UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE EDUCAÇÃO SUPERIOR DO OESTE – CEO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

MARIANE FICAGNA

USO DO PROBIÓTICO *Enterococcus faecium E297* NA DIETA DE FRANGOS DE CORTE EM SUBSTITUIÇÃO A MELHORADORES DE CRESCIMENTO CONVENCIONAIS: EFEITOS SOBRE DESEMPENHO ZOOTÉCNICO, SAÚDE ANIMAL, QUALIDADE DE CARNE.

PINHALZINHO
2021

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de Concentração Propriedades e Segurança dos Alimentos da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de Mestre em Ciência e Tecnologia de Alimentos.
Orientador (a): Liziane Schittler Moroni
Co-orientador(s): Aleksandro Schafer da Silva

PINHALZINHO

2021

Ficha catalográfica elaborada pelo programa de geração automática da

Biblioteca Setorial do CEO/UDESC,

com os dados fornecidos pelo(a) autor(a)

Ficagna, Mariane
Uso do probiótico Enterococcus faecium E297 na dieta de frangos de corte em substituição a melhoradores de crescimento convencionais: : efeitos sobre desempenho zootécnico, saúde animal, qualidade de carne / Mariane Ficagna. -- 2021.
66 p.

Orientadora: Liziane Schittler Moroni
Coorientador: Aleksandro Schafer da Silva
Dissertação (mestrado) -- Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Chapecó, 2021.

1. Aditivos. 2. Avicultura. 3. Melhoradores de desempenho. 4. Probióticos. 5. Produção Animal. I. Schittler Moroni, Liziane . II. Schafer da Silva, Aleksandro . III. Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. IV. Titulo.

Universidade do Estado de Santa Catarina
UDESC Oeste
Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

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Elaborada por
Mariane Ficagna

como requisito parcial para obtenção do grau de
Mestre em Ciência e Tecnologia de Alimentos

Comissão Examinadora:

Liziane Schittler Moroni
Prof. Dra. Liziane Schittler Moroni- Orientadora (UDESC Oeste – Pinhalzinho / SC)

Aniela P. Kempka
Prof. Dra. Aniela Pinto Kempka (UDESC Oeste – Pinhalzinho / SC)

Tiago Goulart Petrolli
Prof. Dr. Tiago Goulart Petrolli (UNOESC - Xanxerê / SC)

Pinhalzinho, 12 de novembro de 2021

AGRADECIMENTOS

Gratidão é recognição pelas situações e dádivas que a vida lhe proporcionou e proporciona. É reconhecer o auxílio de pessoas boas pela nossa trajetória. É entender que precisamos ter humildade na vida pessoal e profissional. É saber agradecer os muitos feitos conquistados e as pessoas que contribuíram para o sucesso deste, pois ninguém é capaz sozinho, Deus sempre coloca as pessoas certas em nossas vidas, é nosso dever saber reconhecer e agradecer.

Nesse momento, tenho muitos a quem agradecer:

Primeiramente à Deus, sem ele nada seria. Obrigada por guiar meu caminho, me amparar nas dificuldades e provar que jamais estou sozinha. Por me reerguer todas as vezes que cai e mostrar-me o quão forte o senhor me tornou.

Aos meus pais Rosa e Dirceu e meu irmão Michael, pela compreensão e ensinamentos. Sem vocês, eu não teria conseguido. Obrigada pelo apoio e incentivo em todos os momentos da minha vida. Por acreditarem em mim, e não medirem esforços para a concretização dos meus sonhos. Sem vocês, nada seria possível. Amo vocês com amor eterno!

Ao meu noivo Ismael, que Deus colocou em meu caminho para que eu pudesse entender que tudo que acontece na vida tem um significado. Obrigado meu amor, por todo incentivo e apoio. Por me acalmar todas as vezes que me desesperei achando que não daria conta. Te amo com todo meu coração.

A minha doce avó Olivia (*in memorian*), não tenho palavras para agradecer tudo o que a senhora foi na minha vida. Impossível escrever sobre ti sem deixar cair as lágrimas. Você foi a mulher mais guerreira que eu conheci em toda a minha vida. O significado perfeito de amor e coragem. A ti, minha gratidão é eterna e apenas é superada pelo amor que sinto por você.

A UDESC, ao Curso de Pós-graduação pela oportunidade, acolhimento e amplo conhecimento obtido, meu muito obrigada.

Aos professores, Liziane Schittler Moroni e Aleksandro Schafer da Silva orientadora e coorientador meu muito obrigado pela dedicação e paciência em me passar os ensinamentos necessários, pela oportunidade oferecida e confiança depositada durante o mestrado.

Aos membros dos Laboratório de Microbiologia (MicroLab) e Laboratório de Anatomia e Parasitologia que auxiliaram nos experimentos da dissertação. Um obrigado muito especial e com muito carinho.

À empresa DMS Nutrição e Saúde Animal por ter apoiado e contribuído com a realização desta pesquisa.

Enfim, a todos que colaboraram, obrigada por toda a ajuda neste período.

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos
Universidade do Estado de Santa Catarina

Uso do probiótico *Enterococcus faecium E297* na dieta de frangos de corte em substituição a melhoradores de crescimento convencionais: efeitos sobre desempenho zootécnico, saúde animal, qualidade de carne

AUTOR: Mariane Ficagna
ORIENTADOR(A): Liziane Schittler Moroni
Pinhalzinho, 12 de novembro de 2021

Antibióticos são administrados na dieta de frangos de cortes como estratégia para melhorar o desempenho zootécnico. Com a tendência de proibição do uso de antibióticos na alimentação de animais como melhorador de desempenho, as indústrias têm estimulado a pesquisa de aditivos como por exemplo, os probióticos. Este estudo teve como objetivo avaliar se a adição do probiótico *Enterococcus faecium* E297 e o probiótico *Enterococcus faecium* SF68 em substituição ao antibiótico na alimentação de frangos de corte tem efeitos positivos sobre desempenho zootécnico e qualidade da carne. Para isto, foram utilizados 224 pintos de corte macho, Cobb 500, de um dia de idade, distribuídos aleatoriamente em 16 boxes com cama de aviário, e submetidos a 4 tratamentos, com 4 repetições de 14 aves cada. Os tratamentos consistiram em: T1 - controle positivo (ração basal mais antibiótico enramicina (Enradin F80), 10mg / kg na dieta basal); T2 - controle negativo (ração basal, sem enramicina e sem probiótico); T3 - adição de probiótico *E. faecium* E.297, 2,5 mL / kg de ração, (10^9 UFC / g) na dieta basal; T4 - adição do probiótico *E. faecium* chamada SF68, 15mg / kg de ração (2,0 x 10^9 UFC / g). A adição de probióticos (E297 e SF68) aumentou o consumo de ração e ganho de peso dos animais. Observou-se aos 41 dias melhor conversão alimentar nos animais alimentados com ração basal e que receberam o probiótico E297, quando comparado aos grupos com antibiótico e o probiótico SF68. No leucograma, observou-se aumento dos leucócitos, linfócitos, heterofilos e monócitos ($p<0,001$) nos esfregaços sanguíneos dos animais alimentados com adição de antibiótico, SF68 e E297 em relação ao controle negativo. Os níveis de globulina sérica e triglicerídeos foram significativamente maiores ($p <0,05$) no soro de frangos com a dieta suplementada com antibiótico do que com adição dos probióticos. O menor nível de colesterol sérico foi observado nos animais alimentados com a ração basal do que nos demais grupos. Houve redução significativa na concentração de gordura total na carne dos frangos, onde os animais receberam na dieta os probióticos quando comparado aos demais tratamentos. O pH da carne e os níveis de proteína foram significativamente maiores ($p<0,001$) nos animais alimentados com os probióticos e ração basal em comparação aos grupos com adição de antibiótico na dieta. Em relação a microbiota, não houve diferenças significativas ($p >0,05$) nas contagens de bactécias ácido láticas (BAL) na cama aviária e cloaca dos animais em relação aos diferentes tratamentos. Não houve diferenças significativas nas contagens de enterobactérias, BAL's, mesófilos e E. coli nas fezes de frangos aos 41 dias, alimentados com probiótico, antibiótico e ração basal. A inclusão do probiótico E297 na alimentação de frangos de corte melhorou ganho de peso, conversão alimentar e exerceu efeitos positivos na saúde dos animais, aumentou a relação entre villo/cripta intestinal e estimulou o sistema imune e modular. O probiótico E297 mostrou-se potencial substituto dos antibióticos melhoradores de desempenho na alimentação de animais em ambiente com desafio sanitário.

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Palavras-chave: Aditivos. Avicultura. Melhoradores de desempenho. Probióticos. Produção Animal.

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos
Universidade do Estado de Santa Catarina

Use of probiotic *Enterococcus faecium* E297 in the diet of bearing chickens in replacement of conventional growth impropers: effects on zootechnical performance, animal health, meat quality

AUTHOR: Mariane Ficagna
ADVISER: Liziane Schittler Moroni
Pinhalzinho, 12 de novembro de 2021

Antibiotics are administered in the diet of broiler chickens as a strategy to improve zootechnical performance. With the trend towards banning the use of antibiotics in animal feed as a performance enhancer, industries have encouraged research into additives such as probiotics. This study aimed to evaluate whether the addition of the probiotic Enterococcus faecium E297 and the probiotic Enterococcus faecium SF68 to replace the antibiotic in broiler chicken feed has positive effects on zootechnical performance and meat quality. For this, 224 one-day-old male Cobb 500 broiler chicks were used, randomly distributed in 16 litter boxes, and developed to 4 treatments, with 4 replicates of 14 birds each. Treatments consisted of: T1 - positive control (basal chow plus enramycin antibiotic (Enradin F80), 10mg/kg basal diet); T2 - negative control (basal diet, without enramycin and without probiotic); T3 - addition of probiotic E. faecium E.297, 2.5 mL / kg of feed, (10^9 CFU / g) in the basal diet; T4 - addition of the probiotic E. faecium called SF68, 15mg / kg of feed (2.0 x 10^9 CFU / g). The addition of probiotics (E297 and SF68) increased animal feed intake and weight gain. At 41 days, a better feed conversion was observed in the animals fed with basal chow and that received the probiotic E297, when compared to the groups with antibiotic and probiotic SF68. The white blood cell count showed an increase in leukocytes, lymphocytes, heterophils and monocytes ($p < 0.001$) in the blood smears of animals fed with the addition of antibiotics, SF68 and E297 in relation to the negative control. Serum globulin and triglyceride levels were obtained higher ($p < 0.05$) in the serum of chickens with the diet supplemented with antibiotics than with the addition of probiotics. The lowest level of serum cholesterol was observed in animals fed with the basal diet than in the other groups. The reduction decreased in the concentration of total fat in chicken meat, where the animals received probiotics in the diet when compared to the other treatments. Meat pH and protein levels were higher ($p<0.001$) in animals fed with probiotics and basal chow compared to groups with antibiotic addition to the diet. Regarding the microbiota, there were no significant differences ($p> 0.05$) in the lactic acid bacteria (LAB) counts in the poultry litter and cloaca of the animals in relation to the different treatments. There were no significant differences in the counts of enterobacteria, BAL's, mesophiles and E. coli in chicken feces at 41 days, fed with probiotic, antibiotic and basal chow. The inclusion of the probiotic E297 in the feed of broiler chickens improved weight gain, feed conversion and had positive effects on animal health, increased the relationship between villo / intestinal crypt and stimulated the immune and modulation system. The E297 probiotic was shown to be a potential replacement for performance-enhancing

antibiotics in animal feed in a sanitary environment.

