (MARTÍNEZ-MIRÓ et al., 2016).

Gestação do tipo coletiva é uma alternativa à individual já implantada em vários países, e recomendada por permitir maiores oportunidades de movimento para fêmeas, melhorando a condição corporal e reduzindo problemas associados com danos nos aprumos, como claudicações e problemas nos cascos (KIM et al., 2016). Morgan et al. (2018) observaram que tais benefícios podem afetar a redução do tempo de parto e aumentar o número de leitões nascidos vivos. Entretanto, desvantagens como possíveis situações de disputas por dominância podem ocorrer neste sistema, causando lesões na pele, estresse ou abortos espontâneos (SPOOLDER et al., 2015).

No que diz respeito a instalações na fase de lactação, as baias convencionais são amplamente utilizadas, pois permitem maior otimização de mão de obra e reduzem mortes de leitões por esmagamento, porém as mesmas também comprometem a movimentação das matrizes, além de não permitirem a expressão de comportamentos naturais da espécie, como a formação de ninhos, e minimizam chances de interação materna com a progênie (CHIDGEY et al., 2017). Tais exemplos podem levar ao aumento do estresse e comprometer o bem-estar de fêmeas (KINANE et al., 2022).

Ainda sobre os fatores extrínsecos, além da qualidade nutricional, a frequência de alimentação é estudada na tentativa de melhorar o aproveitamento dos nutrientes e o bem estar das porcas (MANU et al., 2019). No entanto, Jung et al. (2023) ao avaliarem o efeito de uma única oferta diária de ração em contraponto a duas, para porcas multíparas gestantes, notaram que a frequência alimentar não foi um fator de influência sobre a expressão de estereotipias, assim como o nível de cortisol salivar não diferiu entre os tratamentos. Resultados similares já haviam sido encontrados por Holt et al. (2006). Segundo Jung et al. (2023), o prejuízo para o bem-estar animal, especificamente do ponto de vista alimentar, somente ocorre caso o preenchimento intestinal e necessidades nutricionais não sejam atendidas, o que provavelmente não aconteceu nesses estudos.

No contexto de bem-estar torna-se importante enfatizar que existe uma ampla variabilidade de formas de conceitualização na literatura (MOROTA et al., 2018). Porém, de maneira unificada o bem-estar positivo pode ser traduzido em bom *status* de saúde física, assim como a possibilidade do animal ter acesso ao que quer (DAWKINS et al., 2021). O bem-estar animal é mensurável por meio de distintas formas, como através da longevidade, variáveis de eficiência reprodutiva, padrões comportamentais, por meio de variáveis fisiológicas como frequência cardíaca, temperatura corporal, níveis hormonais e muitas outras formas (DAWKINS et al., 2021). Portanto, quanto mais o animal se distancia do *status* de bem-estar positiva, mais submetido ele estará ao estresse.

Em conclusão, fatores intrínsecos e extrínsecos a porcas podem contribuir para o aumento do estresse e suas consequências são inúmeras. Para reduzir essa condição, estratégias nutricionais com efeitos antioxidantes, microbianos e neurais podem ser oportunidades neste cenário, os probióticos se encaixam nessa categoria (LI et al., 2022).

2.2 Inclusão de bactérias probióticas nas rações para matrizes suínas

Os mecanismos pelos quais os efeitos benéficos dos probióticos ocorrem são abrangentes e não foram ainda totalmente elucidados. Diferentes cepas podem influenciar o ambiente intestinal de maneiras distintas. Os modos de ação mais discutidos são descritos a seguir.

2.2.1 Efeitos sobre a microbiota intestinal

Os meios de ação dos probióticos podem se dar por exclusão competitiva, definida como a ação normal da microbiota que protege o intestino contra o estabelecimento de microrganismos prejudiciais (BAJAGAI et al., 2016). O conceito de exclusão competitiva indica que as culturas de microrganismos benignos selecionados (probióticos) competem com microrganismos prejudiciais no intestino por locais de adesão e substratos orgânicos (LIAO; NYACHOTI., 2017). Logo, a adesão de probióticos à parede intestinal poderia prevenir a colonização por cepas patogênicas (YANG et al., 2015).

A exclusão competitiva é uma barreira física, na qual os probióticos podem impedir a proliferação de bactérias patogênicas ao competir pelo mesmo sítio de ligação, os quais se localizam principalmente nas vilosidades intestinais, nas células caliciformes e nas criptas intestinais (HALLORAN; UNDERWOOD., 2019). Ademais, as bactérias probióticas podem excluir as patogênicas ao competir por nutrientes e energia (ASML et al., 2015). Além disso, probióticos *Bacillus* podem produzir uma ampla gama de nutrientes como polissacarídeos, vitaminas e exoenzimas que podem estimular o crescimento de microrganismos benéficos (CAI et al., 2020).

A inibição antimicrobiana direta trata-se de mais um mecanismo ao nível de microbiota, no qual os probióticos, após seu estabelecimento no intestino, produzem substâncias bactericidas ou bacteriostáticas inibindo a colonização de microrganismos patogênicos, incluindo bactérias gram-negativas (BAJAGAI et al., 2016). Testes bacteriostáticos *in vitro* mostram a inibição de patógenos Gram-negativos como *Escherichia coli* e Gram-positivos como *Staphylococcus aureus* por meio da bacteriocina produzida por *Bacillus subtilis* (ABD; LUTI., 2017; CAHYA et al., 2019). A capacidade de *Lactobacillus plantarum* em atacar a enterotoxina *Escherichia coli,* presente em leitões desmamados, pode ser explicada pela adesão do probiótico nas células da mucosa intestinal e sua produção de metabólitos antimicrobianos, como peptídeos de defesa (WANG et al., 2018). Hu et al. (2021) observaram redução da população de *E. coli* ao suplementar matrizes com mix de cepas probióticas durante a fase de lactação. Porém, resultados não significativos também são encontrados, nos quais a inclusão dietética de *Lactobacillus* não foi capaz de afetar a população microbiana em porcas (BARBA-VIDAL et al., 2019; WANG et al., 2014).

2.2.2 Efeitos sobre a modulação da resposta imune

Efeitos imune dos probióticos são altamente específicos para cada cepa, ou seja, diferentes cepas pertencentes à mesma espécie podem apresentar estruturas antigênicas distintas e influenciar o sistema imunológico de maneira diferenciada (HILL et al., 2014). Por essa razão, as misturas de múltiplas cepas podem ser mais eficazes do que as cepas isoladas, exercendo atividades sinérgicas (TIMMERMAN et al., 2004).

Além disso, algumas cepas microbianas afetam não apenas os mecanismos imune humoral e celular, mas também estimulam uma resposta imune inespecífica, aumentando a atividade das células fagocíticas e células natural killer (YIRGA et al., 2015), que são a primeira linha de defesa contra patógenos (MATHIPA; THANTSHA., 2017).

De maneira geral, metabólitos componentes da parede celular e DNA de probióticos podem estimular o sistema imune através de interações com o epitélio, células dendríticas e com monócitos, macrófagos e linfócitos por meio de mecanismos de respostas da imunidade inata ou adaptativa (ASML., 2015). Porcas gestantes alimentadas com dietas contendo *Bacillus subtilis* e *Bacillus amyloliquefaciens* a partir de 90 dias de gestação até o final da lactação (28 dias), tiveram maior concentração de imunoglobulinas G no sangue ao parto, e por conseqüência, as concentrações de imunoglobulinas M no sangue de leitões ao desmame também foi maior do que no grupo controle (KONIECZKA et al., 2023).

Um dos principais modos de ação do *Bacillus spp.* em suínos pode se apresentar por suas propriedades imunomoduladoras. O tratamento probiótico com *B. toyonensis*, por exemplo, demonstrou uma abundância de células T citotóxicas intraepiteliais e imunoglobulinas A fecal em leitões desmamados, o que pode conferir proteção contra a colonização de patógenos (SCHAREK et al., 2007; SCHIERACK et al., 2009). A capacidade de acionar a síntese de interleucinas anti-inflamatórias já foi observada em *Lactobacillus rhamnosus*, reduzindo a liberação de mediadores pró-inflamatórios (CRISTOFORI., 2021). O mesmo efeito foi observado por Jorjão (2012) e Asml (2015).

2.2.3 Efeitos sobre o sistema neuroendócrino

Bactérias probióticas são capazes de modular comportamentos associados ao estresse (MINDUS et al., 2021). Esses últimos, geralmente apresentam sinais de ansiedade e depressão, pois o sistema nervoso central e estados cognitivos estão afetados (PETRA et al., 2015). Alguns estudos demonstram os efeitos positivos dos probióticos frente ao comportamento de cobaias em experimentação e também em seres humanos (HUANG., 2019).

A comunidade microbiana intestinal pode se comunicar com o cérebro através do nervo vago ou pela modulação do sistema imune, do eixo hipotálamo-pituitária-adrenal (HPA), pela produção de neurotransmissores ou pela produção de ácidos graxos de cadeia-curta (AGCC), que são metabólitos com função neural (DALILE et al., 2019). Bactérias entéricas são capazes de alterar a sensibilidade e processo de resposta do HPA frente a fatores estressores (ANSARI et al., 2023). O estresse psicológico está associado à disbiose da microbiota intestinal, e o contrário também ocorre, pois estas desrregulações influenciam distúrbios mentais (LIU et al., 2019).

A produção de hormônios do estresse é regulada pelo sistema neuroendócrino por meio da ativação de dois eixos, o HPA, resultando na liberação de glicocorticóides que são produzidos e secretados pelo córtex adrenal (estrutura externa que compõe a glândula adrenal) e o eixo simpático adrenal medular (SAM - estrutura interna que compõe a glândula adrenal), que secreta catecolaminas, como norepinefrina e epinefrina (KOGUT et al., 2022). O eixo HPA pode ser ativado por meio de vários estressores extrínsecos e intrínsecos ao animal (neurotransmissores, citocinas e/ou danos moleculares) (LEISTNER; MENKE., 2020). Em situações de estresse agudo, os glicocorticóides estimulam a resposta imune prevenindo a inflamação. Caso o estresse seja crônico a ação dos glicocorticóides é reduzida (KOGUT et al., 2022).

Ácidos graxos de cadeia curta (butirato, acetato e propionato) podem ser uma via de comunicação entre cérebro e intestino, pois promovem a produção intestinal de hormônios e neurotransmissores como o GABA (ácido gama-aminobutírico) e a serotonina, que interagem com células enteroendócrinas e provocam o aumento da circulação destas substâncias (MARTIN-GALLAUSIAUX et al., 2021). Estudos com camundongos evidenciam a ação do butirato como antidepressivo, agindo no restabelecimento cognitivo e social (SILVA et al., 2020). Considerando funções neuroendócrinas, bactérias probióticas podem ser classificadas como psicobióticos (BARBOSA; NETO., 2021). Como exemplo, tem-se que *Lactobacillus helveticus* e *Bifidobacterium longum* reduzem sintomas de ansiedade e depressão em humanos (KARAKULA-JUCHNOWICZ et al., 2019).

Distúrbios na microbiota (disbioses) provocam aumento do cortisol, da permeabilidade de membrane celular, alteram os níveis de neurotransmissores como serotonina e dopamina e de metabólitos bacterianos (HOLZER; FARZI., 2014; LOWIES et al., 2020). Com a integridade da membrana desfavorecida, a entrada e proliferação de patógenos e inflamação podem aumentar. Além disso, citocinas inflamatórias rompem a barreira hematoencefálica, o que leva a maior intensidade de respostas inflamatórias e neurodegenerativas (KIM et al., 2018). Os probióticos tornam-se vantajosos neste contexto, devido sua capacidade em reduzir as reações ao estresse causadas pelo eixo HPA, em reduzir corticosteróides, e ainda aumentar serotonina, GABA, dopamine (intrinsicamente ligada ao aparecimento de comportamentos estereotipados em suínos) e acetilcolina, substâncias que afetam *status* emocional e perfil comportamental (DALIRI et al., 2016).

Enfim, dados da literatura demonstram que a administração de probióticos para porcas visa a melhoria da saúde, do bem-estar e desempenho reprodutivo (LIU et al., 2018). Benefícios relacionados ao consumo de ração e produção de leite também têm sido reportados. Barba-Vidal. (2019) demonstrou que porcas suplementadas com probióticos tiveram maior consumo alimentar na lactação e gestação. Hayakawa et al. (2016) e Jeong et al. (2015), ao incluírem os probióticos *Bacillus mesentericus, Clostridium butyricum e Enterococcus faecalis* a partir do terço final da gestação, também notaram aumento do consumo diário de fêmeas gestantes e lactantes.

Distintas dosagens e cepas probióticas já demonstram interferir na qualidade e quantidade de colostro e leite produzido por porcas (DOMINGOS et al., 2021; PENG et al., 2020). A melhoria das características do colostro e leite afeta o peso de leitões neonatos e permite maior chance de sobrevivência mesmo aos leitões de baixa viabilidade (ZHANG et al., 2020).

2.3 Estudo do eixo intestino-microbiota-cérebro para o bem-estar de fêmeas reprodutoras

Um dos mecanismos de ação dos probióticos é a interação do eixo intestino-microbiota-cérebro (IMC), como abordado inicialmente no tópico anterior.

Pesquisas iniciais datam dos anos 80 e são base para os conhecimentos sobre o eixo IMC (IVAN PAVLOV., 2024). Pesquisas com animais "*germ-free*" mostrando que a ausência de microrganismos pode afetar o cérebro e estudos sobre as diferenças comportamentais entre indivíduos colonizados por distintas cepas bacterianas, evidenciaram a importância de um terceiro componente na comunicação bidirecional entre cérebro e intestino: a microbiota (STRASSER et al., 2017; NEUFELD et al., 2011). Atualmente, a respeito das funções exercidas pelo eixo IMC pode-se dizer que a literatura ainda está "engatinhando" na compreensão completa de tudo que está relacionado ao eixo. É sabido que o eixo IMC está envolvido com a expressão de padrões sociais e comportamentais, assim como condições de medo, ansiedade, estresse ou depressão, porém os caminhos que tornam isso possível ainda não são totalmente entendidos (CRYAN et al. 2019).

Pesquisadores notaram que ao administrar *Lactobacillus reuteri* em ratos, houve aumento do comportamento social em contraponto ao grupo controle (ZHANG et al., 2022). Parece haver relação da microbiota sobre alterações funcionais da amígdala, sendo essa região um importante ponto no circuito neural para modular o comportamento (BUFFINGTON et al., 2016). Voltando à produção de suínos, sabe-se que uma melhor sociabilidade, é uma característica de grande interesse dentro da cadeia produtiva considerando a estreita aproximação de animais e tratadores em diversos manejos diários (arraçoamento, inseminação, transferências de instalações, intervenções em processos de parto e amamentação, etc), assim como a relação entre as próprias fêmeas confinadas (POL et al., 2021).

Pouco se sabe a respeito da influência da microbiota sobre indicadores de estresse em fêmeas suínas reprodutoras. Entretanto, trabalhos com outras espécies, como aves, demonstram possíveis efeitos positivos na redução do comportamento de canibalismo e possível aumento na expressão de genes relacionados ao metabolismo da serotonina, com suplementação de *Lactobacillus rhamnosus* (HUANG et al., 2023). Negm et al. (2023) notaram efeito positivo no comportamento de frangos submetidos a estresse no transporte pré-abate, suplementados com *Bacillus subtilis.* Foi notado redução de marcadores de estresse, como corticosterona, e constataram menor tempo de duração da imobilidade tônica (quando estressadas permanecem por mais tempo em imobilidade) em aves suplementadas. Em ratos, *Lactobacillus fermentum* melhoraram distúrbios emocionais, estabilizando desordens cognitivas relacionadas ao estresse, ansiedade e depressão (PARK et al., 2020). Em suínos, testes comportamentais mostraram que a suplementação dietética com *Lactobacillus* para leitões lactentes evitou aumento de comportamento associado à ansiedade, quando expostos a uma ameaça auditiva (VERBEEK et al., 2021).

Até o presente momento não foram encontrados estudos que tivessem testado especificamente bactérias probióticas sobre o comportamento de porcas gestantes e/ou lactantes. Porém outras substâncias capazes de modular a microbiota afetaram positivamente o desempenho da progênie desses animais, em testes de memória (KRAIMI et al., 2019). Além disto, porcas gestantes multíparas recebendo fibra dietética eubiótica reduziram a frequência de estereotipias (falsa mastigação e lamber o chão) e a expressão de comportamento agressivo com outras fêmeas, quando comparadas ao grupo controle (ODAKURA et al., 2023), demonstrando assim a susceptibilidade cognitiva da espécie suína a alterações microbianas.

A variabilidade dos dados demonstrados sobre o efeito de bactérias probióticas para fêmeas reprodutoras, enfatizam a necessidade de maiores esclarecimentos e consistência nas respostas, especialmente aquelas relativas ao comportamento e bem-estar de porcas e sua leitegada, tendo em vista a ampla gama de cepas, dosagem, estágios da gestação para início da suplementação e duração de tempo de administração do probiótico.

Estudos que objetivam avaliar o efeito da microbiota sobre o cérebro e comportamento e consequentemente bem-estar de porcas gestantes e lactantes, assim como a transferência probiótica materna para leitões, ainda são escassos. Porém, há muita chance de que a modulação da microbiota por meio da inclusão de bactérias probióticas possa afetar positivamente as variáveis produtivas, reprodutivas e de bem-estar de fêmeas suínas reprodutoras. Este manejo pode se tornar uma estratégia efetiva para aumentar competividade de mercado e melhorar a qualidade de vida dos animais, tópico esse cada vez mais exigido pelos consumidores.

3. Hipóteses e objetivos

Hipóteses:

1. Probióticos influenciam o eixo microbiota-intestino-cérebro de matrizes suínas e são capazes de modificar o comportamento destas últimas e de sua progênie, afetando a produção de marcadores de estresse, eficiência de partos e a vitalidade de neonatos.

Objetivos:

1. Avaliar o bem-estar de matrizes suplementadas com probióticos por meio da eficiência durante o parto e da vitalidade dos neonatos.

2. Avaliar os efeitos da inclusão de mix de cepas probióticas nas dietas de matrizes suínas através de caminhos endócrinos e comportamentais.

CAPÍTULO II

ARTIGO 1

Artigo científico nas normas da revista *Plos One,* parcialmente adaptado para tese

EFFECT OF MATERNAL SUPPLEMENTATION WITH *BACILLUS SUBTILIS* AND *BACILLUS LICHENIFORMIS* ON FARROWING EFFICIENCY AND OFFSPRING VITALITY

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ABSTRACT

Probiotic bacteria have the potential to improve animal welfare, which is especially crucial for sows during the parturition phase, when significant metabolic changes occur. Therefore, this study investigated the effects of *Bacillus* spp. administered to sows during gestation and lactation on farrowing traits, vitality of newborn piglets, colostrum intake, in addition to maternal constipation risk and feed intake during lactation. Multiparous sows were fed a diet without probiotics (n = 17) or a diet containing *Bacillus subtilis* and *B. licheniformis* (n = 18) during gestation and lactation. Farrowing kinetics (body temperature, duration, and intervals) and piglet vitality (presence of meconium, umbilical cord rupture, glucose, blood pH, blood oxygen saturation, and heart rate) were monitored individually. In addition, piglet weights (at birth, 24 hours after farrowing) were registered to estimate colostrum intake. Feed intake and fecal scores (in an attempt to infer on the constipation risk) were recorded for the sows. The data were subjected to analysis of variance and interpreted considering the α levels of 5% (significant) or 10% (marginal significance). Probiotics tended to reduce piglet birth interval (P = 0.065) and farrowing duration (P = 0.095). Piglet vitality variables were not affected. However, colostrum intake of piglets from sows that received probiotics was 25% higher (P = 0.001). Supplemented sows tended to have a lower frequency of wet feces (P = 0.078) and consumed 8% more feed than the control sows during lactation (P = 0.006). Therefore, it is observed that the inclusion of probiotics for sows does not alter the vitality of the progeny, but tends to improve the dynamics of farrowing and increases feed intake during lactation.

INTRODUCTION

An intensive animal protein production system requires increasingly high standards of performance, especially in the reproductive phase of the chain. Numerous feed additives are used to achieve these standards, and probiotics are widely known examples in this scenario.

Although not all the action mechanisms of probiotic microorganisms have been completely clarified, it is known that their benefits in health, behavior, welfare, and productivity may come from the modulation of the intestinal microbiota, in addition to the benefits on immunity and gut health (competition for nutrients, inhibition of epithelial invasion by pathogens, and production of microbial substances) [1].

Bacillus strains are among the most popular bacteria used as probiotics. These bacteria have the ability to sporulate and have shown interesting results in both *in vivo* and *in vitro* studies [2,3]. Furthermore, *Bacillus* spp. have antioxidant, antimicrobial, and immunomodulatory abilities that are of high interest in the agricultural and livestock industries [4].

Bacillus subtilis and *Bacillus licheniformis* have already shown positive effects in several phases of pig production, but the use of probiotics during reproductive phases is particularly interesting. The perinatal period is critical for sows because of the impactful metabolic changes, which can lead to an exacerbated increase in the inflammatory status and stress condition [5]. Probiotics can alleviate this challenge by playing an important role via the gut-microbiota-brain (GMB) axis and, consequently, mitigate these negative effects on the productivity and welfare of sows and piglets [5,6].

Previous studies already reported improvements in quality and quantity of milk production [7] and increasing weight gain of suckling piglets when the sows received diets containing probiotics [8]. The effect of *Bacillus* spp. in reducing the farrowing duration was also reported [9].

The perinatal period is very critical for sows, because impactful metabolic changes occur that can lead to an exacerbated increase in the inflammatory status and stress condition, partially due to disorders of the intestinal sow microbiota [5]. Probiotics can play an important role via the GMB axis mitigating the negative effects of this phase on productivity and welfare of sows and piglets [5,6].

Considering that farrowing kinetic is closely related to the newborn vitality, it is possible to hypothesize that piglets born from sows supplemented with probiotics could have higher viability. While the condition has been discussed in the literature, it has not been fully clarified. In addition, most of the available studies did not supplemented the sow during the entire gestation and the effects of adding probiotics in the long term still need to be further studied. This study aimed to evaluate the effects of *B. subtilis* and *B. licheniformis* supplied to gestating and lactating sows on farrowing traits, vitality of newborn piglets, colostrum intake, in addition to maternal constipation risk and feed intake during lactation.

MATERIALS AND METHODS

The experiment was conducted on a commercial farm (Maratá, Rio Grande do Sul, Brazil) and the protocols were carried out following the Brazilian guidelines for animal care and use. The temperature and humidity index of the gestation and lactation periods were calculated using datalogger equipment (Datalogger Instrutherm HT-70, Rio Grande do Sul, Brazil). The calculation was made according to the following equation [10]:

$$THI = 0.8\ T\alpha + (RH/100) \times (T\alpha - 14.3) + 46.4$$

Where: $T\alpha$ – ambient air temperature (°C); RH – relative humidity (%).

Animals, treatments, and facilities

Large-white × Landrace sows (Pic Camborough, Agroceres-PIC, São Paulo, Brazil) with parity orders varying from 2 to 9, were used in the experiment. Five sows were removed from the study, due to the return to estrus.

Treatments tested: 1) Control (17 sows), without probiotic supplementation; and 2) Bacillus (sows = 18), with commercially available product (Bioplus 2B, Chr. Hansen, São Paulo, Brazil) containing B. subtilis (1.6 x 109 CFU/g) and B. licheniformis (1.6 x 10^9 CFU/g), during the entire gestation (approximately 116 days) and lactation (21 days) periods.

Treatments were randomly assigned to sows within each parity order. The number of replications was defined according to Sakomura and Rostagno., 2016 [11] considering the dispersion of the main variables in previous studies. Probiotics were supplied daily via industrial gelatin capsules to each sow, to prevent cross-contamination among sows, from the feed to the piglets, and in the environment. These capsules were them provided once a day to each sow during the trial and it was placed on the top of the feed served. Colorful capsules were used to allow easy observation of its intake by the sow.

Daily amount was weighted individually, to represent an inclusion of 400 g of the additive per ton of feed, which is the concentration recommended by the supplier (Chr. Hansen, São Paulo, Brazil), which resulted in a mean daily supply of 0.8 g during gestation and 3.2 g during lactation per sow. On gestation phase, 1.9 kg of feed was provided once a day (morning) per sow (when it was necessary adjustments of this amount were performed for sows with inadequate body condition scores), with daily feed intake varying from 1.8 to 2.0 kg. On the lactation phase, the amount was supplied *ad libitum*, with daily feed intake gradually increasing during the first week, reaching an average of 7 to 8 kg per animal. A commercial diet based on corn and soybean meal was offered for both phases, only phytase and mycotoxin binders were use as additives (Table 1). Water was provided ad libitum during the entire trial.

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[2]	0.500	0.500
L-Lys (65%)	0.168	
L-Valine		0.158
L-Thr	0.100	0.128
DL- Methionine	0.049	0.100
Tryptophan	0.005	0.050
Phytase[3]	0.005	0.005
Carbohydrase[4]		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
[3] Axtra PHY GOLD, Danisco Nutrition.
[4] Rovabio, Adisseo.

Pregnant sows facilities contained a semi-automatic feeding system and a trough-shaped drinking system (the water flow was not continuous). Sows were kept in entirely concrete individual cages (1.2 m^2) and during lactation each sows were kept individually in a conventional farrowing stall (1.2 m^2) with half of the floor slatted, located in the center of a pen with an available space of 4.2 m^2 with access only for piglets with a fully slatted floor.

Farrowing assessment

Births were monitored individually and following variable were registred: birth interval and the farrowing duration (since first piglet expelled until expulsion of the last piglet). The farrowing duration was classified as ‘long’ when exceeded 300 minutes, or normal when lower than 300 minutes [12]. The interventions were registered (vaginal palpation or oxytocin administration). Sow rectal temperature was recorded (Veterinary thermometer IP67, INCOTERM, Rio Grande do Sul, Brazil) immediately after the birth of each piglet.

In order to evaluate the constipation risk, the feces were observed every morning and afternoon during five days after farrowing and an average was calculated between both periods. The evaluation was visual and was always performed by the same observer, using the following classification [13, Fig 1]. The fecal score was analyzed by individual days, and the frequency of each score was merged and classified into dry (scores 1 and 2), ideal (score 3), and wet (scores 4 and 5) categories.

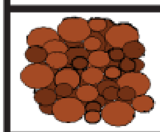
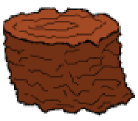
	0	Absence of faeces
	1	Dry and pellet-shaped (unformed)
	2	Between dry and normal (pellet-shaped and formed)
	3	Normal and soft, but firm and well formed
	4	Between normal and wet; still formed, but not firm
	5	Very wet faeces, unformed and liquid

Figure 1. Illustration of fecal score used to classify sows feces [13].

During the entire lactation intake was recorded daily. These data was analyzed considering the first week and the entire lactation period. During 24-hours period feed supplied were quantified, at the end of this period, leftovers were disregarded to determine the daily quantity consumed.

Piglet vitality

Vitality variable was evaluated immediately after birth. The piglet was visually inspected to the presence/absence of meconium in the skin and umbilical cord integrity (presence/absence of rupture). After that, oxygenation and heart rate were measured (Veterinary Oximeter R40, RZVet, São Paulo, Brazil). Umbilical cord blood sample was collected for the assessment of glucose (Monitor Accu-chek Performa, Roche, São Paulo, Brazil) and pH (Akso, Rio Grande do Sul, Brazil). After, each piglet was weighted, identified with ear tags, and returned to the sow. Piglets were weighed again 24 hours after birth to estimate colostrum intake using the equation proposed by Devillers et al., 2004 [14]:

$$C_{int} = -217{,}4 + 0{,}217 \times \mathrm{t} + 1861019 \times \frac{BW_{24}}{t} + BW_b \times$$

$$(54{,}8 - \frac{1861019}{t} \times (0{,}9985 - 3{,}7 \times 10^{-4} \times t_{fs} + 6{,}1 \times 10^{-7} \times t_{fs}^2$$

where C_{int}= colostrum intake (g); t= time from birth to weight measurement (minutes); t_{fs}= time from birth to first suckling (minutes); BW_{24} = body weight at 24 hours (kg); BW_b= body weight at birth (kg). Piglets were then classified into three categories based on the individual colostrum intake, which were: ‘low intake’ when lower than 290 g, ‘average intake’ when between 291 and 439 g; and ‘high intake’ when higher than 440 g [15]. The frequency of pigs in each class was calculated. The vitality variables were chosen based on the studies of Herpin et al., 1996 [16] and the Apgar score program of Alonso-Spilsbury et al., 2005 [17].

Statistical analysis

Statistical analyses were performed using the SAS software (SAS Institute Inc., Cary, NC, USA), through the GLIMMIX procedure. The effects of parity order, and litter size were tested in the model and when not significant. The piglet was considered the experimental unit for the vitality and colostrum responses; however, the data were grouped within the sow (random effect). Residuals were evaluated for normality using univariate procedures and the Shapiro-Wilk test. The treatment means were separated using the PDIFF option and the differences were interpreted at the significance levels of 5% (significant effects) and 10% (marginally significant).

RESULTS AND DISCUSSION

Considering all thermal control methods used on the farm, the THI remained within the value considered safe for sows, that is, below 74. Probiotics tended to reduce the piglet expulsion interval (P=0.065; Table 2) and, consequently, the farrowing duration (P=0.095). These are positive results as they can be associated with lower mortality risk, especially due to oxygen deprivation [18].

Table 2. Effect of probiotic supplementation to gestating-lactating sows on the farrowing duration, required interventions and rectal temperature.

Variables	**Treatments***		***P*-value**[1]
	Control	**Bacillus**	
Interval time, min	26.50 (3.559)	17.43 (3.018)	0.065
Farrowing time, min	342 (29.11)	269 (31.85)	0.095
Interventions, n/litter	4.07 (0.80)	2.28 (0.63)	0.090
Interventions, %[2]	30.0	20.9	-
Sow rectal temperature, °C	38.44 (0.142)	39.17 (0.158)	0.001

[1] Probability of treatment effects.
[2] Number of interventions per litter during total farrowing time.
* Means with standard errors in the parentheses.

The rectal temperature sow supplemented with probiotics was higher than that of control sows (P=0.001). However, the temperature values for both treatments were in line with normality, as shown in previous studies in which pre-and postpartum temperatures were measured [19,20].

The frequency of sows with prolonged farrowing (>300 min) was 45% higher in the control treatment comparing to the group that received probiotics (Fig 2). These results are similar to those of other studies that evaluated the influence of a diet containing *B. subtilis* and *B. licheniformis* used in the last third of pregnancy [9,21]. As expected, the number of interventions at farrowing also tended to decrease (P=0.090) in the group supplemented with probiotics, probably because of the shorter farrowing duration.

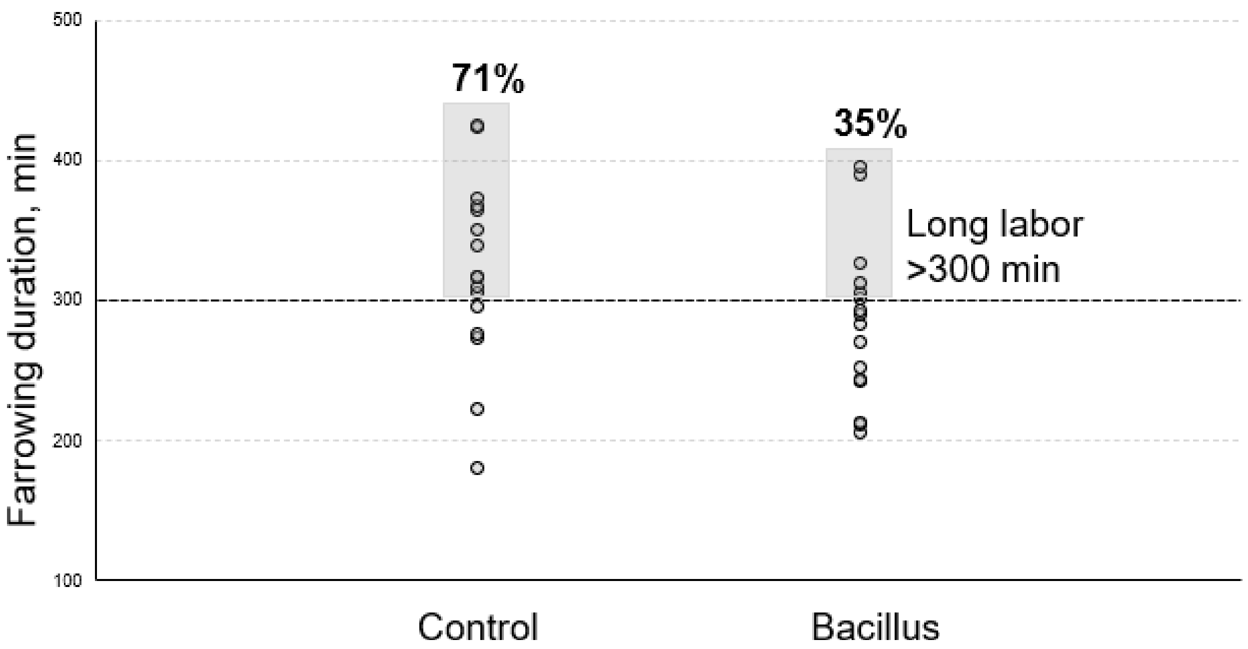


Figure 2. Effect of Bacillus probiotic supplementation to gestating-lactating sows on the farrowing duration and frequency of sows with a long labor.

Farrowing time can be affected by numerous factors, such as the parity order, type of facility, sow body condition, number of piglets born, and/or constipation status [22]. Also, during the peripartum period, females experience metabolic disorders and an increase in inflammatory status that can be associated with oxidative stress [5]. The probiotics used in this study probably improved the intestinal health of the sows [9,23], which can be related to numerous indirect effects on the animal. As an example, *B. subtilis* has been shown to reduce concentrations of malondialdehyde (a product of lipid peroxidation and an important indicator of oxidative stress) and increase the concentration of antioxidant enzymes during farrowing [19,24]. Probiotics are also associated with reduction of stress, which may be another possible mechanism that lead to shorter delivery time.

Recently, the bidirectional relationship between microorganisms and the central nervous system has received considerable attention, as probiotics influence the functionality of the GMB axis through the production of neuroactive substances such as dopamine, serotonin, and y-aminobutyric acid (GABA) [25]. Several studies have shown an increase in these neurotransmitters in piglets and mice receiving probiotics [25,26]. Thus, it is possible that the increase in welfare-related compounds, as neurotransmitters may have affected the duration of parturition by alleviating the stress status of the females.

The feed intake of group supplemented with probiotics was 8% higher than control during lactation (P=0.006), while feed intake during the first postpartum week was not affected by treatment (Table 3). This result can be explained by considering that the first week of lactation is known as a period of stabilization of intake, considering that it will have a gradual increase after farrowing due to the increase in the sow's energy demand.

The higher feed intake observed in supplemented sows may be related to the overall stress levels in the sows, as previously stated for farrowing duration. Some modifications in the intestinal microbiota, which can be related to the probiotic supplementation, may also explain the feeding behavior modulation. Bacteria of the phylum Firmicutes were already reported to have an effect on appetite regulation mechanisms, which may occur via energy balance and/or through the modulation of the central nervous system [6]. Studies indicated that probiotic bacteria could affect appetite through serotonergic signaling, through reward motivation, or through an action on the dopaminergic mesolimbic rewards circuit [26]. This circuit is directly involved in feed reward and impulsive behavior [27].

A recent study showed that supplementation with *B. subtilis* from 90 days of gestation did not affect feed intake during lactation [21]. However, other authors [28] showed an increase in feed intake in the group receiving probiotics when supplementation started from day 30 of gestation. It is noted that this variable is subject to variation, and perhaps new studies that evaluate the effect of the period of the beginning of supplementation are necessary.

Table 3. Effect of probiotic supplementation to gestating-lactating sows in the feed intake (FI) during the lactation period.

Variables	**Treatments***		***P*-value[1]**
	Control	**Bacillus**	
Overall FI, kg/day	5.446 (0.156)	5.905 (0.175)	0.006
FI during 1st week, kg/day	4.506 (0.224)	4.751 (0.222)	0.401

[1] Probability of treatment effects.
* Means with standard errors in the parentheses. Means represent 32 sows.

There was no influence of treatment on the postpartum fecal score (Table 4). Postpartum constipation is frequent in sows due to intestinal tract compression caused by the volume of fetuses at the end of gestation and by the action of autocrine hormones that prevent peristalsis [9]. The results are in agreement with other findings [29]. In the assessment, low scores were associated with drier and harder feces. Thus, constipation can impair animal welfare, arising from the discomfort of the female [30], and also the compromise the productive performance, if it led to prolapses.

Table 4. Effect of probiotic supplementation to gestating-lactating sows on their fecal score[1] during five days after farrowing.

Evaluation period	Treatments*		*P*-value[1]
	Control	Bacillus	
Score			
Day 1	0.333 (0.107)	0.222 (0.107)	0.471
Day 2	0.727 (0.241)	0.437 (0.283)	0.441
Day 3	0.800 (0.198)	0.400 (0.198)	0.162
Day 4	0.772 (0.243)	1.210 (0.262)	0.229
Day 5	1.090 (0.248)	0.947 (0.266)	0.695
Frequency			
Dry, %	80	90	0.182
Ideal, %	2	4	0.529
Wet, %	17	4	0.078

[1] Scores from 0 (attributed to absence of feces) to 5 (attributed to very wet, loose, and liquid feces).
[2] Probability of treatment effects.
* Means with standard errors in the parentheses.

The *B. licheniformis* strain has already been shown to influence the fecal score of sows during the last week of pregnancy, keeping it in the category considered ideal, that is, soft with moderate particle sizes [9]. When the frequency of scores was analyzed within the dry, ideal, and wet categories, a tendency toward minor percentage of wet feces was observed in the supplemented females (P=0.078). Probiotic bacteria may have acted by preventing the growth of enterotoxin-releasing pathogens that cause diarrhea.

The piglet vitality was not affected by the treatments (Table 5). The reduced duration of farrowing was expected to result in higher vitality scores for the piglets. Perhaps the set of variables chosen to express the vitality of the piglets was insufficient. The assessment of piglet vitality is an emerging area of research, and there is currently a lack of standardization in the studies that seek to evaluate it. Complementation with other assessments (physiological and behavioral) could be planned in future studies [31].

The increase in the frequency of Firmicutes, which was already reported in previous studies with similar probiotic supplementation (6), may enhances the body ability to acquire energy from the diet and improves the utilization of other nutrients, which would allow for an advantage in milk production of the supplemented sows [32]. For Bacillus piglets, the same explanation can be valid after the transfer and colonization of probiotic bacteria via maternal, during farrowing, and later in breastfeeding. The strains used in this experiment improved the average weight gain of piglets from supplemented mothers from 110 days of gestation, during intervals from 0 to 7 days and 7 to 14 days of lactation [29]. In another study, with the inclusion of the probiotic additive two weeks before farrowing, a greater weight was also observed at 14 days in piglets [33].

Table 5. Effect of probiotic supplementation to gestating-lactating sows on piglet vitality.

Variables	**Treatments***		***P*-value[1]**
	Control	**Bacillus**	
Meconium presence, n/litter	2.791 (0.393)	2.256 (0.358)	0.315
Umbilical cord rupture, n/litter	30.63 (0.147)	30.61 (0.137)	0.661
Glucose, mg/dL	57.13 (2.797)	50.77 (2.729)	0.104
Blood pH	8.084 (0.023)	8.101 (0.022)	0.421
Blood oxygen saturation, %	98.75 (0.717)	99.16 (0.777)	0.682
Heart rate, bpm	123.1 (5.474)	124.8 (5.751)	0.834

[1] Probability of treatment effects.
* Means with standard errors in the parentheses.

The probiotics improved the colostrum intake by piglets (P=0.001; Table 6). Similarly, the frequency of piglets that consumed a low amount of colostrum was lower in the supplemented group than in the control treatment (Fig 3).

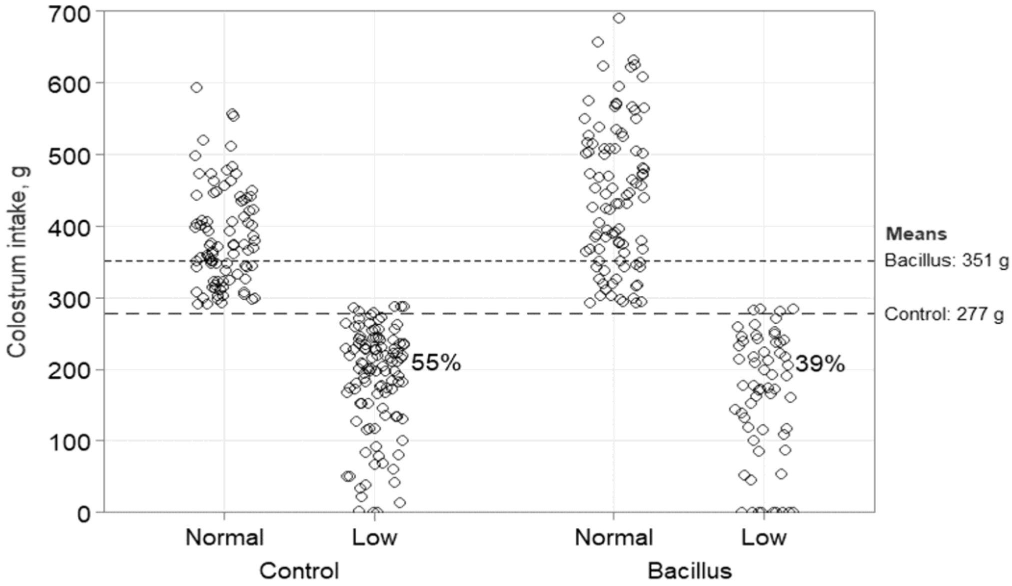


Figure 3. Effect of Bacillus probiotic supplementation to gestating-lactating sows on the colostrum intake of piglets (classified as low or normal).

As already mentioned, this result can probably be explained by the greater milk production capacity of the sows supplemented with probiotics [32], with a greater supply of colostrum and milk, consequently piglets coming from supplemented sows become heavier than control piglets to breastfeed. Unlike the effect observed in the sows, it is not probable that probiotics have affected the feeding behavior of neonates, given that these animals are expected to be born with great innate voracity, in order to meet their needs for energy [34]. It is more likely that the higher colostrum intake in piglets born from supplemented sows had been related to the higher birth weight. However, until the time of writing this paper, no studies have tested this hypothesis in newborn piglets.

Table 6. Effect of probiotic supplementation to gestating-lactating sows on colostrum intake of the piglets

Variables	**Treatments***		***P*-value**[1]
	Control	**Bacillus**	
Colostrum intake per piglet, kg	0.280 (0.013)	0.349 (0.016)	0.001
Colostrum intake per litter, kg	3.697 (0.340)	4.929 (0.330)	0.014
Piglets with low intake, %	55 (3.760)	39 (4.490)	0.051
Piglets with average intake, %	35 (3.368)	30 (4.341)	0.161
Piglets with high intake, %	10 (3.076)	31 (3.674)	<0.001

[1] Probability of treatment effects.
* Means with standard errors in the parentheses.

Conclusion

In conclusion, this study showed that dietary supplementation with *B. subtilis* and *B. licheniformis* for sows from the beginning of pregnancy to the end of lactation increased the reduced farrowing time and increased feed intake of lactating sows. These results indicate that the strains used also can act through neural pathways, affecting the energy balance and behavior. Probiotics reduced the frequency of wet sow feces and did not affect the viability of piglets. However, it improved colostrum piglets intake capacity.

REFERENCES

1. Devaraja G, Prashantkumar CS, Mahanthesh V. Current insights on the modulation of gut microbiome and its effect on human health. In: Sayyed RZ Khan M, editors. Microbiome-gut brain axis. Singapure: Springer, 2022. p. 29-52.

2. Saladrigas-García M, Solà-Oriol D, López-Vergé S, D'Angelo M., Collado MC, Nielsen B, et al. Potential effect of two Bacillus probiotic strains on performance and fecal microbiota of breeding sows and their piglets. J Anim Sci [Internet]. 2022 Jun [cited 2022 Oct 12];100(6): 163. Available from: https://pubmed.ncbi.nlm.nih.gov/35512239/. doi: 10.1093/jas/skac163.

3. He Y, Jinno C, Kim K, Wu Z, Tan B, Li X, et al. Dietary Bacillus spp. enhanced growth and disease resistance of weaned pigs by modulating intestinal microbiota and systemic immunity. J Anim Sci Biotechnol [Internet]. 2020 Sep [cited 2022 Oct 12];11(1): 101. Available from: https://ncbi.nlm.nih.gov/pmc/articles/PMC7491085/. doi: 10.1186/s40104-020-00498-3

4. Elshaghabee FM, Rokana N, Gulhane RD, Sharma C, Panwar, H. Bacillus as potential probiotics: status, concerns, and future perspectives. Front Microbiol [Internet]. 2017 Sep [cited 2022 Oct 12];10(8): 1490. Available from: https://pubmed.ncbi.nlm.nih.gov/28848511/. doi: 10.3389/fmicb.2017.01490

5. Ayala L, Bocourt R, Castro M, Martínez M, Herrera M. Effect of the probiotic additive Bacillus subtilis and their endospores on milk production and immune response of lactating sows. Cuba J Agric Sci [Internet]. 2016 Sep [cited 2022 Oct 12];49(1): [about 4 p.]. Available from https://cjascience.com/index.php/CJAS/article/view/550.

6. Jeong J, Kim J, Lee S, Kim, I. Evaluation of Bacillus subtilis and Lactobacillus acidophilus probiotic supplementation on reproductive performance and noxious gas emission in sows. Ann. Anim. Sci [Internet]. 2015 Jul [cited 2022 Oct 12];15(3): 699-709. Available from https://www.researchgate.net/publication/282563410. doi: 10.1515/aoas-2015-0018

7. Gu X, Chen J, Li H, Song Z, Chang L, He X, et al. 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 [Internet]. 2021 Mar [cited 2022 Oct 12];7(1): 72-83. Available from: https://pubmed.ncbi.nlm.nih.gov/33997334/. doi: 10.1016/j.aninu.2020.06.010

8. Cheng C, Wei H, Yu H, Xu C, Jiang S, Peng J. Metabolic syndrome during perinatal period in sows and the link with gut microbiota and metabolites. Front Microbiol [Internet]. 2018 Aug [cited 2022 Oct 12];24(9): 1989. Available from: https://pubmed.ncbi.nlm.nih.gov/30197635/. doi: 10.3389/fmicb.2018.01989

9. Van de Wouw M, Schellekens H, Dinan TG, Cryan JF. Microbiota-gut-brain axis: modulator of host metabolism and appetite. Nutr J [Internet]. 2017 May [cited 2022 Oct 12];147(5): 727-745. Available from: https://pubmed.ncbi.nlm.nih.gov/28356427/. doi: 10.3945/jn.116.240481

10. Sakomura, NK, Rostagno HS. Métodos de pesquisa em nutrição de monogástricos. 2. ed. Jaboticabal: FUNEP; 2016. 425 p.

11. Islas-Fabila P, Mota-Rojas D, Martínez-Burnes J, Mora-Medina P, González-Lozano M, Roldan-Santiago P, et al. Physiological and metabolic responses in newborn piglets associated with the birth order. Anim Reprod Sci [Internet]. 2018 Oct [cited 2022 Oct 12];197(12): 247-256. Available from: https://pubmed.ncbi.nlm.nih.gov/30193776/. doi: 10.1016/j.anireprosci.2018.08.037

12. Devillers N, Van Milgen J, Prunier A, Ledividich J. Estimation of colostrum intake in the neonatal pig. Anim Sci [Internet]. 2004 Aug [cited 2022 Oct 12];78(2): 305-313. Available from: https://www.researchgate.net/publication/259640919. doi: 10.1017/S1357729800054096.

13. Devillers N, Le Dividich J, Prunier A. Influence of colostrum intake on piglet survival and immunity. Animal [Internet]. 2011 Aug [cited 2022 Oct 12];5(10): 1605-1612. Available from: https://pubmed.ncbi.nlm.nih.gov/22440352/. doi: 10.1017/S175173111100067X.

14. Herpin P, Le Dividich J, Hulin JC, Fillaut M, De Marco F, Bertin R. Effects of the level of asphyxia during delivery on viability at birth and early postnatal vitality of newborn pigs. JAS [Internet]. 1996 Sep [cited 2022 Oct 12];74(9): 2067-75. Available from: https://pubmed.ncbi.nlm.nih.gov/8880407/. doi: 10.2527/1996.7492067x.

15. Alonso-Spilsbury M, Mota-Rojas D, Villanueva-García D, Martínez-Burnes J, Orozco, H. Ramírez-Necoechea, R. Trujillo, M. E. Perinatal asphyxia pathophysiology in pig and human: a review. Anim Reprod Sci [Internet]. 2005 Sep [cited 2022 Oct 12];90(2): 1-30. Available from: https://pubmed.ncbi.nlm.nih.gov/16257594/. doi: 10.1016/j.anireprosci.2005.01.007.

16. Nam NH. Risk factors for prolonged postparturient vaginal discharge in sows. Thai J Vet Med [Internet]. 2020 Mar [cited 2022 Oct 12];50(1): 57-63. Available from: https://he01.tci-thaijo.org/index.php/tjvm/article/view/243257. doi: 10.1038/nrendo.2014.91.

17. King GJ, Willoughby RA, Hacker RR. Fluctuations in rectal temperature of swine at parturition. Can Vet J [Internet]. 1972 Mar [cited 2022 Oct 12];13(3): 72-74. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1695641/.

18. Zhang Q, Li J, Cao M, Li Y, Zhuo Y, Fang Z, et al. Dietary supplementation of Bacillus subtilis PB6 improves sow reproductive performance and reduces piglet birth intervals. Anim Nutr [Internet]. 2020 Mar [cited 2022 Oct 12];6(3): 278-287. Available from: https://pubmed.ncbi.nlm.nih.gov/33005761/. doi: 10.1016/j.aninu.2020.04.002.

19. Farmer C. The gestating and lactating sow. Wageningen Academic Publishers. 2015 [cited 2022 Oct 12] Edition 1. doi: 10.3920/978-90-8686-803-2.

20. Lei K, Li YL, Wang Y, Wen J, Wu HZ, Yu DY, et al. Effect of dietary supplementation of Bacillus subtilis B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice. J Zhejiang Univ Sci B [Internet]. 2015 Jun [cited 2022 Oct 12];16(6): 487-495. Available from: https://pubmed.ncbi.nlm.nih.gov/26055910/. doi: 10.1631/jzus.B1400342.

21. Rajput IR, Li Wf, Li Yl, Lei J, Wang Mq. Application of probiotic (Bacillus subtilis) to enhance immunity, antioxidation, digestive enzymes activity and hematological profile of broiler. Pak Vet J [Internet]. 2013 Jan [cited 2022 Oct 12];33(1): 69-72. Available from: https://www.researchgate.net/publication/236872018.

22. Cao G, Tao F, Hu Y, Li Z, Zhang Y, Deng B. Positive effects of a Clostridium butyricum-based compound probiotic on growth performance, immune responses, intestinal morphology, hypothalamic neurotransmitters, and colonic microbiota in weaned piglets. Food Funct [Internet]. 2019 Oct [cited 2022 Oct 12];10(5): 2926-2934. Available from: https://pubs.rsc.org/en/content/articlelanding/2019/fo/c8fo02370k. doi:10.1039/c8fo02370k.

23. Feng S, Meng C, Hao Z, Liu H. Bacillus licheniformis Reshapes the Gut Microbiota to Alleviate the Subhealth. Nutrients [Internet]. 2022 Apr [cited 2022 Oct 12];14(14): 1642. Available from: https://pubmed.ncbi.nlm.nih.gov/35458204/. doi: 10.3390/nu14081642.

24. Voigt JP, Fink H. Serotonin controlling feeding and satiety. Behav Brain Res [Internet]. 2015 Jan [cited 2022 Oct 12];15(277): 14-31. Available from: https://pubmed.ncbi.nlm.nih.gov/25217810/. doi: 10.1016/j.bbr.2014.08.065.

25. Murray S, Tulloch A, Gold MS, Avena NM. Hormonal and neural mechanisms of food reward, eating behaviour and obesity. Nat Rev Endocrinol [Internet]. 2014 Sep [cited 2022 Oct 12];10(9): 540-52. Available from: https://pubmed.ncbi.nlm.nih.gov/24958311/. doi: 10.1038/nrendo.2014.91.

26. Menegat MB, Gourley KM, Braun MB, DeRouchey JM, Woodworth JC, Bryte J, et al. Effects of a Bacillus-based probiotic on sow performance and on progeny growth performance, fecal consistency, and fecal microflora. Kans Agric Exp Stn Res Rep [Internet]. 2018 Nov [cited 2022 Oct 12];4(9): 10-4148. Available from: https://newprairiepress.org/kaesrr/vol4/iss9/4/. doi: 10.4148/2378-5977.7652.

27. Hu J, Kim YH, Kim IH. Effects of two bacillus strains probiotic supplement on reproduction performance, nutrient digestibility, blood profile, fecal score, excreta odor contents and fecal microflora in lactation sows, and growth performance in sucking piglets. Livest Sci [Internet]. 2021 Feb [cited 2022 Oct 12];244(19): 1-27. Available from: https://pubag.nal.usda.gov/catalog/7142550. doi: 10.1016/j.livsci.2020.104293.

28. Oliviero C, Kokkonen T, Heinonen M, Sankari S, Peltoniemi O. Feeding sows with high fibre diet around farrowing and early lactation: impact on intestinal activity, energy balance related parameters and litter performance. Res Vet Sci [Internet]. 2009 Sep [cited 2022 Oct 12];86(2): 314-319. Available from: https://pubmed.ncbi.nlm.nih.gov/18725160/. doi: 10.1016/j.rvsc.2008.07.007.

29. Nuntapaitoon M, Muns R, Tummaruk P. Newborn traits associated with pre-weaning growth and survival in piglets. Asian-Australas J Anim Sci [Internet]. 2018 Feb [cited 2022 Oct 12];31(2): 237–244. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5767506/. doi: 10.5713/ajas.16.0962.

30. Turnbaugh PJ, Ley RE, Mahowald MA, Vincent M, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature [Internet]. 2006

Dec [cited 2022 Oct 12];21(444): 1027-1031. Available from: https://pubmed.ncbi.nlm.nih.gov/17183312/. doi: 10.1038/nature05414.

31. Link R, Reichel P, Kyzeková P. The influence of probiotics on reproductive parameters of sows and health of their sucklings. Folia Vet [Internet]. 2016 Oct [cited 2022 Oct 12];60(3): 43-46. Available from: https://sciendo.com/article/10.1515/fv-2016-0028. doi: 10.1515/fv-2016-0028.

32. Diehl B, Oster M, Vernunft A, Wimmers K, Bostedt H. Intrinsic challenges of neonatal adaptation in swine. Arch. Anim. Breed [Internet]. 2022 Nov [cited 2024 Jan 17]. 65 (4):427-438. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9752711/.

ARTIGO 2

Artigo científico nas normas da revista *Journal of Animal Science*

Effects of maternal probiotic supplementation on farrowing efficiency and colostrum intake of piglets

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LAY SUMMARY

During the gestation and lactation periods, sows are subject to various circumstances that can lead to heightened levels of stress. Impaired welfare, with consequent damage to farrowing performance and vitality of piglets, is one of the possible consequences. Probiotic microorganisms may be helpful in this phase, as these additives can benefit welfare through action on the gut-brain axis. The objective of this study was to evaluate the effects of sow supplementation with probiotics on farrowing kinetic and newborn vitality. Forty sows were assigned to the following treatments: control, without supplementation, and probiotics. The probiotics were composed of *Lactobacillus acidophilus, Lactobacillus bulgarius, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum*, *Enterococcus faecium*, and *Streptococcus thermophilus*. The probiotic treatment resulted in a lower frequency of long-term deliveries (>300 min), and the sow feed intake was 10% higher than control. Piglets from supplemented sows showed colostrum intake 25% higher than the control. In conclusion, probiotics had no influence on the stool consistency of sows after farrowing. Probiotics can be promising to some reproductive responses.

TEASER TEXT

Supplying multistrain probiotic to sows during gestation and lactation is beneficial for farrowing kinetic, and colostrum intake in neonates.

ABSTRACT

Stress can affect the farrowing kinetics of sows and the vitality of newborn piglets. Probiotics can be beneficial in this context as these additives are able to affect physiological and behavioral processes through multiple pathways, such as the gut-microbiota-brain axis (GMB. This study was developed to evaluate the effects of probiotic supplementation to sows during gestation-lactation and piglet vitality. Forty multiparous sows (parity orders from 2 to 9) were attributed to the following treatments: control, without supplementation; and probiotics, in which a commercially available probiotic was provided to the sows from the beginning of gestation until the end of lactation. The probiotic product was composed of *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium bifidum, Enterococcus faecium,* and *Streptococcus thermophilus*. Variables related to farrowing kinetic and piglet vitality were evaluated. Colostrum intake was estimated by piglet weight at birth and 24h. Sow feed intake and fecal score were also analyzed. Data were analyzed by variance analysis. Probiotic treatment had a lower frequency of long-term deliveries (>300 min), and their piglets had higher colostrum intake (26%; P=0.036). The overall feed intake of the sows supplemented with probiotic was higher than the control sows (10%; P=0.067). The treatments did not influence feed intake in the first week of lactation, the fecal score of the sows, or the vitality of the piglets. According to these results, the probiotic additive seems promising for optimizing farrowing and colostrum intake capacity.

Keywords: gut-microbiota-brain axis, newborn, bacteria, sows, stress

INTRODUCTION

In addition, the influence of probiotics on the gut-brain axis is currently one of the most discussed issues. Studies have shown that the environment and challenges faced by sows, can cause metabolic changes and modify behavioral patterns (Berchieri-Ronchi et al., 2011). Several factors considered critical, can compromise their sows welfare and, consequently, their colostrum and milk production, maternal hability and longevity, in addition to progeny vitality (Barba-vidal et al, 2019). Thus, is beneficial the inclusion of feed additives, such as probiotics, that can influence the modulation of the intestinal microbiota, which can stimulate the production of neurochemical substances and contribute to the improvement of the sow's welfare condition (Lyte and Lyte, 2019).

Probiotics are widely used in swine farming and numerous studies have been carried out on their effects both *in vitro* and *in vivo* (Mathipa and Thantsha, 2017). Its anti-inflammatory, enzymatic, and antioxidative properties have received attention (Quinto et al., 2014). In addition, probiotics are able to modulate the intestinal microbiota, improving gut-health status and affect the neuroendocrine system, through a lot of pathways (Lu, 2021). Understanding the intestinal microbiota in sows is important due to its relationship with feed efficiency, reduction of oxidative stress, and improvement of responses related to farrowing and vitality of neonates (Hasan et al., 2019). Studies have already shown that probiotic strains can positively influence metabolic dysfunctions (i.e. inflammatory status, oxidative stress) in the gestational period which are capable of strongly impairing reproductive variables (Cheng et al., 2018).

There is great variability in the final results when pigs are supplemented with probiotics. This is because different strains, supplementation periods and/or host individualities can affect the final responses (Hill et al., 2014). There are minimal studies testing probiotics for sows and the understanding of the different modes of action of these additives has not yet been fully clarified. The influence of supplementation throughout the gestational and lactation phase also needs to be further studied as most studies begin supplementation in the final thirds of pregnancy (Liu et al., 2018).

Therefore, this study aimed to evaluate the effects of the inclusion of probiotic additives in the diets of pregnant and lactating sows on the reproductive aspects and vitality of newborn piglets.

MATERIALS AND METHODS

The experiment was conducted on a commercial farm (Maratá, Rio Grande do Sul, Brazil) and the protocols were carried out following the Brazilian guidelines for animal care and use. Temperature e humidity index (THI) were analyzed using datalogger equipment (Datalogger Instrutherm HT-70, Rio Grande do Sul, Brazil). Values were classified as a safe if THI ≤ 74, critical, if 74 < THI < 79, dangerous if 79 ≤ THI < 84 and emergency if THI ≥ 84 (Botto et al., 2014). The calculation was made according to the following equation:

$$THI = 0.8\ T\alpha + (RH/100) \times (T\alpha - 14.3) + 46.4$$

Where: Tα – ambient air temperature (°C); RH – relative humidity (%).

Animals and experimental design

Forty Large-white × Landrace (Pic Camborough, Agroceres-PIC, São Paulo, Brazil) sows were used, with parity orders varying from 2 to 9. Due to the return to estrus, five sows were removed from the study. Two treatments were tested: 1) Control (n = 17), sows that did not receive probiotic supplementation; 2) Probiotic (n = 18): sows that received a commercially available product (Protexin™ Concentrated, Elanco™ Animal Health, São Paulo, Brazil) containing *Lactobacillus acidophilus (*2.06 x 10^8 CFU/g*), Lactobacillus bulgaricus (*2.06 x 10^8 CFU/g*), Lactobacillus plantarum (*1.26 x 10^8 CFU/g*), Lactobacillus rhamnosus (*2.06 x 10^8 CFU/g*), Bifidobacterium* *bifidum (*2.00 x 10^8 CFU/g*)*, *Enterococcus faecium (*6.46 x 10^8 CFU/g*)*, and *Streptococcus thermophilus (*4.10 x 10^8 CFU/g*)*, during the entire gestation and lactation (21 days) periods.

Treatments were randomly assigned to sows within each parity order. Consequently, each treatment had the same number of sows in each parity order. The number of replications was defined according (Sakomura and Rostagno, 2016), considering the dispersion of the main variables in previous studies. To prevent cross-contamination (among sows, from the feed to the piglets, and in the environment), probiotics were supplied daily via industrial gelatin capsules to each sow. These capsules were them provided to each sow during the trial. The capsule was placed on the top of the feed served to each sow in the morning. Colorful capsules were used to allow easy observation of its intake by the sow.

Despite being supplied in the gelatin capsules, the daily amount was weighted individually to represent an inclusion of 50 g of the additive per ton of feed, which is the concentration recommended by the supplier (Chr. Hansen, São Paulo, Brazil), which resulted in a mean daily supply of 0.1 g during gestation and 0.4 g during lactation per sow. 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 daily feed intake varying from 1.8 to 2.0 kg. During the lactation phase, the amount was supplied ad libitum, with daily feed intake gradually increasing during the first week, reaching an average of 7 to 8 kg per animal. A commercial diet based on corn and soybean meal was offered for both phases with no other additives except for phytase and mycotoxin binders (Table 1). Water was provided *ad libitum* during the entire trial.

The facilities had a semi-automatic feeding system for pregnant sows and a trough-shaped drinking system, that is, the water flow was not continuous. During pregnancy the sows were kept in concrete individual cages (1.2 m^2) and during lactation each one was kept in a conventional farrowing stall (1.2 m^2) with a semi-slatted floor, located in the center of a pen with an available space of 4.2 m^2 with access only for piglets with a fully slatted floor.

Sows assessment

All births were monitored individually. The birth interval and the farrowing duration were registered. The farrowing duration was classified as ‘long’ when exceeded 300 minutes, or normal when lower than 300 minutes (Islas-Fabila et al., 2018). The interventions were registered (vaginal palpation or oxytocin administration). Sow rectal temperature was recorded (Veterinary thermometer IP67, INCOTERM, Rio Grande do Sul, Brazil) immediately after the birth of each piglet .

In order to evaluate the constipation risk, the feces were observed every morning and afternoon during five days after farrowing and an average was calculated between both periods. The evaluation was visual and was always performed by the same person. The scores were 0 (absence of feces), 1 (dry and pellet-shaped), 2 (between dry and normal), 3 (normal and soft, but firm and well-formed), 4 (between normal and wet; still formed, but not firm), and 5 (very wet feces, unformed, and liquid) (Oliveiro., 2010) (Fig. 1). The fecal score was analyzed by individual days, and the frequency of each score was merged and classified into dry (scores 1 and 2), ideal (score 3), and wet (scores 4 and 5) categories. Feed intake was recorded daily during the entire lactation. Later, the data was analyzed considering the first week and the entire lactation period.

Piglet vitality responses

Vitality responses of piglets born were evaluated immediately after birth. The presence or absence of meconium in the skin and umbilical cord integrity (presence or absence of rupture) were registered. Oxygenation and heart rate were measured (Veterinary Oximeter R40, RZVet, São Paulo, Brazil). A small sample of blood was collected in the umbilical cord for quantification of glucose (Monitor Accu-chek Performa, Roche, São Paulo, Brazil) and pH evaluation (Akso, Rio Grande do Sul, Brazil). These animals were identified, weighed, and returned to the sow.

The rectal temperature of the sow was registered. Piglets were weighed again 24 hours after birth to estimate the colostrum intake. The following equation proposed by Devillers et al. (2004) was used:

$$C_{int} = -217,4 + 0,217 \times t + 1861019 \times \frac{BW_{24}}{t} + BW_b \times \left(54,8 - \frac{1861019}{t} \times \left(0,9985 - 3,7 \times 10^{-4} \times t_{fs} + 6,1 \times 10^{-7} \times t_{fs}^2\right.\right.$$

in which: C_{int}= colostrum intake (grams); t= time from birth to weight measurement (minutes); t_{fs}= time from birth to first suckling (minutes); BW_{24} = body weight at 24 hours (kilograms); BW_b= body weight at birth (kilograms). After, the piglets were divided into three categories of colostrum intake: which were: 'low intake' when lower than 290 g, 'average intake' when between 291 and 439 g; and 'high intake' when higher than 440 g (Devillers et al., 2011). The frequency of pigs in each class was then calculated. For physiological variables, the reference parameters used were based on studies of Herpin et al. (1996) and on Apgar score program of Alonso-Spilsbury et al. (2005).

Statistical analysis

Statistical analyses were performed using the SAS software (SAS Institute Inc., Cary, NC, USA). Responses were analyzed using the GLIMMIX procedure. The effects of parity order, and litter size were tested and removed from the final model when not significant. The piglet was considered the experimental unit for the vitality and colostrum responses; however, the data were grouped within the sow (random effect). Residuals were evaluated for normality using univariate procedures and the Shapiro-Wilk test. The treatment means were separated using the PDIFF option and the differences were interpreted at the significance levels of 5% (significant effects) and 10% (marginally significant).

RESULTS

Considering all thermal control methods used on the farm, the THI remained within the value considered safe for sows, that is, below 74.

Sows assessment

Farrowing duration (P = 0.004) and piglet expulsion interval (P = 0.048; Table 2) were shorter in sows supplemented with probiotics compared to control animals. The non-supplemented group had 36% more deliveries lasting more than 300 minutes (Fig. 2).

There was no difference between treatments for feed consumption during the first postpartum week (P = 0.252). During the entire 21-day period of lactation (P = 0.067) there was a tendency for higher intake in the supplemented ones (Table 3). No significant differences were observed in any of the five days postpartum fecal score analyses (Table 4).

Piglet vitality

Meconium presence, umbilical cord rupture, blood pH, blood glucose, blood oxygen saturation, and heart rate were not influenced by the treatments (Table 5). However, the estimated colostrum intake per piglet was improved by 12% by the probiotics (P = 0.014). Colostrum intake per litter was also 19% higher in supplemented sows (P = 0.011; Table 6). The control group had a higher prevalence of piglets with low colostrum consumption (P = 0.008; Fig. 3), whereas in the probiotic treatment, there was a higher prevalence of piglets with average colostrum intake (P = 0.005). The treatments had similar frequency of piglets with high colostrum intake (P = 0.764).

DISCUSSION

Sow assessment

At a practical level, reducing the birth interval can reduce the need for drug or physical intervention in the sow; however, in this study, no effect was observed on this variable. Probiotics are known to act via the production of antimicrobial substances, modulation of the intestinal microbiota and host immune system, and competitive exclusion between probiotics and pathogenic bacteria (Asml et al., 2015). The results between studies vary greatly because of the various mechanisms of probiotic action, the individuality of the microbial community of each animal, and the specificity of the strains used (Bajagai et al. 2016). In recent decades, there has been increasing discussion regarding the role of microbiota in communication between the gut and the brain (Petra et al., 2015).

Substances arising from the GMB axis could explain why the interval between births and the consequent length of farrowing was longer in control sows, considering that they would not have sufficient stimulation to reduce hormones related to stress and anxiety, such as in supplemented. Probiotic microorganisms have already shown the ability to influence emotional and cognitive state through endocrine, neural, and immune pathways (Rios et al., 2017). *In vivo* studies in mice and humans have reported that probiotic strains can reduce stress-associated behavior and chronic anxiety (Davis et al., 2017; Schnorr and Bachner, 2016). Pregnant sows undergo changes in the microbiota during the perinatal period, and this dysbiosis can cause metabolic changes, such as intestinal inflammation, increased cortisol levels, and increased stress levels (Cheng et al., 2018). Wang et al., (2019) suggested that metabolic changes cause oxidative stress in sows and impact on the increase in farrowing duration and number of stillbirths. Communication between the GMB axis can contribute to this context in a few ways. For example, strains belonging to the *Bifidobacterium* and *Lactobacillus* groups restore plasma concentrations of adrenocorticotropic hormone (ACTH), which is synthesized by adenohypophysis cells and is responsible for stimulating cortisol synthesis and secretion (Kogut et al., 2022). These strains can also reduce corticotropin-releasing hormone (CRF) levels; both CRF and ACTH undergo changes under chronic stress conditions (Laval et al., 2015). Furthermore, maternal stress hormones can activate the hypothalamic-pituitary-adrenal (HPA) axis, preventing the regulation of glucocorticoid levels in the placenta (Sheng et al., 2021). This leads to the activation of the fetal HPA axis, increasing glucocorticoid levels and affecting the contraction of the myometrium, increasing the interval between births (Li et al., 2014). However, the *Bifidobacterium* group can regulate the production of the neurotransmitter serotonin, which is mainly related to the limbic system, and may control anxiety and stress reactions through an increase in plasma tryptophan (Desbonnet et al., 2010).

Probiotic microorganisms can positively stimulate the development and proliferation of beneficial bacteria, reducing highly virulent bacteria that release toxins and cause diarrhea (Asml et al., 2015). It has already been reported that probiotic strains were able to reduce the incidence of diarrhea in the second postpartum week (Giang et al., 2010). However, Hu et al. (2021) tested *Bacillus* strains in pregnant sows and found no effect of treatment on the fecal score after farrowing, similar to the results of this study. Jeong et al. (2015) also did not observe a difference in the fecal score of sows in the first week after farrowing with additives containing *Bacillus subtilis* and *Lactobacillus acidophilus*.

The influence of the treatments on feed intake may have occurred indirectly by improving the integrity of the intestinal barrier, one of the beneficial effects of probiotics (Bajagai et al., 2016). Increased intestinal permeability is known to result from dysbiosis in the intestinal environment during the perinatal period (Bjorkman et al., 2023). In addition to stimulating a systemic inflammatory response, this leads to an excessive reduction in insulin sensitivity, which can result in a reduction in food intake (Xu et al., 2020). Lower feed intake also impacts inefficient milk production, for example (Strathe et al., 2017). Studies have shown that probiotics, such as *Lactobacillus acidophilus,* can regulate the expression of proteins that are components of tight junctions in an experiment with mice (Al-Sadi et al., 2021; Rose et al., 2021). Improving the integrity of tight junctions allows for the regulation of intestinal permeability. In the current study, the control sows had a significantly lower feed intake than the sows receiving probiotics. The results of this study corroborate those of Bohmer et al. (2006) and Hayakawa et al. (2016), because sows supplemented with probiotics containing the *Enterococcus faecium* strain and a product containing a mixture of *Bacillus mesentericus*, *Clostridium butyricum,* and *Enterococcus faecalis* strains, respectively, showed an increase in daily intake. Both studies added an additive effect from the last third of gestation until weaning. Jeong et al. (2015) noted the same response in feed intake during lactation. The first postpartum week is a period known for stabilization of feed intake; therefore, it is likely that the absence of a significant result can be explained by this factor.

Piglet vitality assessment

Analyzes performed on newborn piglets, such as meconium presence, umbilical cord rupture, blood glucose, blood pH, blood oxygen saturation, heart rate, were done as an indirect way of also evaluating the effect of probiotic supplementation. These variables are often used to determine the viability of newborn piglets (Pandarzi et al. 2013). It was hypothesized that the improvement of the sow's emotional condition would impact the efficiency of farrowing, and consequently, this would be positive for the piglets' vitality. The vitality of newborn piglets is directly associated with the intensity of asphyxia during the farrowing process, and this is correlated with the duration of farrowing (Mota-Rojas et al., 2018). As already explained, the duration of delivery was most likely affected by treatment; however, vitality variables were not affected. In future studies, it might be beneficial to complement the vitality assessment to assess the interval between birth and positioning with all four limbs erect, as well as the interval between birth and first breath (Mota-Rojas et al., 2012). These variables can be useful for evaluating neurological and olfactory damage caused by hypoxia, helping assess vitality (Mota-Rojas et al., 2018). Perhaps, the variable evaluation used in this study was not sufficient to demonstrate an effect on the vitality of newborn piglets. Previous studies have reported a relationship between physiological variables of vitality and an increase in piglet expulsion time (Van Dijk et al., 2006; Islas-Fabila et al., 2018; Mota-Rojas et al., 2014).

The absence of difference between sow’s rectal temperature may be positive, considering that high values could indicate activation of the immune system, resulting in fever (Böhmer et al., 2006). There was no climate challenge during the experiment to produce such a condition.

It has already been reported that *Lactobacillus*, *Enterococcus,* and *Streptococcus* increase the body's ability to acquire energy from the diet. This process may favor supplying energy to sows during lactation (Bajagai et al., 2016; Cheng et al., 2018). Therefore, probiotic sows were probably able to produce more colostrum and milk from the beginning of lactation. It is likely that piglets with greater food availability were heavier than those from the control sows. These results corroborate those of Jeong et al. (2015), who reported that *Bacillus* supplementation increased the weight of suckling piglets.

In summary, the farrowing process affects the productivity and welfare of sows, the quality of piglets, and the entire dynamics of the farm. Probiotic is bacteria capable of benefiting from this context are necessary and of great interest. This study highlights the need to deepen knowledge about the function of the microbiota throughout the animal organism. It is noted that microorganisms can be the key to increasing productivity directly or indirectly.

CONCLUSION

The inclusion of multistrain probiotics to gestating and lactating sows is promising to improve farrowing kinetics. Probiotics are not able to improve vitality variables in neonatal piglets. Multistrain probiotics do not affect sows fecal score. However, piglets from supplemented sows are more efficient in their capacity to consume colostrum.

LITERATURE CITE

Alonso-Spilsbury. M., Mota-Rojas, D. Villanueva-García, D. Martínez-Burnes, J. Orozco, H. Ramírez-Necoechea, R. Trujillo, M. E. 2005. Perinatal asphyxia pathophysiology in pig and human: a review. Animal reproduction Science. 90:1-30. doi: 10.1016/j.anireprosci.2005.01.007.

Al-Sadi, R., Nighot, P. Nighot, M. Haque, M. Rawat, M. Ma, T. Y. 2021. Lactobacillus acidophilus induces a strain-specific and toll-like receptor 2–dependent enhancement of intestinal epithelial tight junction barrier and protection against intestinal inflammation. The American Journal of Pathology. 191:872-884. doi: 10.1016/j.ajpath.2021.02.003.

Asml, A., Agazzi, A. Invernizzi, G. Bontempo, V. Savoini, G. 2015. The beneficial role of probiotics in monogastric animal nutrition and health. Journal of Dairy, Veterinary & Animal Research. 2:116-132. doi: 10.15406/jdvar.2015.02.00041.

Barba-vidal, E., Martín-orúe, S. M, Castillejos, L. 2019. Practical aspects of the use of probiotics in pig production: A review. Livestock Science. 223: 84-96. doi: 10.1016/j.livsci.2019.02.017.

Bajagai, Y. S., Klieve, A.V., Dart, P.J., Bryden, W.L. 2016. Probiotics in animal nutrition: Production, impact and regulation. FAO. Anim. Prod. Health. 56:978-992.

Berchieri-Ronchi, C. B., Kim, S. W. Zhao, Y. Correa, C. R. Yeum, K. J. Ferreira, A. L. A. 2011. Oxidative stress status of highly prolific sows during gestation and lactation. Animal. 5:1774-1779. doi: 10.1017/S1751731111000772.

Böhmer, B.M., Kramer, W. Roth – Mayer, D.A. 2006. Dietary probiotic supplementation and resulting effects on performance, health status, and microbial characteristics of primiparous sows. J. Anim. Physiol. Anim. Nutr. 90:309–315. doi: 10.1111/j.1439-0396.2005.00601.x.

Björkman, S., Kauffold, J., Kaiser M. Ø. 2022. Reproductive health of the sow during puerperium. Mol. Reprod. Dev. 90:561-579. doi: 10.1002/mrd.23642.

Botto, L., Lendelová, J., Strmeňová, A., Reichstädterová, T. The effect of evaporative cooling on climatic parameters in a stable for sows. Res. Agr. Eng. 60:85-91. 2014.

Cheng, C., Wei, H. Yu, H. Xu, C. Jiang, S. Peng, J. 2018. Metabolic syndrome during perinatal period in sows and the link with gut microbiota and metabolites. Frontiers in microbiology, 9, 1989. doi: 10.3389/fmicb.2018.0198.

Davis, D.J., Hecht, P.M. Jasarevic, E. Beversdorf, D.Q. Will, M.J. Fritsche, K. Gillespie, C.H. 2017. Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. Brain Behav Immun. 59:38–48. doi: 10.1016/j.bbi.2016.09.003.

Desbonnet, L., Garrett, L. Clarke, G. Kiely, B. Cryan, J.F. Dinan, T. G. 2010. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience. 170:1179–1188. doi: 10.1016/j.neuroscience.2010.08.005.

Devillers, N., Van Milgen, J. Prunier, A. Ledividich, J. 2004. Estimation of colostrum intake in the neonatal pig. Animal Science. 78:305-313. doi: 10.1017/S1357729800054096.

Devillers, N., Le Dividich, J. Prunier, A. 2011. Influence of colostrum intake on piglet survival and immunity. Animal. 5:1605-1612. doi: 10.1017/S175173111100067X.

Giang, H. H., Viet, T. Q. Ogle, B. Lindberg, J. E. 2010. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. Livest. Sci. 129:95–103. doi: 10.1016/j.livsci.2010.01.010.

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. 87:1501-1510. doi: 10.1111/asj.12565.

Hasan, S., Saha, S. Junnikkala, S. Orro, T. Peltoniemi, O. Oliviero, C. 2019. Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota. Animal. 13:1599-1606. doi: 10.1017/S1751731118003518.

Herpin, P., Le Dividich, J. Hulin, J. C. Fillaut, M. De Marco, F. Bertin, R. 1996. Effects of the level of asphyxia during delivery on viability at birth and early postnatal vitality of newborn pigs. Journal of Animal Science. 74:2067-2075. doi: 10.2527/1996.7492067x.

Hill, C., Guarner, H., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., Sanders, M. E. 2014 Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews. Gastroenterology & Hepatology, London 11:506–514. DOI: 10.1038/nrgastro.2014.66.

Hu, J., Kim, Y. H; Kim, I. H. 2021 Effects of two bacillus strains probiotic supplement on reproduction performance, nutrient digestibility, blood profile, fecal score, excreta odor contents and fecal microflora in lactation sows, and growth performance in sucking piglets. Livestock Science. 244:104-293. doi: 10.1016/j.livsci.2020.104293.

Islas-Fabila, P., Mota-Rojas, D. Martínez-Burnes, J. Mora-Medina, P. González-Lozano, M. Roldan-Santiago, P. Orozco-Gregorio, H. 2018. Physiological and metabolic responses in newborn piglets associated with the birth order. Animal reproduction Science. 197:247-256. doi: 10.1016/j.anireprosci.2018.08.037.

Kogut, M. H, Zhang, G. 2022 Gut Microbiota, immunity and health in production animals. 1th rev. ed. Stillwater: Springer, Berlim, DE.

Oliviero, C. Successful farrowing in sows. Animal Reproduction Science. 2010.

Laval, L., Martin, R. Natividad, J. N. Chain, F. Miquel, S. De Maredsous, C.D. Capronnier, S. Sokol, H. Verdu, E. F. Van Hylckama Vlieg, J. E. T. Bermudez-Humaran, L. G. 2015. Lactobacillus rhamnosus CNCM I-3690 and the commensal bacterium Faecalibacterium prausnitzii A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice. Gut Microbes. 6:1–9. doi: 10.4161/19490976.2014.990784.

Li, X. Q., Zhu, P. Myatt, L. Sun, K. 2014. Roles of glucocorticoids in human parturition: a controversial fact? Placenta. 35:291-296. doi: 10.1016/j.placenta.2014.03.005.

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. 18:335-350, Lyte, J. M.; Lyte, M. 2019. Microbial endocrinology: intersection of microbiology and neurobiology matters to swine health from infection to behavior. Animal. 13:2689-2698. doi: 10.1017/S1751731119000284.

Lu, S. 2021. The role of Lactic Acid Bacteria in Gut Microbiota and Mucosal Immune System: El papel de las bacterias del ácido láctico en la microbiota intestinal y el sistema inmunitario de la mucosa. South Florida Journal of Development. 2:3818-3825. doi: 10.46932/sfjdv2n3-009.

Mathipa, M. G., Thantsha, M. S. 2017. Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. Gut Pathogens. 9:28-36. doi: 10.1186/s13099-017-0178-9.

Mota-Rojas, D., Fierro, R. Roldan-Santiago, P. Orozco-Gregorio, H. Gonzalez-Lozano, M. Bonilla, H. Ramirez-Necoechea, R. 2014. Outcomes of gestation length in relation to farrowing performance in sows and daily weight gain and metabolic profiles in piglets. Animal Production Science, 55:93-100. doi: 10.1071/AN13175.

Mota-Rojas, D., López, A. Martínez-Burnes, J. Muns, R. Villanueva-García, D. Mora-Medina, P. Ramírez-Necoechea, R. 2018. Is vitality assessment important in neonatal animals. CAB Rev. 13:1-13. doi: 10.1079/PAVSNNR13036.

Mota-Rojas, D., Villanueva-Garcia, D. Hernandez-Gonzalez, R. Roldan-Santiago, P. Martinez-Rodriguez, R. Gonzalez-Meneses, B. 2012. Assessment of the vitality of the newborn: an overview. Scientific Research and Essays. 7:712–8. doi: 10.5897/SRE.9000221.

Panzardi, A., Bernardi, M. L. Mellagi, A. P. Bierhals, T. Bortolozzo, F. P. Wentz, I. 2013. Newborn piglet traits associated with survival and growth performance until weaning. Preventive Veterinary Medicine. 110:206-213. doi: 10.1016/j.prevetmed.2012.11.016.

Petra, A. I., Panagiotidou, S., Hatziagelaki, E., Stewart, J.M., Conti, P., Theoharides, T.C. 2015. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. Clin. Ther. 37:984–995. doi: 10.1016/j.clinthera.2015.04.002.

Quinto, E. J., Jiménez, P. Caro, I. Tejero, J. Mateo, J. Girbés, T. 2014. Probiotic lactic acid bacteria: a review. Food and Nutrition Sciences. 5:17-23. doi: 10.4236/fns.2014.518190.

Rios, A. C., Maurya, P. K. Pedrini, M. Zeni-Graiff, M. Asevedo, E. Mansur, R. B. Wieck, A. Grassi, O. R. McIntyre, R. S. Hayashi, M. A. Brietzke, E. 2017. Microbiota abnormalities and the therapeutic potential of probiotics in the treatment of mood disorders. Rev Neurosci. 28:739–749. doi: 10.1515/revneuro-2017-0001.

Rose, E. C., Odle, J. Blikslager, A. T. Ziegler, A. L. 2021. Probiotics, prebiotics and epithelial tight junctions: a promising approach to modulate intestinal barrier function. International journal of molecular sciences. 22:67-29. doi: 10.3390/ijms22136729.

Sakomura, N. K.; Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. In: editor, FUNEP, 2 ed. Jaboticabal, SP. p. 365-425.

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. 2021. The hypothalamic-pituitary-adrenal axis: development, programming actions of hormones, and maternal-fetal interactions. Front. Behav. Neurosci. 14:1-21. doi: 10.3389/fnbeh.2020.601939.

Schnorr, S. L., Bachner, H. A. 2016. Focus: microbiome: integrative therapies in anxiety treatment with special emphasis on the gut microbiome. Yale J Biol Med. 89:397-422.

Strathe, A. V., Bruun, T. S., & Hansen, C. F. 2017. Sows with high milk production had both a high feed intake and high body mobilization. Animal, 11:1913-1921. doi:10.1017/S1751731117000155.

Van Dijk, A. J., Van der Lende, T. Taverne, M. A. M. 2006. Acid–base balance of umbilical artery blood of liveborn piglets at birth and its relation with factors affecting delivery of individual piglets. Theriogenology. 66:1824-1833. doi: 10.1016/j.theriogenology.2006.04.035.

Xu, C., Peng, J. Zhang, X. Peng, J. 2020. Inclusion of soluble fiber in the gestation diet changes the gut microbiota, affects plasma propionate and odd-chain fatty acids levels, and improves insulin sensitivity in sows. Int J Mol Sci. 21:635-640. doi: 10.3390/ijms21020635.

Wang, H., Hu, C. Cheng, C. Cui, J. Ji, Y. Hao, X. Tan, C. 2019. Unraveling the association of fecal microbiota and oxidative stress with stillbirth rate of sows. Theriogenology. 136:131-137. doi: 10.1016/j.theriogenology.2019.06.028.

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[2]	0.500	0.500
L-Lys (65%)	0.168	
L-Valine		0.158
L-Thr	0.100	0.128
DL- Methionine	0.049	0.100
Tryptophan	0.005	0.050
Phytase[3]	0.005	0.005
Carbohydrase[4]		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
[3] Axtra PHY GOLD, Danisco Nutrition.
[4] Rovabio, Adisseo.

Table 2. Effect of probiotic supplementation to gestating-lactating sows on the farrowing duration, required interventions and rectal temperature.

Variables	Treatments*		*P*-value[1]
	Control	Probiotic	
Interval time, min	28.28 (3.397)	20.07 (2.853)	0.048
Farrowing time, min	379.0 (26.36)	273.7 (24.13)	0.004
Interventions, n/litter	4.076 (1.261)	3.461 (1.261)	0.733
Interventions, %	30.08	20.83	-
Sow rectal temperature, °C	38.60 (0.047)	38.64 (0.048)	0.546

[1] Probability of treatment effects.

* Means with standard errors in the parentheses.

Table 3. Effect of probiotic supplementation to gestating-lactating sows on their feed intake (FI) during the lactation period.

Variables	**Treatments***		***P*-value[1]**
	Control	**Probiotic**	
Overall FI[2], kg/day	5.528 (0.216)	6.149 (0.293)	0.067
FI[2] during 1st week, kg/day	4.685 (0.195)	4.992 (0.212)	0.252

[1] Probability of treatment effects.

[2] Feed intake.

* Means with standard errors in the parentheses. Means represent 32 sows.

Table 4. Effect of probiotic supplementation to gestating-lactating sows on their fecal score[1] during five days after farrowing.

Evaluation period	Treatments*		*P*-value[2]
	Control	Probiotic	
Day 1	0.333 (0.109)	0.250 (0.103)	0.583
Day 2	0.727 (0.221)	0.318 (0.221)	0.197
Day 3	0.800 (0.267)	1.000 (0.255)	0.591
Day 4	0.772 (0.245)	0.909 (0.245)	0.696
Day 5	1.090 (0.271)	1.270 (0.271)	0.638

[1] Scores from 0 (attributed to absence of feces) to 5 (attributed to very wet, loose and liquid feces).

[2] Probability of treatment effects.

* Means with standard errors in the parentheses.

Table 5. Effect of probiotic supplementation to gestating-lactating sows on piglet vitality.

Variables	Treatments*		*P*-value[1]
	Control	Probiotic	
Meconium presence, n/litter	2.682 (0.286)	2.690 (0.266)	0.983
Umbilical cord rupture, n/litter	1.280 (0.037)	1.285 (0.039)	0.914
Glucose, mg/dL	56.84 (2.519)	56.93 (2.384)	0.980
Blood pH	8.085 (0.019)	8.100 (0.020)	0.530
Blood oxygen saturation, %	98.76 (0.195)	99.05 (0.226)	0.287
Heart rate, bpm	124.3 (4.942)	128.1 (5.405)	0.568

[1] Probability of treatment effects.

* Means with standard errors in the parentheses.

Table 6. Effect of probiotic supplementation to gestating-lactating sows on colostrum intake of the piglets.

Variables	Treatments*		*P*-value[1]
	Control	Probiotic	
Colostrum intake per piglet, kg	0.279 (0.011)	0.317 (0.012)	0.014
Colostrum intake per litter, kg	3.697 (231.9)	4.577 (231.9)	0.011
Piglets with low intake, %	55 (3.730)	38 (4.361)	0.008
Piglets with average intake, %	35 (3.798)	51 (4.440)	0.005
Piglets with high intake, %	10 (2.572)	11 (3.007)	0.764

[1] Probability of treatment effects.

* Means with standard errors in the parentheses.

Figure 1. Illustration of fecal score used to classify sows feces (Oliveiro., 2010).

Figure 2. Effect of probiotic supplementation to gestating-lactating sows on the farrowing duration and frequency of sows with a long labor.

Figure 3. Effect of probiotic supplementation to gestating-lactating sows on the colostrum intake of piglets (classified as low or normal, which includes average and high intake).

Figure 1

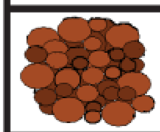
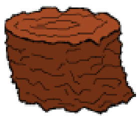
	0	Absence of faeces
	**1**	Dry and pellet-shaped (unformed)
	**2**	Between dry and normal (pellet-shaped and formed)
	**3**	Normal and soft, but firm and well formed
	**4**	Between normal and wet; still formed, but not firm
	5	Very wet faeces, unformed and liquid

Figure 2

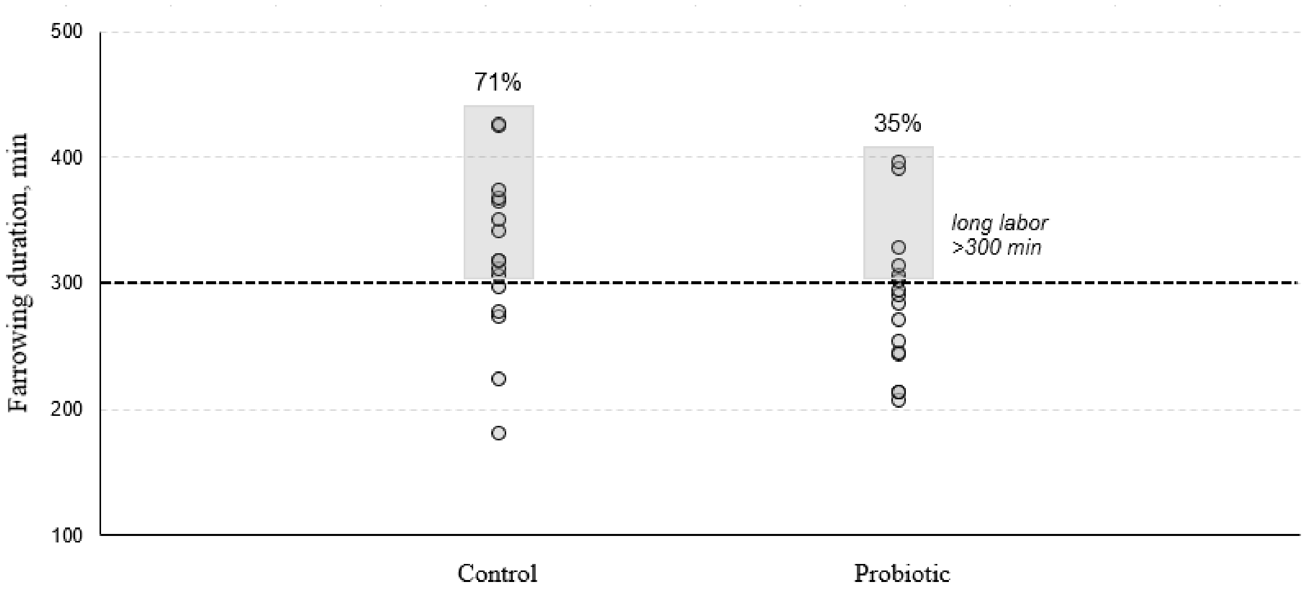
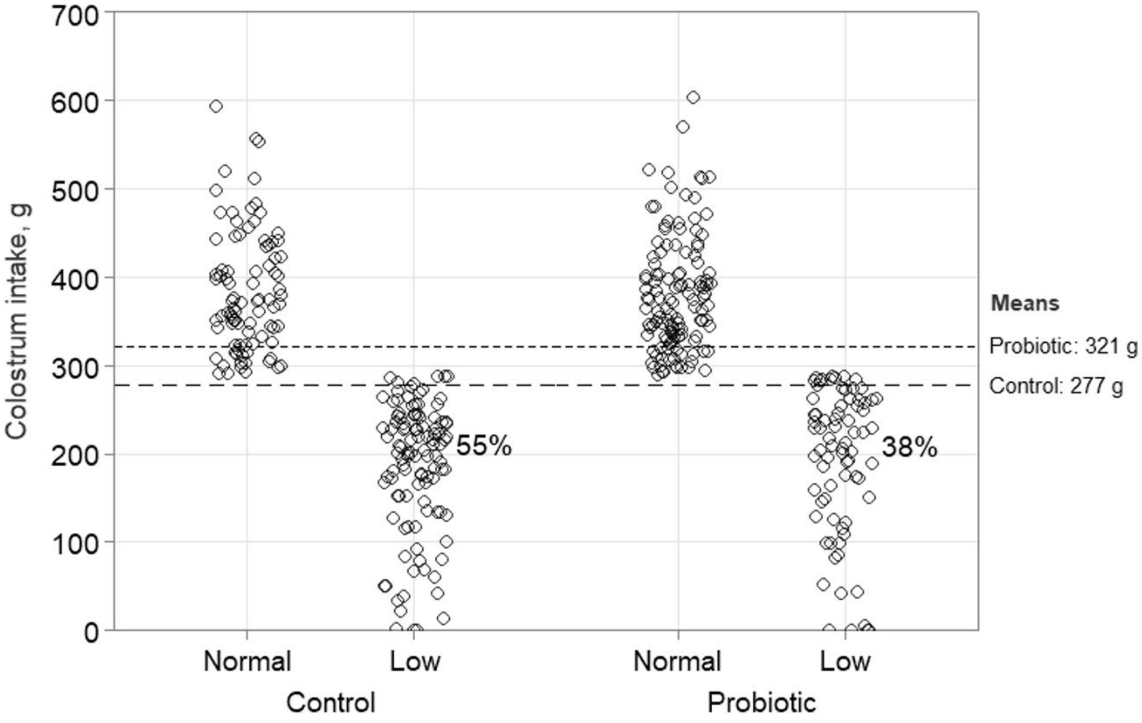

500
400
300
200
100
Farrowing duration, min
71%
35%
long labor
>300 min
Control
Probiotic

Figure 3


700
600
500
400
300
200
100
0
Colostrum intake, g
Normal
Low
Control
Normal
Low
Probiotic
55%
38%
Means
Probiotic: 321 g
Control: 277 g

CAPÍTULO III

ARTIGO 3

Artigo científico nas normas da revista *Animals*

Effects of multistrain probiotic supplementation on sows' emotional and cognitive states and the progeny welfare

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Simple Summary: Feeding technologies, such as probiotics, are widely used in animal industry for purposes beyond nutritional needs. Probiotic bacteria have been shown to have multiple effects on sow physiology during gestation and lactation, making them a subject of great interest when it comes to animal behavior and welfare. In this study, the probiotics mitigated the apathetic state of gestating sows housed in individual cages, in addition to reducing fear and aversion towards humans during interaction tests. The influence was also observed in the progeny, as piglets from sows supplemented with probiotics were less resistant in fear assessments, which may indicate lower stress levels. The findings indicate that the use of probiotics during gestation and lactation can be used as a tool in enhancing animal welfare.

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Abstract: The intensification of production systems has resulted in detrimental effects on sow welfare, which, can have an adverse influence on their offspring. Considering the relevance of microbiota-gut-brain axis, probiotics can mitigate such impacts. To investigate the effects of the dietary inclusion of probiotics on the welfare of sows and piglets, 147 multiparous sows were randomly assigned two groups: control group or group supplemented with multistrain probiotic from the beginning of pregnancy to end of lactation. The human-animal relationship (HAR), stereotypic behavior, position changes, salivary cortisol, and plasma serotonin levels were assessed in the sows. Piglets back-test and organ weight were analyzed. Probiotic-supplemented sows exhibited a better HAR index (P=0.017), which indicated reduced aversion towards humans. The frequency of stereotypies was not influenced by the treatments. However, supplemented sows spent more time standing (P=0.054) and less time lying down (P=0.008). The cortisol level of supplemented sows was 50% lower (P=0.047), and serotonin levels were 11% higher (P=0.034) than control animals. Multistrain piglets were more passive and less resistant (P=0.076) in the back-test. The organ weights were not influenced by treatments. In conclusion, sows supplemented with probiotics showed less fear and more motivation indicators, while their piglets showed less aggression behaviors.

Keywords: microbiota metabolites; feed additive; gut-brain axis; piglets

1. Introduction

The intensive production systems were developed to improve productive indices and its ongoing progress is noticed year after year. However, it is essential to acknowledge that some intrinsic factors of these production models can potentially lead to negative impacts on animal welfare and subsequently influence animal physiology [1,2].

Various studies have consistently evidenced the beneficial impact of incorporating probiotics in the diets of sows on the overall performance of the animals. These effects include enhancing piglet birth weight, the quality and quantity of colostrum, and feed consumption during lactation, with positive consequences for body score at weaning [3,4]. Recently, the hypothesis that probiotic bacteria are also a promising strategy to mitigate the adverse effects on animal welfare caused by intensive farming practices has garnered significant interest within the industry.

Research has provided evidence that feed additives capable of modulating the intestinal microbiota can produce positive effects on the expression of stereotypies [5,6,7]. This type of behavioral pattern is associated with the occurrence of apathy, lack of motivation, and may cause negative results in social interactions among sows and between sows and handlers. These effects not only increase the occurrence of injuries to those involved, but exacerbates the already established stress [8].

The microbiota-gut-brain axis is already well stablished in other research areas as an intersection of microbiology and neuroscience. This concept relies on the influence that intestinal bacteria have on neural processes, activation of the hypothalamus-pituitary axis (HPA), and behavior expressions [9]. These communication networks are complex, involving the exchange of immunological, neuronal, and chemical signals [10]. However, the bidirectional communication between gut bacteria and the brain certainly plays a critical role in the current understanding of animal behavior. Therefore, lactic acid bacteria can alter an individual's behavior by affecting the brain through the regulation of hormonal (as serotonin, dopamine, cortisol and more) patterns via the HPA [11]; by inducing anti-inflammatory cytokines and reducing proinflammatory cytokines [12]; by regulating the pattern of neurotransmitters in the body; and by the production of metabolites such as short chain fatty acids [13].

Notably, the use of probiotics belonging to the *Lactobacillus* strains have shown effects on the reduction of behaviors associated with anxiety and depression in mice [9,14]. In addition to the direct effects in the supplemented animals, probiotics administered to sows may confer advantages to their offspring as well. Previous studies already proved the great maternal influence on the first microbial colonization of the piglets [15]. Complementary, certain behavioral conditions can also be transferred from mothers to offspring. For instance, more stressed and aggressive sows can consequently produce more stressed and aggressive piglets [16,17]. Considering the relevance of the topic for human and animal sciences, this study was carried out to evaluate the impact of including a multistrain probiotic additive in the diets of gestating and lactating sows on various aspects of behavior and welfare of both sows and their litters.

2. Materials and Methods

The experimental protocol was approved by the institutional Animal Care and Use Committee of Universidade Federal do Rio Grande do Sul (register number: 39736).

2.1. Farm, animals, housing, and management

This study was conducted on a commercial farm located in the Brazilian state of Rio Grande do Sul, which experiences a subtropical climate.

A total of 147 sows (Pic Camborough, Agroceres-PIC, São Paulo, Brazil) were used in the trial. The initial number were 166, but some animals were removed during the study due to mortality or reproductive failures. Animals with parity orders from 2 to 9 were used in the trial, but equally distributed within each treatment.

The gestating and lactating sows were housed in different facilities, which naturally ventilated and on gestation house an evaporative cooling system was activated whether necessary. Gestation housing consisted of standard individual crates (1.2 m^2), equipped with automatic feeding and drinking systems. During pregnancy, temperature control was performed by using curtains and fans. Farrowing housing consisted of a crate (1.2 m^2) for each sow, complemented with a circulation space (4.2 m^2) plus a creep heated area (1 m^2) for the piglets. Manual feeders and nipple drinkers were used. During lactation, the temperature was controlled by using curtains and an air conduction heating system with thermostat and for piglets, floor heating and incandescent lamps.

During the study, almost all existing animal husbandry practices on the farm were upheld, including feeding practices, farrowing attendance, and weaning ages. These practices were representative for modern and intensive pig production in Brazil. However, some extra activities were implemented to warranty the scientific quality of the study. For instance, the accuracy of the automatic feeders was checked weekly in the gestation phase and the amount of feed offered during the lactation period was quantified. In addition, when implementing the cross-fostering management (already practiced in the farm to equalize litter size), extra attention was undertaken to ensure that animals were not transferred outside their designated treatment.

2.2. Experimental design, treatments and feeding practices

Each sow/piglet was considered an experimental unit in this study. The number of replicates used in each test was defined for each response considering the data dispersion observed in previous trials and using the tools available on Minitab Software (v. 20, Minitab Inc., State College, PA).

The treatments were randomly assigned to the experimental units within each block (parity order) in such a way that each treatment had the same number of animals of each parity order. The final arrangement led to a parity order distribution very similar between the treatments (mean = 4.45 x 4.39; mode = 4 in both groups; first quartile = 3 in both groups; and last quartile = 6 in both groups).

Two treatments were evaluated: control, in which animals did not receive any feed additive; 2) probiotic, in which sows received a probiotic (Protexin™ Concentrated, Elanco™ Animal Health, São Paulo, Brazil) with *Lactobacillus acidophilus (*2.06 x 10^8 CFU/g*), Lactobacillus bulgaricus (*2.06 x 10^8 CFU/g*), Lactobacillus plantarum (*1.26 x 10^8 CFU/g*), Lactobacillus rhamnosus (*2.06 x 10^8 CFU/g*), Bifidobacterium bifidum (*2.00 x 10^8 CFU/g*), Enterococcus faecium (*6.46 x 10^8 CFU/g*),* and *Streptococcus thermophilus (*4.10 x 10^8 CFU/g*)*.The supplementation was provided only to the sows, lasting from right after the artificial insemination until the weaning of the piglets at 21 days of age. Piglets were not supplemented. To prevent cross-contamination (among sows, from the feed to the piglets, and in the environment), pharmaceutical gelatin capsules were filled up with the specified amount of the probiotic to each sow in each day. These capsules were them provided to each sow during the trial. The capsule was placed on the top of the feed served to each sow in the morning. Red capsules were used to allow easy observation of its intake by the sow. It was verified in all cases that the animals consumed the capsule.

A feed based on corn and soybean meal was used in both phases. Formulation was performed following conventional nutritional levels for Brazilian pig production [18]. 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 daily feed intake varying from 1.8 to 2.0 kg. During the lactation phase, the amount was supplied *ad libitum*, with daily feed intake gradually increasing during the first week, reaching an average of 7 to 8 kg per animal. Despite being supplied in the gelatin capsules, the daily amount was weighted individually to represent an inclusion of 50 g of the additive per ton of feed, which is the concentration recommended by the supplier (Elanco™ Animal Health, São Paulo, Brazil), which resulted in a mean daily supply of 0.1 g during gestation and 0.4 g during lactation per sow.

2.3. Sow assessments

The repertory of stereotypic behaviors presented in the farm was built in a preliminary study that lasted for a week. The following behaviors were registered: sham chewing, tongue rolling, bar biting, and floor licking. The test was conducted out in the mornings (one hour after feeding) during the final week of gestation (before transfer to farrowing facilities). Each sow was observed for three minutes, with the first minute disregarded. A score of 0 was assigned when no stereotyped behaviors were observed. A score of 1 was attributed if any stereotyped behavior were observed during the observation time. To disregard the "novelty" factor, a single evaluator was used, always wearing the same clothes. During the observation time, the evaluator noted on a specific form whether stereotypies occurred.

The body posture was also observed and registered during the same observation period [adapted from 19]. Sows were classified as: standing, lying, lean forward, and sitting. Changes of posture during the period were also registered. In this regard, a score 0 was attributed to none change in posture, while 1 was attributed when a change in posture was observed.

The human-animal relationship (HAR) observational test was also conducted out in the mornings and afternoons during the final week of gestation (before transfer to farrowing facilities), right after the previous described test. A simulation was performed in the week before, so that the sows could get used to the analysis and the novelty factor would not affect the animal response and to understand some behavior patterns, as grunts. The test consisted of three distinct steps. In the first step, the evaluator approached the sow from the front, positioning himself slightly to the right of the sow for 10 seconds. After, the evaluator crouched down in front of the sow and remain motionless for 10 seconds. Lastly, the evaluator reached out and attempt to touch the sow between the ears for 10 seconds. An external observer registered the result as ‘0’ if the sow allows the evaluator to touch her between the ears or ‘1’ if the sow does not allow the evaluator to touch her between the ears [adapted from 19]. Additionally, the vocalizations emitted by the sows during the test were evaluated as ‘0’ if there was no vocalization and ‘1’ if there was vocalization. At the beginning of the test, At the beginning of the test, the first grunt was disregarded, and the following ones were in fact used to register that there was vocalization.

Saliva samples were collected in the mornings, five days after farrowing from a subset of sows (n = 14) for cortisol quantification. These sows were chosen randomly inside the blocks (parity order). Cotton ropes were utilized for saliva sampling, tied to the side of the farrowing crate, at an accessible height for the sows to moisten the material for 30 minutes. After this time, through manual pressure on the rope, the saliva was collected and kept in −80 ºC until analysis [20]. Cortisol was measured using commercial kits (Cortisol ELISA Kit, ELK Biotechnology, Wuhan, China) in a spectrophotometer (DR-200BN, Kasuaki, Stockholm, Sweden).

Blood samples were collected for the serotonin quantification in the same animals after the saliva sampling. The animals were individually restrained by nose snaring positioned behind the canine teeth and held in the correct position with the neck lifted upward to facilitate access to the vein. The collection was performed by puncture of the cranial vena cava using hypodermic needles and syringes with EDTA. Blood was centrifuged at 3,000 rpm for 10 min and plasma separated and frozen at – 20 º C until analysis. The quantitative determination of plasma serotonin concentration was performed via enzyme immunoassay (5-Hydroxytryptamine ELISA Kit, ELK Biotechnology, Wuhan, China) in a spectrophotometer (DR-200BN, Kasuaki, Stockholm, Sweden).

2.4. Piglet assessments

At 14 days of age, a back-test was conducted in all piglets in the litter. For the test, each animal was individually positioned in dorsal decumbency using a table positioned in front of each pen. The animal was observed for 60 seconds, during which the number of escape attempts, the latency to first vocalization, the number of vocalizations, and the frequency of vocalization throughout the test were registered [21,22]. Grunt, squeal and scream were classified. Subsequent vocalizations had to be separated at least by 1 s of rest to be treated as two separate vocalizations. One person (evaluator 1) performed all the testing. During the test the evaluator 1 placed one hand on the piglet's chest and the other hand supporting piglet's hip. The four members were free. A second evaluator was responsible for timing the test and registering the data.

At the end of the experimental period (weaning), a total of 11 piglets were randomly selected (1 animal per sow), weighted, and euthanized. Immediately after, the abdominal cavity was opened, and the gut segments were isolated from each other. Spleen, thymus, heart, and adrenal glands were weighted, and the obtained value was relativized to the body weight of each piglet [23].

2.5. Statistical analysis

Data were analyzed using SAS (v. 9.4, SAS Institute Inc., Cary, NC). Responses were subjected to variance analysis using the GLIMMIX procedure. All statistical models included the effect of treatment. Models with blocks (i.e., parity order) and their interactions were tested for each response and maintained in the final model when significant ($P<0.10$). The binary responses (scores) were denoted through the utilization of a particular option. Residual of the final analysis were stored and evaluated for normality using the Shapiro-Wilk test. Results were interpreted at 5% (significant results) and 10% (tendencies) levels of significance.

3. Results

3.1. General observations

Sows and piglets performed as expected for modern genetics with no health concerns detected during the study. The treatment effect on the performance was reported in a previous publication (24). Briefly, no effects were observed on the number of total born alive, stillborn, and mummified piglets. However, probiotics improved birth weight alive (1.342 kg x 1.404 kg; $P<0.05$) and weaning weight (5.725 kg x 5.329 kg; $P<0.001$).

3.2. Sow assessment

Sows supplemented with probiotics presented 33% lower HAR scores ($P=0.005$; Table 1) or, in other words, were less reactive to the presence of humans. This effect was also observed in the afternoons, when the probiotic supplemented sows exhibited a 31% better HAR score ($P=0.017$). The probiotic still tended to improve (57%; $P=0.075$) the HAR scores if only the results obtained in the mornings were considered. However, the results obtained in the morning presented greater variability (among sows) compared to the afternoon. There was no effect of the treatments on the vocalizations during the HAR test.

Table 1. Effect of multistrain probiotic supplementation on human-animal relationship (HAR) and vocalizations of gestating sows.

Variables	Treatments[1]		*P*-value[2]
	Control	Probiotic	
Sows, number	76	71	-
HAR[3] score – morning	0.923 (0.221)	0.400 (0.178)	0.075
Vocalization HAR[4] – morning	0.153 (0.095)	0.111 (0.081)	0.736
HAR score – afternoon	0.627 (0.056)	0.433 (0.058)	0.017
Vocalization HAR – afternoon	0.538 (0.035)	0.529 (0.036)	0.859
HAR score – morning + afternoon	0.645 (0.054)	0.430 (0.055)	0.005
Vocalization HAR – morning + afternoon	0.514 (0.034)	0.492 (0.035)	0.659

[1] Least squares means with standard errors in the parentheses.
[2] Probability of treatment effects.
[3] Score 0 was attributed when the sow allows the evaluator to touch her between the ears, and score 1 when the sow does not allows the evaluator to touch her between the ears.
[4] Score 0 there was no vocalization, while 1 was attributed there was vocalization.

The frequency of stereotypies was not influenced by the treatments (Table 2). However, sows supplemented with probiotics presented more standing positions (25%; P=0.054) and less lying position (-15%; P=0.008) in comparison to the control group. The frequency of the other positions and posture changes were similar between the treatments.

Table 2. Effect of multistrain probiotic supplementation the on stereotypes and posture assessment of gestating sows.

Variables	Treatments[1]		*P*-value[2]
	Control	Probiotic	
Sows, number	66	56	-
Stereotypes[3]			
Sham chewing	0.798 (0.050)	0.771 (0.055)	0.716
Tongue rolling	0.127 (0.026)	0.173 (0.029)	0.245
Bar biting	0.127 (0.025)	0.135 (0.028)	0.831
Floor licking	0.116 (0.024)	0.122 (0.026)	0.873
Posture[4]			
Standing	0.277 (0.024)	0.347 (0.026)	0.054
Lying	0.780 (0.023)	0.662 (0.025)	0.008
Lean forward	0.044 (0.011)	0.045 (0.012)	0.998
Sitting	0.034 (0.010)	0.051 (0.011)	0.284
Change in posture	1.137 (0.028)	1.102 (0.031)	0.408

[1] Least squares means with standard errors in the parentheses.
[2] Probability of treatment effects.
[3] Score 0 was attributed to none observed stereotyped behavior, while 1 was attributed to observed stereotyped behavior.
[4] Score 0 was attributed to none change in posture, while 1 was attributed when a change in posture was observed.

Sows treated with probiotics had 50% lower concentration of salivary cortisol than control sows at the end of gestation (P=0.047; Table 3). In addition, the probiotics were able to improve by 10% the serotonin levels in the sows (P=0.034).

Table 3. Effect of multistrain probiotic supplementation on the hormonal concentration of gestating sows.

Variables	Treatments*		*P*-value[1]
	Control	Probiotic	
Sows, number	7	7	-
Salivary cortisol, mcg/dL	0.665 (0.084)	0.335 (0.094)	0.047
Blood serotonin, ng/dL	151.5 (3.456)	166.5 (4.340)	0.034

[1] Probability of treatment effects.
* Least squares means with standard errors in the parentheses.

3.3. Piglet assessment

Piglets whose mothers were supplemented with probiotics tended to present the first vocalization earlier (P=0.076), as well as showed a lower time of vocalization throughout the test (P=0.065) compared to the control group (Table 4). However, the number of vocalizations and escape attempts were not influenced by the treatments. Piglet organ weights were also not influenced by treatments (Table 5).

Table 4. Effect of maternal probiotic supplementation during gestation and lactation on the piglet back-test evaluated.

Variables	**Treatments**[1]		***P*-value**[2]
	Control	**Probiotic**	
Piglets, number	71	90	-
Escape attempts, number	2.014 (0.161)	1.833 (0.143)	0.404
Time to first vocalization, sec	20.32 (2.662)	14.05 (2.292)	0.076
Vocalizations, number	1.971 (0.131)	2.211 (0.116)	0.175
Vocalizations, % of time	34.36 (4.478)	23.42 (3.824)	0.065

[1] Least squares means with standard errors in the parentheses
[2] Probability of treatment effects.

Table 5. Effect of maternal probiotic supplementation during gestation and lactation on the relative piglet organ weights.

Variables	**Treatments**[1]		***P*-value**[2]
	Control	**Probiotic**	
Piglets, number	6	6	-
Thymus, % BW	0.211 (0.033)	0.216 (0.036)	0.911
Heart, % BW	0.565 (0.032)	0.609 (0.035)	0.386
Spleen, % BW	0.519 (0.238)	0.211 (0.261)	0.406
Average adrenal[3], % BW	0.141 (0.017)	0.163 (0.019)	0.426

[1] Least squares means with standard errors in the parentheses.
[2] Probability of treatment effects.
[3] Mean of the left and right adrenal glands.

4. Discussion

Factors such as limited space within confinement and high productivity rates are inherent to intensified production systems, such as the one where the study was developed [25,26]. These factors can affect the animal at behavioral, emotional, and physiological levels. Increased stress levels emerge as one of the consequences, triggering disturbances in the body's homeostasis and increasing abnormal behaviors [27]. The gut microbiota plays a role in brain function by regulating stress and cognition [28]. The mechanisms that connect intestinal bacteria and behavioral patterns are numerous, but not all of them are known or fully understood [11]. Through the production of metabolites, regulation of the immune system, hormonal patterns and modulation of neurotransmitters, lactic acid bacteria are capable of modifying host brain functions and thereby shaping their behavior [29].

The HAR is well recognized as an effective test to assess the fear of animals towards humans [19,30]. A previous study showed that confident sows exhibited great numbers of piglets born and weaned, indicating a positive association between sow behavior and reproductive performance [8]. In addition, sows that exhibit better HAR responses are also more likely to accept the daily handling practices, which can minimize their susceptibility to injuries resulting from aversive or aggressive behavior towards humans [31]. Consequently, factors that may benefits the human-animal relationship hold significant importance both for the animal and the production chain.

The HAR assessment might be influenced by different periods of the day (morning and evening) because cortisol secretion follows a circadian rhythm, with peak occurring in the morning followed by a decline during the day [32]. In the control group, a typical biological pattern (i.e., in accordance with the cortisol secretion pattern) was observed, with higher HAR values in the morning and a decrease in the afternoon. The probiotic group, on the other hand, exhibited a decrease in aversion and reactivity behaviors, as well as a reduction in the circadian fluctuations of these behaviors.

The vocalization measures can also be used as biological markers of emotional reactivity, which is defined as the predisposition that an individual has in the face of a challenging situation [33]. However, in this study, vocalizations were not affected by the probiotic supplementation.

Stereotyped behavior has been widely recognized as a manifestation of frustration, apathy, or a lack of motivation [26]. Stereotypies are observed in sows especially in environments of intensive production, absence of stimuli, or restrictions on the animal's freedom to engage in natural behaviors [34]. These behaviors have no clear purpose and can lead into injuries to the animals, further exacerbating their stress level [25].

In a previous study, authors hypothesized that the expression of stereotypies might not be correlated with the increase in salivary cortisol concentrations during the first third of pregnancy [26]. This hypothesis suggested that stereotyped behaviors could be a form of relief, compensating for the absence of external stimuli. However, the relationship between these factors was not established as it failed to persist throughout the remaining sections of pregnancy during subsequent assessments [26]. In the present investigation, no relationship was established between probiotics and stereotyped behaviors, despite the considerable impact of the additive on cortisol levels.

In this study, supplemented sows spent more time standing and less time lying down, which may indicate that the probiotic acted to mitigate the depressive and apathetic state of supplemented sows. Probiotics can modulate the intestinal microbiota, which has been linked to changes in behavioral patterns [10]. It is known that stressed sows change position more frequently [35]. No effects of probiotics on position changes were found in the study. However, in confined conditions, sows take longer to transition from one position to another because of both intrinsic and extrinsic factors [36,37]. Thus, the likelihood of the treatment having an effect could be reduced by the fact that postural changes were minimal also in the control group.

Decreased motivation, anhedonia, and physical exercise are behavioral patterns commonly observed in human and rat diagnosed with depression and anxiety disorders [38]. These patterns are attributed to the reduced sympathetic nerve function, which is involved in the unconscious mechanisms of warning signals and energy expenditure [39]. Studies that evaluated the action of *Lactobacillus casei* on the autonomic nervous system in rats subjected to stress, observed suppression of the afferent sympathetic nerve output to the adrenal gland and suppressing the activation of the hypothalamic-pituitary-adrenal axis [40,41]. In the current study, the probiotic supplementation might likely had an impact on the autonomic nervous system, leading to a reduction in symptoms associated to lethargy and demotivation. Consequently, the sows displayed increased activity, as evidenced by their greater tendency to stand and reduced inclination to lie down.

Bifidobacterium and *Lactobacillus* were able to reduce the levels of corticotropin-releasing hormone [42]. This hormone is the main stimulator of the release of adrenocorticotropic hormone, responsible for inducing the release of cortisol by the adrenal cortex [43]. The effects of probiotics on cortisol are particularly important for intensive pig production because long-term strict confinement can induce apathy and boredom in sows [44], leading them to express depressive behavior, often associated with increased cortisol levels [38]. Cortisol can cross the blood-brain barrier at the hypothalamic-pituitary-adrenal axis and affect the regulation of the brain region that controls emotions [25].

It has already been elucidated host-microbial imbalance is associated with mental disorders and stress-related illnesses in humans [45]. Probiotics plays an important role in the regulation of the intestinal microbiota, thereby mitigating the effects of stress [2]. Certain strains, like *Lactobacillus* and *Bifidobacterium*, have the ability to influence brain functions [46] by participating in the communication axis between the brain and intestine through different paths, including the expression of neurotransmitters, dopamine, and serotonin [47]. Notably, the increase in plasmatic serotonin can be explained due the synthesis of serotonin being dependent on several families of bacteria in the intestinal microbiota [48]. The microbiota can act on enterochromaffin cells, present in the intestinal epithelium and specialized in the production of serotonin [49]. Being an important neurotransmitter for the communication of the microbiota-gut-brain axis, serotonin acts on the central nervous system through the serotonergic system, responsible for aspects such as stress response, emotional states, food impulse, circadian rhythm, between others [50].

Typically, piglets exhibit a greater degree of activity and display a more extensive range of behaviors. The back-test is one of the assessments that can be applied in this category and is widely used to evaluate fear, resistance, personality, stress responses, and coping style [21,22]. Longer and numerous vocalizations during the test are generally viewed as indicative of a proactiveness, indicating also a higher level of resistance [51]. Piglets from supplemented sows showed less reactive behavior, which is characterized by less resistance, increased passivity, and milder responses.

Previous studies have associated the proactive behavioral profile, characterized by higher vocalization percentage and longer initial vocalization duration during the back-test, with increased aggressiveness in animals introduced to a new social group [52, 53,16]. The expression of aggressive behavior is related to the occurrence of fights, difficulty during handling, and elevated risk of injuries, that can depreciate the carcasses [54]. Other authors also pointed out that a higher frequency and duration of vocalizations during piglet behavior tests can be an indication of stress, as the expression of certain types of vocalizations are affected by adrenaline discharges [28].

Bacteria resident in the gut affect the central nervous system through bidirectional communication via the microbiota-gut-brain axis. For newborn piglets, the maternal microbiota is one of the main sources for the establishment of their own microbial community [51,34]. Previous research attested that the behavioral and physiological profile of the sow can affect the profile of the progeny. For instance, sows that react with fear to humans tends to produce piglets that also react with fear [16]. Therefore, current findings on the effects of probiotics on piglet behavior could be explained by both the direct maternal influence on the piglet behavior (because probiotics modulated sow behavior) and also the great importance of the mother on the bacterial colonization of the offspring (because probiotics modulated the sow gut microbiota), considering the transfer of maternal microbiota to the piglets at the time of birth and during breastfeeding.

Furthermore, studies show that probiotics affect the neuroendocrine system [16], therefore, supplemented sows could present calmer behavior and consequently better maternal ability, which make the stressful farrowing moment faster, which benefit the survival of piglets after birth as it reduces the number of uterine contractions and the consequent chance of hypoxia in intrauterine neonates [55]. Piglets that suffered less stress during birth search for food quickly [56]. In addition, regarding the relationship between behavior of sows with piglets’ responses in the back-test, it is understood that the link is based on the combination of genetic, epigenetic, and environmental effects [57; 58].

It was also hypothesized that the weights of certain organs could be affected by stress due to the existing association between physiological changes, such as anxiety conditions, as previously demonstrated in rats supplemented with *Bifidobacterium longum* [23]. However, this condition was not found in the current trial.

Chronic stress in sows emerged due to the challenging conditions of the traditional intensive production system worsens welfare status and, consequently, their production rates. The study of the potential of probiotics bacterias to modulate the neuroendocrine system allows to better understand neurological disorders and their various consequences, in order to mitigate them. In this context, probiotics can be the “key” to positively contribute to the quality of life of sows.

5. Conclusions

The use of multistrain probiotic proved to be a beneficial nutritional strategy to enhance the welfare of pregnant sows. Supplemented sows showed better human-animal interaction, without interfering on the occurrence of stereotypies. Probiotics reduced the cortisol levels and increased serotonin in the sows. Piglets from supplemented sows tend to vocalize less during back-test. These findings provide a theoretical basis for understanding maternal-progeny integration through a microbial link. More research is needed to fully evaluate the effects of probiotics on welfare in different environments and production systems (e.g., collective pens).

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References

1. Hayakawa, T.; Masuda, T.; Kurosawa, D.; Tsukahara, T. 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. *Anim. Sci. J*. **2016**, 87, 1501-1510; DOI: 10.1111/asj.12565.

2. Zhang, N.; Zhang, Y.; Li, M.; Wang, W.; Liu, Z.; Xi, C.; Huang, X.; Liu, J.; Huang, J.; Tian, D.; et al. Efficacy of probiotics on stress in healthy volunteers: a systematic review and meta-analysis based on randomized controlled trials. *Brain and Behav*. **2020**, 10, 1-14; DOI: 10.1002/brb3.1699.

3. 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. *Veteri. Worl*. **2023**, 10), 2055–2062; DOI: 10.14202/vetworld.2023.2055-2062.

4. Kritas, S.K.; Marubashi, T.; Filioussis, G.; Petridou, E.; Christodoulopoulos, G.; Burriel, A.R.; Tzivara, A.; Theodoridis, A.; Pískoriková, M. Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (Bacillus subtilis C-3102). *Anim. Sci. J*. **2015**, 93, 405–413; DOI: 10.2527/jas.2014-7651.

5. Dam, S.A. From belly to brain: investigating the role of gut bacteria in neurodevelopmental disorders using rodent models. Doctoral dissertation, Radbound University Nijmegen, Netherlands, December 15, **2021**.

6. Budiño, F.E.L.; Vieira, R.F.N.; Mello, S.P.; Duarte, K.M.R. Behavior and performance of sows fed different levels of fiber and reared in individual cages or collective pens. *An. Acad. Bras. Cienc*. **2014,** 86, 2109–2119; DOI:10.1590/0001-3765201420140301.

7. Sapkota, A.; Marchant-Forde, J.N.; Richert, B.T.; Lay, D.C. Including dietary fiber and resistant starch to increase satiety and reduce aggression in gestating sows. *J. Anim. Sci.* **2016**, 94, 2117–2127; DOI:10.2527/jas.2015-0013.

8. Pol, F.; Kling-Eveillard, F.; Champigneulle, F.; Fresnay, E.; Ducrocq, M.; Courboulay, V. Human–animal relationship influences husbandry practices, animal welfare and productivity in pig farming. *Animal*. **2021**, 15, 100103; DOI:10.1016/j.animal.2020.100103.

9. Kraimi, N.; Dawkins, M.; Gebhardt-Henrich, S.G.; Velge, P.; Rychlik, I.; Volf, J.; Leterrier, C. Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: A review. *Physiol. Behav*. **2019**, *210*, 112658; DOI:10.1016/j.physbeh.2019.112658.

10. Kennedy, P.J.; Cryan, J.F.; Dinan, T.G.; Clarke, G. Irritable bowel syndrome: A microbiome-gut-brain axis disorder? *World J. Gastroenterol*. **2014**, 20, 14105–14125; DOI:10.3748/wjg.v20.i39.14105.

11. Olorocisimo, J.P.; Diaz, L.A.; Co, D.E.; Carag, H. M., Ibana, J.A., Velarde, M.C. Lactobacillus delbrueckii reduces anxiety-like behavior in zebrafish through a gut microbiome–brain crosstalk. *Neuropharmacol.* **2023**, 225, 109401; DOI:10.1016/j.neuropharm.2022.109401.

12. Yang, F.; Hou, C.; Zeng, X.; Qiao, S. The use of lactic acid bacteria as a probiotic in swine diets. *Pathogens*. **2015,** 1, 34-45; DOI:10.3390/pathogens4010034.

13. Thananimit, S.; Pahumunto, N.; Teanpaisan, R. Characterization of short chain fatty acids produced by selected potential probiotic lactobacillus strains. *Biomolec*. **2022**, 12, 1829. DOI:10.3390/biom12121829.

14. Liang, S,T.; Wang, X.; Hu, J.; Luo, W.; Li, X.; Wu, Y.; Duan, F. Administration of Lactobacillus Helveticus NS8 improves behavioral, cognitive and biochemical aberrations caused by chronic restraint stress. *NeuroSci*. **2015,** 310, 561–577; DOI:10.1016/j.neuroscience.2015.09.033.

15. Chen, X.; Xu, J.; Ren, E.; Su, Y.; Zhu, W. Co-occurrence of early gut colonization in neonatal piglets with microbiota in the maternal and surrounding delivery environments. *Anaerobe*. **2018**, 49, 30-40; DOI: 10.1016/j.anaerobe.2017.12.002.

16. Rooney, H.B.; Schmitt, O.; Courty, A.; Lawlor, P.G.; O'Driscoll, K. Like mother like child: Do fearful sows have fearful piglets? *Animals*. **2021**, 11, 1-17; DOI:10.3390/ani11051232.

17. Rutherford, K.M.; Piastowska-Ciesielska, A.; Donald, R.D.; Robson, S.K.; Ison, S.H.; Jarvis, S.; Lawrence, A.B. Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig. *Physiol. Behav*. **2014**, 129, 255-264; DOI: 10.1016/j.physbeh.2014.02.052.

18. Rostagno, H.S.; Albino, F.L.T.; Hannas, M.I.; Donzele, J.L.; Sakomura, N.K.; Perazzo, F.G.; Saraiva, A.; Oliveira, R.F.; Barreto, S.L.T. Tabelas Brasileiras para Aves e Suínos - Composição de alimentos e exigências nutricionais. 4ª Ed. Universidade Federal de Viçosa; **2017**. 1-469.

19. Blokhuis, H. J. Welfare Quality. Welfare Quality: Assessment protocol for pigs (sows and piglets, growing and finishing pigs). Welfare Quality Consortium, Lelystad, The Netherlands. **2009**.

20. Morgan, L.; Klement, E.; Novak, S.; Eliahoo, E.; Younis, A.; Sutton, G.A.; Raz, T. Effects of group housing on reproductive performance, lameness, injuries and saliva cortisol in gestating sows. *Prev. Vet. Med*. **2018**, 160, 10-17; DOI: 10.1016/j.prevetmed.2018.09.026.

21. Bolhuis, J.E.; Parmentier, H.K.; Schouten, W.G.P.; Schrama, J.W.; Wiegant, V.M. Effects of housing and individual coping characteristics on immune responses of pigs. *Physiol. Behav*. **2003**, 79: 289–296; DOI:10.1016/s0031-9384(03)00090-8.

22. Melotti, L.; Oostindjer, M.; Bolhuis, J.E.; Held, S.; Mendl, M. Coping personality type and environmental enrichment affect aggression at weaning in pigs. *Appl. Anim. Behav. Sci.* **2011**, 133, 144–153; DOI:10.1016/j.applanim.2011.05.018.

23. Savignac, H.M.; Kiely, B.; Dinan, T.G.; Cryan, J.F. Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterol. Motil*. **2014**, 26, 1615-1627; DOI: 10.1111/nmo.12427.

24. Franceschi, C.H.; Pereira, M.M.C.; Stefanello, T.B.; Mariani, A.B.; Camargo, N.T.; Cony, B.S.L.; Furtado, J.C.V.; Kipper, M.; Andretta, I. Effect of multi-strain probiotic on the performance of sows and their progeny. SINSUI, **2022**.

25. Zhang, M.Y.; Li, X.; Zhang, X.H.; Liu, H.G.; Li, J.H.; Bao, J. Effects of confinement duration and parity on stereotypic behavioral and physiological responses of pregnant sows. *Physiol. Behav*. **2017,** 179, 369-376; DOI: 10.1016/j.physbeh.2017.07.015.

26. Tatemoto, P.; Bernardino, T.; Rodrigues, F.A.M.L.; Zanella, A.J. Does high stereotypic behavior expression affect productivity measures in sows? *Rev. Bras. de Zootec*. **2019**, 48; DOI:10.1590/rbz4820180135.

27. Wang, H.; Braun, C.; Murphy, E.F.; Enck, P. *Bifidobacterium longum* 1714™ strain modulates brain activity of healthy volunteers during social stress. The *Am. J. Gastroenterol*. **2019**, 114, 1152; DOI:10.14309/ajg.0000000000000203.

28. Mayer, E.A.; Tillisch, K.; Gupta, A. Gut/brain axis and the microbiota. *Journ Clinic. Investig*. **2015**, 3, 926-938; DOI: 10.1172/JCI76304.

29. Agirman, G.; Hsiao, E.Y. SnapShot: The microbiota-gut-brain axis. *Cell*. **2021**, 184(9), 2524-2524; DOI: 10.1016/j.cell.2021.03.022.

30. Powell, C.; Hemsworth, L.M.; Rice, M.; Hemsworth, P.H. Comparison of methods to assess fear of humans in commercial breeding gilts and sows. *Appl. Anim. Behav. Sci*. **2016**, 181, 70–75; DOI:10.1016/j.applanim.2016.05.027.

31. Prunier, A.; Tallet, C. Endocrine and behavioural responses of sows to human interactions and consequences on reproductive performance. In The gestating and lactating sow, 1st ed.; Farmer, C. Wageningen Academic: Wageningen, Netherlands, **2015**; Volume 1, pp. 279–295.

32. Munsterhjelm, C.; Brunberg, E.; Heinonen, M.; Keeling, L.; Valros, A. Stress measures in tail biters and bitten pigs in a matched case-control study. *Anim. Welf.* **2013**, 22, 331-338; DOI:10.7120/09627286.22.3.33.

33. Leliveld, L.M.C.; Düpjan, S.; Tuchscherer, A.; Puppe, B. Vocal correlates of emotional reactivity within and across contexts in domestic pigs (Sus scrofa). *Physiol. Behav*. **2017**, 181, 117–126; DOI:10.1016/j.physbeh.2017.09.010.

34. Tatemoto, P.; Bernardino, T.; Morrone, B.; Queiroz, M. R.; Zanella, A.J. Stereotypic behavior in sows is related to emotionality changes in the offspring. *Front. Vet. Sci*. **2020**, 7, 79; DOI:10.3389/fvets.2020.00079.

35. Muns, R.; Malmkvist, J.; Larsen, M.L.V.; Sørensen, D.; Pedersen, L.J. High environmental temperature around farrowing induced heat stress in crated sows. *J.Anim. Sci*. **2016**, 94, 377-384; DOI:10.2527/jas.2015-9623.

36. Zhang, J.; Yu, L.; Yin, G. Evaluation of behavior and affective state of different-parity sows with strong/weak pupil light reflex. *Animals.* **2022**, 12, 1-12; DOI:10.3390/ani12091184.

37. Marchant, J.N.; Broom, D.M. Factors affecting posture-changing in loose-housed and confined gestating sows. *Anim. Sci*. **1996**, 63, 477–485; DOI:10.1017/s135772980001537x.

38. Nandam, L.S., Brazel, M., Zhou, M., Jhaveri, D.J. Cortisol and major depressive disorder—translating findings from humans to animal models and back. *Front. Psychiatry*. **2020**, 10, 974; DOI: 10.3389/fpsyt.2019.00974.

39. Kandola, A.; Lewis, G.; Osborn, D.P.J.; Stubbs, B.; Hayes, J.F. Depressive symptoms and objectively measured physical activity and sedentary behaviour throughout adolescence: a prospective cohort study. *Lancet. Psychiatry.* **2020**, 7, 262-271; DOI:10.1016/S2215-0366(20)30034-1.

40. Takada, M.; Nishida, K.; Katoaka-Kato, A.; Gondo, Y.; Ishikawa, H.; Suda, K.; Kawai, M.; Hoshi, R.; Watanabe, O.; Igarashi, T.; et al. Probiotic Lactobacillus casei strain Shirota relieves stress-associated symptoms by modulating the gut–brain interaction in human and animal models. *Neurogastroenterology & Motility*. **2016**, 28, 1027-1036; DOI:10.1111/nmo.12804.

41. Tanida, M.; Imanishi, K.; Akashi, H.; Kurata, Y.; Chonan, O.; Naito, E.; Kunihiro, S.; Kawai, Kato-kataoka, A.; Shibamoto, T. Injection of *Lactobacillus casei* strain S hirota affects autonomic nerve activities in a tissue-specific manner, and regulates glucose and lipid metabolism in rats. *Journal of Diabetes Investigation*. **2014**, 5, 153-161; DOI:10.1111/jdi.12141.

42. Laval, L.; Martin, R.; Natividad, J.N.; Chain, F.; Miquel, S.; De Maredsous, C.D.; Capronnier, S.; Sokol, H.; Verdu, E.F.; Van Hylckama Vlieg, J.E.T.; Bermudez-Humaran, L. G. *Lactobacillus rhamnosus* CNCM I-3690 and the commensal bacterium *Faecalibacterium prausnitzii* A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice. *Gut Microbes*. **2015**, 6:1–9; DOI:10.4161/19490976.2014.990784.

43. Inda, C.; Armando, N.G.; dos Santos Claro, P.A.; Silberstein, S. Endocrinology and the brain: corticotropin-releasing hormone signaling. *Endocr. Connect*. **2017,** 6, 6; DOI:https://doi.org/10.1530/EC-17-0111.

44. Hales, J.; Moustsen, V.A.; Nielsen, M.B.F.; Hansen, C. F. The effect of temporary confinement of hyperprolific sows in Sow Welfare and Piglet protection pens on sow behaviour and salivary cortisol concentrations. *Appl. Anim. Behav. Sci*. **2016**, 183, 19-27; DOI:10.1016/j.applanim.2016.07.008.

45. Kim, J.S.; de La Serre, C.B. Diet, gut microbiota composition and feeding behavior. *Physiol. Behav*. **2018**, 192, 177–181; DOI:10.1016/j.physbeh.2018.03.026.

46. Petra, A.I., Panagiotidou, S., Hatziagelaki, E., Stewart, J.M., Conti, P., Theoharides, T.C. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. *Clin. Ther*. **2015**, *37*, 5, 984-995; DOI: 10.1016/j.clinthera.2015.04.002

47. Cryan, J.F.; Kenneth J.O.; Caitlin, S.M.; Cowan, K.V.; Sandhu, T.F.S.; Bastiaanssen, M.B.; Martin, G.; Codagnone, S.C.; Fulling, C.; Gulubeva, A.V.; et al. The microbiota-gut-brain axis. *Physiol. Rev.* **2019**, 99, 1877–2013; DOI:10.1152/physrev.00018.2018.

48. Legan, T.B., Lavoie, B., Mawe, G.M. Direct and indirect mechanisms by which the gut microbiota influence host serotonin systems. *Neurogastroenterol. Motil*. **2022**, 34, 10; DOI:10.1111/nmo.14346.

49. Simpson, C.A.; Diaz-Arteche, C.; Eliby, D.; Schwartz, O.S.; Simmons, J.G.; Cowan, C.S.M. The Gut microbiota in anxiety and depression – A systematic review. *Clin. Psychol. Rev.* **2021**, 83, 1-79; DOI: 10.1016/j.cpr.2020.101943.

50. Carhart-Harris, R.L.; Nutt, D.J. Serotonin and brain function: A tale of two receptors. *J. Psychopharmacol*. **2017**, 31, 1091–1120; DOI:10.1177/0269881117725915.

51. Choudhury, R.; Middelkoop, A.; Bolhuis, J.E.; Kleerebezem, M. Exploring the association between microbiota and behaviour in suckling piglets. *Sci. Rep.* **2022**, 12, 1-9; DOI:10.1038/s41598-022-16259-3.

52. Horback, K.M. Personality in swine. In Personality in nonhuman animals, 1st ed.; Jennifer Vonk, California, **2017**; Volume 1, pp. 185-204; DOI:10.1007/978-3-319-59300-5.

53. Horback, K.M.; Parsons, T.D. Ontogeny of behavioral traits in commercial sows. *Animal*. **2018,** 12, 11; DOI:10.1017/S1751731118000149.

54. O'Malley, C.I.; Turner, S.P.; D'Eath, R. B.; Steibel, J.P.; Bates, R.O.; Ernst, C.W.; Siegford, J. M. Animal personality in the management and welfare of pigs. *Applied Animal Behaviour Science*. **2019**, 218, 1-17; DOI:10.1016/j.applanim.2019.06.002.

55. Blavi, L.; Solà-Oriol, D.; Llonch, P.; López-Vergé, S.; Martín-Orúe, S. M.; Pérez, J.F. Management and feeding strategies in early life to increase piglet performance and welfare around weaning: A review. *Animals*. **2021**, 2, 302; DOI:10.3390/ani11020302 .

56. Trujillo-Ortega, M.E.; Mota-Rojas, D.; Juárez, O.; Villanueva-García, D.; Roldan-Santiago, P.; Becerril-Herrera, M.; Hernández_González, R.; Mora-Medina, P.; Alonso-Spilsbury, M.; Rosales, A.M.; et al. Porcine neonates failing vitality score: Physio-metabolic profile and latency to the first teat contact. *Czech J. Anim. Sci*. **2011**, 56, 499–508; DOI:10.17221/3839-CJAS.

57. Zebunke, M.; Repsilber, D.; Nürnberg, G.; Wittenburg, D.; Puppe, B. The backtest in pigs revisited–an analysis of intra-situational behaviour. *Appli. Anim. Behavi. Sci.* **2015** 169, 17-25; DOI:10.1016/j.applanim.2015.05.002

58. Curley, J.P.; Branchi, I.G.O.R. Ontogeny of stable individual differences. Animal Personalities: Behavior, Physiology, and Evolution. eds Carere C, Maestripieri D), Ed.; Chicago and London: Chicago, USA; 2013; Vol. 1, Chapter 10, p 279-316.

CAPÍTULO IV

4. CONSIDERAÇÕES FINAIS

Por inúmeros motivos intrínsecos e extrínsecos aos animais, matrizes suínas passam por períodos de estresse nas fases de gestação e lactação. Distúrbios metabólicos podem acarretar alterações intensas na microbiota intestinal, que por sua vez, pode acarretar em estresse oxidativo e aumento da inflamação sistêmica. Todos estes fatores somados ao ambiente e condições de instalações inadequadas em que as fêmeas se encontram, afetam negativamente o bem-estar animal.

Aditivos probióticos são grande foco de estudo frente ao contexto de uso racional de substâncias antibióticas, além do favorecimento da eficiência de parto, e com consequente aumento da vitalidade de leitões neonatos por meio de melhor *status* de bem-estar da matriz. Em vias de compreender como o cérebro e microbiota podem ajudar a solucionar problemas neurológicos, modelos animais são muito utilizados no objetivo de entender doenças da medicina humana como transtorno de depressão, transtorno de ansiedade generalizado, mal de alzheimer, etc. Ratos e leitões são os principais animais neste quesito. Porém, tratando-se de avaliar variáveis comportamentais, fisiológicas e hormonais com foco no bem-estar de porcas, os trabalhos são escassos. Entende-se também que existe uma imensa pressão social que recai sobre a comunidade científica e agroindústria para melhorar a qualidade de vida de animais de produção.

Com base nos dados deste estudo, observamos que probióticos são benéficos para porcas na melhoria do bem-estar por meio do melhor índice na relação humano-animal, menor tempo de duração do parto, menor nível de cortisol pós-parto e maior nível de serotonina ao desmame. Os probióticos aumentam indiretamente o consumo de colostro, sugerindo influência positiva também na produção leiteira, além de afetar padrões cognitivos de leitões durante o back-test.

Um fator limitante no trabalho com porcas é de fato a alta demanda por espaço físico, mão-de-obra e recursos financeiros, entende-se que um número amostral maior poderia contribuir na observação de efeito dos probióticos sobre a expressão de estereotipias e vitalidade dos leitões.

Em ambos estudos, as porcas foram suplementadas desde o início da gestação ao final de 21 dias de lactação. Portanto, o uso dos probióticos pode ser oneroso pela duração do uso, sendo assim, faz-se necessário estudos que avaliem tratamentos com diferentes períodos de início e término da suplementação, durante a gestação e lactação. Além disto, o contínuo acompanhamento do grupo amostral no ciclo reprodutivo subsequente é importante para entender se existe efeito residual dos probióticos sobre variáveis multifatoriais de bem-estar.

Em ambos os presentes estudos, as porcas já foram consideradas em prejuízo frente ao bem-estar animal, devido às características convencionais do sistema em total confinamento, assim como provam inúmeros estudos. Porém, em adição, ao iniciar um estudo de bem-estar seria fator de grande auxílio, determinar dentre os indivíduos da amostra, grupos com diferentes condições de bem-estar, esta análise crítica é essencial para embasar experimentos futuros.

O estudo de probióticos é extenso devido às particularidades de cada cepa e de seu hospedeiro. Ainda, a inclusão de um aditivo alimentar torna-se uma despesa a mais dentro do sistema produtivo, neste estudo a realização de uma análise econômica forneceria mais um fator prático necessário, tendo em vista o longo período de suplementação imposto. Sendo assim, torna-se necessário o entendimento do assunto por meio de uma abordagem integrada, considerando também metabólitos microbianos, produção hormonal e regulação da imunidade. Em suma, os resultados deste trabalho mostram que existe viabilidade de uso dos probióticos para alcançar melhores padrões de bem-estar, porém o sucesso deste aditivo também exige a manutenção da microbiota benéfica residente, incluindo pontos básicos como alimentação balanceada, água limpa e disponível, boas condições de biosseguridade e todos os demais aspectos que favorecem uma produção com ou sem bactérias probióticas.

5. REFERÊNCIAS

ABD, F. N.; LUTI K. J. K. Detection, purification and characterization of a bacteriocin produced by *Bacillus subtilis* NK16 exhibits a significant antimicrobial activity against clinical *Staphylococcus* spp. **Baghdad Science Journal**, Baghdad, v. 14, n. 3, p. 524-538, 2017. DOI:10.21123/bsj.14.3.524-538. Disponível em: https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/2392. Acesso em: 13 dez. 2023.

ANSARI, F.; NESHAT, M.; POURJAFAR, H.; JAFARI, S.; SAMAKKHAH, S. A.; MIRZAKHANI, E. The role of probiotics and prebiotics in modulating of the gut-brain axis. **Frontiers in Nutrition**, Lausanne, v. 10, [art.] 1173660, [p. 1-15], 2023. DOI: 10.3389/fnut.2023.1173660. Disponível em: https://www.frontiersin.org/articles/10.3389/fnut.2023.1173660/full. Acesso em: 17 jan. 2023.

ASML, A.; INVERNIZZI, A. A.; SAVOINI, B. The beneficial role of probiotics in monogastric animal nutrition and health. **Journal of Dairy, Veterinary & Animal Research**, Budapest, v. *2*, n. 4, p. 1-20, 2015. DOI: 10.15406/jdvar.2015.02.00041. Disponível em: https://medcraveonline.com/JDVAR/the-beneficial-role-of-probiotics-in-monogastric-animal-nutrition-and-health.html. Acesso em: 12 nov. 2023.

BAXTER, E. M.; ANDERSEN, I. L.; EDWARDS, S. A. Sow welfare in the farrowing crate and alternatives. *In*: ŠPINKA, M. (ed.). **Advances in pig welfare**. Cambridge: Elsevier, Woodhead Publishing, 2018. p. 27–72. DOI: 10.1016/B978-0-08-101012-9.00002-2. Disponível em: https://pure.sruc.ac.uk/en/publications/sow-welfare-in-the-farrowing-crate-and-alternatives. Acesso em: 5 nov. 2023.

BAJAGAI, Y. S.; KLIEVE, A. V.; DART, P. J.; BRYDEN, W. L. **Probiotics in animal nutrition:** production, impact and regulation. Rome: FAO, 2016. (Animal Production and Health, 56). Disponível em: https://www.fao.org/documents/card/en?details=e6232d34-e38e-4b4c-9a45-70fa75f7da23/. Acesso em: 2 nov. 2023.

BARBA-VIDAL, E.; MARTIN-ORÚE, S. M.; CASTILLEJOS, L. Practical aspects of the use of probiotics in pig production: a review. **Livestock Science**, Amsterdam, v. 223, p. 84–96, 2019. DOI: 10.1016/j.livsci.2019.02.017. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1871141318302646. Acesso em: 13 dez. 2023.

BARBOSA, G. D. A.; DUARTE, J. M. W. N. Psicobióticos e suas influências nos tratamentos de depressão e ansiedade. **Nutrivisa - Revista de Nutrição e Vigilância em Saúde**, Fortaleza, v. 8, n. 1, p. 1-9, 2021. DOI: 10.59171/nutrivisa-2021v8e9633 Disponível em: https://revistas.uece.br/index.php/nutrivisa/article/view/9633. Acesso em: 19 nov. 2023.

BERCHIERI-RONCHI, C. B. Oxidative stress status of highly prolific sows during gestation and lactation. **Animal**, Cambridge, v. 5, n. 11, p. 1774-1779, 2011. DOI: 10.1017/S1751731111000772. Disponível em: https://pubmed.ncbi.nlm.nih.gov/22440418/. Acesso em: 19 jan. 2023.

BJÖRKMAN, S.; KAUFFOLD, J.; KAISER M. Ø. Reproductive health of the sow during puerperium. **Molecular Reproduction and Development**, Hoboken, v. 90, n. 7, p. 561-

579, 2023. DOI: 10.1002/mrd.23642. Disponível em: https://pubmed.ncbi.nlm.nih.gov/36054784/. Acesso em: 16 dez. 2023.

CAI, G.; WU, D.; LI, X.; LU, J. Levan from *Bacillus amyloliquefaciens* JN4 acts as a prebiotic for enhancing the intestinal adhesion capacity of *Lactobacillus reuteri* JN101. **International Journal of Biological Macromolecules**, Amsterdam, v. 146, p. 482–487, 2020. DOI: 10.1016/j.ijbiomac.2019.12.212. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0141813019394644. Acesso em: 10 jan. 2023.

CAHYA, V. A.; HANIM, C.; YUSIATI, L. M.; BACHRUDDIN, D.; ERWANTO, Y. Growth optimization of *Bacillus subtilis* 11A isolated from Indonesian native chicken (*Gallus domesticus*) for bacteriocin production. **IOP Conference Series: Earth and Environmental Science**, Bristol, v. 387, [art.] 012013, [p. 1-5], 2019. DOI: 10.1088/1755-1315/387/1/012013. Disponível em: https://iopscience.iop.org/article/10.1088/1755-1315/387/1/012013/pdf. Acesso em: 10 jan. 2023.

CHENG, C.; WEI, H.; YU, H.; XU, C.; JIANG, S.; PENG, J. Metabolic syndrome during perinatal period in sows and the link with gut microbiota and metabolites. **Frontiers in Microbiology**, Lausanne, v. 9, [art.] 1989, [p. 1-13], 2018. DOI: 10.3389/fmicb.2018.01989. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6117386/. Acesso em: 13 dez. 2023.

CHIDGEY, K. L.; MOREL, P. C. H.; STAFFORD, K. J.; BARUGH, I, W. Sow and piglet behavioral associations in farrowing pens with temporary crating and in farrowing crates. **Journal of Veterinary Behavior**, New York, v. 20, p. 91-101, 2017. DOI: 10.1016/j.jveb.2017.01.003. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1558787817300126. Acesso em: 26 jan. 2023.

CRISTOFORI, F.; DARGENIO, V. N.; DARGENIO, C.; MINIELLO, V. L.; BARONE, M.; FRANCAVILLA, R. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. **Frontiers in Immunology**, Lausanne, v. 12, [art.] 578386, [p.1-21], 2021. DOI: 10.3389/fimmu.2021.578386. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33717063/. Acesso em: 13 dez. 2023.

CRYAN, J. F.; O'RIORDAN, K. J.; COWAN, C. S. M.; SANDHU, K, V.; BASTIAANSSEN, T. F. S.; BOEHME, M.; CODAGNONE, M. G.; CUSSOTO, S.; FULLING, C.; GOLUBEVA, A. V.; MARTIN, J.A.; MOLINERO-PEREZ, A.; MOLONEY, G.; MORELLI, E.; MORILLAS, E.; O'CONNOR, R.; CRUZ-PEREIRA, J.; PETERSON, V.L.; REA, E.; RITZ, N. L.; SHERWIN, S.; SPICHAK, S.; TEICHMAN, E. M.; DE WOWN, M. V.; VENTURA-SILVA, A. P.; WALLACE-FITZSIMONS, S. E.; HYLAND, N.; CLARKE, G.; DINAN, T. G. The microbiota-gut-brain axis. **Physiological Reviews**, Bethesda, v. 99, n. 4, p. 1877-2013, 2019. DOI: 10.1152/physrev.00018.2018. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31460832/. Acesso em: 13 dez. 2023

DALIRI, E.; OH, D.; LEE, B. Psychobiotics; a promise for neurodevelopmental therapy. **Journal of Probiotics & Health**, Los Angeles, v. 4, [art.] 2, [p. 1-4], 2016. DOI: 10.4172/2329-8901.1000146. Disponível em: https://www.longdom.org/open-access-pdfs/psychobiotics-a-promise-for-neurodevelopmental-therapy-2329-8901-1000146.pdf. Acesso em: 5 nov. 2023.

DALILE, B.; OUDENHOVE, L. V.; VERVLIET, B.; VERBEKE, K. The role of short-chain fatty acids in microbiota–gut–brain communication. **Nature Reviews. Gastroenterology & Hepatology**, London, v. 16, n. 8, p. 461–478, 2019. DOI: 10.1038/s41575-019-0157-3. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31123355/.pdf. Acesso em: 31 jan. 2023.

DOMINGOS, R. L.; SILVA, B. A. N.; BRAVO DE LAGUNA, F.; ARAUJO, W. A. G.; GONÇALVES, M. F.; REBORDÕES, F. I. G.; MOTTA, S. A. B. *Saccharomyces cerevisiae* var. Boulardii CNCM I-1079 during late gestation and lactation improves voluntary feed intake, milk production and litter performance of mixed-parity sows in a tropical humid climate. **Animal Feed Science and Technology**, Amsterdam, v. 272, p. 1-8, 2021. DOI: 10.1016/j.anifeedsci.2020.114785. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0377840120306908. Acesso em: 1º nov. 2023.

DAWKINS, M. S.; WANG, L.; ELLWOOD, S. A.; ROBERTS, S. J.; GEBHARDT-HENRICH, S. G. Optical flow, behaviour and broiler chicken welfare in the UK and Switzerland. **Applied Animal Behaviour Science**, v. 234, [art.] 105180, [p. 1-6], 2021. DOI:10.1016/j.applanim.2020.105180. Disponível em: https://www.researchgate.net/publication/347212459. Acesso em: 21 mar. 2024.

DAWKINS, M. S. Does smart farming improve or damage animal welfare? Technology and what animals want. **Frontiers in Animal Science**, v. 2, [art.] 736536, [p. 1-6], 2021. DOI:10.3389/fanim.2021.736536. Disponível em: https://www.frontiersin.org/articles/10.3389/fanim.2021.736536/full. Acesso em: 21 mar. 2024.

EGLI, P. T.; SCHÜPBACH-REGULA, G.; NATHUES, H.; ULBRICH, S. E.; GRAHOFER, A. Influence of the farrowing process and different sow and piglet traits on uterine involution in a free farrowing system. **Theriogenology**, New York, v. 182, p. 1-8, 2022. Disponível em: https://www.researchgate.net/publication/358255350. Acesso em: 13 dez. 2023.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO. **Probiotics in food:** health and nutritional properties and guidelines for evaluation. Rome: FAO, 2006. (FAO food and nutrition paper, 85). Disponível em: https://www.fao.org/3/a0512e/a0512e.pdf. Acesso em: 28 nov. 2023.

GOUMON, S.; ILLMANN, G.; MOUSTSEN, V. A.; BAXTER, E. M.; EDWARDS, S. A. Review of temporary crating of farrowing and lactating sows. **Frontiers in Veterinary Science**, Lausanne, v. 9, [art.] 811810, [p. 1-25], 2022. DOI: 10.3389/fvets.2022.811810. Disponível em: https://www.frontiersin.org/articles/10.3389/fvets.2022.811810/full. Acesso em: 8 nov. 2023.

GOURLEY, K. M.; DEROUCHEY, J. M.; TOKACH, M. D.; DRITZ, S. S.; GOODBAND, R. D.; WOODWORTH, J. C. Associations between piglet umbilical blood hematological criteria, birth order, birth interval, colostrum intake, and piglet survival. **Journal of Animal Science**, Champaign, v. 98, n. 10, [art.] skaa329, 2020. DOI: 10.1016/j.anifeedsci.2020.114785. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33031551/. Acesso em: 13 dez. 2023.

HALES, V. A.; MOUSTSEN, A. M.; DEVREESE, M. B. F.; NIELSEN, C. F.; HANSEN, H. Comparable farrowing progress in confined and loose housed hyper-prolific sows.

Livestock Science, Amsterdam, v. 171, p. 64-72, 2015. DOI: 10.1016/j.livsci.2014.11.009. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33031551/. Acesso em: 13 dez. 2023.

HALLOWELL, A.; PIERDON, M. Effects of lameness on productivity and longevity for sows in pen gestation. **Journal of Swine Health and Production**, Perry, v. 30, n. 4, p. 223-229, 2022. DOI: 10.54846/jshap/1271. Disponível em: https://www.aasv.org/shap/issues/v30n4/v30n4p223.pdf. Acesso em: 27 jan. 2023.

HALLORAN, K.; UNDERWOOD, M. A. Probiotic mechanisms of action. **Early Human Development**, Limerick, v. 135, p. 58-65, 2019. DOI: 10.1016/j.earlhumdev.2019.05.010. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31174927/. Acesso em: 27 jan. 2023.

HAYAKAWA, T.; MASUDA, T.; KUROSAWA, D.; TSUKAHARA, T. 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**, Richmond, v. 87, n. 12, p. 1501-1510, 2016 DOI: 10.1111/asj.12565. Disponível em: https://onlinelibrary.wiley.com/doi/abs/10.1111/asj.12565. Acesso em: 26 nov. 2023.

HILL, C.; GUARNER, H.; REID, G.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.; FLINT, H. J.; SALMINEN, S.; CALDER, P. C.; SANDERS, M. E. Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. **Nature Reviews. Gastroenterology & Hepatology**, London, v. 11, p. 506–514, 2014. DOI: 10.1038/nrgastro.2014.66. Disponível em:https://pubmed.ncbi.nlm.nih.gov/24912386/. Acesso em: 18 dez. 2023.

HOLT, J. P.; JOHNSTON, L, J.; BAIDOO, S. K.; SHURSON, G. C. Effects of a high-fiber diet and frequent feeding on behavior, reproductive performance, and nutrient digestibility in gestating sows. **Journal of Animal Science**, Champaign, v. 84, n. 4, p. 946-955, 2006. DOI: 10.2527/2006.844946x. Disponível em: https://academic.oup.com/jas/article-abstract/84/4/946/4804229. Acesso em: 1º dez. 2023.

HOLZER, P.; FARZI, A. Neuropeptides and the microbiota-gut-brain axis. **Advances in Experimental Medicine and Biology**, New York, v. 817, p. 195–219, 2014. DOI: 10.1007/978-1-4939-0897-4_9. Disponível em: https://pubmed.ncbi.nlm.nih.gov/24997035/. Acesso em: 13 nov. 2023.

HMAR, E. B. L.; PAUL, S.; SHARMA, H. K. An Insight into the combination of probiotics and their implications for human health. **Endocrine, Metabolic & Immune Disorders Drug Targets**, Sharjah, v. 24, n. 1, p. 1-12, 2024. DOI: 10.2174/1871530323666230502141717. Disponível em: https://www.ingentaconnect.com/content/ben/emiddt/2024/00000024/00000001/art00003. Acesso em: 13 nov. 2023.

HUANG, C.; YUE, Q.; SUN, L.; DI, K.; YANG, D.; HAO, E.; WANG, D.; CHEN, Y.; SHI, L.; ZHOU, R.; ZHAO, G.; CHEN, H. Restorative effects of *Lactobacillus rhamnosus* LR-32 on the gut microbiota, barrier integrity, and 5-HT metabolism in reducing feather-pecking behavior in laying hens with antibiotic-induced dysbiosis. **Frontiers in Microbiology**, Lausanne, v. 14, [art.] 1173804, 2023. DOI:

10.3389/fmicb.2023.1173804. Disponível em: https://pubmed.ncbi.nlm.nih.gov/37180262/. Acesso em: 13 nov. 2023.

HUANG, T. T.; LAI, J. B.; DU, Y. L.; XU, Y.; RUAN, L. M.; HU, S. H. Current understanding of gut microbiota in mood disorders: an update of human studies. **Frontiers in Genetics**, Lausanne, v. 10, [art.] 98, [p. 1-12], 2019. DOI: 10.3389/fgene.2019.00098. Disponível em: https://www.frontiersin.org/articles/10.3389/fgene.2019.00098/full. Acesso em: 13 nov. 2023.

HU, J.; KIM, Y. H.; KIM, I. H. Effects of two bacillus strains probiotic supplement on reproduction performance, nutrient digestibility, blood profile, fecal score, excreta odor contents and fecal microflora in lactation sows, and growth performance in sucking piglets. **Livestock Science**, Amsterdam, v. 244, [art.] 104293, [p. 1-6], 2021. DOI: 10.1016/j.livsci.2020.104293. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1871141319315306. Acesso em: 13 nov. 2023.

ISLAS-FABILA, P.; MOTA-ROJAS, D.; MARTÍNEZ-BURNES, J.; MORA-MEDINA, P.; GONZÁLEZ-LOZANO, M.; ROLDAN-SANTIAGO, P. OROZCO-GREGORIO, H. Physiological and metabolic responses in newborn piglets associated with the birth order. **Animal Reproduction Science**, Amsterdam, v. 197, p. 247-256, 2018. DOI: 10.1016/j.anireprosci.2018.08.037. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0378432018304469. Acesso em: 13 nov. 2023.

ISON, S. H.; JARVIS, S.; HALL, S. A.; ASHWORTH, C. J.; RUTHERFORD, K. M. Periparturient behavior and physiology: further insight into the farrowing process for primiparous and multiparous sows. **Frontiers in Veterinary Science**, Lausanne, v. 5, [art.] 122, 2018 Disponível em: https://www.frontiersin.org/articles/10.3389/fvets.2018.00122/full. Acesso em: 13 nov. 2023.

IVAN PAVLOV: facts. Stockholm: The Nobel Foundation, 7 Feb 2024. Disponível em: https://www.nobelprize.org/prizes/medicine/1904/pavlov/facts/. Acesso em: 19 nov. 2023.

JEONG, J.; KIM, J.; LEE, S.; KIM, I. Evaluation of *Bacillus subtilis* and *Lactobacillus acidophilus* probiotic supplementation on reproductive performance and noxious gas emission in sows. **Annals of Animal Science**, Warsaw, v. 15, n. 3, p. 69-89, 2015. Disponível em: https://sciendo.com/article/10.1515/aoas-2015-0018. Acesso em: 13 nov. 2023.

JORJÃO, A. L. **Efeitos do *Lactobacillus rhamnosus* e seus produtos sobre a liberação de mediadores pró e anti-inflamatórios em macrófagos de camundongo**. 2012. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, São José dos Campos, 2012. Disponível em: https://pesquisa.bvsalud.org/portal/resource/pt/biblio-867518. Acesso em: 13 nov. 2023.

JUNG, S. W.; DO, S.; JANG, J. C.; HONG, J.; KIM, Y. Y. Effect of feeding frequency on reproductive performances and stress responses in gestating sows. **Journal of Animal Science and Technology**, Seoul, v. 66, n. 1, p. 135-144, 2024. Disponível em: https://www.ejast.org/archive/view_article?pid=jast-2023-e42. Acesso em: 13 jan. 2023.

KAISER, M.; JACOBSON, M.; ANDERSEN, P. H.; BÆKBO, P.; CERÓN, J. J.; DAHL, J.; ESCRIBANO, D.; JACOBSEN, S. Inflammatory markers beforeand after farrowing in healthy sows and in sows affected with postpartum dysgalactia syndrome. **BMC Veterinary Research**, London, v. 14, [art.] 83, [p. 1–15], 2018. Disponível em: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-018-1382-7. Acesso em: 28 jan. 2023.

KARAKULA-JUCHNOWICZ, H.; ROG, J.; JUCHNOWICZ, D.; ŁONIEWSKI, I.; SKONIECZNA-ZYDECKA, K.; KRUKOW, P.; FUTYMA-JEDRZEJEWSKA, M.; KACZMARCZYK, M. The study evaluating the effect of probiotic supplementation on the mental status, inflammation, and intestinal barrier in major depressive disorder patients using gluten-free or gluten-containing diet. **Nutrition Journal**, London, v. 18, n. 1, [art.] 50, 2019. DOI: 10.1186/s12937-019-0475-x. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31472678/. Acesso em: 6 nov. 2023.

KELLS, N. J. The five domains model and promoting positive welfare in pigs. **Animal**, Cambridge, v. 16, [art.] 100378, 2022. Supl. 2. DOI: 10.1016/j.animal.2021.100378. Disponível em: https://www.sciencedirect.com/science/article/pii/S1751731121002214. Acesso em: 8 nov. 2023

KIERNAN, D. P.; O’DOHERTY, J. V.; SWEENEY, T. The effect of maternal probiotic or synbiotic supplementation on sow and offspring gastrointestinal microbiota, health, and performance. **Animals**, Basel, v. 13, n. 19, [art.] 2996, 2023. DOI: 10.3390/ani13192996. Disponível em: https://www.mdpi.com/2076-2615/13/19/2996. Acesso em: 26 jan. 2023.

KIM, J. S.; DE LA SERRE, C. B. Diet, gut microbiota composition and feeding behavior. **Physiology & Behavior**, Oxford, v. 1, n. 192, p. 177–18, 2018. DOI: 10.1016/j.physbeh.2018.03.026. Disponível em: https://pubmed.ncbi.nlm.nih.gov/29605585/. Acesso em: 13 nov. 2023.

KIM, S. W.; WEAVER, A. C.; SHEN, Y. B.; ZHAO, Y. Improving efficiency of sow productivity: Nutrition and health. **Journal of Animal Science and Biotechnology**, London, n. 4, n. 26, p. 1-8, 2013. DOI: 10.1186/2049-1891-4-26. Disponível em: https://www.researchgate.net/publication/252323642. Acesso em: 13 jan. 2023.

KIM, K. H.; HOSSEINDOUST, A.; INGALE, S. L.; LEE, S. H.; NOH, H. S.; CHOI, Y. H.; ET, A. L. Effects of gestational housing on reproductive performance and behavior of sows with different backfat thickness. **Asian-Australasian Journal of Animal Science**, Seoul, v. 29, n. 1, p. 142-148, 2016. DOI: 10.5713/ajas.14.0973. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4698681/. Acesso em: 11 jan. 2023.

KINANE, O.; FIDELMA, B.; KEELIN O. Freedom to move: free lactation pens improve sow welfare. **Animals**, Basel, v. 12, n. 14, [art.] 1762, [p. 1-13], 2022. DOI:10.3390/ani12141762. Disponível em: https://www.mdpi.com/2076-2615/12/14/1762. Acesso em: 13 nov. 2023

KONIECZKA, P.; FERENC, K.; JØRGENSEN, J. N.; HANSEN, L. H.; ZABIELSKI, R.; OLSZEWSKI, J.; LIPIŃSKI, K. Feeding Bacillus-based probiotics to gestating and lactating sows is an efficient method for improving immunity, gut functional status and biofilm formation by probiotic bacteria in piglets at weaning. **Animal Nutrition**, Beijing, v. 13, p. 361-372, 2023. Disponível em: https://www.mdpi.com/2076-2615/12/14/1762. Acesso em: 18 nov. 2023

KOGUT, M. H. **Gut Microbiota, immunity and health in production animals**. 4th ed. Stillwater: Springer, 2022.

KRAIMI, N.; DAWKINS, M.; GEBHARDT-HENRICH, S. G.; VELGE, P.; RYCHLIK, I.; VOLF, J.; LETERRIER, C. Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: a review. **Physiology & Behavior**, Oxford, v. 210, p. 1-9, 2019. DOI: 10.1016/j.physbeh.2019.112658. Disponível em: https://www.sciencedirect.com/science/article/pii/S003193841930561X. Acesso em: 13 nov. 2023.

LAGODA, M. The implications of chronic stress during gestation for sow reproductive performance and welfare. 2022. Thesis (Doctoral) - Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Warszawa, 2022. Disponível em: https://www.igbzpan.pl/uploaded/FSiBundleContentBlockBundleModelTranslatableBlockTranslatableFilesElement/filePath/2295/autoreferat-martyna-lagoda.pdf. Acesso em: 13 nov. 2023.

LAUFFER, A. Efeito do estresse agudo, crônico e ambos combinados na permeabilidade intestinal de ratos. 2015. Tese (Doutorado) – Programa de Pós-graduação: Ciências em Gastroenterologia e Hepatologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul. Porto Alegre, 2015.

LEE, J.; SHIN, H.; JO, J.; LEE, G.; YUN, J. Large litter size increases oxidative stress and adversely affects nest-building behavior and litter characteristics in primiparous sows. **Frontiers in Veterinary Science**, Lausanne, v. 10, [art.] 1219572, 2023. DOI: 10.3389/fvets.2023.1219572. Disponível em: https://www.frontiersin.org/articles/10.3389/fvets.2023.1219572/full. Acesso em: 13 out. 2023.

LEISTNER, C.; MENKE, A. Hypothalamic–pituitary–adrenal axis and stress. **Handbook of Clinical Neurology**, Amsterdam, v. 175, p. 55-64, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/33008543/. Acesso em: 1º nov. 2023.

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. Disponível em: https://pubmed.ncbi.nlm.nih.gov/29767089/. Acesso em: 22 out. 2023.

LI, Q.; YANG, S.; CHEN, F.; GUAN, W.; ZHANG, S. Nutritional strategies to alleviate oxidative stress in sows. **Animal Nutrition**, Beijing, v. 9, p. 60-73, 2022. DOI: 10.1016/j.aninu.2021.10.006. Disponível em: https://www.sciencedirect.com/science/article/pii/S240565452100192X. Acesso em: 1º nov. 2023.

LIPPI, I. C. C.; CALDARA, F. R.; MORAIS, H. B.; VARGAS, L. B.; ODAKURA, A. M.; BURBARELLI, M. F. C.; SANTOS, L. S. Effects of auditory enrichment on welfare and performance of sows during pregnancy and farrowing/lactation periods. **Journal of Applied Animal Welfare Science**, London, 1-17, May 2023. Disponível em: https://pubmed.ncbi.nlm.nih.gov/37224626/. Acesso em: 20 jan. 2023.

LIU, W. C.; YE, M.; LIAO, J. H.; ZHAO, Z. H.; KIM, I. H.; AN, L. L. Application of complex probiotics in swine nutrition–a review. **Annals of Animal Science**, Warsaw, v. 18, n. 2, p. 335-350, 2018. Disponível em: https://www.sciencedirect.com/science/article/pii/S240565452100192X. Acesso em: 26 nov. 2023.

LIU, H.; HOU, C.; LI, N.; ZHANG, X.; ZHANG, G.; YANG, F.; QIAO, S. Microbial and metabolic alterations in gut microbiota of sows during pregnancy and lactation. **The FASEB Journal**, Bethesda, v. 33, n. 3, p. 4490-4501, 2019. Disponível em: https://www.sciencedirect.com/science/article/pii/S240565452100192X. Acesso em: 1º fev. 2023.

LIU, R. T.; WALSH, R. F.; SHEEHAN, A. E. Prebiotics and probiotics for depression and anxiety: a systematic review and meta-analysis of controlled clinical trials. **Neuroscience and Biobehavioral Reviews**, New York, v. 102, p. 13–23, 2019. DOI: 10.1016/j.neubiorev.2019.03.023. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31004628/. Acesso em: 13 fev. 2023.

LIU, H. Y.; ZHU, C.; ZHU, M.; YUAN, L.; LI, S.; GU, F.; CAI, D. Alternatives to antibiotics in pig production: looking through the lens of immunophysiology. **Stress Biology**, [Shaanxi], v. 4, [art.] 1, [p. 1-23], 2024. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10758383/. Acesso em: 2 fev. 2023.

LOUWIES, T.; JOHNSON, A. C.; OROCK, A.; YUAN, T.; GREENWOOD-VAN MEERVELD, B. The microbiota-gut-brain axis: an emerging role for the epigenome. **Experimental Biology and Medicine**, Maywood, v. 245, p. 138–145, 2020. Disponível em: https://pubmed.ncbi.nlm.nih.gov/31805777/. Acesso em: 9 nov. 2023.

LUCY, M. C.; SAFRANSKI, T. J. Heat stress in pregnant sows: thermal responses and subsequent performance of sows and their offspring. **Molecular Reproduction and Development**, Hoboken, v. 84, n. 9, p. 946-956, 2017. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1002/mrd.22844. Acesso em: 3 jan. 2023.

LYTE, J. M.; LYTE, M. Microbial endocrinology: intersection of microbiology and neurobiology matters to swine health from infection to behavior. **Animal**, Cambridge, v. 13, n. 11, p. 2689-2698, 2019. Disponível em: https://pubmed.ncbi.nlm.nih.gov/30806347/. Acesso em: 25 dez. 2023.

MANU, H.; LEE, S.; REN, P.; PANGENI, D.; YANG, X.; BAIDOO, S. K. Effect of feeding frequency and sow parity based on isocaloric intake during gestation on sow performance. **Journal of Animal Science**, Champaign, v. 97, n. 5, p. 2154-2164, 2019. DOI: 10.1093/jas/skz099. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6488309/. Acesso em: 13 nov. 2023.

MARTÍNEZ-MIRÓ, S.; TECLES, F.; RAMÓN, M.; ESCRIBANO, D.; HERNÁNDEZ, F.; MADRID, J.; CERÓN, J. J. Causes, consequences and biomarkers of stress in swine: an update. **BMC Veterinary Research**, London, v. 12, [art.] 171, [p. 1-9], 2016. DOI: 10.1186/s12917-016-0791-8. Disponível em: https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-016-0791-8. Acesso em: 19 nov. 2023.

MARTIN-GALLAUSIAUX, C.; MARINELLI, L.; BLOTTIÈRE, H. M.; LARRAUFIE, P.; LAPAQUE, N. SCFA: mechanisms and functional importance in the gut. **The Proceedings of the Nutritional Society**, Wallingford, v. 80, n. 1, p. 37–49, 2021. DOI: 10.1017/S0029665120006916. Disponível em: https://pubmed.ncbi.nlm.nih.gov/32238208/. Acesso em: 13 dez. 2023.

MATHIPA, M. G.; THANTSHA, M. S. Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. **Gut Pathogens**, **London,** v. 9, [art.] 28, 2017. DOI: 10.1186/s13099-017-0178-9. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5422995/. Acesso em: 8 dez. 2023.

MCEWEN, B. S. Neurobiological and systemic effects of chronic stress. **Chronic Stress**, Thousand Oaks, v. 1, Apr. 2017. DOI: 10.1177/2470547017692328. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28856337/. Acesso em: 29 nov. 2023.

MINDUS, C.; ELLIS, J.; VAN STAAVEREN, N.; HARLANDER-MATAUSCHEK, A. *Lactobacillus*-based probiotics reduce the adverse effects of stress in rodents: a meta-analysis. **Frontiers in Behavioral Neuroscience**, Lausanne, v. 15, [art.] 642757, 2021. DOI: 10.3389/fnbeh.2021.642757. Disponível em: https://www.frontiersin.org/articles/10.3389/fnbeh.2021.642757/full. Acesso em: 13 nov. 2023.

MORGAN, L.; KLEMENT, E.; NOVAK, S.; ELIAHOO, E.; YOUNIS, A.; SUTTON, G. A.; ABU-AHMAD, W.; RAZ, T. Effects of group housing on reproductive performance, lameness, injuries and saliva cortisol in gestating sows. **Preventive Veterinary Medicine**, Amsterdam, v. 160, p. 10-17, 2018. DOI: 10.1016/j.prevetmed.2018.09.026. Disponível em: https://pubmed.ncbi.nlm.nih.gov/30388991/. Acesso em: 2 nov. 2023.

MOROTA, G., VENTURA, R. V., SILVA, F. F., KOYAMA, M., AND FERNANDEZ, S. C. Machine learning and data mining advance predictive big data analytics in precision animal agriculture. **Journal of Animal Science,** Champaign. v. 96, p. 1540–1550, 2018. DOI: 10.1093/jas/sky014. Disponível em: https://pubmed.ncbi.nlm.nih.gov/29385611/. Acesso em: 21 mar. 2024.

MUSAZZI, L., TORNESE, P., SALA, N., & POPOLI, M. Acute or Chronic? A Stressful Question. **Trends in Neurosciences**, v. 40, n. 9, p. 525–535, 2017. DOI:10.1016/j.tins.2017.07.002. Disponível em: https://europepmc.org/article/med/28778394. Acesso em: 21 mar. 2024.

NEGM, E. A.; EL SAYED, M. A.; ABDELRAHMAN, M. A.; MOHAMMED, A. A.; DARWISH, M. H. Probiotic effects on behavior, stress indicators and antioxidant activity of transported broiler chicken. **Alexandria Journal for Veterinary Sciences**, Alexandria, v. 76, n. 1, p. 48-57, 2023. DOI: 10.5455/ajvs.1186.Disponível: https://www.ejmanager.com/mnstemps/31/31-1669657447.pdf?t=1702988698. Acesso em: 7 nov. 2023.

NEUFELD, K. M.; KANG, N.; BIENENSTOCK, J.; FOSTER, J. A. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. **Neurogastroenterology Motility**, Oxford, v. 23, p. 255–264, 2011. DOI:10.1111/j.1365-2982.2010.01620.x. Disponível em: https://pubmed.ncbi.nlm.nih.gov/21054680/. Acesso em: 11 nov. 2023.

ODAKURA, A. M.; CALDARA, F. R.; BURBARELLI, M. F. D. C.; PAZ, I. C. D. L. A.; GARCIA, R. G.; SANTOS, V. M. O.; SILVA, M. I. L. Dietary Supplementation of Eubiotic Fiber Based on Lignocellulose on Performance and Welfare of Gestating and Lactating Sows. **Animals**, Basel, v. 13, n. 4, [art.] 695, 2023. DOI: 10.3390/ani13040695. Disponível: https://www.mdpi.com/2076-2615/13/4/695. Acesso em: 10 nov. 2023.

OTTEN, W.; KANITZ, E.; TUCHSCHERER, M. The impact of pre-natal stress on offspring development in pigs. **The Journal of Agricultural Science**, London, v. 153, n. 5, p. 907–919, 2015. DOI: 10.1017/s0021859614001361. Disponível em:

https://www.cambridge.org/core/journals/journal-of-agricultural-science/article/abs/impact-of-prenatal-stress-on-offspring-development-in-pigs/44E6B677C9C11AA23D5F595B467F8411#access-block. Acesso em: 19 nov. 2023.

PARK, M. R.; SHIN, M.; MUN, D.; JEONG, S. Y.; JEONG, D. Y.; SONG, M.; OH, S. Probiotic *Lactobacillus fermentum* strain JDFM216 improves cognitive behavior and modulates immune response with gut microbiota. **Scientific Reports**, London, v. 10, [art.] 21701, [p. 1-13], 2020. DOI: 10.1038/s41598-020-77587-w. Disponível em: https://www.nature.com/articles/s41598-020-77587-w. Acesso em: 19 nov. 2023.

PELTONIENI, O.; HAN, T.; YUN, J. Coping with large litters: management effects on welfare and nursing capacity of the sow. **Journal of Animal Science and Technology**, Seoul, v. 63, n, 2, p. 199-210, 2021. DOI: 10.5187/jast.2021.e46. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8071737/pdf/jast-63-2-199.pdf. Acesso em: 19 nov. 2023.

PENG, X.; YAN, C.; HU, L.; HUANG, Y.; FANG, Z.; LIN, Y.; XU, S.; FENG, B.; LI, J.; ZHUO, Y.; WU, D.; CHE, L. Live yeast supplementation during late gestation and lactation affects reproductive performance, colostrum and milk composition, blood biochemical and immunological parameters of sows. **Animal Nutrition**, Beijing, v. 6, n. 3, p. 288–292, 2020. DOI: 10.1016/j.aninu.2020.03.001. Disponível em: https://www.sciencedirect.com/science/article/pii/S2405654520300330. Acesso em: 9 nov. 2023.

PETRA, A. I.; PANAGIOTIDOU, S.; HATZIAGELAKI, E.; STEWART, J. M.; CONTI, P.; THEOHARIDES, T. C. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. **Clinical Therapeutics**, Belle Mead, v. 37, p. 984–995, 2015. DOI: 10.1016/j.clinthera.2015.04.002. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S014929181500226X. Acesso em: 19 out. 2023.

POL, F.; KLING-EVEILLARD, F.; CHAMPIGNEULLE, F.; FRESNAY, E.; DUCROCQ, M.; COURBOULAY, V. Human–animal relationship influences husbandry practices, animal welfare and productivity in pig farming. **Animal**, Cambridge, v. 15, n. 2, [art.] 100103, [p. 1-10], 2021. DOI: 10.1016/j.animal.2020.100103. Disponível em: https://www.sciencedirect.com/science/article/pii/S1751731120301051. Acesso em: 19 nov. 2023.

ROONEY, H. B.; SCHMITT, O.; COURTY, A.; LAWLOR, P. G.; O'DRISCOLL, K. Like mother like child: do fearful sows have fearful piglets? **Animals**, Basel, v. 11, n. 5, [art.] 1232, 2021. DOI: 10.3390/ani11051232. Disponível em: https://www.sciencedirect.com/science/article/pii/S1751731120301051. Acesso em: 13 jul. 2023.

RUTHERFORD, K. M.; PIASTOWSKA-CIESIELSKA, A.; DONALD, R. D.; ROBSON, S. K.; ISON, S. H.; JARVIS, S.; BRUNTON, P. J.; RUSSELL, J. A.; LAWRENCE, A. B. Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig. **Physiology & Behavior**, New York, v. 129, p. 255-264, 2014. DOI: 10.1016/j.physbeh.2014.02.052. Disponível em: https://pubmed.ncbi.nlm.nih.gov/24631303/.Acesso em: 23 out. 2023.

SCHAREK, L.; GUTH, J.; FILTER, M.; SCHMIDT, M. F. Impact of the probiotic bacteria *Enterococcus faecium* NCIMB 10415, SF68 and *Bacillus cereus* var. toyoi NCIMB

40112 on the development of serum IgG and faecal IgA of sows and their piglets. **Archives of Animal Nutrition**, Abingdon, v. 61, p. 223-234, 2007. DOI: 10.1080/17450390701431540. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/17450390701431540.Acesso em: 19 nov. 2023.

SCHIERACK, P.; FILTER, M.; SCHAREK, L.; TOELKE, C.; TARAS, D.; TEDIN, K.; HAVERSON, K.; LÜBKE-BECKER, A.; WIELER, L. H. Effects of *Bacillus cereus* var toyoi on immune parameters of pregnant sows. **Veterinary Immunology and Immunopathology**, Amsterdam, v. 127, p. 26-37, 2009. DOI: 10.1016/j.vetimm.2008.09.002. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0165242708003541.Acesso em: 19 nov. 2023.

SILVA, W. C. D.; SILVA, J. A. R. D.; CAMARGO-JÚNIOR, R. N. C.; SILVA, É. B. R. D.; SANTOS, M. R. P. D.; VIANA, R. B.; LOURENÇO-JÚNIOR, J. D. B. Animal welfare and effects of per-female stress on male and cattle reproduction—A review. **Frontiers in Veterinary Science**, Lausanne, v. 10, [art.] 1083469, 2023. DOI: 10.3389/fvets.2023.1083469. Disponível em: https://www.frontiersin.org/articles/10.3389/fvets.2023.1083469/full. Acesso em: 19 nov. 2023.

SILVA, Y. P.; BERNARDI, A.; FROZZA, R. L. The role of short-chain fatty acids from gut microbiota in gut-brain communication. **Frontiers in Endocrinology**, Lausanne, v. 11, [art.] 25, [p. 1-14], 2020. DOI: 10.3389/fendo.2020.00025. Disponível em: https://www.frontiersin.org/articles/10.3389/fendo.2020.00025. Acesso em: 19 nov. 2023.

SPOOLDER, H. A. M.; VERMEER, H. M. Gestation group housing of sows. *In:* FARMER, C. **The gestating and lactating sow**. Wageningen: Wageningen Academic, 2015. p. 47-72. DOI: 10.3920/978-90-8686-803-2_3.

SEQUEIRA, C. M. L. M.; HENGSTBERGER, C.; ENCK, P.; MACK, I. Effect of probiotics on psychiatric symptoms and central nervous system functions in human health and disease: a systematic review and meta-analysis. **Nutrients**, Basel, v. 14, n. 3, [art.] 621, [p. 1-28], 2022. Disponível em: https://www.mdpi.com/2072-6643/14/3/621. Acesso em: 20 nov. 2023.

STRASSER, B.; BECKER, K.; FUCHS, D.; GOSTNER, J. M. Kynurenine pathway metabolism and immune activation: peripheral measurements in psychiatric and co-morbid conditions. **Neuropharmacology**, Oxford, v. 112, p. 286–296, 2017. DOI: 10.1016/j.neuropharm.2016.02.030. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S002839081630065X. Acesso em: 2 jan. 2023.

TIMMERMAN, H. M.; KONING, C. J. M.; MULDER, L.; ROMBOUTS, F. M.; BEYNEN, A. C. Monostrain, multistrain and multispecies probiotics—a comparison of functionality and efficacy. **International Journal of Food Microbiology**, Amsterdam, v. 96, p. 219–233, 2004. DOI: 10.1016/j.ijfoodmicro.2004.05.012. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S0168160504002855. Acesso em: 29 nov. 2023.

TUMMARUK, P.; DE RENSIS, F.; KIRKWOOD, R. N. Managing prolific sows in tropical environments. **Molecular Reproduction and Development**, Hoboken, v. 90, n. 7, p.

533–545, 2023. DOI: 10.1002/mrd.23661. Disponível em: https://pubmed.ncbi.nlm.nih.gov/36495558/. Acesso em: 18 nov. 2023.

VERBEEK, E.; DICKSVED, J.; KEELING, L. Supplementation of *Lactobacillus* early in life alters attention bias to threat in piglets. **Scientific Reports**, London, v. 11, [art.] 10130, [p. 1-10], 2021. DOI: 10.1038/s41598-021-89560-2. Disponível em: https://www.nature.com/articles/s41598-021-89560-2#citeas. Acesso em: 13 nov. 2023.

WANG, J.; JI, H. F.; HOU, C. L.; WANG, S. X.; ZHANG, D. Y.; LIU, H.; WANG, Y. M. Effects of *Lactobacillus johnsonii* XS4 supplementation on reproductive performance, gut environment, and blood biochemical and immunological index in lactating sows. **Livestock Science**, Amsterdam, v. 164, p. 96-101, 2014. DOI: 10.1007/978-1-4939-0897-4_9. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S1871141314001383. Acesso em: 16 dez. 2023.

WANG, J.; ZENG, Y.; WANG, S.; LIU, H.; ZHANG, D.; ZHANG, W.; JI, H. Swine-derived probiotic *Lactobacillus plantarum* inhibits growth and adhesion of enterotoxigenic *Escherichia coli* and mediates host defense. **Frontiers in Microbiology**, Lausanne, v. 9, [art.] 1364, [p. 1-11], 2018. DOI: 10.3389/fmicb.2018.01364 Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6028558/. Acesso em: 20 dez. 2023.

YANG, F.; WANG, A.; ZENG, X.; HOU, C.; LIU, H.; QIAO, S. *Lactobacillus reuteri* I5007 modulates tight junction protein expression in IPEC-J2 cells with LPS stimulation and in newborn piglets under normal conditions. **BMC Microbiology**, London, v. 15, [art.] 32, [p. 1-11], 2015. DOI: 10.1186/s12866-015-0372-1. Disponível em: https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-015-0372-1#citeas. Acesso em: 5 jan. 2023.

YIRGA, H. The use of probiotics in animal nutrition. **Journal of Probiotics & Health**, Los Angeles, v. 3, n. 2, p. 1–10, 2015. DOI:10.4172/2329-8901.1000132. Disponível em: https://www.researchgate.net/publication/282904768_The_Use_of_Probiotics_in_Animal_Nutrition. Acesso em: 13 nov. 2023.

ZHANG, M. Y.; LI, X.; ZHANG, X. H.; LIU, H. G.; LI, J. H.; BAO, J. Effects of confinement duration and parity on stereotypic behavioral and physiological responses of pregnant sows. **Physiology & Behavior**, Oxford, v. 179, p. 369-376, 2017. DOI: 10.1016/j.physbeh.2017.07.015. Disponível em: https://pubmed.ncbi.nlm.nih.gov/28705536/. Acesso em: 29 jan. 2023.

ZHANG, Q.; LI, J.; CAO, M.; LI, Y.; ZHUO, Y.; FANG, Z.; WU, D. 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. DOI: 10.1016/j.aninu.2020.04.002. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7503085/. Acesso em: 18 nov. 2023.

ZHANG, W.; HUANG, J.; GAO, F.; YOU, Q.; DING, L.; GONG, J.; ZHANG, Y. *Lactobacillus reuteri* normalizes altered fear memory in male Cntnap4 knockout mice. **EBioMedicine**, Amsterdam, v. 86, [art.] 104323, 2022. DOI: 10.1016/j.ebiom.2022.104323. Disponível em: https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(22)00505-9/fulltext. Acesso em: 13 dez. 2023.

ANEXOS

Parecer da Comissão de Ética na Utilização de Animais (CEUA) – UFRGS

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA
Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 39736
Título: Efeitos de probióticos no desempenho e saúde de matrizes suínas e suas leitegadas

Vigência: 01/11/2020 à 01/11/2022

Pesquisadores:

Equipe UFRGS:

FRANCIELE MABONI SIQUEIRA - coordenador desde 01/11/2020
INES ANDRETTA - coordenador desde 01/11/2020
GABRIELA MERKER BREYER - desde 01/11/2020
CAROLINA HAUBERT FRANCESCHI - desde 01/11/2020
Paula Gabriela da Silva Pires - pesquisador desde 01/11/2020

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 07/12/2020 via Webconferência - Mconf UFRGS, em seus aspectos éticos e metodológicos, para a utilização de 300 matrizes suínas, provenientes de granja comercial localizada no município de Maratá – RS, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Sexta-Feira, 18 de Dezembro de 2020

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética