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Dissertação

Suplementação de butirato para prevenção de diarreia e no desenvolvimento gastrointestinal e corporal de bezerros leiteiros

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Suplementação de butirato para prevenção de diarreia e no desenvolvimento gastrointestinal e corporal de bezerras leiteiras

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Resumo

NICOLA, Murilo Scalcon. **Suplementação de butirato para prevenção de diarreia e no desenvolvimento gastrointestinal e corporal de bezerras leiteiras**. 2022. 69f. Dissertação (Mestrado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2022.

As primeiras semanas após o nascimento são marcadas por diversas adaptações no organismo dos ruminantes, uma delas, se não a principal é o desenvolvimento do trato gastrointestinal, que é fundamental, para o rápido crescimento corpóreo assim como para a desenvolvimento da resistência contra enfermidades, principalmente as gastrointestinais. O presente estudo teve como objetivo avaliar a capacidade do butirato de sódio em estimular o desenvolvimento do trato gastrointestinal e prevenir diarreias em bezerras leiteiras. Para isso, 124 animais da raça Holandesa, foram distribuídos em dois grupos: GB - Butirato - que recebeu 4g de uma produto contendo 90% de butirato de sódio no leite integral do 1º ao 90º dia de vida, além de concentrado *ad libitum* (50 fêmeas e 12 machos, n=62), e GC - Controle - alimentados com a mesma dieta, mas com leite integral não-suplementado (50 fêmeas e 12 machos, n=62). A consistência das fezes foi avaliada diariamente nos primeiros 30 dias e os animais foram monitorados quanto à ocorrência de diarreia, sendo determinada a sua morbidade, recorrência, mortalidade e letalidade. O desempenho zootécnico dos animais foi monitorado durante os primeiros 60 dias de vida (D1, D8, D15, D22, D29, D45, D60), e aos 90 dias (desmame). Avaliações histológicas e de expressão gênica do trato gastrointestinal foram realizadas a partir de amostras coletadas de machos eutanasiados aos 15 e aos 30 dias de vida. Os resultados demonstraram que a suplementação de butirato no leite reduziu a morbidade (GB = 30% *vs.* GC = 50%) e a recorrência (GB = 26,67% *vs.* GC = 60%) da diarreia neonatal, além do número de dias com fezes anormais, em relação ao grupo não-suplementado (GB = 4,64 ± 0,47 dias *vs.* GC = 8,6 ± 0,65 dias). A suplementação também influenciou o ganho médio diário de peso (GMD), que tendeu a ser maior aos 30 dias (GB =0,441 ± 0,018 *vs.* GC = 0,394 ± 0,018) e aos 60 dias de vida (GB =.0,555 ± 0,016 *vs.* GC = 0,516 ± 0,016). Além disso, a morfometria do TGI aos 30 dias revelou maior comprimento (GB = 560,92 ± 10,28 µm *vs.* GC = 388,67 ± 10,28 µm) e maior área superficial (GB = 0,276 ± 0,008 mm^2 *vs.* GC = 0,184 ± 0,008 mm^2) de papilas ruminais nos animais suplementados. Na porção duodenal do intestino, o comprimento das vilosidades (GB = 488,14 ± 4,76 *vs.* GC = 446,87 ± 4,76 µm) e a profundidade das criptas (GB = 254,96 ± 2,75 µm *vs.* GC = 231,32 ± 2,75 µm) foram maiores no GB. Ao comparar os resultados da análise de expressão duodenal dos genes *LCT* e *GLP2* de animais com 30 dias de vida que tiveram um episódio de diarreia (GB e GC - com diarreia) com os de animais que não tiveram episódios (GB e GC - sem diarreia), foi possível observar que os níveis transcritos no GB com diarreia, foi semelhante aos encontrados nos grupos GB e GC sem a enfermidade. Em conjunto, esses resultados permitem concluir que a suplementação contínua com butirato de sódio melhora o desenvolvimento gastrointestinal, reduz a ocorrência de diarreias e torna os quadros clínicos mais brandos e a recuperação mais rápida. Todos esses fatores tendem a favorecer um

maior GMD nos primeiros 60 dias de vida, sendo uma alternativa interessante para acelerar o desenvolvimento inicial e melhor a saúde de bezerras leiteiras.

Palavras Chave: aleitamento; expressão gênica; fezes anormais; ganho de peso; histologia.

Abstract

NICOLA. Murilo Sacalcon. **Butyrate supplementation for diarrhea prevention and gastrointestinal and bodily development of dairy calves.** 2022. 69f. Dissertation (Master degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2022.

The first weeks after birth are marked by several adaptations in the ruminant organism, one of them, if not the main one, is the development of the gastrointestinal tract, which is essential for rapid body growth as well as for the development of resistance against diseases, especially the gastrointestinal ones. The present study aimed to evaluate the ability of sodium butyrate to stimulate the development of the gastrointestinal tract and prevent diarrhea in dairy calves. For this, 124 Holstein animals were divided into two groups: GB - Butyrate - which received Sodium Butyrate in whole milk for 90 days, in addition to concentrate *ad libitum* (50 females and 12 males, n=62), and GC - Control - fed the same diet, but with unsupplemented whole milk (50 females and 12 males, n=62). The consistency of the feces was evaluated daily for the first 30 days and the animals were monitored for the occurrence of diarrhea, and its morbidity, recurrence, mortality and lethality were determined. Growth was evaluated during the first 60 days of life (D1, D8, D15, D22, D29, D45, D60), and at 90 days (weaning). Histological and gene expression evaluations of the gastrointestinal tract were performed from samples collected from males euthanized at 15 and 30 days of age. The results showed that butyrate supplementation in milk reduced morbidity (GB = 30% vs. GC = 50%) and recurrence (GB = 26,67% vs. GC = 60%) of neonatal diarrhea, in addition to the number of days with abnormal feces, compared to the non-supplemented group (GB = 4.64 ± 0.47 days vs. GC = 8.6 ± 0.65 days). Supplementation also influenced the average daily gain (ADG), which tended to be greater at 30 days (GB = 0.441 ± 0.018 vs. CG = 0.394 ± 0.018) and at 60 days of life (GB = 0.555 ± 0.016 vs. GC = 0.516 ± 0.016). In addition, TGI morphometry at 30 days revealed greater length (GB = 560.92 ± 10.28 µm vs. GC = 388.67 ± 10.28 µm) and greater surface area (GB = 0.276 ± 0.008 mm^2 vs. GC = 0.184 ± 0.008 mm^2) of rumen *papillae* in the supplemented animals. In the duodenal portion of the intestine, the length of the *villi* (GB = 488.14 ± 4.76 vs. GC = 446.87 ± 4.76 µm) and the depth of the crypts (GB = 254.96 ± 2.75 µm vs GC = 231.32 ± 2.75 µm) were greater in GB. When comparing the results of the analysis of duodenal expression of the LCT and GLP2 genes of 30-day-old animals that had an episode of diarrhea (GB and GC - with diarrhea) with those of animals that did not have episodes (GB and GC - without diarrhea), it was possible to observe that the transcription levels in the GB with diarrhea were similar to those found in the GB and GC groups without the disease. Taken together, these results allow us to conclude that continuous supplementation with sodium butyrate improves gastrointestinal development, reduces the occurrence of diarrhea and makes clinical conditions milder and recovery faster. All these factors tend to favor a greater ADG in the first 60 days of life, being an interesting alternative to accelerate the initial development and improve the health of dairy calves.
Keywords: abnormal feces; average daily gain; gene expression; histology; milk feeding.

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Lista de Abreviaturas e Siglas

Cm	Centímetros
cm^2	Centímetros Quadrados
CFMV	Conselho Federal de Medicina Veterinária
d	Dias
dL	Decilitro
DNA	Ácido Desoxirribonucleico
FTIP	Falha de Transferência na imunidade passiva
g	Gramas
GLP-2	Peptídeo 2 Semelhante ao Glucagon
GMD	Ganho Médio Diário
GB	Grupo Butirato
GC	Grupo Controle
IGF-1	Fator de Crescimento Semelhante a Insulina-1
Kg	Quilogramas
L	Litro
LCT	Lactase
mm^2	Milimetros quadrados
N	Número de Animais
Ng	Nanogramas
PPT	Proteína Plasmática Total
RNA	Ácido Ribonucleico
TGI	Trato Gastrointestinal
µm	Micrômetro

Lista de Símbolos

<	Menor
>	Maior
±	Mais ou menos
%	Porcentagem
°C	Grau Celsius
*	Asterisco
®	Marca Registrada

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1 Introdução

Um sistema de produção leiteira é formado por diversos segmentos, os quais são fundamentais para o sucesso da atividade, qualquer falha de manejo seja ele sanitário ou nutricional irá afetar o sucesso e a lucratividade da atividade. Um dos segmentos é a criação de bezerras que é uma das mais desafiadoras, uma vez que os animais nesta fase não apresentam um sistema imunológico e digestório completamente desenvolvido, aumentando as probabilidades de desenvolvimento, de doenças e atrasos no crescimento (Lopes e Vieira, 1998).

A criação de bezerras é fundamental dentro do sistema, visto que, estas se tornarão as futuras vacas, então quanto mais rápido e eficiente for este processo, mais rápido o animal chegará à vida produtiva e maior será sua produção futura (Spadetto, 2013). Diante disso, diversas tecnologias de manejo e nutricionais vem sendo estudadas e desenvolvidas para acelerar o desenvolvimento e minimizar os atrasos de crescimento nesta fase, como a suplementação com probióticos, prebióticos, extratos vegetais, butirato e sistemas de monitoramento e alimentação automática (Xiao et al, 2016; Giannenas et al, 2011; Górka et al, 2014; Duthie et al, 2021).

As bezerras logo após o nascimento não apresentam o Trato Gastrointestinal (TGI) totalmente desenvolvido, esse processo se dá ao longo das primeiras semanas de vida, mediado pela alimentação que o animal recebe (Heinrichs, 2005). A suplementação com butirato de sódio vem sendo estudada como um eficiente estimulador do desenvolvimento gastrointestinal dos bezerros nestas primeiras semanas de vida (Górka et al, 2014).

Naturalmente o ácido butírico será produzido através da fermentação ruminal e é uma importante fonte de energia para o desenvolvimento das células epiteliais do trato gastrointestinal (Bergman, 1990), porém a síntese só iniciará quando o consumo de alimentos sólidos se tornar significativo (Hill et al., 2010).

O butirato de sódio quando suplementado nas primeiras semanas de vida, através da dieta líquida terá um efeito importante sobre o desenvolvimento do trato digestivo inferior visto que a goteira esofágica desvia o leite ingerido pelos bezerros diretamente para o abomaso, porém seus efeitos benéficos quando suplementado

desta forma sobre o trato digestivo superior também são relatados através de mecanismos indiretos (Górka et al., 2018).

O desenvolvimento do trato gastrointestinal pode refletir diretamente no aproveitamento dos nutrientes e no crescimento corpóreo das bezerras, além de refletir em um animal mais saudável que será naturalmente resistente à ocorrência de doenças dentre elas as diarreias, além disso também é relatado um possível efeito antibacteriano do butirato (Cherrington,1991), os quais juntos contribuirão para um desenvolvimento acelerado e uma rápida evolução do animal para a vida produtiva.

Diante do apresentado a suplementação com butirato de sódio nas primeiras semanas de vida pode ser uma alternativa interessante para acelerar o desenvolvimento e crescimento nesta fase, porém estudos são necessários para elucidar os reais efeitos e benefícios desta molécula no desenvolvimento TGI, crescimento e redução da ocorrência de diarreias.

2 Revisão da Literatura

2.1 Trato Gastrointestinal de Bezerras

O TGI dos ruminantes em geral não está plenamente desenvolvido nas primeiras semanas de vida, em especial as cavidades pré-gástricas como rúmen, retículo e omaso, impossibilitando a ingestão e aproveitamento de alimentos sólidos neste momento (Heinrichs, 2005). Esse processo de desenvolvimento se dá substancialmente entre as 3 e 4 semanas de vida, momento em que o consumo de alimentos sólidos começa a se tornar significativo (Heinrichs, 2005; Hill et al., 2010). O desenvolvimento do rúmen só se dará por completo com vários meses de vida (Bailey, 1986), porém o quanto ele se desenvolve no período pré desmame pode afetar significativamente o desenvolvimento do animal e sua produtividade futura (Khan et al., 2016).

Antes do desenvolvimento do rúmen, ocorre o desenvolvimento e maturação do abomaso e intestino, além de sua colonização bacteriana que são fundamentais para o pleno crescimento corpóreo, aproveitamento de nutrientes e sanidade dos animais (Guilloteau et al., 2009). O desenvolvimento intestinal e abomasal, diferente do rúmen, é fundamental não apenas para a digestão de alimentos sólidos, mas também para a digestão e aproveitamento de alimentos líquidos, como o leite integral

e sucedâneo lácteo, que é a principal fonte nutricional nas primeiras semanas de vida dos ruminantes (Górka et al, 2011).

Visto que o desenvolvimento de todo TGI é fundamental para um melhor aproveitamento de nutrientes que resultam em um acelerado crescimento corpóreo e maior resistência à ocorrência de doenças, é importante investir em alternativas que melhorem tanto o desenvolvimento ruminal quanto intestinal. Em ruminantes são conhecidos os efeitos benéficos do ácido butírico no desenvolvimento e maturação do epitélio ruminal (Mentschel et al.,2001). Além disso, estudos mostram efeitos benéficos da suplementação dietética de butirato não só no crescimento ruminal, mas também no desenvolvimento abomasal, intestinal e pancreático em bezerros (Guilloteau et al, 2009; Górka et al, 2014), e não só em ruminantes, também alguns estudos relatam efeitos benéficos no desenvolvimento do TGI em suínos recém nascidos (Kotunia et al, 2004; Le Gall et al, 2009) e aves (Moquet et al., 2016).

2.2 Fontes Naturais de Butirato

O ácido butírico é um ácido graxo de cadeia curta, naturalmente produzido através da fermentação bacteriana no rumem e intestino grosso (Bergman, 1990). Ele é o menos abundante entre os três principais produzidos a nível ruminal (propionato, acetato e butirato), variando entre 5 a 20% dependendo da dieta. Em grande parte, é absorvido na parade ruminal, servindo como fonte de energia para o próprio desenvolvimento das papilas ruminais (Bergman, 1990).

O butirato também está presente, mesmo que em pequena quantidade, no leite integral, sendo este alimento a principal fonte da molécula para bezerros recém nascidos que ainda não estão ingerindo alimentos sólidos (Guilloteau et al., 2010). Sua concentração em forma livre no leite integral é baixa, cerca de 0,16 gramas por litro, porém, segundo Guilloteau et al. (2010), esta quantidade já pode ser suficiente para afetar o desenvolvimento do TGI. Além da forma livre, o butirato pode ser obtido através da quebra de moléculas de gordura do leite no abomaso, a qual contem cerca de 2 a 4% de butirato (Chilliard et al, 2009).

2.3 Fontes Artificiais de Butirato

O butirato é fornecido na dieta em forma de sais ou ésteres, essas formas se tornam vantajosas em relação ao ácido butírico em forma livre, por apresentar uma maior estabilidade, uma redução do cheiro forte e característico do ácido butírico, além de facilitar o manuseio e fornecimento em larga escala na dieta dos animais (Guilloteau et al.,2010).

A forma mais comum encontrada do butirato para ser fornecido na dieta é na forma de sais de sódio, por questões econômicas e por ser altamente solúvel em água e prontamente liberado a nível ruminal quando fornecido na dieta sólida sem proteção, ou a nível abomasal e intestinal quando fornecido na dieta líquida (Mallo et al., 2012). Já o butirato de cálcio, que é menos comum, é mais insolúvel em água tendo uma maior resistência a dissolução sendo liberação mais lenta ao longo do trato, porém também sua taxa de perda, sem ser absorvido, se torna maior (Mallo et al., 2012).

O butirato tanto de sódio quanto de cálcio pode também ser encontrado de forma protegida, geralmente encapsulado por uma matriz lipídica (Moquet et al, 2016). Esse envoltório reduz a velocidade de dissociação e liberação do butirato no trato digestivo, fazendo que uma pequena parte seja liberada no rúmen e uma maior parte liberada no abomaso e intestinal, devido a ação das lipases presentes no fluido abomasal e ação do suco pancreático a nível intestinal, isso quando fornecido em dieta sólida (Piva et al., 2007).

O fornecimento do butirato de forma protegida em dietas líquidas não é usual, pois primeiramente o envoltório lipídico dificulta a diluição do produto no leite e também pois o principal objetivo de proteger é para o mesmo não ser completamente liberado a nível ruminal, então como o alimento líquido naturalmente é desviado do rúmen pela goteira esofágica, a proteção não se faz necessária (Górka, et al. 2011). Porém, estudos são necessários para verificar possíveis benefícios do fornecimento da forma protegida em dietas líquidas, visto que apenas um estudo até o momento realizou o fornecimento desta forma (Nazari et al., 2012).

2.4 Suplementação na Dieta Líquida

Como dito acima, os estudos até o momento com fornecimento em dieta líquida são utilizando o butirato em sua forma desprotegida. Além disso, a grande maioria dos

estudos são suplementando o butirato em dietas líquidas a base de substituto lácteo, visto que neste, o butirato não está presente e na maioria das formulações a fonte de gordura é provinda de gorduras vegetais que são pobres em butirato. Diferente do leite integral, que como dito anteriormente possui uma concentração de butirato livre e outra associada às moléculas de gordura (Guilloteau et al. 2010, Chilliard et al, 2009). Isso tudo justifica então que a suplementação com butirato se faz necessária principalmente em sucedâneo. Porém, não se descarta e são necessários mais estudo para verificar se a suplementação com butirato em dietas a base de leite integral proporciona um melhor desenvolvimento do TGI, corpóreo e redução de doenças.

Em estudos a suplementação de butirato no sucedâneo lácteo provocou efeitos mais pronunciados a nível de intestino delgado e na função pancreática, com redução dos índices apoptóticos, aumento do tamanho de vilosidades e espessura de mucosa (Guilloteau et al, 2009; Górka et al, 2011), assim como aumentou a secreção de suco pancreático, quimotripsinas e lipases (Guilloteau et al, 2010). Porém, esses resultados são relatados em estudos utilizando substituto lácteo, onde a suplementação de butirato tornou o desenvolvimento das bezerras semelhante ao observado em animais alimentados com leite integral (Górka et al, 2011). Mais estudos são necessários para verificar se existem efeitos benéficos da suplementação também em animais alimentados com leite integral.

2.5 Suplementação na Dieta Sólida

O butirato quando fornecido na dieta sólida sem proteção é rapidamente dissociado, e deverá afetar predominantemente as estruturas ruminais. Estudos mostram que nestes casos ocorre um aumento do comprimento de papilas no rúmen e um maior consumo de alimentos, favorecendo o crescimento e melhorando o Ganho Médio Diário (GMD) dos animais (Cavini et al., 2015). No entanto nestes casos os resultados relacionados a melhora do desenvolvimento intestinal e secreção de suco gástrico não são relatados afirmativamente, apesar de estudos mostrarem que quanto maior a ingestão de alimentos e melhora na função ruminal, melhor será a atividade intestinal.

Já em casos em que o butirato é fornecido em forma protegida espera-se que o mesmo seja liberado em maior quantidade e tenha seu principal local de ação o intestino delgado. Além disso essa forma de suplementação permite uma liberação

controlada e por maior tempo que além de possibilitar que o mesmo atinja o trato inferior, proporciona um maior aproveitamento do suplemento (Górka et al, 2011; Kowalski et al, 2015).

Como dito a principal forma de gerar essa proteção é a inclusão do butirato em invólucros lipídicos, esta técnica de proteção é utilizada para muitas outras moléculas (Rossi et al., 2003). Esta proteção reduz significativamente a degradação a nível ruminal pela fermentação microbiana e as microcápsulas de gordura com butirato, são facilmente transportadas pelo reticulo, rumen e omaso, fato o qual também permite que o mesmo seja liberado em maior quantidade a nível intestinal e não unicamente a nível ruminal (Kowalski et al, 2015). Porém ainda não existem estudos que comparem de forma controlada os efeitos das duas apresentações (protegida e desprotegida), na suplementação em dieta sólida de bezerros.

2.6 Mecanismo de Ação

O mecanismo de ação do butirato ainda é bastante discutível, com efeitos diretos por ser uma fonte de energia para o desenvolvimento das estruturas internas do trato gastrointestinal (Wiese et al, 2013), mas também são relatados efeitos indiretos, através da estimulação de expressão genica e secreção de hormônios e fatores de crescimento como o IGF-1, insulina e GLP-2 (Herrick et al, 2017). Os efeitos indiretos são mais aceitos visto que com doses muito baixas que são insignificantes pensando em aporte energético, como menos de 0,3% da MS, já são observados efeitos positivos na promoção do desenvolvimento de papilas e vilosidades (Cavini et al., 2015).

Relacionado a expressão gênica o butirato tem efeito sobre a acetilação e desacetilação das histonas (Canani et al., 2011), através do bloqueio das enzimas histonas desacetilases. O bloqueio desta enzima, permite uma maior acetilação das histonas, resultando na descompactação da cromatina o que aumenta a expressão gênica e reduz a apoptose (De Ruijter et al., 2003). Esta maior expressão pode explicar em partes o maior desenvolvimento de papilas, por um efeito direto relacionado ao crescimento tecidual local, mas também por um efeito indireto relacionado a maior expressão de genes relacionados a estimulação do apetite dos animais (Guilloteau et al, 2010).

2.7 Efeitos da Suplementação no Crescimento e Redução de Diarreias

O maior consumo de alimentos em animais que recebem o butirato é relatado em diversos estudos (Burakowska et al., 2017; Davarmanesh et al., 2015; Górka et al, 2014; Mccurdy et al., 2019; Ślusarczyk et al., 2010), esse maior consumo pode explicar o melhor desempenho de crescimento e GMD relatado nos animais suplementados com butirato (Davarmanesh et al., 2015; Hill et al, 2007; Liu et al. 2021; Ślusarczyk et al., 2010). Além do maior consumo, o acelerado desenvolvimento de papilas e estruturas do trato gastrointestinal, podem melhoram e digestão e o aproveitamento dos nutrientes resultando também em um maior crescimento corpóreo.

A diarreia é uma das principais causas de mortalidade em bezerros e também de perdas econômicas na pecuária, principalmente por retardo de crescimento e número de óbitos, o qual pode chegar a 20% (Mota et al., 2000). Esta é considerada uma doença multifatorial, podendo envolver o animal, o ambiente, a nutrição e agentes infecciosos (Buzinaro et al., 2003; Fontes et al., 2006), podendo o butirato de sódio ser uma alternativa para reduzir estes prejuízos.

É relatado em alguns estudos uma redução nos dias com fezes anormais e uma menor ocorrência de diarreia em animais suplementados com butirato (Hill et al, 2007; Górka et al, 2011). Isso pode estar relacionado com a melhor condição nutricional e de desenvolvimento gastrointestinal proporcionada pela suplementação, assim como o efeito antioxidante relatado por Liu et al (2021). Além de um possível efeito antibacteriano gerado por uma alteração nos gradientes eletroquímicos e uma redução no pH intestinal (Cherrington,1991; Drackley et al, 2008), o que limita o desenvolvimento de bactérias patogênicas. Este efeito também já foi relatado em humanos (Krokowicz et al., 2014).

3 OBJETIVOS

3.1 Objetivo geral

Avaliar a eficácia da administração contínua de butirato de sódio não protegido em dieta líquida na prevenção de diarreias, desenvolvimento corpóreo e do trato gastrointestinal em bezerros neonatos.

3.2 Objetivos específicos

1. Avaliar o desempenho zootécnico de bezerras leiteiras submetidas à administração contínua de butirato na dieta líquida;
2. Avaliar o grau de desenvolvimento das células do trato gastrointestinal (tamanho das papilas e superfície absortiva) dos bezerros que receberam administração contínua de butirato na dieta líquida;
3. Avaliar a expressão gênica, em nível intestinal, para fatores de crescimento e apoptose em bezerros machos submetidos à administração contínua de butirato na dieta líquida;
4. Determinar a morbidade, recidivas e mortalidade por diarreia em bezerras leiteiras submetidas à administração contínua de butirato na dieta líquida;
5. Determinar o escore de fezes de bezerras leiteiras submetidas à administração contínua de butirato na dieta líquida

4 Artigo

Butyrate supplementation in the liquid diet of dairy calves leads to a rapid recovery from diarrhea and reduces its occurrence and relapses in the preweaning period

M.S. Nicola, A.L. Kalb, A.A. Barbosa, B.E.S. Velasquez, J.A.A. Rincon, J.O. Feijó, E.N. Dellagostin, A.W.S. Martins, E.B. Blödorn, W.B. Domingues, F. Lopes, W. Quinteiro, R.G. Mondadori, V.F. Campos , V.R. Rabassa, Komninou E.R., Corrêa M.N.

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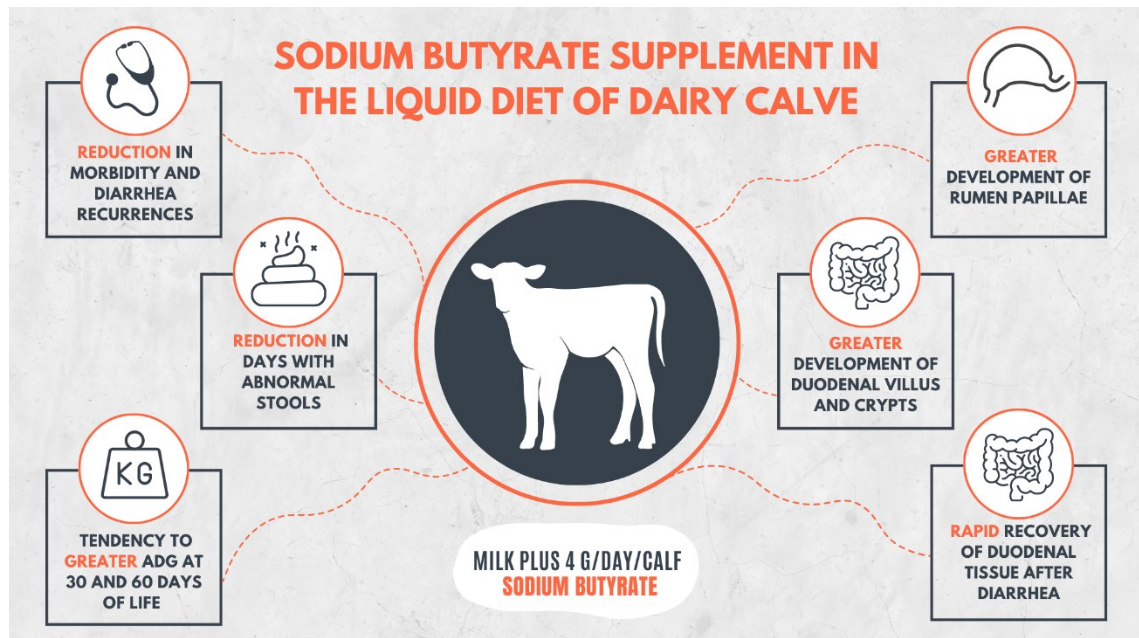
1 **RUNNING TITLE:**
2 **BUTYRATE AND CALF DIARRHEA**
3
4 ## Interpretive Summary

5 Sodium butyrate supplementation is been studied as an alternative to improve gastrointestinal
6 development and reduce the occurrence of diarrhea in dairy calves. Diarrhea is a very
7 recurrent disease during the calf rearing phase, bringing damages related to growth
8 retardation, cost of antibiotics and death of animals. Our study shows that sodium butyrate
9 supplementation in the liquid diet was able to reduce the occurrence of diarrhea and accelerate
10 gastrointestinal development, resulting in better animal health and well-being, reduced
11 antibiotic use and greater profitability.
12
13 By Nicola et al.
14
15 **Graphical abstract:**



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Butyrate supplementation in the liquid diet of dairy calves leads to a rapid recovery from diarrhea and reduces its occurrence and relapses in the preweaning period

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ABSTRACT

The present study aimed to evaluate the effect of continuous butyrate administration in dairy calves' liquid diet considering diarrhea, metabolic profile, gastrointestinal development, and corporal growth. Immediately after birth, calves were randomly allocated into 2 groups of 62 calves (50 females and 12 males): the Butyrate Group (BG) received 4 g/day of sodium butyrate (Admix® Easy - Adisseo) diluted in the whole milk, and the Control Group (CG) received whole milk with no supplementation. The product was administrated from day one of life until the weaning at 90 days. Feces consistency was assessed daily for the first 30 d and characterized by scores from 0 to 4 (0 and 1 for normal and 2, 3, and 4 for abnormal feces). Diarrhea was diagnosed when the animals had abnormal feces and fever. Morbidity, recurrence, mortality, and lethality data were recorded and compared between the groups. Average daily weight gain (ADG) and corporal growth (body weight, thoracic perimeter, height at the withers, and croup width) were evaluated weekly, from the first day to the 30th, and later at the 45th, 60th, and 90th d of life. Blood samples were taken weekly for up to 30 d to determine the main plasma parameters for a metabolic profile. The males were euthanized at 15 (n = 6 per group) and 30 d (n = 6 per group) for morphometric, histological, and gene expression analysis of the gastrointestinal tract. The results showed that the BG had a lower rate of morbidity (BG = 30% *vs*. CG = 50%) and recurrence (BG = 26.7% *vs.* CG = 60%) of diarrhea than CG. In addition, the BG had abnormal feces for a shorter period (BG = 4.64 ± 0.47 d *vs.* CG = 8.6 ± 0.65 d). The ADG tended to be higher in BG than CG up to 30 and 60 days. Metabolic evaluations showed the lowest levels of glucose and highest levels of NEFA in BG. On day 30 of life, rumen papillae length, papilla area, duodenum villus length, and crypt depth were higher in BG than in CG. The duodenal gene expression at 30 d showed that animals with diarrhea episodes that did not receive butyrate had the highest levels of transcripts for the *LCT* and *GLP2* genes. In addition, in different ways, both butyrate and neonatal diarrhea affected the expression of transporter-related genes *SLC5A1* and *AQP3* and appetite-related genes *PYY* and *GHRL*. These results allow us to conclude that continuous supplementation with sodium butyrate improves gastrointestinal development, reduces the occurrence of diarrhea, and makes clinical conditions milder with faster recovery, favoring a higher ADG in the first 30 and 60 d of life. We conclude that sodium butyrate can be indicated for liquid diet supplementation to accelerate gastrointestinal tract development and prevent severe cases of neonatal diarrhea, tending to improve average daily gain until the weaning.

86 **Keywords:** gastrointestinal development, gene expression, feces, metabolism.

87

88 ## INTRODUCTION

89

90 The rearing of dairy heifer calves is essential in the life production cycle of the dairy
91 cow. In this process, the neonatal period is a critical moment in which nutrition and
92 management practices are crucial to prevent diseases and ensure development and survival
93 until the weaning (Volpato et al., 2017). After birth, the new environment, loaded with
94 potential pathogens, can predispose to the development of neonatal diarrhea, which is the
95 leading cause of morbidity and mortality in calves (Rosa et al., 2018; Urie et al., 2018).
96 Growth delay, increased susceptibility to other diseases, costs with the prevention, treatment,
97 genetic losses (Cho and Yoon, 2014), reproductive efficiency, and milk production
98 throughout life (Urie et al., 2018) are the main consequences of diarrhea in heifer calves. In
99 addition, the occurrence of diarrhea is related to the delay in the development and bacterial
100 colonization of the gastrointestinal tract in the pre-weaning period (Dias et al., 2018)
101 The development and maturation of the gastrointestinal tract occur initially under the
102 stimulation of a liquid diet (colostrum and milk) and later by solid foods (roughage and
103 concentrate) (Guilloteau et al., 2009). Recently, butyrate has been identified as an essential
104 player in these processes in calves (Dias et al., 2018).
105 Butyrate is naturally produced in ruminants' rumen and large intestines (Górka et al.,
106 2018). These intestinal portions are composed of a great diversity of bacteria, fungi, archaea,
107 and viruses, influenced by internal and external factors that modulate their composition and
108 function (Moraes *et al.* 2014), which can play a role in hormone production and subsequent
109 fertility (Wijdeveld et al., 2020). These have revealed that the microbiota can affect behaviors,
110 including feeding and modulating digestive/absorptive processes, metabolism, and immune
111 response, with repercussions on energy, homeostasis, health, and reproduction of hosts
112 (Wijdeveld et al., 2020).
113 An increase in size and number of ruminal papillae and on development of intestinal
114 epithelium villi and crypts were observed in response to butyrate supplementation in newborn
115 calves (Hill et al., 2007). Therefore, favoring the digestibility of food, improvement of the
116 intestinal absorptive area, better fecal consistency, and the growth of the animals were
117 reported (Hill et al., 2007). In addition, the increase in pancreatic juice production, anti-
118 secretory and anti-inflammatory effects in the intestine, the influence on the somatotropic
119 axis, and energy metabolism have been observed in response to the supply of sodium butyrate

120 in the liquid diet of cattle in the initial phases of the development (Guilloteau et al., 2009;
121 Canini et al., 2011; Gorka et al., 2018).
122 Although the influence of butyrate on the development of the gastrointestinal tract has
123 already been reported in several studies (Guilloteau et al., 2009; Górka et al., 2011), a
124 possible preventive action in cases of neonatal diarrhea associated with its use as a food
125 supplement, has not yet was investigated. We hypothesized that in addition to promoting
126 gastrointestinal development, butyrate might act on preventing diarrheal conditions,
127 contributing to the growth-promoting effect.
128 The present study aimed to evaluate the effect of continuous administration of butyrate
129 in the liquid diet of dairy calves on diarrhea, metabolic profile, gastrointestinal development,
130 and corporal growth.
131
132 **METHODOLOGY**
133
134 ***Experimental Design***
135
136 All procedures in this study were approved by the Animal Ethics and Experimentation
137 Committee of the Federal University of Pelotas (nº CEUA 9466-2020). The study was
138 conducted on a commercial farm with an intensive milk production system in Rio Grande -
139 RS, Brazil (32º 16 'S, 52° 32' E). 124 Holstein animals were used for the study (100 females
140 and 24 males). Soon after birth, identification was performed with earrings, and the animals
141 were housed in individual pens containing rice husk bedding. Four liters of colostrum with a
142 quality equal to or greater than 25 degrees Brix were provided for the calves within the first
143 12 hours after birth. Also, 10% iodine was applied to the umbilical twice daily for the first 3
144 d. After that, the animals were transferred to elevated wooden hutches and raised individually
145 until 90 d, fed daily with six liters of milk, and with access to water and a solid diet *ad*
146 *libitum*. The milk supplied had a dry matter average of 12%. Dry matter content (g/kg)
147 analyzed chemical composition (g/kg dry matter), and the guarantee levels of the solid ration
148 provided during the experiment are shown in Supplemental Table S1.
149
150 ***Experimental groups***
151
152 The 124 animals were homogeneously randomized into two groups by birth order and
153 weight. The Butyrate Group (BG) (50 females and 12 males, n = 62) received daily, a

154 commercial product containing 90% of sodium butyrate (Admix Easy® - Adisseo) added to
155 the milk, throughout the preweaning period, following the manufacturer's recommendation
156 (4g/day, 0.2% of total DM). The Control Group (CG) (50 females and 12 males, n = 62)
157 consisted of animals that received only milk without any additive.
158 The failure of passive immunity transfer (FPT) was used to exclude animals. Between
159 24 and 48 h of life, blood was collected by jugular venipuncture using a Vacutainer System
160 (BD Diagnostics, São Paulo, Brazil) and tubes containing EDTA. The whole blood was
161 centrifuged to obtain and analyze the total plasmatic protein concentration, and values of at
162 least 5.5 g/dL of PPT (corresponding to 1 g/dL of serum immunoglobulin G) were used as a
163 cut-off point (Tyler et al., 1996)
164
165 ***Feces score evaluation***
166
167 Feces consistency was determined daily up to 30 d of age (n = 100), using the
168 following score: 0 (normal), 1 (loose), 2 (watery), 3 (profuse diarrhea with liquefied), and 4
169 (profuse diarrhea with liquefied and bloody feces). Based on the feces score, animals with
170 values equal to or above 2 (2 to 4) were considered to have diarrheal wastes (McGuirk, 2008).
171 Also, other possible clinical changes, such as fever or dehydration (Radostits et al., 2006),
172 were considered for diagnosing diarrhea. The day diarrhea ended was considered the day at
173 the animal again presented feces scores of 0 or 1.
174
175 ***Disease monitoring during the preweaning period***
176
177 The females were monitored up to 90 d of age for the occurrence of diarrhea,
178 determining: morbidity (number of animals that became ill /total number of animals in the
179 experiment), mortality (number of animals that died / total number of animals), lethality
180 (number of animals that died from diarrhea/number of animals that had diarrhea) and
181 recurrence (number of animals that became sick twice or more during the period considered).
182 Diarrhea in males was also monitored until 15 and 30 d of age.
183 The occurrence of respiratory diseases, as well as mortality from this disease, was also
184 determined. Furthermore, other neonatal diseases were evaluated, generating the incidence of
185 each condition and the overall mortality (total number of animals that died / total number of
186 animals) (Radostits et al., 2006).
187

188 *Zootechnical evaluations*
189
190 Assessments of weight, thoracic perimeter, height at the withers, and rump width of
191 the animals were performed at birth and weekly until 30 d of age and later at 45, 60, and 90 d
192 of age. The average daily weight gain (ADG) of animals that completed 90 d of age (100
193 animals, minus deaths) was determined through body weight.
194
195 *Collection of tissue fragments from the gastrointestinal tract*
196
197 At 15 and 30 d of age, six males from each group were euthanized to evaluate the
198 development of the gastric and intestinal compartments, totaling 12 animals per group. The
199 performance of euthanasia followed the recommendations of the Federal Council of
200 Veterinary Medicine (CFMV) expressed in Resolution No. 1000, of May 11, 2012. After
201 confirmation of death, rumen-reticulum, omasum, and abomasum were individualized,
202 emptied, washed repeatedly with water, dried, and weighed. Tissues of 1cm^2 were collected in
203 duplicate for histological and molecular analysis. The location of the tissue collection
204 included the sac of the rumen; mid-ventral portion of the abomasum; duodenum (about 10
205 centimeters distal to the pyloric sphincter); jejunum (10 centimeters distal to the duodenal-
206 jejunal ligament); ileum (10 cm proximal to the ileocecal junction), and colon (medial
207 portion) (Liu et al., 2018). All samples were collected in duplicate for histological and
208 molecular analysis.
209 The fragments were fixed in 4% formalin for 48 hours for histological analysis and
210 stored in 70% alcohol. For gene expression analysis, the collected tissues were quickly
211 washed in sterile saline solution at 0.9%, inserted into cryotubes free of DNAse and RNAse,
212 frozen in liquid nitrogen, and subsequently stored at -80 ºC.
213
214 *Histological analyzes*
215
216 The samples were sequentially dehydrated in 80%, 90%, and absolute alcohol, cleared
217 in xylene, and incorporated into paraffin blocks during processing. Thick sections (10 sections
218 of each sample) were taken from each piece with a microtome (RM 2245, Leica Biosystems
219 Nussloch GmbH®, Germany). These were distended on microscopy slides and stained with
220 hematoxylin and eosin to be mounted (Entellan®, Merck, Germany) in coverslips onto
221 microscope slides and later covered with coverslips.

222 Images from slides were captured by a camera (Moticam 5, 5.0 MP, USB, Motic®, China) attached to a microscope (Eclipse E200, Nikon®, Japan). The morphometry of the rumen papillae (length, width, surface, and density) and intestinal villi (height, depth of the crypt, and villus-crypt ratio) were determined in 30 papillae (rumen) and 30 villi with corresponding crypts (intestine) using the image analysis of the software (Motic Images Plus 2.0, Motic®, China). The length of the ruminal papilla was measured from the apex to the base, while the width was in the middle of the papilla (Hofmann and Schnorr, 1982). Density was evaluated with a magnifying glass (SM45TR, Physis®) attached to the video camera (Moticam 5, 5.0 MP, USB, Motic®, China) and determined further using the Image J (Image J 1.44 software, National Institutes of Health, Bethesda, USA). The surface of the papillae per cm^2 was determined as length × width × 2. In the intestine, the villus height was measured from the villus tip to the villus-crypt interface, while the crypt depth was calculated from the villus-crypt to the lamina propria opening at the base of the villi (Schaff et al., 2018).

235

236 ***Gene expression analysis***

237

238 After histological evaluation, it was observed that the main morphometric changes in response to butyrate supplementation occurred in the duodenal tissue at 30 d of life. Thus duodenal tissues from animals of the CG and BG, diagnosed with and without diarrhea during the neonatal period (n = 3 animals per group), had transcripts levels for the genes Glucagon-like peptide 2 (*GLP2*), Lactase (*LCT*), Insulin-like growth factor 1 (*IGF1*), Solute Carrier Family 5 Member 1 (*SLC5A1*), Aquaporin 3 (*AQP3*), Ghrelin (*GHRL*), Pro-opiomelanocortin (*POMC*) and Peptide YY (*PYY*) analyzed (Supplemental Table S2).

245 RNA extraction was performed using the PureLink® kit (Invitrogen, Carlsbad, USA), with minor adaptations to the manufacturer's instructions (30 mg of tissue, 600 µL of lysis buffer, and final elution with 30 µL in DEPC water). Total RNA quantification was performed using a NanoVue™ spectrophotometer (GE Healthcare, Chicago, IL, USA). Through the absorbance ratio of 260/280 nm, a purity index of 2.0 to 2.04 was observed in all samples. Complementary DNA (cDNA) synthesis was conducted using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, Massachusetts, USA) with an input of 1 µg of total RNA, to a final volume of 20 µL, following the manufacturer's instructions.

254 Real-time PCR was performed in the StepOne system (Applied Biosystems, Waltham, Massachusetts, USA), using 1 µL of pure cDNA, qPCR Mix, and CXR reference probe

256 (GoTaq qPCR MasterMix, Promega, Wisconsin, Madison, USA), mixed to specific primer
257 pairs (Exxtend, Paulínia, São Paulo, Brazil) with 0.25 µM concentration. The final volume of
258 the qPCR reaction was 12.5 µL, and all reactions were performed in duplicate. Thermocycling
259 conditions followed the manufacturer's instructions.

260 The differences in the gene expression levels of duodenal samples from groups BG,
261 CG, diarrheal BG, and non-diarrheal CG at 30 d of age (n = 3 animals per group) were
262 determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The relative expression of
263 the target genes was normalized with the *RN18S1* gene (Table S2).

264

265 ***Blood collections and metabolic assessments***

266

267 Blood collections were performed through jugular venipuncture on d 1, 8, 15, 22, and
268 29 from 20 animals in each group. Tubes without anticoagulants were used to assess serum
269 levels of total calcium, phosphorus, chloride, bicarbonate, BHBA (beta-hydroxybutyrate), and
270 NEFA (non-esterified fatty acids), and tubes with potassium fluoride for lactate and glucose
271 assessment. Immediately after collection, blood samples were centrifuged at 3,500 rpm for 10
272 minutes to obtain serum and plasma. Subsequently, they have placed in 1.5 ml Eppendorf-
273 type microtubes (in duplicate) and frozen. The collected serum and plasma samples were
274 evaluated using Plenno enzyme kits (Labtest Diagnóstica, MG, Brazil) in a Labmax Plenno
275 automatic biochemical analyzer (Labtest Diagnóstica, MG, Brazil).

276

277 ***Statistical analysis***

278

279 All data were normal (F>0.90) using the Shapiro-Wilk distribution test. Continuous
280 variables with multiple collections such as zootechnical evaluations, gastrointestinal
281 morphometries, and metabolic parameters were evaluated using the GLIMMIX model,
282 considering the animal, group, collection time, and interactions. Continuous variables with a
283 single collection, such as the ADG, were analyzed using the T-test. Categorical variables were
284 evaluated using the chi-square test. All the above analyses were performed in the SAS
285 statistical program (JMP Pro 14.0, SAS Institute Inc., Cary, NC, USA). The results of
286 differential gene expression, considering the group and the occurrence of neonatal diarrhea,
287 were obtained by two-way analysis of variance (Two-way ANOVA) using the GraphPad
288 Prism v.8 software (San Diego, CA, USA). P values < 0.05 were considered statistical
289 difference and p values > 0.05 and < 0.1 considered trends.

RESULTS

Occurrence of disease and mortality

When comparing the averages of diarrhea morbidity, considering the total preweaning period (90 d), a difference of 20% was observed between the Control and Butyrate groups (CG =50% vs. BG = 30%, $p=0,04$). There was also a lower recurrence of cases in BG compared to Control (CG = 60% vs. BG = 26.67%, $p=0,04$). The comparison results between morbidity, mortality, lethality, and disease recurrence rates can be seen in Table 1.

Feces score

The feces score was used to characterize the time that the calves of each group remained diarrheic (score ≥ 2) during the first 30 d of life. As a result, the butyrate group showed a reduction in d with diarrhea ($p < 0.001$) compared to the Control group (Figure 1).

Zootechnical evaluation

In the zootechnical evaluations, there was a tendency ($p = 0.07$) towards a higher ADG when considering the neonatal period, up to 28 d, and a trend ($p = 0.09$) from 0 to 60 d. In the other periods, the ADG of the Butyrate group was equivalent to that of the Control group ($p > 0.1$) (Table 3). The other zootechnical parameters of croup width, thoracic perimeter, and height at the withers were not influenced by butyrate supplementation in the calves' diet ($p > 0.1$) (Table 3).

The achievement of twice the birth weight at 60 d and at 90 d was evaluated. Twice birth weight is the index used as a parameter for weaning. At 60 d of age, there was a tendency ($p = 0.06$) for a higher percentage of animals in the Butyrate group to reach twice their birth weight than in the Control group (25% vs. 10.64%). The property weaned most of the animals at 90 d of age, and at this moment, 93.75% of the animals in the Butyrate group and 91.3% of the Control group presented double or more than birth weight, with no difference between groups ($p > 0.1$).

Morphometric assessments of the gastrointestinal tract

324

325 The histological evaluations aimed to measure the development of the gastrointestinal
326 tract. Size, density per cm^2, width, area of the ruminal papillae, villi size, and depth of the
327 crypts in the duodenum, jejunum, and ileum were evaluated. The results with *p* values for the
328 difference between groups, age, and interaction, are shown in Table 4 and Figure 2.
329 In the rumen, a greater papilla length ($p < 0.01$) was observed in BG (560.92 ± 10.28
330 µm) compared to CG (388.67 ± 10.28 µm) at 30 d of life (Figure 2A), which reflected in the
331 largest area of papilla (BG 0.276 ± 0.008 vs. CG 0.184 ± 0.008 mm2, $p < 0.01$) (Figure 2C).
332 The density of papillae per cm^2 was also higher in BG (206.33 ± 3.28) compared to CG
333 (163.17 ± 3.28; $p < 0.01$). As for the width of the papillae, there were no differences between
334 the groups ($p > 0.05$), only between the ages (Figure 2B).
335 In the small intestine portion, a difference was observed between the groups only in
336 the duodenum portion, where the villus length in BG was 488.14 ± 4.76 µm and 446.87 ± 4.76
337 µm in the CG, being statistically higher in the BG ($p < 0.01$) at 30 d of life (Figure 2D). The
338 same was observed for the crypt depth, which was 254.96 ± 2.75 µm in BG versus 231.32 ±
339 2.75 µm in CG ($p < 0.01$) (Figure 2E).
340 Regarding the total weight of the gastric compartments (reticulum, rumen, omasum,
341 and abomasum), there were no differences between the groups (CG = 690.25 ± 24.14g vs. BG
342 = 733.5 ± 24.14g, p = 0, 22).
343

344 ***Gene expression analysis***

345

346 The relative expression of *IGF1*, *LCT*, and *GCG* genes in the duodenum are shown in figure 3,
347 and the relative expressions of the SLC5A1, *AQP3*, *POMC,* and *PYY* genes, are shown in figure
348 4.

349

350 ***Metabolic Assessments***

351

352 In the metabolic evaluations, a higher concentration of glucose ($p<0.01$) was observed
353 in the control group in relation to butyrate when observed in all analyzed periods, whereas the
354 serum concentration of NEFA was higher ($p<0.01$) in the butyrate (figure 5). The other
355 analyzed markers showed no difference between the groups. Figure 6 shows the results of
356 Glucose and NEFA, also considering the occurrence or not of diarrhea during the period.

357

358 ## DISCUSSION

359

360 In our study, sodium butyrate supplementation in the liquid diet reduced morbidity and
361 diarrhea recurrences, similar to that previously found by Hill et al. (2007) and Górka et al.
362 (2011). This finding can be attributed to a possible antibacterial effect provided by sodium
363 butyrate. According to Cherrington (1991), butyrate changes electrochemical gradients and
364 reduces intestinal pH, affecting the colonization of pathogenic gram-negative bacteria, the
365 leading cause of diarrhea (Drackley et al., 2008).
366 A shorter period with abnormal feces was also observed, similar to Hill et al. (2007).
367 The explanation may be related to a possible reduction in the infective pressure of pathogenic
368 bacteria at the gastrointestinal level in response to supplementation (Guilloteau et al., 2010).
369 Another reason could be related to the earlier development of the rumen and duodenum
370 observed in the histological evaluation of BG. Better use of the food and consequently
371 improved metabolic condition, allowing a more significant energy intake, enabling an
372 accelerated recovery from diarrhea (Guilloteau et al., 2010). As well as a reduction in
373 intestinal permeability (Wu et al., 2022)
374 Our results suggest that despite sodium butyrate not passing through the rumen, it
375 indirectly enhanced rumen papillae's earlier development in GB. These results agree with
376 Guilloteau (2010) and Górka (2011). They associated butyrate supplementation with an
377 increase in the secretion of peptides and hormones from the gastrointestinal tract, which could
378 explain the development of the rumen, even with the predominance of a liquid diet.
379 The greater villi length and crypt depth increase the absorption surface area.
380 Consequently, there is greater use of the ingested nutrients, as previously reported in other
381 studies by Guilloteau et al. (2010), Frieten et al. (2017), and Gerbert et al. (2017) with
382 butyrate supplementation in bovine diets during pre-weaning. This effect can also explain this
383 more remarkable development in appetite and greater consumption observed by Mccurdy et
384 al. (2019). Still, unfortunately, this was not evaluated in our study.
385 In our study, at 30 d of life, villi development was remarkable in the duodenum due to
386 butyrate supplementation. Also, the IGF1 gene, which regulates growth, was less expressed in
387 calves supplemented with butyrate than in the Control group. Significant development of
388 intestinal villi and lower expression of IGF1 in calves supplemented with butyrate was also
389 reported by Koch et al. (2019). These results suggest that proliferation and development in
390 this tissue are probably not mediated by the IGF1 gene.

391 The lactase gene (LCT) is related to consuming a diet rich in lactose since it encodes
392 the lactase enzyme, which is part of the intestinal secretion of young mammals and essential
393 for the digestion of milk (Le Huerou et al., 1992). In addition, the increase in the depth of the
394 intestinal crypts, an indicator of intestinal maturation, is related to a reduction in lactase
395 activity, occurring gradually, from birth to weaning. The highest levels of transcripts for the
396 LCT gene in our study were observed in the GC with diarrhea, composed of animals with
397 neonatal diarrhea and not supplemented with butyrate. This result contrasts with the much
398 lower expression pattern of animals diagnosed with diarrhea from GB that, despite having
399 suffered from neonatal diarrhea, had levels of LCT transcripts similar to those of the control
400 CG that have no diarrhea in the first 30 d of life. Considering the importance of lactase in
401 milk digestion (Le Huerou et al., 1992), the reduced expression of LCT found at 30 d of life in
402 calves may indicate a reduction in the demand for intestinal lactase, proportional to the
403 development of the gastrointestinal tract and the consumption of solid foods (Nicola et al.,
404 2022). Unfortunately, as stated above, evaluating individual feed consumption in the present
405 study was not possible. Still, the results suggest a predominance of solid diet consumption
406 over a liquid diet, both in the CG that did not have diarrhea and in the BG that had neonatal
407 diarrhea.
408 The GLP2 gene encoding the hormone GLP-2 is related to tissue development and
409 repair (Burrin et al., 2005). When administered exogenously in healthy animals of
410 monogastric species, this hormone promotes intestinal epithelium growth by increasing villi
411 and crypts, reducing epithelial cell apoptosis, and improving gut blood flow, nutrient
412 absorption, and epithelial barrier function. Also stimulating intestinal blood flow, and
413 producing anti-inflammatory effects, regardless of their cell proliferation actions (Dubé &
414 Brubaker 2007).
415 The pattern of GLP2 gene expression in the present study was very similar to that
416 found for the LCT gene. Both were higher in the group with neonatal diarrhea, differing from
417 the pattern of the animals with diarrhea from BG, which presented gene expression values
418 similar to those of animals that did not have diarrhea in the neonatal period. Despite the role
419 of GLP-2 in normal intestinal function in monogastric species, its functional role in ruminants
420 has not been well investigated and remains poorly understood (Connor et al., 2010). Our
421 results indicate a positive correlation between the LCT and GLP2 genes. Considering what
422 has already been discussed about lactase, the animals in the groups with and without neonatal
423 diarrhea, supplemented with butyrate, had more remarkable intestinal development at 30 d
424 and therefore would consume a predominantly solid diet. In this case, these two genes seem to

425 act synergistically as markers of intestinal immaturity since their expression is positively
426 correlated with milk consumption. Furthermore, recent studies have suggested that GLP-2 has
427 a protective effect on intestinal permeability and the subsequent infiltration of endotoxins
428 (Kvidera et al., 2017). This may explain the high expression in the Control group with
429 neonatal diarrhea. Also, in this sense, considering that the high GLP2 expression is due to a
430 demand for local tissue repair caused by the inflammatory process and increased intestinal
431 permeability in the animals that had diarrhea, in the group supplemented with butyrate, the
432 reduced expression of GLP2 may suggest that the demand has been smaller.

433 Since our study found that the GB animals remained with diarrheal feces for fewer
434 days, it is possible that the increase in intestinal permeability and the local inflammatory
435 stimulus in this group were minimal. The low expression of GLP2 on day 30 of life suggests
436 that tissue repair after diarrhea took place in a shorter period in GB, leading this group of
437 animals to have a status in terms of gene expression equivalent to that of animals that crossed
438 the entire neonatal period in the absence of diarrhea (Nicola et al., 2022).

439 The SLC5A1 gene is involved in transporting glucose from the intestinal lumen to the
440 body (Wright et al., 2013). Our findings indicate that its higher expression was stimulated by
441 a compensatory effect on the occurrence of diarrhea, unlike other studies (Rosa et al., 2018),
442 which found a negative regulation of diarrhea on gene expression. This may have occurred in
443 our study, as the collections were not carried out on the day of diarrhea. Still, at 30 days of
444 life, when the conditions were no longer in the clinical course, the gene expression was higher
445 due to the possible more tremendous need for energy to restore intestinal tissues damaged by
446 the disease.

447 In the same way as the SLC5A1, the AQP3 expression was greatly affected by the
448 occurrence of diarrhea. The AQP3 gene is a stimulator of forming aquaporins, channels
449 responsible for transporting water through the intestinal wall (Rojek et al., 2008). In our study
450 was downregulated in the animals that have neonatal diarrhea. This negative regulation was
451 previously found in rats with colitis by Zhao et al. (2014) and in calves with mild
452 inflammatory diarrhea by Rosa et al. (2018), indicating a reduced water exchange between the
453 intestinal lumen and the epithelium in this case.

454 The POMC and PYY genes control satiety (Schwartz et al., 2000; Batterham et al.,
455 2002). POMC was not affected by butyrate supplementation nor by diarrhea. In contrast,
456 PYY, which reduces the appetite, was downregulated by butyrate in animals without diarrhea
457 and upregulated in animals with diarrhea. Based on our results, butyrate reduces satiety in
458 healthy animals and increases satiety in animals with diarrhea.

459 The GHRL gene, on the other hand, encodes the hormone ghrelin, the hunger
460 hormone, stimulating appetite (Romero, 2006). The gene was more expressed in the GC
461 without diarrhea, indicating that butyrate reduced hunger in healthy animals but was not
462 affected in animals with diarrhea.

463 A lower serum glucose concentration in the GB was also found in the study by Hatew
464 (2019), who observed, in addition to the glucose reduction, an increase in insulin
465 concentrations, the hormone responsible for stimulating the entry of glucose into the cells,
466 thus justifying the lower concentration. Allied to this, the concentration of NEFA was higher
467 in the GB, possibly due to a greater energy demand generated by the superior growth of the
468 body, papillae, and villi, observed in the GB. Or also by a compensatory reflex of the lower
469 glucose concentration to meet the greater energy demand for growth and development
470 required by GB animals. However, this higher concentration of NEFA was not enough to
471 generate a change in BHBA that could harm the body. In addition, although different between
472 groups, the parameters remained within the physiological levels of the species (Yu et al.,
473 2019), and the other analyzed markers did not differ, indicating that butyrate supplementation
474 did not negatively affect mineral metabolism and the acid-base balance.

475 Considering whether the animals presented diarrhea or not (figure 6), the glucose
476 concentration was only lower in the GB without diarrhea, indicating that in the animals that
477 passed through diarrhea, the insulin stimulation by butyrate may be reduced or a
478 compensatory effect. The highest expression of the SLC5A1 gene in duodenal tissue at 30
479 days of life may have improved glucose uptake and contributed to enhancing glucose
480 circulating levels in GB animals.

481 Concerning the growth of the animals, a tendency for more significant weight gain
482 was identified at 30 and 60 days of life, and a more substantial number of animals with twice
483 the weight at 60 days of life was determined. These findings are quite contradictory in some
484 studies, and Kato et al. (2011), Araújo et al. (2015), and Frieten et al. (2017) found no
485 difference between supplemented and non-supplemented groups. However, similar to our
486 results, Hill et al. (2007) and Guilloteau et al. (2009) observed a more significant weight gain
487 in animals, which can be explained in these studies and ours by better use of nutrients made
488 possible by better ruminal and duodenal development and by the lower occurrence and
489 accelerated recovery of diarrhea.
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CONCLUSION

Overall, the results allow us to conclude that supplementation with butyrate in the liquid diet improved the development and maturation of rumen and duodenum, reduced morbidity, recurrence, and days with diarrheal feces in dairy calves in the preweaning period. These results indicate that supplementation with butyrate stimulates the development of the gastrointestinal tract and reduces the losses associated with diarrhea, reflecting a trend towards better average daily weight gain. Further studies evaluating solid feed intake may help determine whether the mechanism behind the growth and development of dairy calves supplemented with butyrate improves feed efficiency or increased feed intake.

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699 **Table 1:** Morbidity, mortality, lethality, and disease recurrence rates, from birth to weaning, in
700 calves fed with milk supplemented or not with butyrate.

Parameter	groups		p-value
	Control	butyrate	
Morbidity from neonatal diarrhea (%)**	36% (18/50)	26% (13/50)	0.28
Diarrhea morbidity (%)*	50% (25/50)	30% (15/50)	0.04
Respiratory disease morbidity (%)*	74% (37/50)	76% (38/50)	0.82
Morbidity from other diseases (%)*	20% (10/50)	20% (10/50)	1.00
Mortality from diarrhea (%)*	2% (1/50)	0% (0/50)	0.50
Mortality from respiratory disease (%)*	2% (1/50)	4% (2/50)	0.38
Overall mortality (%)*	6% (3/50)	4% (2/50)	0.32
Diarrhea lethality (%)*	4% (1/25)	0% (0/15)	0.62
Diarrhea recurrence (%)*	60% (15/25)	26.7% (4/15)	0.04

701 **First 30 days of life. * 90 days of life.
702 shows a significant statistical difference ($p < 0.05$).
703

704 **Table 2:** Mean daily weight gain (ADG) (in kg) of animals supplemented or not supplemented
705 with butyrate during lactation, broken down by period (n = 95).

Parameter	groups				p-value
	Control		butyrate		
	Mean	EPM[1]	Mean	EPM[1]	
ADG up to 8 d	0.340	0.036	0.401	0.028	0.17
ADG from 8 to 15 d	0.364	0.040	0.417	0.035	0.33
ADG from 15 to 22 d	0.477	0.040	0.548	0.039	0.22
ADG from 22 to 29 d	0.492	0.043	0.524	0.050	0.63
ADG from 29 to 45 d	0.533	0.043	0.551	0.030	0.66
ADG from 45 to 60 d	0.740	0.030	0.761	0.029	0.67
ADG from 60 to 90 d	0.835	0.037	0.798	0.035	0.50
ADG from 0 to 29 d	0.394B	0.018	0.441A	0.018	0.07
ADG from 0 to 60 d	0.516B	0.016	0.555A	0.016	0.09
ADG from 29 to 90 d	0.743	0.024	0.727	0.024	0.64
ADG during preweaning	0.627	0.019	0.632	0.017	0.84

706 [1]Standard error of mean A, B Different letters indicate trend P<0.1

707 **Table 3:** Zootechnical parameters of calves fed with milk supplemented or not with butyrate
708 for 90 days of life (n = 95).

Parameter	Groups				p-value		
	Control		butyrate		Group	Day	Group*Day
	Average	EPM[1]	Average	EPM[1]			
Thoracic Perimeter[2]	85.09	0.39	85.63	0.39	0.33	<0.001	0.99
Height at withers[2]	82.67	0.35	82.67	0.34	0.99	<0.001	0.59
Croup width[2]	21.80	0.13	22.04	0.13	0.18	<0.001	0.73

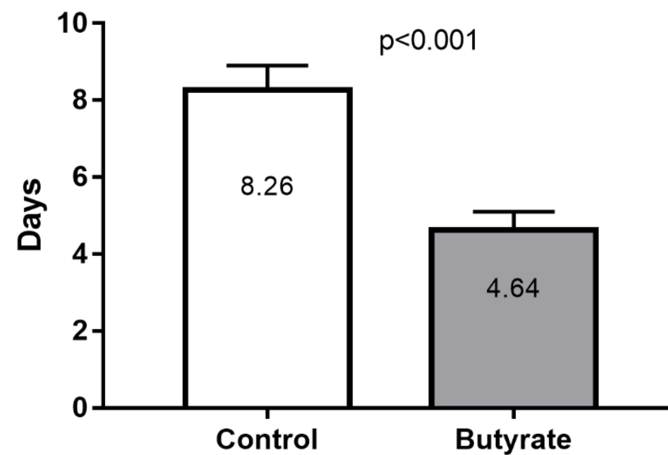
709 [1]Mean standard error
710 [2] Mesures in centimeters
711

712 **Table 4:** Rumen and intestinal development parameters of calves fed milk supplemented or not
713 with butyrate.

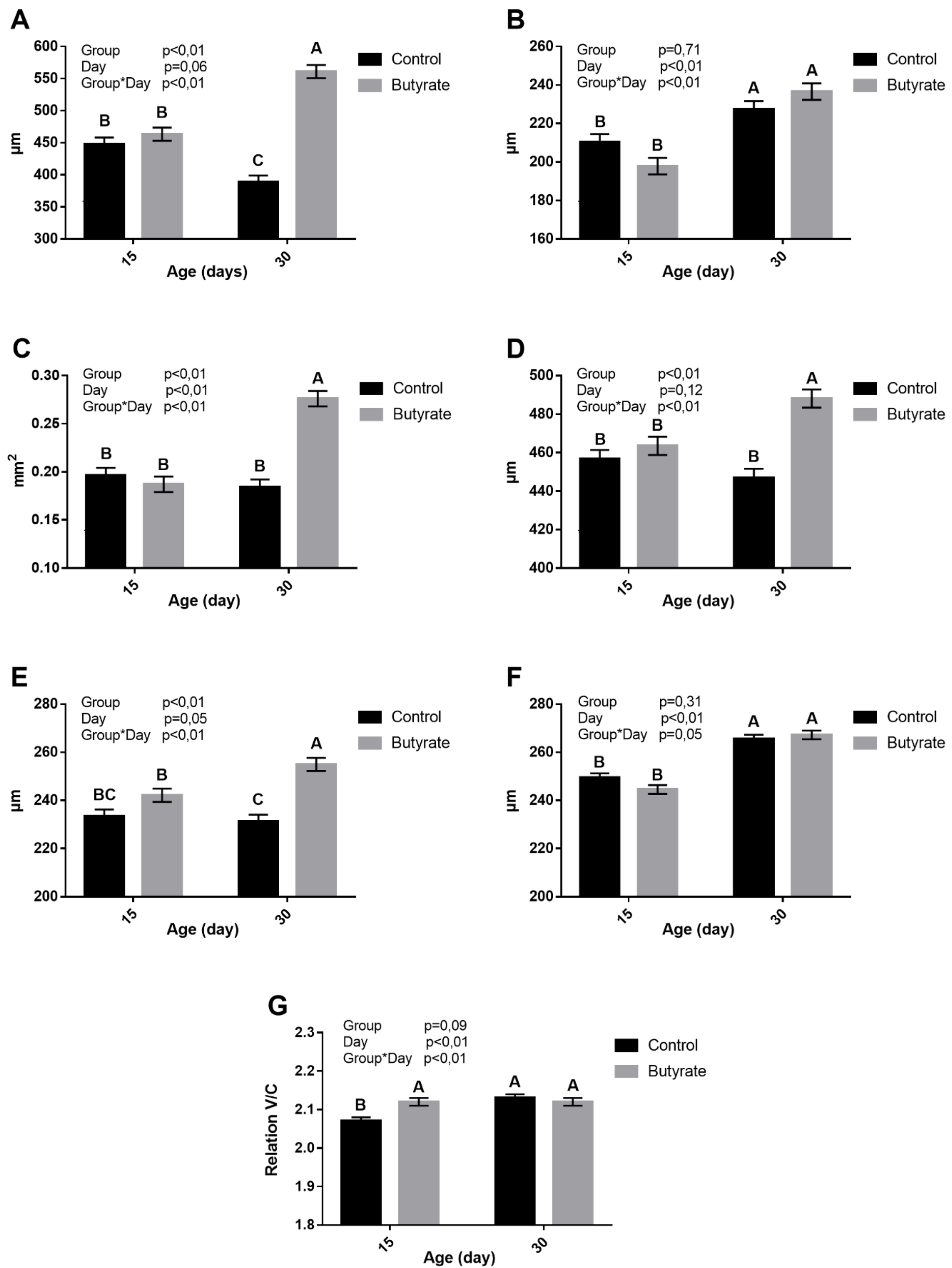
Gastrointestinal Portion	Group			p-value		
	Control	butyrate	EP[1]	Group	Age	Group x Age
Rumen						
Papilla Length (µm)	418.25[b]	512.15[a]	7.27	<0.01	0.06	<0.01
Papilla width (µm)	218.81	217.20	3.04	0.71	<0.01	0.01
Area per papilla (mm2)	0.190[b]	0.232[a]	0.006	<0.01	<0.01	<0.01
Papillae Density / cm2	180.50[b]	223.67[a]	2.32	<0.01	<0.01	1.0
Duodenum						
Villus Length (µm)	451.73[b]	475.86[a]	3.37	<0.01	0.12	<0.01
Crypt Depth (µm)	232.40[b]	248.54[a]	1.94	<0.01	0.05	<0.01
Villus/crypt relationship	1.96	1.94	0.01	0.16	0.55	0.60
Jejunum						
Villus Length (µm)	540.31	541.02	2.68	0.85	<0.01	0.52
Crypt Depth (µm)	257.70	255.91	1.26	0.31	<0.01	0.05
Villus/crypt relationship	2.10	2.12	0.01	0.09	0.01	<0.01

Ileum						
Villus Length (µm)	517.16	519.37	2.18	0.47	<0.01	0.06
Crypt Depth (µm)	247.22	246.35	1.01	0.54	0.65	<0.01
Vilo/crypt relationship	2.10	2.11	0.01	0.06	<0.01	0.27

714 [1]Mean standard error
715 Different letters on the same line indicate the statistical difference.

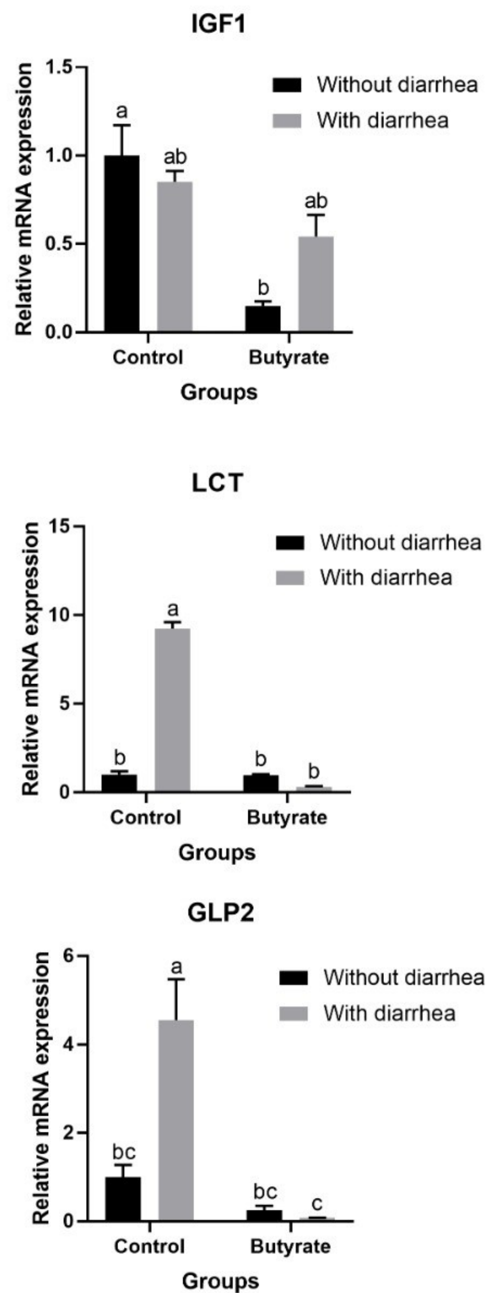


716
717 **Figure 1.** Average days with abnormal feces* in dairy heifers supplemented or not with
718 butyrate during the first 30 days of life (n = 100).
719 *Feces were considered abnormal when scores ≥2 were identified on the following scale: 0
720 (normal feces), 1 (loose feces), 2 (watery feces), 3 (profuse diarrhea with liquefied feces), and
721 4 (profuse diarrhea with liquefied feces and bloody content).



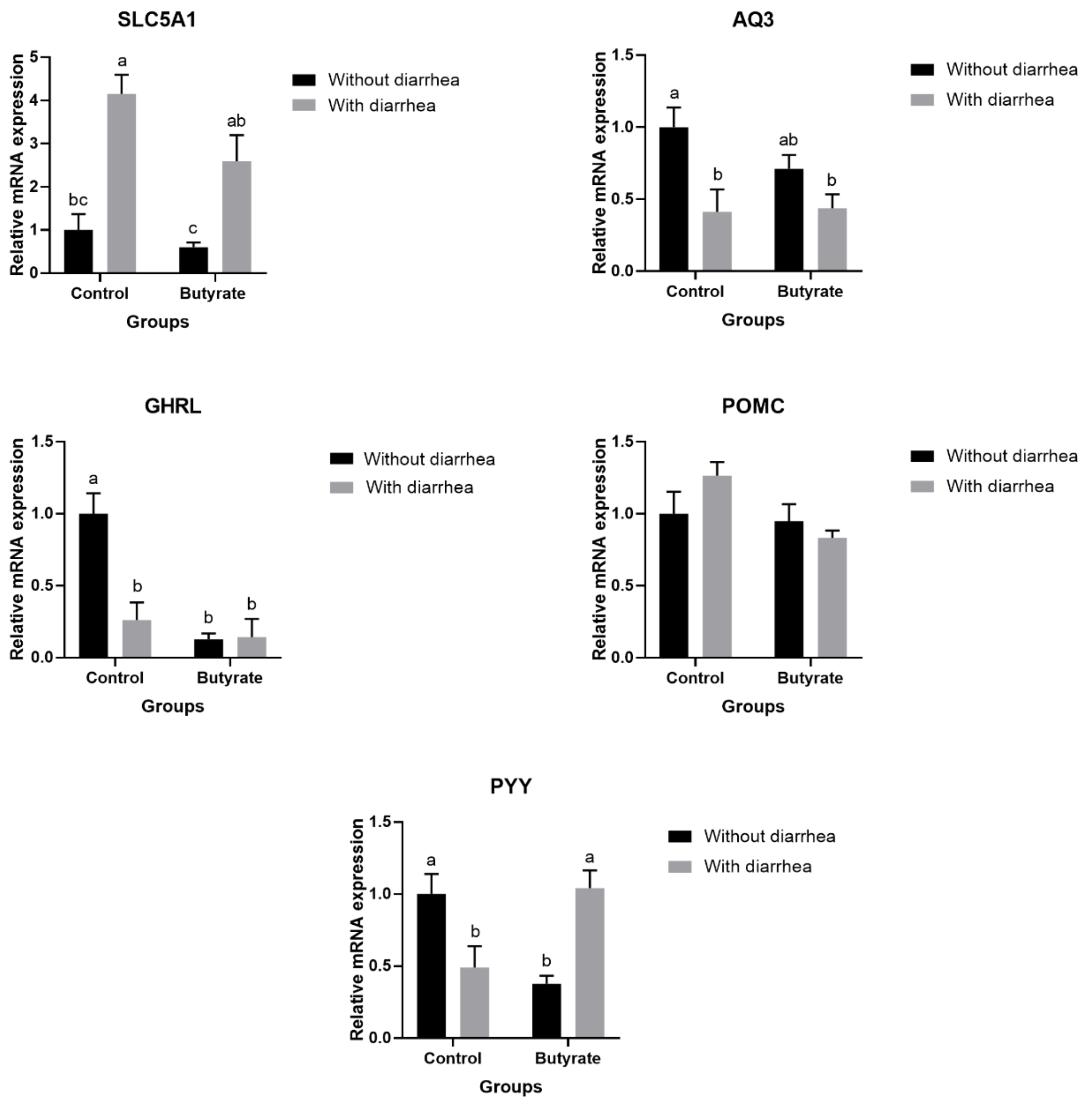
722

723 **Figure 2. Evaluation of the gastrointestinal development of calves fed milk**
724 **supplemented with butyrate (Butyrate) or not (Control)**. A (ruminal papilla length); B
725 (ruminal papilla width); C (Ruminal papilla area); D (Duodenal villus length); E (Duodenal
726 crypt depth); F (Jejunal crypt depth); G (Villus/jejunal crypt ratio).



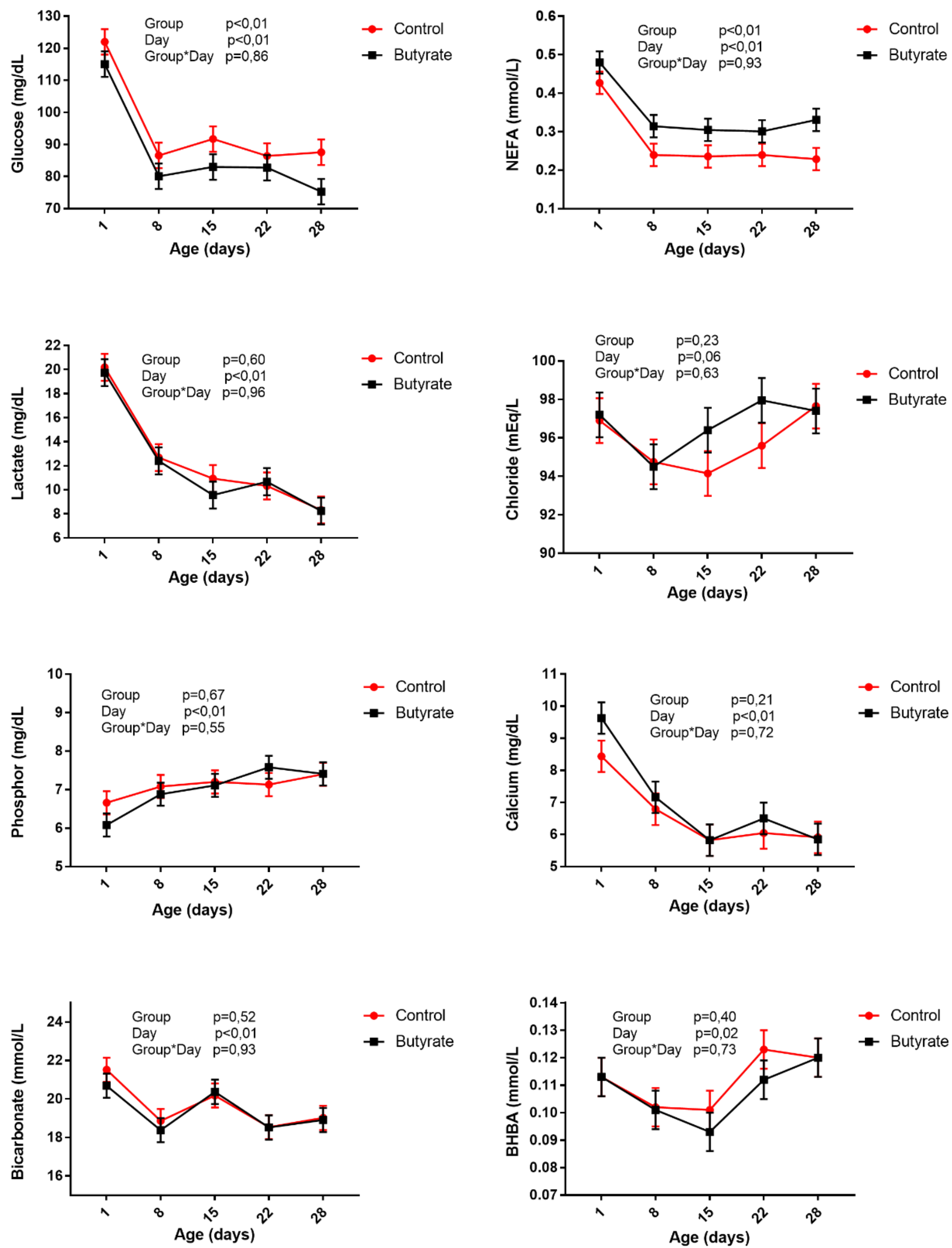
727

728 **Figure 3.** Relative expression of *IGF1*, *LCT*, and *GLP-2* transcripts in the duodenum of
729 calves at 30 days of age. Groups of animals fed with milk (Control) or milk supplemented
730 with butyrate (Butyrate) were diagnosed with and without diarrhea during the neonatal period.



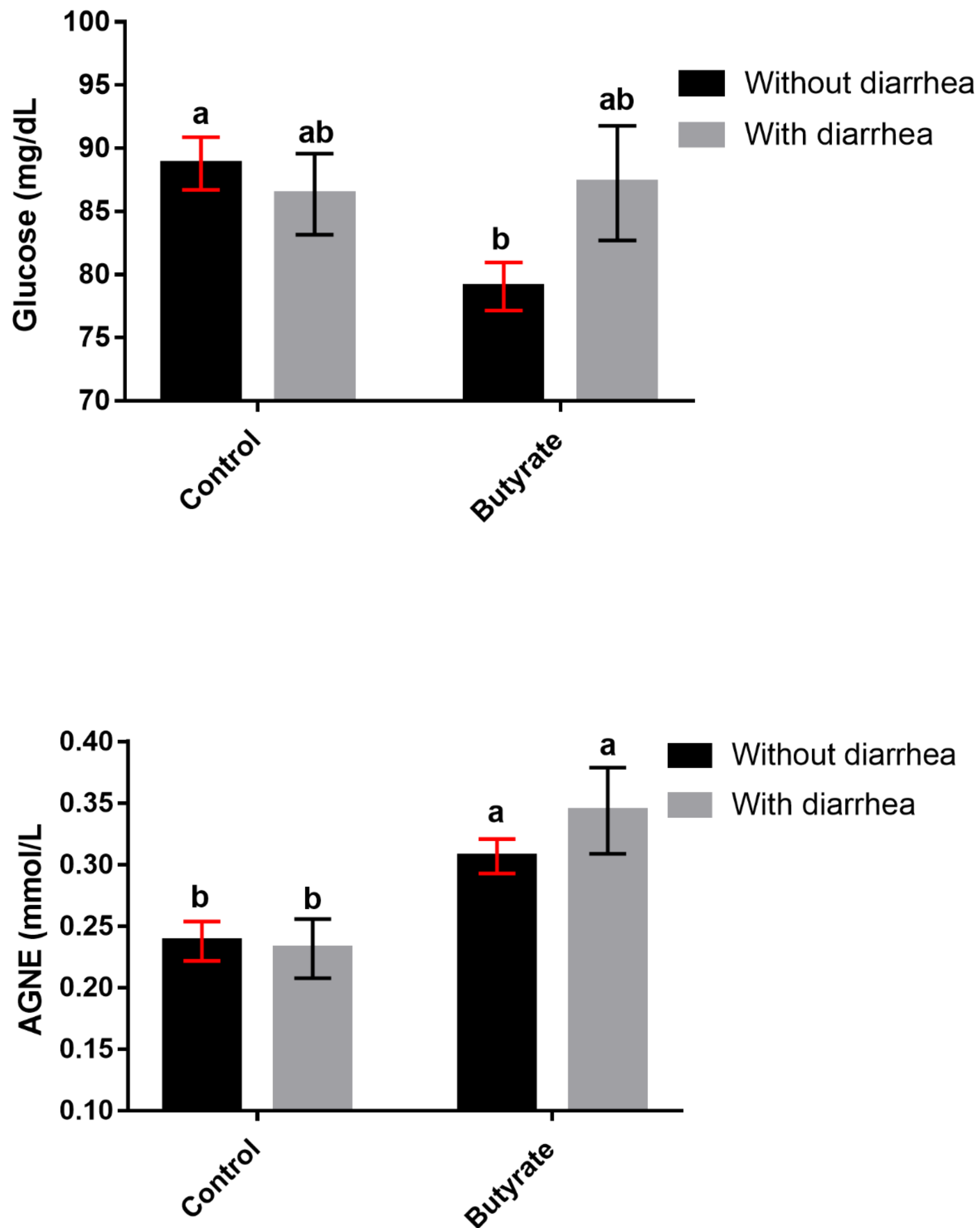
731

732 **Figure 4.** Relative expression of transcripts from SLC5A1, AQP3, POMC, and PYY in
733 the duodenum of calves at 30 days of age. Groups consisting of animals fed with milk
734 (Control) or milk supplemented with butyrate (Butyrate), diagnosed with and without
735 diarrhea during the first 30 days.



736

737 **Figure 5.** Evaluation of the serum metabolic profile (glucose, NEFA, lactate, BHBA,
738 calcium, phosphorus, chloride, bicarbonate) of calves fed with milk supplemented with
739 butyrate (Butyrate) or not (Control).



740

741 **Figure 6.** Evaluation of the serum metabolic profile (glucose, NEFA) in dairy heifers during
742 the first 30 days of life. Groups of animals fed with milk (Control) or milk supplemented with
743 butyrate (Butyrate) were diagnosed with and without diarrhea during the neonatal period.

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748 **Supplementary Material**

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750 **Supplemental Table S1.** Dry matter content (g/kg), analyzed chemical composition (g/kg dry
751 matter) of the solid diet fed during the experimental period (90 days)

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Ingredients	Level of Warranty
Estimated TDN	740 g/kg
raw fiber	60 g/kg
ADF	80 g/kg
Crude Protein	200 g/kg
Ethereal Extract	30 g/kg
Mineral Matter	80 g/kg
Calcium	12 g/kg
Phosphor	7.000 mg/kg
Monensin Sodium	30 mg/kg
bacillus cereus	2,5 $x10^8$ CFU/kg
Lactobacillus acidophilus	2 $x10^8$ CFU/kg
Ruminobacter amylophilum	2 $x10^8$ CFU/kg
Saccharomyces cerevisiae	2,3 $x10^6$ CFU/kg

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764 **Supplemental Table S2.** List of analyzed genes and normalizing gene primers with the
765 accession number and forward and reverse primer sequences.

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Gene	Accession number	Sequence 5'→ 3'	Reference
GLP2	NM_173916.2	F: CCGATGGCTCTTTCTCTGAT	(Alam et al., 2012)
		R:TTTTCGTCTGAAGCAACCAG	
LCT	NM_001205787.1	F: AAGGTGCGGTCATCTCCATC	(Koch et al., 2019)
		R: TCAGGGACGCGAGACTTGTT	
IGF1	NM_001077828.1	F: TCGCATCTCTTCTATCTGGCCCTGT	(Pfaffl et al., 2002)
		R: GCAGTACATCTCCAGCCTCCTCAGA	
SLC5A1	NM_174606.2	F: CAGTCAGCACAGAGTGGACAG	(Koch et al., 2019)
		R: AAGAGGGAGACAGCCAGGA	
AQP3	NM_001079794.1	F: GGGTTGTATTACGATGCGATCTG	(Rosa et al., 2018)
		R: AAAGATGCCAGCTGTGCCATTG	
GHRL	NM_174067.2	F: CAGAGGACGAGCTGGAAATC	(Alam et al., 2012)
		R: TCCCAAAGGATGTCCTGAAG	
POMC	NM_174151.1	F: CCTTGTCACGCTGTTCAAAA	(Alam et al., 2012)
		R: AGGCCTTCAGGGTCAACTTT	
PYY	NM_001040587.2	F: AAACGCGACTTTTCAGAAGC	(Alam et al., 2012)
		R: ATGGGGTTGGTGTCTTAGCA	
RN18S1	NR_036642.1	F: CCTTCCGCGAGGATCCATTG	(Rovani et al., 2017)
		R: CGCTCCCAAGATCCAACTAC	

767 *RN18S1* = 18S ribosomal 5; *GLP2* = Glucagon-like peptide 2; *LCT* = Lactase; *SLC5A1* =
768 Solute carrier family 5 member 1; *AQP3* = Aquaporin 3; *GHRL* = Ghrelin and obestatin
769 prepropeptide; *POMC* = Proopiomelanocortin; *PYY* = Peptide YY.
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775 **Supplemental Table S3.** Information required by the Minimum Information for Publication of
776 Quantitative real-time PCR Experiments (MIQE) to ensure quality control.
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Item to check	Importance*	
		Nucleic acid extraction
Source of additional reagents used	D	TRI Reagent® BD (Sigma Aldrich)
Contamination assessment (DNA or RNA)	E	Reverse transcription controls (without enzyme) were performed to assess the absence of DNA in the RNA sample. For that purpose, RNA was processed as a standard sample in the RT step, except that no reverse transcriptase was added to the reaction mixture (see "complete reaction conditions" in Reverse Transcription).
Nucleic acid quantification	E	RNA concentration was determined by measuring the absorbance at 260 nm UV light
Instrument and method	E	NanoDrop ND-1000 (NanoDrop Technologies)
Purity (A260/A280)	D	RNA purity was determined by measuring the absorbance ratio 260/280
RNA integrity: method/instrument	E	AATI Fragment Analyzer (Agilent Technologies)
RIN/RQI or Cq of 3' and 5' transcripts	E	All samples had an RNA integrity score greater than 8.0
Electrophoresis traces	D	
Inhibition testing (Cq dilutions, spike, or other)	E	The standard curve has been considered sufficient to rule out the presence of inhibitors of the reverse-transcription activity or PCR
		Reverse transcription
Complete reaction conditions	E	RNA isolated from whole blood was used for RT-qPCR analysis. The cDNA synthesis was performed using 100 ng of RNA standardized by dilution in nuclease-free water. The total RNA was mixed using a first mix (Mix 1) containing 1 µL random primers (Roche Applied) and 9 µL nuclease-free water. The RNA+Mix 1 was incubated at 65°C for 5 min and

		kept on ice for 3 min. A second mix (Mix 2) consisted of 4 µL 5x First-Strand Buffer (Thermo Fischer Scientific), 1 µL Oligo dT18 (Integrated DNA Technologies), 2 µL 10 mmol/L dNTP mix (Invitrogen), 0.25 µL 200 IU/µL of Revert aid (Thermo Fischer Scientific), 0.125 µL 20 U/µL of RNase inhibitor (Thermo Fischer Scientific), and 1.625 µL nuclease-free water. After adding Mix 2 to the RNA+Mix 1 sample, the reaction was performed in a Mastercycler Gradient (Eppendorf) using the following temperature program: 25 °C for 5 min, 42 °C for 60 min, and 70 °C for 5 min. cDNA was then diluted 1:4 with DNase/RNase-free water.
Amount of RNA and reaction volume	E	Amount of RNA: 100 ng; Reaction volume: 20 µL
Priming oligonucleotide (if using GSP) and concentration	E	Not applicable
Reverse transcriptase and concentration	E	Revert aid (Thermo Fischer): 2.5 IU/µL
Temperature and time	D	Specified in "Complete reaction conditions-Reverse transcription”
Manufacturer of reagents	D	Specified in "Complete reaction conditions-Reverse transcription”
Cqs with and without reverse transcription	D	
Storage conditions of cDNA	D	-20°C

778

		qPCR protocol
Complete reaction conditions	E	Quantitative PCR was performed in a MicroAmp Optical 384- Wel Reaction Plate (Applied Biosystems) using 4 µL diluted 1:4 cDNA and 6 µL SYBR Green mixture (Applied Biosystems) with 0.4 µL 10 µM forward and reverse primers, and 0.2 µL nuclease-free water. Each gene was run in triplicate on a single plate with a 7-point standard curve plus the negative control.
Reaction volume and amount of cDNA/DNA	E	Reaction volume: 10 µL; amount of cDNA: 1:4 dilution.
Additives	E	Specified in "Complete reaction conditions-qPCR protocol"
Manufacturer of plates/tubes	D	Specified in "Complete reaction conditions-qPCR protocol"
Complete thermocycling parameters	E	2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C (denatur and 1 min at 60 °C (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95 °C for 15 s plus 65 °C for 15 s.
Reaction setup (manual/robotic)	D	Robotic
Manufacturer of qPCR instrument	D	Specified in "Complete reaction conditions-qPCR protocol"
		qPCR validation
Evidence of optimization (from gradients)	D	
Specificity (gel, sequence, melt, or digest)	E	Melting curve analysis, ramping from 55°C to 95°C, where fluoresc data are measured continuously (measured melting temperature val are provided as supplementary data). Gene-specific amplification was confirmed by a single band in 2% agarose gel electrophoresis stained with ethidium bromide. No template controls (no cDNA in PCR) were run for each gene to detect unspecific amplification and primer dimerization.
For SYBR Green, Cq of the NTC	E	The signal of the amplification plot never crossed the threshold (Cq > 40) and therefore, there was a high Cq value difference betw the negative control and all the cDNA sample.
Calibration curves with slope and y	E	The slopes are provided in Supplemental Table S2

intercept		
PCR efficiency calculated from slope	E	Provided in Supplemental Table S2
Cis for PCR efficiency or SE	D	
r2 of calibration curve	E	Provided in Supplemental Table S2
		Data analysis
qPCR analysis program	E	Specified in "Complete reaction conditions-qPCR protocol"
Method of Cq determination	E	The threshold is determined using the Amplification-based Threshold method. The threshold is used to specify Cq values of samples
Outlier identification and disposition	E	None of the Cq values were discarded
Results for NTCs	E	The signal of the amplification plot never crossed the threshold (Cq > 40), and therefore there was a high Cq value difference between the negative control and all the cDNA sample.
Justification of number and choice of reference genes	E	The use of *GAPDH*, *RPS9,* and *ACTB* as reference genes for whole blood leucocytes gene expression has been successfully used by Lopes et al. (2020, J. Dairy Sci. 104:2266-2279), Vailati-Riboni et al. (2019, J. Dairy Sci. 102:8343-8351) and Lopreiato et al. (2019, J. Dairy Sci. 102:10395-10410)

Description of normalization method	E	As described in the main body
Number and concordance of biological replicates	D	
Number and stage (RT or qPCR) of technical replicates	E	qPCR reactions were performed in triplicate
Repeatability (intraassay variation)	E	The mean coefficient of variation of triplicates was lower than 5%
Reproducibility (interassay variation, CV)	D	
Power analysis	D	
Statistical methods for results significance	E	As described in the main body
Software (source, version)	E	As described in the main body
Cq or raw data submission with RDML	D	
		qPCR target information
Gene symbol	E	Provided in Supplemental Table S2
Sequence accession number	E	Provided in Supplemental Table S2
Location of amplicon	D	
Amplicon length	E	As reported in Zhou et al. (2018, J. Dairy Sci. 101:10374-10382)
In silico specificity screen (BLAST, and so on)	E	As reported in Zhou et al. (2018, J. Dairy Sci. 101:10374-10382)
Pseudogenes, retropseudogenes, or other homologs?	D	
Sequence alignment	D	
Secondary structure analysis of amplicon	D	
		qPCR oligonucleotides
Primer sequences	E	Provided in Supplemental Table S2

RTPrimerDB identification number	D	
Probe sequences	D	
Location and identity of any modifications	E	No modifications were done
Manufacturer of oligonucleotides	D	IDT Oligo
Purification method	D	Desalted

*E = Essential information; D = Desirable information.

5. Considerações Finais

As primeiras semanas de vida das bezerras são fundamentais para o desenvolvimento do trato gastrointestinal, visto que os ruminantes não nascem com o mesmo plenamente desenvolvido. Então estratégias como a suplementação com butirato se tornam uma aliada para acelerar este processo e mimetizar possíveis transtorno que possam a vir causar algum atraso no desenvolvimento destes animais.

Com isso a partir dos dados observados neste trabalho é possível concluir que a suplementação com butirato de forma contínua na dieta líquida melhora o desenvolvimento e a maturação ruminal e duodenal, reduz a morbidade e as recidivas de diarreia, além de reduzir os dias com fezes anormais. Ainda, a expressão dos genes *LCT* e *GLP2* sugere que o desenvolvimento e a maturação do epitélio intestinal, assim como o consumo de dieta sólida não foram prejudicados nos animais do GB que tiveram diarreia neonatal.

O conjunto desses resultados indica que a suplementação de bezerros leiteiros com butirato além de estimular o desenvolvimento do trato gastrointestinal, reduz os prejuízos associados à diarreia, refletindo em um melhor ganho médio diário de peso e um acelerado desenvolvimento, tornando-se uma alternativa para aumentar melhorar a eficiência desta fase de criação.

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Anexos

Anexo I - Documento da Comissão de Ética e Experimentação Animal

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **44/2020/CEEA/REITORIA**
PROCESSO Nº 23110.009466/2020-81

Certificado

Certificamos que a proposta intitulada **"Uso de butirato na prevenção de diarreias neonatais e desenvolvimento do trato gastrointestinal de bezerras leiteiras "**, registrada com o n° 23110.009466/2020-81**,** sob a responsabilidade de **Viviane Rohrig Rabassa** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **03 de junho de 2020**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	01/07/2020 a 01/06/2022
Espécie/linhagem/raça	Bovina/Holandês
N° de animais	112
Idade	0-90 dias
Sexo	12 machos e 100 fêmeas
Origem	Granja 4 irmãos, Rio Grande/RS

Código para cadastro nº **CEEA 9466-2020**

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 05/06/2020, às 09:59, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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