Keywords: Additives. Poultry farming. Performance enhancers. Probiotics. Animal production.

SUMÁRIO

1 CAPÍTULO I
REVISÃO DE LITERATURA

1.1 INTRODUÇÃO

1.1.1 A avicultura de corte brasileira

A avicultura industrial brasileira surgiu no final da década de 1950, quando os aviários passaram a utilizar novos métodos de manejo e os institutos de pesquisa intensificaram os estudos no combate às doenças e controle sanitário geral (SCHMIDT; SILVA, 2018). Em 1970, o desenvolvimento da avicultura se efetivou, surgiram os sistemas de Integração Vertical, uma parceria entre indústria (frigoríficos) e os produtores. O avicultor integrado passou a contar com o apoio da indústria com o fornecimento de insumos, como ração e medicamentos, além de assistência técnica e reposição de lotes (animais pintinhos) (ZEN et al., 2015).

As grandes modificações no sistema produtivo, como o alto nível tecnológico e a integração avicultor/agroindústria, deram maior dinamismo à atividade, colocando-a em posição privilegiada em relação a outras atividades pecuárias no Brasil. O novo sistema de produção, contribuiu para o desenvolvimento da avicultura nacional, principalmente nos quesitos relacionados à biosseguridade, sanidade, qualidade dos animais e da carne de frango. A conquista do mercado externo veio com a comprovação da qualidade sanitária dos animais (SCHMIDT; SILVA, 2018; ZEN et al., 2015).

A avicultura, é o setor do agronegócio que apresenta maior expansão entre os principais setores produtivos de proteína de origem animal. O Brasil, compete no mercado mundial (SANTOS; MADUREIRA, 2019), no ano de 2019 produziu 13.245 milhões de toneladas de carne de frango, classificando o país como o terceiro maior produtor. Desta produção, 68% foram destinados ao mercado interno e 32% ao mercado externo, equivalendo a 4,2 milhões de toneladas de carne para expostação (ABPA, 2020).

Para SCHMIDT; SILVA, (2018) a posição conquistada pelo Brasil como maior exportador mundial de carne de frango se deve a uma tríade básica: *status* sanitário, custo baixo e diferenciação pela qualidade. Essa combinação de fatores confere à carne de frango brasileira qualidade superior frente à dos demais competidores.

O sistema produtivo adotado pelos produtores, para atender a demanda por proteína animal, torna os plantéis mais densos, com maior volume de aves, com isto, há maior exposição à agentes patogênicos que podem estar presentes na cama, na ração, na água, no ar, além da contaminação através do contato entre elas (ZANINELLI et al., 2018). Os animais devem apresentar boa saúde, e por sua vez, uma rápida ativação e resposta do sistema imune, haja vista, que na produção de aves, as doenças entéricas possuem alto impacto, causando perdas na produtividade (CHAVEZ; LÓPEZ; PARRA, 2016).

1.1.2 Nutrição de frangos de corte

A formulação de dietas balanceadas é de extrema importância para proporcionar máxima eficiência alimentar e melhor desempenho produtivo, visto que a nutrição é responsável pela maior parcela dos custos de produção (MARTINS; ASSUNÇÃO, 2018). A nutrição adequada dos frangos de corte depende de conhecimento técnico sobre nutrientes, energia, aminoácidos, minerais, vitaminas, ácidos graxos e água, devendo ser atendidas as exigências energéticas dos frangos de acordo com a fase produtiva.

Os principais nutrientes são: as proteínas, as gorduras, os carboidratos, os minerais, as vitaminas e a água. Proteínas são sequências de aminoácidos, dos quais, 10 são essenciais (arginina, fenilalanina, histidina, isoleucina, leucina, lisina, metionina, treonina, triptofano e valina), ou seja, as aves precisam ingerir para que suas necessidades sejam supridas, pois estes não são produzidos pelo organismo, devendo ser fornecidos em quantidades adequadas na dieta (GARCIA; GOMES, 2019).

Durante o crescimento das aves, ocorrem alterações nas exigências nutricionais, necessitando o fornecimento de diferentes rações de acordo com a fase de desenvolvimento dos frangos. Os programas nutricionais mais utilizados na produção de frangos de corte são os programas de três fases (inicial, crescimento e terminação), quatro fases (pré-inicial, inicial, crescimento e terminação) e cinco fases (pré-inicial, inicial, crescimento I, crescimento II e terminação). (CARNEIRO, 2018). No Brasil são utilizadas as recomendações nutricionais expressas nas Tabelas Brasileiras para Aves e Suínos – Composição de Alimentos e Exigências Nutricionais, elaboradas por Rostagno e colaboradores (2017).

As etapas de alimentação são divisões feitas para otimizar o uso da ração. Essas divisões são baseadas nos processos fisiológicos e metabólicos do animal, visando fornecer à ave a quantidade necessária de nutrientes em uma determinada idade e evitar desperdícios ou superalimentação (BAILEY, 2019). O conceito de nutrição de precisão é baseado na determinação das necessidades das aves, sem deficiências ou excessos nutricionais. Nutrição de precisão é uma dieta baseada na abordagem de alterar os constituintes da dieta a fim de atingir a máxima eficiência metabólica e nutricional (BAILEY, 2019).

Aditivos nutricionais vem sendo usado nas rações como um incremento ao desenvolvimento produtivo dos frangos de corte para melhorar a eficiência alimentar (ROSA et al., 2018). Aditivos alimentares sãosubstâncias, micro-organismos ou produtos formulados adicionados intencionalmente aos produtos, contendo ou não valor nutricional. Com capacidade de melhorar as características dos produtos destinados à alimentação animal e/ou dos produtos animais, influenciando positivamente na melhora do desempenho animal, conferindo benefícios à saúde intestinal (através de propriedades funcionais digestivas ou equilibradores de flora); status imunológico e eventualmente auxiliando no crescimento e saúde animal (GAGGÌA; MATTARELLI; BIAVATI, 2010a).

1.1.3 Antibióticos na avicultura

O uso de antibióticos como promotores de crescimento, é realizado em doses subterapêuticas na alimentação dos animais, a fim de inibir o crescimento de bactérias que prejudiquem a absorção de nutrientes, melhorando a conversão alimentar devido a diminuição do consumo de energia por esses micro-organismos (PANDOLFI; MOTA, 2020).

Estudos apontam que o uso de antimicrobianos como promotorores de crescimento em aves pode gerar, um problema de saúde pública, haja vista que, o consumo da carne destes animais contribui para a resistência de bactérias causadora de infecções em humanos, dificultando o tratamento (CASTANON, 2007).

A resistência a antimicrobianos causou a restrição do uso de antibióticos em diversos países, inclusive no Brasil. Várias moléculas já estão proibidas, tais quais: organoclorados, avoparcina, arsenicais, antimoniais, cloranfenicol, nitrofuranos, substâncias com efeito tireostático, androgênico, estrogênico, gestagênico e ßagonista em aves, olaquindox, carbadox,

violeta de genciana, anfenicois, tetraciclinas, B-Lactâmicos (penicilinas e cefalosporinas), quinolonas e sulfonamidas sistêmicas, Espiramicina e eritromicina, colistina (como aditivo melhorador de desempenho), 2016 Tilosina, lincomicina e tiamulina (como aditivo melhorador de desempenho) (BRASIL, 2020).

Com a informação da população sobre o uso de antibiótico na produção de carne de frango, gerou uma nova demanda de mercado por alimentos, a de “carne sem antibiótico”. Por isso, os consumidores desempenham um papel fundamental para adoção de práticas de produção com uso racional de antibióticos (PANDOLFI; MOTA, 2020).

Com este cenário, aliados ao Plano Nacional de Controle de Resíduos e Contaminantes PNCRC/ Animal que tem como principal base legal, a Instrução Normativa SDA n° 42, de 20 de dezembro de 1999, inspiram estudos afim de desenvolver produtos alternativos em substituição aos antibióticos, que sejam eficazes no controle de patógenos e no melhor desempenho zootécnico dos animais (KURITZA; WESTPHAL; SANTIN, 2014)

Entre as alternativa, destaca-se os probióticos, que de acordo com GUEVARRA et al., (2019), apresentam potencial na substituição de antimicrobianos promovendo o crescimento e melhorando a saúde animal.

1.1.4 Probióticos

Probióticos são definidos como micro-organismos vivos (bactérias ou leveduras) que quando ingeridos em concentração adequada, exercem diversos efeitos benéficos no hospedeiro. Esses grupos microbianos possuem, a capacidade de resistir a condições desfavoráveis do corpo humano (por exemplo, enzimas salivares, baixo pH e suco pancreático), colonizar células epiteliais intestinais e contribuir para a saúde do ambiente hospedeiro (AZAD et al., 2018).

Para uma bactéria ser considerada probiótica, ela deve possuir alguns requerimentos, tais como: resistência a acidez gástrica e aos sais biliares, atividade antimicrobiana contra bactérias patogênicas, capacidade de reduzir a adesão de patógenos às superfícies, habilidade de aderir as células dos hospedeiros, persistir e se multiplicar, ser seguro, não invasivo, não patogênico e ter capacidade de agregar-se a outras bactérias para formar uma microbiota balanceada (GANGULY, N. K et all., 2011; SALMINEN et al., 1998; BEN BRAÏEK et al., 2018) .

O uso de probióticos, principalmente bactérias produtoras de ácido láctico na alimentação das aves, contribui para a manutenção da integridade e estabilidade da microbiota intestinal, dificultando a multiplicação de micro-organismos prejudiciais, prevenindo o desenvolvimento de doenças e melhorando a produtividade (DÍAZ-LÓPEZ; ÁNGEL-ISAZA; ÁNGEL B., 2017). Com a modulação da microbiota intestinal, o organismo é protegido contra as infecções gastrointestinais (GAGGÌA; MATTARELLI; BIAVATI, 2010b), fazendo com que haja aproveitamento dos nutrientes da dieta, principalmente pela produção de enzimas digestvas e absorção de vitaminas do complexo B.

A cultura probiótica pode ser constituída de um único micro-organismo, ou uma combinação deles. Quando se sabe a composição da cultura esta é classificada como cultura definida, e indefinida quando não se sabe os micro-organismos (CARVALHO et al., 2018; DÍAZ-LÓPEZ; ÁNGEL-ISAZA; ÁNGEL B., 2017).

Os principais gêneros bacterianos probióticos são os *Lactobacillos, Bifidobacterium, Bacillus, Streptococcus, Pediococcus, Enterococcus* e leveduras, como *Saccharomyces cerevisiae, Saccharomyces boulardii* (RAPOSO; DEFENSOR; GRAHL, 2019; ZAGHARI et al., 2015).

Na microbiota do trato gastrointestinal é comum a presença de *Enterococcus* spp. (AUDREY et al., 2017), os quais representam um grupo alternativo com potencial probiótico. Exemplo, o isolado de *Enterococcus faecium* (E297), obtido de leite cru da região oeste de Santa Catarina, foi caracterizado como produtor de bacteriocinas, sobrevivendo em faixa de pH de 2 a 8 (testes gástricos in vitro), sendo que, ao envolver-se em uma matriz alimentar a cepa apresenta maior resistência no transito gastrointestinal; não apresentando fatores de virulência e nem resistência antimicrobiana, bem como potencial probiótico *in vitro,* tendo capacidade de sobrevivência a simulados do trato gastrointestinal (SCHITTLER et al. (2019).

Para que um probiótico seja aceito pela indústria, ele deve apresentar resultados similares ao proporcionado pelos antibióticos melhorados de desempenho (CHAVEZ; LÓPEZ; PARRA, 2016).

1.1.5 Mecanismos de ação dos probióticos

As bactérias probióticas tem a capacidade de aderir-se ás células epiteliais e superfícies

mucosas, estimulando os mecanismos imunes, concominantemente inibem a adesão de patógenos, por meio da exclusão competitiva e redução da área de interação da mucosa com microrganismos indesejáveis, formando uma barreira física contra a fixação de bactérias patogênicas, produzindo susbtâncias antimicrobianas e modulando o sistema imunológico (MELO, 2018; REFELD et al., 2020; YAZDI et al., 2019).

A suplementação da dieta dos animais com agentes microbianos baseia-se no princípio da simbiose, em que há associação de micro-organismos benéficos com o animal hospedeiro (MULLER FERNANDES et al., 2016). A adição de probióticos como suplemento alimentar melhora a modulação da resposta imune e das células imunes da mucosa intestinal (GAGGÌA; MATTARELLI; BIAVATI, 2010b; MELO, 2018).

Os probióticos podem apresentar ação imunoestimulatória aumentando as concentrações séricas de IgM, IgA ou IgG (MA; SUZUKI; GUAN, 2018; SILVA, 2016a), úteis para neutralizar micro-organismos patogênicos (KIM; LILLEHOJ, 2019). Já, LOURENÇO et al. (2013), relatam aumento na expressão de células caliciformes, CD4+ e CD8+ na mucosa intestinal das aves alimentadas com probióticos.

As cepas probióticas podem produzir substâncias antimicrobianas, como ácidos orgânicos (acético e lático), dióxido de carbono e peróxido de hidrogênio que promovem a diminuição do pH intestinal, reduzindo a colonização por micro-organismos patógenos. Além disso, as cepas são competitivas, algumas produzem bacteriocinas, peptídeos antimicrobianos com capacidade bacteriostática ou bactericida, as quais favorecem o processo de exclusão competitiva, impedindo a fixação de patógenos no lúmen intestinal (ABD EL-HACK et al., 2020; BARBIERI, 2015; HALLORAN; UNDERWOOD, 2019; HOSSAIN; SADEKUZZAMAN; HA, 2017; MELO, 2018).

A presença de bactérias probióticas também contribui para o aumento de mucinas (glicoproteínas secretadas pelas células caliceformes presentes no epitélio intestinal) (ABD EL-HACK et al., 2020), formando uma camada protetora no intestino. Para os patógenos intestinais causarem danos, eles precisam aderir-se e invadir as células epiteliais, através da penetração da camada mucosa, portante a integridade desta dificulta a invasão bacteriana e sua aderência (BARBIERI, 2015; HALLORAN; UNDERWOOD, 2019; HOSSAIN; SADEKUZZAMAN; HA, 2017; MELO, 2018).

A adição de probióticos nas dietas proporcionam modificações na permeabilidade e aumento das junções epiteliais intestinais (MELO, 2018), ocasionando um aumento na altura das vilosidades e profundidade das criptas intestinais (ABD EL-HACK et al., 2020).

1.1.6 Desempenho de frangos de corte

O desempenho de animais é avaliado através dos índices zootécnicos, que são dados produtivos, quantitativos e qualitativos, que refletem em números a produção. Algumas variáveis de desempenho são: peso inicial (g); conversão alimentar; ganho de peso diário (g); consumo de ração (g); mortalidade (%); e índice de eficiência produtiva ou fator de produção - IPE (%) (BATTISTI; FREITAS, 2018).

Como descrito pelo autor Pareja Arcila et al., (2018), os índices zootécnicos são obtidos de acordo com as fórmulas:

*Conversão alimentar: através da divisão do consumo total de ração dos animais em determinado período pelo peso médio das aves. A ração consumida pelas aves que morrem é contabilizada, sendo assim, quanto maior a mortalidade, pior a conversão alimentar.

$$\text{Conversão alimentar} = \frac{\text{Consumo total de ração}}{\text{Peso médio das aves}}$$

*Ganho de peso diário Produto da divisão do peso médio do lote pela sua idade, em dias.

$$\text{Ganho de peso médio} = \frac{\text{Peso médio do lote}}{\text{Idade}}$$

*Peso Médio (PM): Obtido dividindo-se o peso total das aves de cada parcela, pelo número médio de aves da parcela

$$\text{Peso Médio} = \frac{\text{Peso total}}{\text{N° de aves}}$$

*Consumo de ração média (CRM) Calculado pela razão entre o consumo de ração total (fornecido – sobra) e o número médio de aves;

$$\text{Consumo de ração média} = \frac{\text{Consumo total de ração}}{\text{N° de aves}}$$

*Índice de Eficiência Produtiva (IEP):

Determina a eficiência produtiva atingida durante a criação, obtido pela fórmula:

$$\text{IEP} = \frac{\text{Ganho de peso diário (KG) x Viabilidade (\%) x 100}}{\text{Conversão alimentar x Idade ao abate}}$$

*Mortalidade Expressa em porcentagem, será verificado diariamente entre as 7h e 19h, se houve morte das aves. A viabilidade (VB) será calculada pela equação: (100 – mortalidade) conforme descrito pela Agência Embrapa de Informação Tecnológica.

Do ponto de vista econômico, a conversão alimentar é o índice de maior importância na atividade avícola devido aos altos custos da alimentação.

1.2 OBJETIVOS:

1.2.1 Geral:

Avaliar se a adição do probiótico *Enterococcus faecium* E297 e o probiótico *Enterococcus faecium* SF68 em substituição ao antibiótico na alimentação de frangos de corte tem efeitos positivos sobre desempenho zootécnico e qualidade da carne.

1.2.2 Específicos:

- Avaliar se adição de E297 é capaz de substituir antibiótico mantendo desempenho zootécnico;
- Analisar se a adição de E297 influencia o sistema imune das aves e morfologia intestinal;
- Caracterizar a carne dos animais suplementados com antibióticos e probióticos;
- Caracterizar a microbiota intestinal dos frangos de corte quanto a concentração de micro-organismos.

2 CAPÍTULO II
ARTIGOS e/ou MANUSCRITO

Os resultados desta dissertação são apresentados na forma de um manuscrito, com sua formatação de acordo com as orientações da (s) revista (s) Microbial Pathogenesis ao qual (is) será (ram) submetido (s):

2.1 MANUSCRITO I

Use of probiotic *Enterococcus faecium* E297 in the diet of bearing chickens in replacement of conventional growth impropers: effects on zootechnical performance, animal health, meat quality

Mariane Ficagna[1], Maiara Marchiori[2], Vinicius Oliveira[2], Alexandra de Carvalho[3], Stéfani Mallmann[3], Tais Cecchin Nunzio[3], Gabriel Danieli[3], Valcenir Júnior Mendes Furlan [4], Roger Wagner [5], Ricardo E. Mendes [6], Marcel M. Boiago[2, 3], Aleksandro S. Da Silva[2], Liziane Shittler Moroni[1,3]

[1.] Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Universidade do Estado de Santa Catarina (UDESC), Pinhalzinho, SC, Brasil.

[2.] Departamento de Zootecnia, UDESC, Chapecó, SC, Brasil.

[3.] Departamento de Engenharia de Alimentos e Engenharia Química, UDESC, Pinhalzinho, SC, Brasil.

[4.] Curso de Ciência e Tecnologia de Alimentos, Universidade Federal do Pampa (UNIPAMPA), Campus Itaqui, Brasil.

[5.] Departamento de Ciencia de Alimentos, Universidade Federal de Santa Maria, Santa Maria, Brasil.

[6.] Instituto Federal Catarinense, campus Concórdia, Brasil.

Autor coresspondencia: L.S. Moroni (lizianeschittler@gmail.com)

ABSTRACT

Antibiotics as growth promoters in the broiler diet are a strategy used to improve zootechnical performance. The banishment trend on antimicrobials spurred industries the develop alternative forms of performance-enhancing additives. The aim of this study was to evaluate the effect of the probiotic *Enterococcus faecium* E297 on the intestinal microbiota, zootechnical performance and meat quality of broiler chickens. For this, 224 one-day-old males chicks Cobb 500 were used and randomly distributed in 16 pens for evaluation of four treatments, with four replications for 42 days. Treatments: T1 - positive control (basal diet with enramycin (Enradin F80), 10mg / Kg in the basal diet); T2 - negative control (basal diet); T3 – basic diet with probiotic E.297, (10^9 CFU/g); T4 – base diet with commercial probiotic (Cylactin, *E. faecium* strain called SF68) (2.0 x 10^9 CFU / g). Zootechnical performance, immunology, biochemistry, and serum histopathology were analyzed in broiler chickens. Characterization of meat and intestinal microbiota were also performed. The use of probiotic in the diet influenced ($P>0.05$) zootechnical performance in the evaluated periods. In the leucogram, an effect of the treatments on the counts of leukocytes, lymphocytes, heterophylls and monocytes was observed ($P<0.01$). Serum globulin and triglyceride levels were significantly higher in the serum of broilers fed diet supplemented with performance enhancer antibioticthan in groups supplemented with probiotic. The lowest serum cholesterol level was observed in the negative control ($P<0.001$). Total protein, albumin, uric acid, and glucose showed no significant difference between treatments ($P>0.05$). The use of probiotic influenced the intestinal morphometric measurements, the villus/crypt ratio was better in broilers fed basal diet supplemented with probiotic E297 and negative control ($P <0.001$). Regarding meat composition, there were significant differences in total fat, protein, pH, and water activity. The effect of treatments on the fatty acid composition of meat was also observed. Regarding the microbial count in the feces of broiler chickens, there were no significant differences in the counts of enterobacteria, lactic acid bacteria (BAL's), mesophiles and *E. coli* between treatments at 42 days of the experiment. For microbiological counts in the poultry bed, no significant differences were observed between treatments for counting of BAL's, but for enterobacteria, significant differences were found in the counts between treatments and between the times evaluated. In the microbiological count of the cloacal swab, no significant difference was found between treatments for the counting of BALs; only one difference between the times

evaluated for the performance enhancer antibiotic group. For the count of enterobacteria in the cloacal smear, a significant difference was found between treatments only at time 7. Significant differences between treatment times were presented for the performance enhancer antibioticgroup at 7 and 21 days. The inclusion of the probiotic *Enterococcus faecium* E297 in broiler chicken feed proved to be a potential replacement for conventional performance enhancer antibiotic, contributing to zootechnical indices, stimulating the immune system and positively modulating the villus/intestinal crypt ratio.

Keywords: Animal Production. Performance enhancer. Poultry. Probiotics. Zootechnical Performance.

Introduction

Antimicrobial resistance has resulted in many restrictions on its use in several countries, the use of antimicrobials as performance enhancers is a public health issue, considering that the consumption of meat from animals that use antibiotics as performance enhancers contributes to resistance of bacteria causing infections in humans, making treatment difficult. [1]. The production system adopted by producers, to meet the higt demand for animal protein, makes the creation denser, with a greater volume of birds, with this, there is greater exposure to pathogens that may be present in bedding, feed, water, air, in addition to contamination through contact between them [2].

The use of probiotic microorganisms, then, emerges as an alternative form of zootechnical enhancer, especially lactic acid-producing bacteria, as it contributes to the maintenance of the integrity and stability of the intestinal microbiota, hindering the multiplication of harmful microorganisms, diseases and better productive yield [3]. Through a modulation of the intestinal microbiota, protecting the body from gastrointestinal infections [4], ensuring health and the use of nutrients in the diet, mainly through digestive enzymes and B vitamins. In the gastrointestinal tract microbiota, the presence of Enterococcus spp. [5], which represent an alternative group with probiotic potential. For example, the isolate of Enterococcus faecium (E297), obtained from raw milk in a study carried out by [6], was characterized as a bacteriocin producer, surviving in a pH range from 2 to 8 (in vitro gastric tests). when involved in a food matrix, the strain presents greater resistance in the gastrointestinal transit; not showing virulence

factors or antimicrobial resistance, as well as probiotic potential in vitro, having the ability to survive in simulated gastrointestinal tract.

According to the literature, the inclusion of microorganisms as probiotics in the diet of broiler chickens proved to be a viable and effective alternative as an alternative source to the use of antibiotics as growth promoters, modulating the microbiota and intestinal architecture, thus producing yields consistent zootechnics [7–9]. Therefore, the objectives of this study were to evaluate the effect of the probiotic *Enterococcus faecium* E297 on the intestinal microbiota as well as the zootechnical performance in broilers and meat quality.

Material e methods

Probiotics

Enterococcus faecium E297 belongs to the bacterial culture collection in Food Microbiology Laboratory (Universidade do Estado de Santa Catarina, Pinhalzinho, SC, Brazil). This isolated was obtained from fresh milk from the west region of Santa Catarina, identified as *E. faecium* based on partial sequencing of 16S rRNA [6]. The isolate was stored at -80 °C in de Man Rogosa and Sharpe (MRS) broth (Oxoid Ltd., Basingstoke, England) supplemented with glycerol at 25% (v/v).

The commercial probiotic called 'Cylactin', composed of *Enterococcus faecium* (SF68) from the company DSM Nutrition and Health Animal. Before the experiments, cultures of *E. faecium* (E297) and commercial culture Cylactin were transferred to MRS (broth or agar) and incubated overnight at 36 ºC.

Ethical committe

This research was carried out in an experimental shed, after approval by the Ethics Committee for the Use of Animals of the Universidade do Estado de Santa Catarina, CEUA nº 1450170920.

Animals and experimental desing

In the experiments, animals were used males (Coob 500), donated by a company in the city of Chapecó, SC, Brazil. Upon arriving at the experimental shed of the Department in Zootechnics, UDESC Oeste Chapecó, the broilers were weighed and randomly distributed in 16 boxes with aviary litter already used, four treatments, four repetitions and 14 birds per repetition: T1 - positive control, plus antibiotic Enramycin (Enradin F80), 10mg/Kg in the basal diet; T2 - negative control (basal ration, no enramycin and no probiotic); T3 - addition of probiotic E297, *E faecium*, 2.5 ml/kg of feed, (10^9 CFU /g) in the basal diet; T4 - addition of probiotic Cylactin, strain of *E. faecium* called SF68, 15mg/Kg of feed (2.0 x 10^9 CFU/g). All groups received the same basal diet; what has changed is the presence or absence of antibiotics and probiotics, being were fed in tubular feeders. The trial period lasted 41 days.

Feed preparation

The E297 was cultivaded in MRS broth at 36 °C by 18 h, the samples were centrifuged, and cells washed in autoclaved distilled water. One kilo of ground corn was sprayed with 2.5 ml of probiotic E297 and mixed with the other feed ingredients using the automatic horizontal mixer (Perozin MH-150), obtaining a concentration of 10^9 CFU / g of feed. For probiotic SF68 culture, the manufacturer's recommendation was used, 15mg / kg of feed (2.0 x 10^9 cfu / g).

All feed (Table 1) were formulated according to the composition of the ingredients and nutritional requirements established in the Brazilian tables for poultry and swine [10]. All formulated feeds were stored in identified buckets and kept at room temperature.

Zootechnical performance

The broilers and left-over feed were weighed on days 1, 7, 21, 35 and 41 of the experiment; this allowed calculation of weight gain (WG), feed intake (FI) (g/bird/day) and feed conversion (FC). Feed intake (FI) (g/bird/day) was calculated as the difference between feed provided at the beginning and the heavy leftovers at the end of each period. Feed conversion (FC) was calculated as the total amount of feed ingested divided by the live weight of the broilers.

Sample collection

Blood samples from six broilers per treatment were collected on day 41. The broilers were manually restrained, and blood was collected from the ulnar veins using insulin syringes. Blood samples were deposited in tubes, one with EDTA (1,5mg/ml sangue), and the other not containing anticoagulant to obtain serum. Blood collected without anticoagulant was centrifuged at 3500 rpm for 10 minutes and the serum was frozen for subsequent biochemical analyses.

At 42 days, one broiler per repetition (four per treatment) were euthanized, according to animal welfare standards and euthanasia. Gut fragments were collected for histology, and the pectoral muscle was analyzed for proteins, pH, and water activity, as well as for fatty acid profile.

Histomorphometry

Intestinal (jejunal) samples were collected and stored in flasks containing 10% formaldehyde. Slides with histological cuts were made and stained with H&E. The villus height and crypt depth were determined according to the methodology described by Caruso and Demonte (2005)[11]. Histological images of the slides were captured using a Digital Electronic Eyepiece Camera Video microcamera, coupled to a biological trinocular microscope model TNB-41T-PL of the OPTON brand and a specific program for capturing histological images. Villus length was determined using a straight line from the tip of the villi to the upper portion of the crypts. To determine crypt depth, another line was drawn from the base of the crypt to its upper portion using ImageJ.

Leucogram

At the time of blood collection, blood smears were made, stained with a Panótipo Rápido to perform differential leukocyte counting using a 1000 × magnification light microscope. The leukocyte count was performed using a Neubauer chamber, according to the method of Thrall et al. (2015) [40]. The results were expressed as numbers of cells/µL.

Serum biochemistries

Biochemical variables total protein, albumin, triglyceride, cholesterol, glucose, and uric acid were measured in the serum, using commercial analytical kits (Analisa®) and a semi-automatic biochemical analyzer BioPlus (Bio-2000®). Globulin values were calculated using the following mathematical formula: globulin = total proteins – albumin.

Total lipids content and fatty acid (FA) profile

Total lipids content and fatty acids profiles were analyzed in chicken and feed samples. Bligh and Dyer (1959)[41] method was used to lipid extraction with some modifications: 4 g of samples, 3.6 mL of water, 16 mL of methanol, and 8 mL of chloroform were added into 50 mL polypropylene tubes, and mechanical shaking was performed for 60 min. After that, 8 mL of chloroform and 8 mL of Na_2SO_4 1.5% solution were added to promote a biphasic system. This mixture was shaken for 2 min, and after centrifugation (5 min at 1200 × g) chloroform phase containing lipids was separated to total lipids analysis by gravimetry and for fatty acid analysis after solvent drying under N_2 flow. FA methylation from the lipid fraction was performed using a transesterification/ esterification method proposed by Hartman and Lago (1973)[43]. Briefly, approximately 40 mg of lipids were added 1 mL of 0.4 M KOH methanolic solution in a test tube and shaken in a vortex for 1 min, and then samples were kept in a water bath for 10 min at boiling point. Subsequently, samples were cooled at room temperature, and 3 mL of 1 M H_2SO_4 methanolic solution was added and shaken in vortex and maintained in a water bath for 10 min. After cooling, 2 mL of iso-octane were added and centrifuged at 3652 × g for 5 min. Finally, iso-octane extract containing fatty acid methyl esters (FAME) was subjected to chromatography analysis.

FAME determination was carried out in a gas chromatograph model 3400CX equipped with a flame ionization detector (Varian, Palo Alto, CA). One microliter of samples was injected in a split/splitless injector, operated in split (1:25) mode at 250 °C. Hydrogen was used as carrier gas at a constant pressure of 25 psi. FAMEs separation was carried out using an HP-88 chromatography column (100 m × 0.25 mm; 0.20 μm film, Agilent, J & W, Folsom, CA, USA). The initial oven temperature was programmed at 50 °C for 1 min and increase to 185 °C, at 15 °C/min. Then, increasing to 195 °C, at a rate of 0.5 °C/min, and finally until 230 °C, increasing 5 °C/min, and maintained for 6 min in isothermal conditions. The detector temperature was kept constant at 250 °C. The FAME compounds were identified by comparing experimental retention

times with those from authentic standards (FAME Mix-37, Sigma Aldrich, St. Louis, MO). The results were presented as a percentage of each FA identified in the lipid fraction, considering the chain size equivalent response factor and a conversion factor of the ester to the respective acid for each FAME applied to FID, according to Visentainer (2012)[42]. Results fatty acid in feed was added in Suplementary Material 1.

Measurement of pH value, water activity, and protein of broiler meat

To measure the pH value, the mPA-210 benchtop pHmeter was used, where samples of 10 grams of chicken breast slaughtered at 42 days were added to 100ml of distilled water and homogenized for reading. Each sample was measured in duplicate and the mean value was used for an analysis statistic. The measurement of water activity was performed with the AquaLab Pre Water Activity Analyzer device.

Protein analysis was performed by outsourced laboratory (LANAL Physical-Chemical - Laboratory of Food Analysis, FIESC - SENAI Chapecó-SC), using the ISO 1871: 2009 methodology.

Microbiological counts

At times 0, 7, 14, 21, 28, 35 and 42 days, the ration was collected to perform the lactic acid bacteria count (BALs). From the aviary beds were collected at times 0, 21, 35 and 42 days and the counting of BALs and enterobacteria was performed. Samples were collected from the cloaca of the animals using a swab moistened in peptone water 0,1% on the 7, 21 and 35 days to perform the count of enterobacteria. At 42 days, cecum feces were collected to count enterobacteria, BALs, mesophiles and *E. coli.*

The samples were diluted in peptone water and inoculated in MRS agar, PCA agar, VRB agar and EMB agar for counting BAL, mesophiles, enterobacteria and *E.coli*. Plates were incubated at 37 °C for 48 hours (BAL, mesophiles) and 24 hours (enterobacteria and *E.coli*). Counts were performed and results were expressed in colony forming units per mL (CFU / mL) [12].

Statistical analysis

The data were evaluated firstly by descriptive statistics for contingency of information and for further assumptions. They were showed as descriptive using mean and standard deviation. The data were tested for normality using the Shapiro-Wilk test and the skewness, kurtosis and homogeneity by the Levene test. A one-way ANOVA was used to analyze all parameters that showed difference comparing groups at each time period (days 21, 35 and 42); and body weight (on days 1, 7, 21, 35 and 42 of age) was analyzed using a post hoc test Tukey's test. It was considered significantly different when $P<0.05$. The statistical process was performed using R-language, v.2.15.1.

Results

Zootechnical performance

Results performance were showed um Table 2, during the first and 7 days of age a higher there was a dietary intake in the broilers was observed in treatments with probiotics than in treatment with enramycin ($P <0.001$). However, there was no difference in weight gain and feed conversion of the animals between treatments. From 1 to 21 days of age of the experiment, in the treatment in which the broilers were fed diets supplemented probiotic SF68 the body weight was higher than in the treatment on what diet supplemented with enramycin and negative control ($P <0.001$). However, there was no significant difference in the body weight gain of the animals fed with ration supplemented with the probiotic E297 when compared to the negative control and the other treatments, basal diet supplemented enramycin and basal diet supplemented probiotic SF68 ($P<0.001$). At 21 days, it was observed that there was better feed conversion of chickens in treatments with probiotic E297, negative control and enramycin ($P <0.028$), when compared to treatment with probiotic SF68.

At 35 days of age, the difference in body weight was presented in relation to treatment with probiotic SF68 and in the negative control ($P <0.001$) where animals supplemented with probiotic SF68 showed greater weight gain when compared to the negative control. Animals supplemented with probiotic SF68 had higher feed intake, compared to other treatments (P

<0.001), whereas the best feed conversion was in the negative control (P <0.016), compared to treatments with probiotic SF68 and enramycin, howeve, in relation to the treatment with probiotic E297, there was no significant difference. From 1 to 41 days of age of the experiment, we recorded higher body weights in treatments with probiotic E297 and probiotic SF68 than in treatments with enramycin and negative control (P <0.001). The best feed conversion was in the negative control (P <0.001), compared to treatment with the enramycin and probiotic SF68, however, there was no significant difference when compared to the basal diet supplemented with probiotic E297.

Leukogram

Results of leukogram was shoewd in Table 3, at 42 days, total leukocytes number in the blood of the broilers were significantly higher in the group treated with enramycin than in the negative control and the group treated with probiotic SF68 (P <0.001), but there was no difference total leukocytes number in the blood of the broilers in relation to the group in which the basal diet was supplemented with probiotic E297. Numbers of lymphocytes was significantly higher in the broilers fed in the basal diet supplemented with enramycin when compared to the negative control, but there was no significant difference when compared to the basal diet supplemented with probiotic SF68 and basal diet supplemented with probiotic E297. The broilers fed the basal diet supplemented with probiotic SF68, probiotic E297 and negative control showed no significant difference between them (p<0.001). The number of heterophiles was higher in the groups treated with enramycin and probiotic E297 when compared to the negative control (P <0.001). The monocytes were significantly higher in the groups in treatment whith enramycin and probiotic SF68 (P <0.001) compared to the negative control. There were no significant differences between treatment in terms of basophil number and eosinophil number.

Biochemistry in serum

The results of the serum biochemistry are presented in Table 4, levels of serum globulin and triglyceride were significantly higher in the serum of broilers fed wiht feed supplemented with enramycin than in animals fed wiht feed supplemented with probiotic E297 and probiotic

SF68. The lowest serum cholesterol level was observed in the negative control, when compared to diets supplemented with enramycin, probiotic E297 and probiotic SF68 ($P < 0.001$). Total protein, albumin, uric acid, and glucose showed no significant difference between treatments ($P > 0.05$).

Histomorphometry

The results of the histomorphometry (villo, crypt and relation villo/crypt of jejunum of broilers) are presented in Table 5, the intestinal villo of broilers was significantly higher in the group fed with feed basal negative control, than the groups of animals that in the diet presented supplementation with enramycin and probiotic E297 ($P < 0.001$) but was no significant difference when compared the intestinal villo of broilers fed probioc SF68. In relation to intestinal crypts of broilers, the size of the crypts was significantly smaller in animals fed a basal diet supplemented with probiotic E297 ($P < 0.001$) compared to the negative control, enramycin and probiotic SF68 treatments. The villus/crypt ratio was better in chickens fed a basal diet supplemented with probiotic E297 and negative control ($P < 0.001$), compared to treatments with enramycin and probiotic SF68.

Measurement of pH value, water activity, and protein in the broiler meat

The measurement results of pH value, water activity, total fat and protein are shown in table 6, there were significant differences in total fat between the groups: values were significantly lower in the groups supplemented with probiotic E297 and probiotic SF68 group than in the groups supplemented with enramycin and negative control. Protein levels were significantly higher in the E297 probiotics supplemented groups, SF68 probiotic group and negative control group compared to the enramycingroup. The same is verified for the pH reading, where the E297 probiotic, SF68 probiotic and negative control groups presented values significantly higher than the group supplemented with enramycin. The groups supplemented with probiotic E297 and probiotic SF68 had significantly higher water activity than the enramycin and negative control groups.

Fatty acids in animal feed and meat

There were no significant differences in terms of percentage of total fat in the ration between treatments (Supplementary material 1). In meat, fatty acid profiles are displayed in Table 7. There was a significant difference for fat levels in meat. The total saturated fatty acids in meat were lower in the group supplemented with enramycinwhen compared to the group treated with probiotic E297. The opposite occurred with respect to total monounsaturated fatty acids; that is, they were lower in the groups treated with E297 probiotic and SF68 probiotic, when compared to the negative control group and the group supplemented with enramycin. On the other hand, the levels of polyunsaturated fatty acids are also lower in the group supplemented with probiotic E297, as well as in the negative control, when compared to the group supplemented with probiotic SF68, which has higher levels, but did not differ earlier when compared to the group supplemented with enramycin. There were significantly lower levels of pentadecanoic acid in the meat of the group supplemented with probiotic E297 when compared to the other groups (enramycin, negative control and probiotic SF68). The NI levels in meat in the enramycin and negative control groups were significantly lower than in the groups supplemented with probiotic E297 and probiotic SF68. At trans isomer levels were significantly lower in the groups treated with probiotic E297 and probiotic SF68, when compared to the enramycin and negative control groups. The γ-linolenic acid and arachidic acid levels were significantly lower in the Cylactin-supplemented group, followed by the probiotic-supplemented group, with the highest levels of this fatty acid found in meat from the enramycinand negative-control groups. For nervonic acid levels, the significantly lower concentration was in the negative control group, followed by the groups treated with probiotic E297 and probiotic SF68, with the highest levels of this fatty acid found in the enramycin group. The cis-4,7,10,13,16,19- docosahexaenoic acid showed significantly lower levels in the group supplemented with probiotic SF68, when compared to groups supplemented with probiotic E297, enramycin and negative control.

Microbiological counts

BAL count in broiler feed

The BAL counts in the feed used to feed the chickens during the 42 days can be seen in Table 8. Statistically, the animals that were fed the feed supplemented with probiotics E297 and SF68 had the highest counts than in the groups supplemented with the enramycin and negative control. It is also possible to verify that there are significant differences in the BAL counts in each treatment at different times. In the enramycin group, the highest BAL counts were verified at times 21 (4.57 log CFU/g) and 28 (4.54 log CFU/g) and the lowest count was verified at 42 (3.25 log CFU g-1) days. In the negative control group, the highest count of BALs (5.72 log CFU/g) was at 0 days and the lowest count (2.07 log CFU/g) at 28 days. For the diet supplemented with probiotic E297, the highest BAL count (15.29 log CFU/g) was verified at 0 days and the lowest BAL count (9.08 log CFU/g) at 14 days. As for the diet supplemented with probiotic SF68, the highest BAL (14.28 log CFU/g) count was at 7 days and the lowest BAL count (6.04 log CFU/g) was verified at 42 days of treatment.

Counting of Enterobacteria, BALs, Total Mesophiles and E. coli in broiler feces

There were no significant differences in the microbiological counts of enterobacteria, BAL's, mesophiles and *E. coli* in the feces of 42-day-old chickens fed with probiotic, enramycin and negative control. (Table 9).

BAL and Enterobacteria counts in aviary litter

The counts of Enterobacteria and BALs in the aviary litter are shown in table 10, as can be seen in the table, the BAL counts varied from 7.17 log CFU/g and 9.01 log CFU/g. There was no significant difference ($p>0.05$) between treatments for the counting of BALs in the aviary litter. In the aviary litter of negative control group, staple diet, BAL counts were significantly lower (7.23 log CFU/g) at 41 days than at time zero (9.54 log CFU/g). The counts of Enterobacteria in the aviary beds varied between 2.74 log CFU/g and 6.13 log CFU/g. For the count of enterobacteria at times 0 and 21, we obtained significantly higher values ($p<0,05$) in the negative control group, animals fed with basal diet than in the groups fed with enramycin, probiotic E297 and probiotic SF68. At 35 days, there was no significant difference in enterobacteria counts between treatments (enramycin, negative control, probiotic E297 and

probiotic SF68). At 41 days it was possible to verify a higher count of enterobacteria in the litter where the group of broilers fed with probiotic E297 were housed than in the negative control group, basic diet. However, there were no significant differences (p>0,05) when comparing the counts obtained from enterobacteria in the aviary litter where the groups fed with probiotic Sf68 and with the enramycin were used. Between the times, it was also possible to observe significant differences (p>0,05) in the counts of enterobacteria in chicken litter. For the group of animals fed with enramycin, there was a significant difference between times 0 and 21, where on day zero (0) there was a lower count of enterobacteria when compared to 21 days. There was no difference (p>0,05) between the times 35 and 41 days in the counts of enterobacteria in the aviary litter in the enramycin group. In the negative control group, animals fed the staple diet, the lowest counts of enterobacteria were obtained at 35 and 41 days, differing significantly from day 0, and these from day 21. In the group in which animals received in the diet, supplemented with probiotic E297, the lowest counts of enterobacteria were obtained at times zero (2.74 log CFU/g) and 35 days (3.24 log CFU/g), differing significantly from the values obtained at 21 (4.86 log CFU/g) and 41 (4.69 log CFU/g) days. The group supplemented with probiotic SF68 had a lower microbiological count for enterobacteria on day 0 (2.77 log CFU/g), however, this did not differ significantly (p>0,05) from the day 35. The highest count of enterobactéria in probiotic SF68 group was at 21 dias (4.83 log CFU/g).

Count of BALs and Enterobacteria in cloacal swab

The BAL counts of the cloacal swab varied between 5.50 log CFU/g and 7.89 log CFU/g. There was no significant difference in BAL counts between treatments: enramycin, negative control, probiotic E297 and probiotic SF68. In the broiler group with diet supplemented with enramycin, there were differences in the cloacal BAL counts between times 7(7.63 log CFU/g) and 35 days (5.92 log CFU/g) (Table 11). The cloacal swab enterobacteria counts ranged from 6.14 log CFU/g and 8.40 log CFU/g. In the counts of enterobacteria in the chicken cloacal swab, there was a significant difference (p<0,05) only at time 7, between treatment probiotic SF68 when compared to the probiotic E297 and negative control. Where, the group fed with probiotic E297 (6.14 log CFU/g) e negative control group (6.18 log CFU/g) had the lowest counts of enterobacteria compared to the probiotic group SF68 (6.42 log CFU/g) and enramycin (6.30 log

CFU/g). In the group of chickens with diet supplemented with enramycin the lowest counts were obtained with significant difference ($p<0,05$) of enterobacteria at times 7 and 21 compared to 35 days. The group supplemented with probiotic E297 had a lower count of enterobacteria the at 7 days, when compared to 21 and 35 days. In the group supplemented with probiotic SF68, the lowest count of enterobacteria was performed the at 7 days, compared to 21 days, and these were lower than at 35 days.

Discussoin

It is possible to verify that during total productive cycle the weight gain of broilers supplemented with probiotics (SF68 and E297) showed significantly better results than that of broilers fed with enramycin supplemented feed and negative control group. However, the best feed conversion was observed in chickens fed with feed supplemented with E297, and negative control. HE et al. (2019)[13] in your study verified in general (days 1 to 42 days) that broilers fed with probiotic (*Bacillus subtilis, Bacillus licheniformis, and Saccharomyces cerevisiae*) showed average daily gain and feed conversion better than broilers fed only with basal diet, and average gain better daily compared to broilers supplemented with antibiotics as growth promoters. LACERDA (2020)[14] can observe that there was no significant difference in weight gain and feed conversion in broilers supplemented with probiotics compared to broilers supplemented with antibiotic. ARAUJO et al. (2019)[15] found that the studied performance variables (weight gain, feed consumption and feed conversion) had only antibiotic effect, that is, the broilers fed with antibiotic-supplemented feed showed better zootechnical performance.

This increase in weight gain and feed conversion may be related to the beneficial effects of probiotics on enramycin and intestinal morphology. ABD EL-HACK et al. (2020)[16] reports that the increase in the height of the villi and the short depth of the crypts in the gastrointestinal tract allows for better digestion and absorption of nutrients, as observed in our study, where we found that the villus height/crypt depth ratio was better in chickens fed with diet baseline supplemented with probiotic E297 and negative control. The function of the intestinal crypts is to multiply new enterocytes, that is, to replace tissue in the villi, the greater the height of the villi, the greater the capacity to absorb nutrients due to the greater number of enterocytes present (each enterocyte is an absorptive unit). The low height of villi indicates intestinal sanitary or pathogenic challenges, as it suggests that the intestine is losing enterocytes at the apex of the villi and the

crypts are not able to produce new enterocytes to renew and restore the desquamation balance. The crypt like any organ, the more it requires, the more it will increase in size (hypertrophy), due to the increased activity. As it is an invagination, it grows downwards (sinks), therefore, the greater the depth, the greater the crypt size and the greater need for enterocyte production. Intestinal morphological measurements, such as increased villus height and short crypt depth, indicate an increase in nutrient absorption by increasing the surface area available for nutrient absorption [17]. In a study of ALAGAWANY et al. (2018)[18], reported that animals supplemented with probiotic containing *L. casei, L. acidophilus, Bifidobacterium thermophilum and Enterococcus faecium*, also showed an increase in the height of the jejunal villi and a decrease in the depth of the crypt.

Research carried out “in vitro”, animal and human models show that probiotics can stimulate both non-specific and specific immune responses [19]. The use of probiotics has been recommended for improving the immunity of broilers and the characteristics of the blood in the short term, the increase in the concentration of leukocytes and lymphocytes acts as effector cells in the mediation of inflammatory processes [12]. In the present study, we can see that the number of leukocytes and lymphocytes was influenced by the diet. Where, the group treated with enramycin showed higher levels of leukocytes in relation to the negative control and the group treated with probiotic SF68, but there was no difference in relation to the group in which the basal diet was supplemented with probiotic E297. For lymphocytes, basaldiets supplemented with enramycin and probiotics SF68 and E297 showed significantly higher values. SILVA (2016)[21] states that lymphocytes are entirely influenced by the modulation of the microbiota, with probiotics being effective on this modulation, lymphocytes are shown as good markers for assessing the immunocompetence of broilers. MELO (2018)[12] in his study did not find such a benefit on broilers treated with microorganisms such as probiotics or antibiotics, but they were also not harmed by the addition of additives. In a study conducted with quails, MIRZA (2020)[22] found that supplementation of animals with commercial *Lactobacillus* animalis and multiprobiotics, positively influenced hematological parameters, significantly increasing the leukocyte and lymphocyte count compared to the control treatment.

The present study revealed relatively lower triglycerides in the broiler serum with the supplementation of the diet with probiotics E297 compared to broilers in which the feed was supplemented with enramycin. There was no significant difference when compared to the groups

in which the feed was supplemented with probiotic SF68 and the negative control group. However, in the present study, there was no drop in cholesterol in the groups treated with probiotics and antibiotics, the lowest value being found in the negative control group. Corroborating with DEV et al. (2020)[23] in relation to triglycerides, but disagreeing in relation to cholesterol, which found in their study that broilers supplemented with probiotic + symbiotic had lower triglycerides and cholesterol than the group supplemented with antibiotics and the negative control group. SHAH et al. (2021)[24] also observed a small depletion in the total cholesterol levels, although there were no significant changes, in relation to triglycerides in the groups supplemented with commercial probiotic and mixture of probiotics in the study, the values were slightly lower than in the group supplemented with antibiotic. Another researcher who detected reductions in serum triglyceride and cholesterol levels in broilers supplemented with probiotics compared to broilers supplemented with antibiotics was NOSRATI et al., (2017)[25]. This reduction in triglyceride levels can be explained by the activation of the immune system, caused by the administration of probiotic strains. An activated immune system, in terms of performance, is not good, because the activation of immune mechanisms requires time and energy, mobilizing energy reserves, impairing animal performance and feed conversion.

Studies show positive impacts of probiotic supplementation in the diet of broilers on the attributes of meat quality, in the chemical composition, the main characteristics affected by the probiotic are presented in the protein and fat content of the meat [26]. In the present study, it was possible to verify that animals supplemented with probiotics had increased protein levels, while the fat content was reduced when compared to the group supplemented with enramycin. Even in the present study, when we compared the negative control group, we noticed that the difference was only presented in the reduction of fat content, where the probiotic groups had lower rates. Regarding protein levels, no difference was found between the groups supplemented with probiotics and the negative control. Corroborating the study by XUE et al., (2019)[27] who noted that there was no difference in the protein contents of animals supplemented with probiotic and negative control, but disagreeing in relation to the fat contents, since there was no significant difference in their study. STĘCZNY; KOKOSZYNSKI, (2019)[28] in a study carried out with Ross 308 broilers, observed higher protein content in animals supplemented with probiotics, when compared to the control group, as well as lower fat content in the groups supplemented with probiotics in relation to the control group. Another author who observed higher protein

content and lower fat in the breast meat of broilers supplemented with probiotics in the diet was ABDURRAHMAN et al. (2016)[29].

The fatty acid composition of meat is one of the most important indicators of meat quality in relation to consumer health. The increase of polyunsaturated fatty acids in meat is desirable by consumers, however, its greater susceptibility to lipid oxidation causes deterioration in terms of flavor, color, texture and nutritional value [30]. Regarding the fatty acid composition, there is little research investigating the influence of probiotics in the diet of broilers on the fatty acid profile of meat [26,31]. Most authors present results that are divergent from those found in this work. Some authors report a decrease in saturated fatty acids and an increase in unsaturated fatty acids, such as SHIRANI et al., (2019)[31] who found in a study that supplementing chicken feed with a probiotic decreased the content of saturated fatty acids (SFA) and increased the amount of unsaturated fatty acids (monounsaturated (MUFA) and polyunsaturated (PUFA)). HUSSEIN; SELIM, (2018)[32] found no differences in total saturated and monounsaturated fatty acids between the treatments tested, but reported an increase in polyunsaturated fatty acids in chicken meat supplemented with probiotics. One of the reasons attributed to the modification of the fatty acid profile by these researchers is due to the positive effect of probiotics on the intestinal microflora, the change in lipid metabolism, the probiotic strain used and the concentration administered. Several other factors can alter the fatty acid profile, such as genetics, breeding system, sex, and slaughter age of birds. This interaction of probiotics added to broiler feed on the fatty acid profile is not clear in current studies requiring further investigation to determine the possible mechanism of action.

The pH, on the other hand, is one of the most important factors in the transformation of muscle into meat and has a decisive effect on the quality of fresh meat and meat products. A live muscle has mean pH values around 7.2. After slaughter, the meat continues in a biochemical process, leading to a drop in the pH of the chicken meat due to acid formation, and the breast meat must have a final pH between 5.7 and 5.9 [33]. In the present study, the mean pH values of meat in the groups were: group fed with enramycin 5.86; negative control group 5.98; group fed with probiotic E297 5.98; group fed with probiotic SF68 5.97. Corroborating with the finding of CRAMER et al., (2018) that when evaluating the pH of the meat of chickens fed with probiotics, they found a significant interaction, where the supplementation of probiotics increased the final

pH of chicken breasts ($P < 0.05$). Just like ABOU-KASSEM et al., (2021)[35] who evaluated the pH of quail meat and found an increase in groups treated with dietary probiotic.

Regarding faecal microbiological counts, our results do not show significant differences (Table 10), corroborating with MELO, (2018)[12] who did not observe a significant effect ($P> 0.05$) of supplementation of probiotics and enramycin in the diet of chickens on count of total mesophiles and faecal coliforms present in bedding. For the count of microorganisms in the aviary litter, there was only a significant difference at 0 and 21 days, where the count of enterobacteria in the chicken litter was lower in the groups supplemented with probiotic E297 and probiotic SF68, when compared to the enramycingroups and negative control, this can be explained by the antimicrobial action of probiotic bacteria; by producing acetic and lactic acid, carbon dioxide, hydrogen peroxide, diacetyl, and bacteriocins, they reduce the colonization of intestinal pathogenic bacteria, so there is less elimination of these bacteria in the faeces into litter [12,36]. Authors report that BAL's in broiler diets reduce the pH and moisture of excreta [37], leading to a less favorable environment for the proliferation of pathogenic microorganisms. The high concentration of organic matter and nutrients, in addition to the population density of broilers, gives the aviary bed features favorable to the maintenance and development of a diversified microbial population [12]. Authors report an increase in BALs and a decrease in coliforms and *E. coli* in faecal and poultry litter counts in chickens supplemented with probiotics [35,38]. The stimulation of the mucosal immune system produces antibodies (IgA) that block receptors and reduce the number of pathogenic bacteria in the intestinal lumen and consequently in the external environment, since the escape of microorganisms contained in the digestive tract of birds to the bed contributes to the characteristics of the poultry bed [8,9].

The effectiveness of a probiotic depends on several factors such as microbiais species composition, dose administered, mode and frequency of administration, diet composition and environmental stressors. In a study by ALMEIDA PAZ et al., (2019)[39], when comparing the effectiveness of a commercial probiotic at different dosages (half recommended dose, recommended dose and double recommended dose), it was found that chickens fed a diet containing probiotic in the dose recommended by manufacturer, better results in weight gain, feed consumption, feed conversion and production efficiency, when compared to treatments with half dose of probiotic and double dose of probiotic. In our study, we noticed that the smallest weight gain of animals fed with probiotic E297 occurred at 21 days. When compared with the

microbiological counts of BAL in the chicken feed, we can see that in this same period the greatest decline in the counts of the probiotic E297 in the feed was observed, with levels of 1.20x10E9 ufg / g in the feed being found. Corroborating the findings of other authors, who state that the best zootechnical indices are found in animals that are offered a recommended dose of probiotic in the feed, in this case based on the indicated dose of the commercial probiotic SF68, which is also a strain of *Enterococcus faecium* and has its guarantee level in the supply of 2x10E10 cfu / g. This sharp decline in the count of probiotic E297 was due to the form of administration, where the strains did not go through any technological process, as the cells were only diluted in sterilized water and applied directly to the feed.

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Tables

Table 1: Ingredients and basal diet used for all experimental groups.

Ingredients (%)	Age (days)		
	1 – 21	22 - 35	36 – 45
Corn	55.15	58.00	62.10
Soybean meal	37.32	33.7	29.82
Soy oil	3.90	4.93	4.97
Bicalcium phosphate	1.27	1.30	1.12
Calcitic limestone	1.14	0.92	0.80
Iodized salt	0.48	0.43	0.43
DL-Methionine – 99%	0.29	0.28	0.25
L-lysine – 78%	0.20	0.19	0.26
L-threomine – 99%	0.05	0.05	0.05
Premix of vitamins and minerals[1]	0.20	0.20	0.20
Calculated chemical composition	100	100	100
Energy (kcal/kg)	3050	3150	3200
Brute protein (%)	21.20	19.80	18.40
Calcium (%)	0.84	0.76	0.66
Available phosphorus (%)	0.40	0.35	0.31
Digestible lysine (%)	1.22	1.13	1.06
Digestible methionine (%)	0.47	0.45	0.42
Digestible methionine + cysteine (%)	0.88	0.83	0.77
Digestible threonine (%)	0.79	0.73	0.69
Digestible tryptophan (%)	0.21	0.20	0.19
Sodium (%)	0.21	0.20	0.19

[1] Minimal vitamin and mineral levels per kg of product: vitamin A (5.000.000 UI); vitamin D3 (1.000.000 IU); vitamin E (15.000 UI); vitamin K3 (1.500 mg); vitamin B1 (1.500 mg); vitamin B2 (3.000 mg); vitamin B6 (2.000 mg); vitamin B12 (7.000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7000 mcg); choline (80 g); biotin (100 mg); Copper (10 g); iron (50 g); iodine (1.000 mg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g).

Table 2. Performance (mean and standard deviation) of broilers fed with Probiotic to replace performance enhancer antibiotic.

Day 1 to 7			
Treatment	Body weight (g)	Feed intake (g)	FC
Enramicyn	146.6 (7.3)[a]	126.7 (9.0)[b]	1.22 (0.04)[a]
Negative control	147.7 (5.7)[a]	134.6 (8.4)[ab]	1.29 (0.13)[a]
Probiotic E297	157.6 (11.7)[a]	139.9 (5.2)[a]	1.22 (0.05)[a]
Probiotic SF68	156.9 (8.1)[a]	140.2 (4.4)[a]	1.24 (0.06)[a]
P-value	0.193	**0.046**	0.297
Day 1 to 21			
Treatment	Body weight (g)	Feed intake (g)	FC
Enramycin	873.1 (39.4)[b]	1116.1 (45.1)[b]	1.34 (0.01)[ab]
Negative control	868.9 (35.1)[b]	1138.5 (45.2)[b]	1.38 (0.06)[a]
Probiotic E297	912.5 (41.4)[ab]	1193.7 (40.9)[ab]	1.37 (0.06)[a]
Probiotic SF68	950.0 (40.5)[a]	1203.5 (48.2)[a]	1.32 (0.02)[b]
P-value	**0.001**	**0.001**	**0.028**
	Day 1 to 35		
Treatment	Body weight (g)	Feed intake (g)	FC
Enramycin	2463.6 (42.2)[ab]	3453 (89)[b]	1.42 (0.01)[b]
Negative control	2411.1 (45.2)[b]	3587 (71)[ab]	1.48 (0.01)[a]
Probiotic E297	2435.2 (98.5)[ab]	3595 (48)[ab]	1.47 (0.04)[ab]
Probiotic SF68	2558.9 (37.4)[a]	3625 (60)[a]	1.44 (0.02)[b]
P-value	**0.001**	**0.001**	**0.016**
	Day 1 to 41		
Treatment	Body weight (g)	Feed intake (g)	FC
Enramycin	3167 (52)[bc]	4830 (111)[a]	1.52 (0.03)[b]
Negative control	3036 (48)[c]	4858 (125)[a]	1.57 (0.02)[a]
Probiotic E297	3215 (98)[ab]	4890 (80)[a]	1.54 (0.04)[ab]
Probiotic SF68	3279 (101)[a]	4863 (14)[a]	1.53 (0.01)[b]
P-value	**0.001**	0.598	**0.001**

Different letters on the same column indicate significant differences between groups using the Tukey test. Note: initial weight were 42,8g (Growth promoter), 42,6 g (Negative control), 43,3 g (Probiotic E297) e 43,1 g (Probiotic Cylactin).

Table 3. Leukogram of broilers feed supplemented probiotic to replace antibiotics.

Treatment	Leukocytes ($x10^3$ µL)	Lymphocyte ($x10^3$ µL)	Heterophil ($x10^3$ µL)	Basophil ($x10^3$ µL)	Monocyte ($x10^3$ µL)	Eosinophil ($x10^3$ µL)
Enramycin	5.68 (1.52)[a]	2.65 (0.85)[a]	1.72 (0.6)[a]	0.0 (0)[a]	1.26 (0.41)[a]	0.03 (0.4)[a]
Negative control	2.18 (1.06)[b]	0.97 (0.47)[b]	0.81 (0.29)[b]	0.01 (0.15)[a]	0.66 (0.21)[b]	0.01 (0.2)[a]
Probiotic E297	3.93 (1.14)[ab]	1.65 (0.75)[ab]	1.31 (0.54)[a]	0.0 (0)[a]	0.95 (0.30)[ab]	0.006 (0.07)[a]
Probiotic SF68	3.33 (1.07)[b]	1.38 (0.63)[ab]	0.92 (0.38)[ab]	0.005 (0.09)[a]	1.64 (0.68)[a]	0.05 (0.1)[a]
P-value	**0.001**	**0.001**	**0.001**	**0.653**	**0.001**	**0.741**

Different letters on the same column indicate significant differences between groups using the Tukey test.

Table 4. Biochemistry in serum of broilers supplemented feed probiotic to replace antibiotics.

Treatment	TP	ALB	GLO	TRI	CHO	UA	GLU
Enramycin	3.91 (0.8)	1.31 (0.3)	2.6 (0.5)^{a}	103 (29)^{a}	332 (116)^{a}	2.93 (0.6)	305 (39)
Negative control	3.30 (0.4)	1.5 (0.1)	1.8 (0.4)^{ab}	86 (19)^{ab}	115 (16)^{b}	3.16 (0.6)	308 (53)
Probiotic E297	3.35 (0.6)	1.7 (0.4)	1.61 (0.5)^{b}	58 (24)^{b}	302 (115)^{a}	4.2 (1.9)	287 (47)
Probiotic SF68	3.10 (0.5)	1.55 (0.2)	1.55 (0.4)^{b}	85 (31)^{ab}	417 (41)^{a}	3.06 (0.5)	274 (51)
P-value	**0.298**	**0.146**	**0.05**	**0.001**	**0.001**	**0.380**	**0.726**

Different letters on the same column indicate significant differences between groups using the Tukey test.

Note: TP – Total protein; ALB – Albumin; GLO – globulin; TRI – triglyceride; CHO – cholesterol; UA – uric acid; GLU – glucose.

Table 5 Villo, crypt and relation villo/crypt of jejunum of broilers feed containing probiotic to replace antibiotics.

Treatment	Villo (µm)	Crypt (µm)	Villo/crypt ratio
Enramycin	952 (35)[b]	216 (21)[a]	4.41[c]
Negative control	1368 (63)[a]	214 (17)[a]	6.38[a]
Probiotic E297	982 (37)[b]	160 (11)[b]	6.10[a]
Probiotic SF68	1173 (54)[ab]	219 (20)[a]	5.35[b]
P-value	**0.001**	**0.001**	**0.001**

Different letters on the same column indicate significant differences between groups using the Tukey test.

Table 6. Total lipids, protein, pH, and water activity in the meat of chickens fed with probiotics as a replacement for the use of growth promoters.

Variables	Enramycin	Negative control	Probiotic E297	Probiotic SF68	Value-P
Total fat (g/kg)	19.9[a]	18.8[a]	14.5[b]	13.5[b]	<0.001
Protein (g/100g)	14.34[b]	21.73[a]	20.47[a]	22.20[a]	<0.001
pH	5.86[b]	5.98[a]	5.98[a]	5.97[a]	<0.001
AW	0.975[b]	0.965[b]	0.994[a]	0.996[a]	<0.001

Note: P≤0.05 indicates difference between treatments, different letters being used on the same line to show the difference between groups.

Table 7. Fatty acid profile in the meat of broilers fed with probiotics as a replacement for the use of growth promoters.

Variables (%)	Enramycin	Negative control	Probiotic E297	Probiotic SF68	Value-P
C14:0 - myristic acid	0,594	0,624	0,572	0,569	0.589
C14:1n5 - myristoleic acid	0,072	0,073	0,046	0,054	0.061
C15:0 - pentadecanoic acid	0,121^{a}	0,103^{bc}	0,097^{c}	0,107^{ab}	0.024
NI	3,038^{b}	2,931^{b}	4,203^{a}	4,073^{a}	<0.001
C16:0 - palmitic acid	28,72^{b}	31,49^{a}	31,85^{a}	31,25^{a}	0.007
C16:1n9	0,415	0,382	0,402	0,394	0.726
C16:1n7 - palmitoleic acid	2,704	3,044	2,262	1,999	0.054
C17:0 - heptadecanoic acid	0,593	0,587	0,745	0,662	0.251
NI	0,129	0,134	0,122	0,137	0.840
C17:1n7 - cis-10-heptadecenoic acid	0,550	0,339	0,572	0,577	0.189
C18:0 - stearic acid	8,984	8,279	9,088	8,380	0.782
C18:1 trans isomer	0,105	0,082	0,086	0,079	0.628
C18:1 cis (n9) - oleic acid	25,26^{a}	24,71^{a}	22,77^{b}	22,51^{b}	0.003
C18:1 cis (n11) - vaccenic acid	1,785	1,777	1,817	1,605	0.872
C18:2 cis (n6) - linoleic acid	22,08	21,36	20,78	23,47	0.087
C18:3n6 - γ-linolenic acid	0,106^{a}	0,086^{ab}	0,049^{b}	0^{c}	<0.001
C20:0 - arachidic acid	0,049^{a}	0,038^{a}	0,021^{b}	0^{c}	<0.001
C18:3n3 - α-linolenic acid	1,322	1,179	1,104	1,223	0.364
C20:1(n9) - cis-11-eicosenoic acid	0,171	0,121	0,121	0,111	0.927
C20:2n6 - cis-11,14-eicosadienoic acid	0,347	0,217	0,311	0,285	0.098
C20:3n6 - cis-8,11,14-eicosatrienoic acid	0,368	0,278	0,365	0,303	0.549
C20:4n6 - arachidonic acid	1,952	1,758	2,130	1,802	0.180
C24:1n9 - nervonic acid	0,408^{a}	0,285^{c}	0,355^{ab}	0,328^{bc}	0.050
C22:6(n3) (DHA) - cis-4,7,10,13,16,19-docosahexaenoic acid	0,106^{a}	0,101^{a}	0,106^{a}	0,056^{b}	<0.001
acidos graxos saturado	39,06^{b}	41,12^{ab}	42,38^{a}	40,97^{ab}	0.035
acidos graxos monosaturados	31,06^{a}	30,43^{a}	28,03^{b}	27,27^{b}	0.002
acidos graxos polissaturados	26,28^{ab}	24,98^{b}	24,85^{b}	27,14^{a}	<0.001

Note: P≤0.05 indicates difference between treatments, different letters being used on the same line to show the difference between groups.

Table 8. Lactic Bacteria Count (LAB) in the broiler ration.

Treatment	**log CFU g^{-1}**						
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Enramycin	4.28(0.03) dB	3.43(0.02) dD	4.33(0.04) cB	4.57(0.02) bA	4.54(0.04) cA	3.85(0) cC	3.25(0.03) cE
Negative control	5.72(0.01) cA	4.28(0.03) cB	3.79(0.01) dC	3.06(0.08) cD	2.07(0.10) dF	3.76(0.02) dC	2.60(0.01) dE
Probiotic E297	15.29(0.05) aA	11.89(0.01) bC	9.08(0.05) bF	10.84(0.01) aD	10.85(0.01) aD	12.81(0.01) bB	10.04(0.06) aE
Probiotic SF68	13.27(0.05) bC	14.28(0.06) aA	11.34(0.03) aD	11.04(0.06) aE	10.04(0.046) bF	13.75(0.02) aB	6.04(0.06) bG
P-value	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**

Different letters tiny on the same column indicate significant differences between groups, different letters capital on the same line indicate significant differences between the time.

Table 9. Count of enterobacteria, lactic acid bacteria (LAB), mesophiles and *Escherichia coli* in broiler feces fed with feed supplemented with probiotics in replacement to the use of antibiotics.

Treatment	**log CFU g^{-1}**			
	Enterobacteria	LAB	Mesophiles	*E.coli*
Enramycin	7.23(1.08)[a]	7.84(0.78)[a]	8.35(1.10)[a]	7.52(0.88)[a]
Negative control	7.03(0.65)[a]	7.71(0.47)[a]	7.46(1.17)[a]	7.13(1.15)[a]
Probiotic E297	6.08(1.42)[a]	8.02(0.64)[a]	7.18(0.94)[a]	7.37(1.41)a
Probiotic SF68	6.13(0.61)[a]	7.72(0.51)[a]	7.76(0.61)[a]	8.24(1.53)[a]
P-value	**0.443**	**0.517**	**0.230**	**0.161**

Different letters on the same column indicate significant differences between groups.

Table 10. Count of lactic acid bacteria (LAB) and enterobacteria from avian litter in broilers supplemented with probiotics in replacement to the use of antibiotics.

Treatment	**LAB (log CFUg^{-1})**			
	Day 0	Day 21	Day 35	Day 41
Enramycin	8.47(0.95)^{aA}	7.77(0.80)^{aA}	7.18(1.46)^{aA}	7.35(1.59)^{aA}
Negative control	9.54(0.34)^{aA}	8.43(0.55)^{aAB}	8.34(0.20)^{aAB}	7.23(0.75)^{aB}
Probiotic E297	8.72(0.91)^{aA}	9.01(1.46)^{aA}	9.01(0.49)^{aA}	8.81(0.13)^{aA}
Probiotic SF68	8.38(0.34)^{aA}	8.02(0.69)^{aA}	8.48(0.85)^{aA}	8.77(0.76)^{aA}
P-value	**0.191**	**0.460**	**0.402**	**0.257**
Treatment	**Enterobacteria (log CFU g^{-1})**			
	Day 0	Day 21	Day 35	Day 41
Enramycin	2.78(0.25)^{bB}	5.07(0.10)^{bA}	3.33(0.89)^{aAB}	4.44(0.01)^{abAB}
Negative control	4.13(0.07)^{aB}	6.13(0.18)^{aA}	3.04(0.37)^{aC}	3.95(0.24)^{bBC}
Probiotic E297	2.74(0.06)^{bB}	4.86(0.36)^{bA}	3.24(0.34)^{aB}	4.69(0.06)^{aA}
Probiotic SF68	2.77(0.10)^{bC}	4.83(0.13)^{bA}	3.68(0.40)^{aBC}	4.08(0.18)^{abAB}
P-value	**0.001**	**0.003**	**0.622**	**0.001**

Different letters tiny on the same column indicate significant differences between groups, different letters capital on the same line indicate significant differences between the time.

Table 11. Quantification of lactic acid bacteria (LAB) and Enterobacteria microorganisms in broiler chickens by cloacal swab.

Treatment	**LAB (log CFU g^{-1})**		
	Day 7	Day 21	Day 35
Enramycin	7.63(0.50)^{aA}	6.85(0.43)^{aAB}	5.92(0.11)^{aB}
Negative control	7.89(0.97)^{aA}	7.33(0.48)^{aA}	5.50(0.71)^{aA}
Probiotic E297	6.84(0.09)^{aA}	7.31(0.33)^{aA}	6.72(0.34)^{aA}
Probiotic SF68	7.36(0.28)^{aA}	7.35(0.66)^{aA}	6.95(2.08)^{aA}
P-value	**0.498**	**0.753**	**0.453**
Treatment	**Enterobacteria (log CFU g^{-1})**		
	Day 7	Day 21	Day 35
enramycin	6.30(0)^{abB}	7.01(0.47)^{aB}	8.19(0.06)^{aA}
Negative control	6.18(0.10)^{bC}	7.37(0.01)^{aB}	8.40(0.06)^{aA}
Probiotic E297	6.14(0.01)^{bB}	7.35(0.29)^{aA}	8.38(0.34)^{aA}
Probiotic SF68	6.42(0.05)^{aC}	7.40(0.05)^{aB}	8.28(0.06)^{aA}
P-value	**0.003**	**0.616**	**0.612**

Different letters tiny on the same column indicate significant differences between groups, different letters capital on the same line indicate significant differences between the time.

Supplementary material 1. Total lipids and fatty acid profile in the ration of chickens fed with probiotics as a replacement for the use of growth promoters.

Variáveis	Enramycin	Negative control	Probiotic E297	Probiotic SF68
Total Fat	7,52	7,34	7,52	7,62
C14:0 - myristic acid	0,102	0,093	0,096	0,110
C16:0 - palmitic acid	16,29	16,38	16,42	15,93
C16:1n7 - palmitoleic acid	0,095	0,101	0,111	0,095
C17:0 - heptadecanoic acid	0,103	0,103	0,103	0,102
C18:0 - stearic acid	4,160	3,947	3,977	4,081
C18:1 cis (n9) - oleic acid	28,47	28,78	29,02	29,03
C18:2 cis (n6) - linoleic acid	46,16	46,12	45,78	46,13
C20:0 - arachidic acid	0,400	0,369	0,391	0,396
C18:3n3 - α-linolenic acid	3,848	3,769	3,747	3,772
C22:0 - behenic acid	0,352	0,310	0,338	0,343

NOTE: there was no statistical difference (P>0.05) between treatments for the variables present in the table.

3 CONSIDERAÇOES FINAIS

A inclusão do probiótico *Enterococcus faecium E297* na alimentação de frangos de corte, mostrou-se um potencial substituto aos antibióticos melhorados de desempenho, por contribuir na melhorara dos índices zootécnicos, proporcionando maior ganho de peso e conversão alimentar nos animais suplementados. Exercendo efeitos positivos na saúde animal, por estimular o sistema imune e modular positivamente a relação villo/cripta intestinal.

Na contagem de BAL's na ração percebemos um declínio maior de microrganismos quando a ração ficou exposta por maior tempo (ração inicial 0 a 21 dias), prognosticando que devesse expor o probiótico a um processo tecnológico que possibilite maior 'conservação' da bactéria na fase log/estacionaria.

Ainda, são relevantes futuras pesquisas sobre ação do probiótico E297 como coccidiostatico, assim como inibidor no desenvolvimento de samoneloses e prevenção e controle de colibacilose.

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CARTA DE APROVAÇÃO DO CETEA (obrigatório)

Projetos que envolveu animal devem digitalizar a carta de aprovação do cetea de seu projeto, e adicionar aqui

ANEXOS (opcional)

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Uso do probiótico Enterococcus faecium E297 na dieta de frangos de corte: desempenho zootécnico e qualidade da carne", protocolada sob o CEUA nº 1450170920 (ID 001228), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 25/09/2020.

We certify that the proposal "Use of the probiotic Enterococcus faecium E297 in the broiler diet: zootechnical performance and meat quality", utilizing 225 Birds (225 males), protocol number CEUA 1450170920 (ID 001228), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 09/25/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 10/2020 a 12/2020 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Aves	sexo:	Machos	idade:	1 a 42 dias	N:	225
Linhagem:	Coob500			Peso:	44 a 3000 kg		

Local do experimento: setor de avicultura da UDESC - Oeste

Lages, 20 de outubro de 2021

José Cristani
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Pedro Volkmer de Castilhos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina